UNIVERSITY OF ZULULAND

An ethnobotanical survey, antimicrobial activity and toxicity evaluation of plant species used for blood purification by rural communities in northern Maputaland, South Africa

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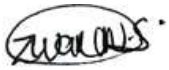
DECLARATION

The research described in this dissertation was carried out in the Department of Botany at the University of Zululand, KwaDlangezwa, under the supervision of Prof. H. de Wet and Prof. S.F van Vuuren. This study has not been submitted for any degree or diploma at any University. Where use has been made of work of others, it is duly acknowledged in the text.



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Nontobeko Zwane

Date: 22 June 2021

I certify that the above statement is correct

Prof H de Wet

Supervisor

Date: 28 June 2021

DEDICATION

This work is dedicated to the memory of my grandmother. Ntombi yaseMabomvini, I thank you for the wonderful family you have provided for me; a loving family that believes, supports and encourages my abilities. I will forever hold the conversations, advice and moments we had, dearly in my heart. Rest in eternal peace Sophathwa! To my Mother, Bhekisephi Xulu, for being my inspiration through the journey of life, for constant love and support. My aunts, Zesulile Zulu and Ntwenhle Xulu, thank you for the moral support and endless love. To my siblings (Khonqo, Nono, Lungelo, Fano, Malibongwe and Sizo) for giving me the burden to lead by example, I hope I am doing good and exceeding your expectations and not your capabilities.

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ABSTRACT

A blood purifier is defined as a substance that removes toxins from the bloodstream via a process known as detoxification. Blood purification (cleansing) is highly crucial for the entire body functioning as the body depends on healthy blood. In South Africa herbal remedies, herbal teas, spices and traditional medicine are all used for the purification of blood. The use of different supplements to purify or strengthen the blood are available in pharmacies and informal trading centres without any prescription from a doctor. Medicinal plant species used for blood purification are a large component of rural peoples' *Materia Medica*. This can be a concern as very few of these products and plant species have been scientifically tested for safety and efficacy. Therefore, ethnobotanical survey was conducted on blood purification plant species used in rural areas of northern Maputaland. Furthermore, an investigation into laypeoples' concept of blood purification and the respective plant species used were investigated. A total of 55 key informants were chosen purposively and interviewed using a structured questionnaire. The information gathered during the interviews was documented along with the plant voucher specimens. Available plant samples were collected for extraction. The study found that the laypeople in northern Maputaland use blood purification as a dual strategy for health care reasons. This technique is used for the prevention and treatment of diseases caused by various pathogens (skin infections, oral infections, urinary tract infections) and spiritual complications. The study documented 65 plant species with *Bridelia cathartica, Catunaregam obovata, Cymbopogon marginatus, Sclerocarya birrea, Senecio serratuloides* and *Terminalia sericea* being the most frequently cited plant species within these communities. Twenty-two plant species and 32 sets of plant species combinations were recorded for the first time as blood purifiers. There were five new vernacular (IsiZulu) names mentioned by the participants (*isikwakwane, upata, umhlakuva, umbungwa* and *uvemvane*) that were not found in literature previously. Most blood purification remedies were prepared as decoctions, and taken orally before breakfast.

Aqueous and organic (1:1 dichloromethane methanol) extracts were prepared from 58 plant samples that were available for collection in northern Maputaland. Antimicrobial

screening using the minimum inhibitory concentration (MIC) assay was performed on selected bacterial pathogens involved in blood-related infections, i.e. *Acinetobacter baumannii*, *Cutibacterium acnes*, *Listeria monocytogenes*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Streptococcus agalactiae* and *S. pyogenes*. The most active aqueous extract came from *Albertisia delagoensis* root with a noteworthy inhibition at 0.13 mg/ml MIC value against *C. acnes*. Whereas the most active organic extract was *Ozoroa engleri* with the MIC values of 0.004 mg/ml against *C. acnes*, 0.01 mg/ml against *S. agalactiae* and 0.02 mg/ml against *L. monocytogenes, S. aureus* and *S. pyogenes* respectively. *Gymnosporia senegalensis* and *O. engleri* had the broadest spectrum of antimicrobial activities with notable MIC values against four and five pathogens respectively. It was worth noting that the antimicrobial activity of *A. delagoensis* and *Sapium integerrimum* was evaluated for the first time and both have significant activities (MIC 0.16 mg/ml) against *C. acnes*.

The antimicrobial activity of 308 plant combinations was evaluated using sum of the fractional inhibitory concentration index (ΣFIC). Once the ΣFIC of plant species combined in equal ratios was calculated, the combinations exhibited synergistic (61%), additive (16%), non-interactive (49%) and antagonistic (29%) effects. The broadest synergistic effect was displayed by *Abrus precatorius* + *B. cathartica* root extracts, where the combination was synergistic against *C. acnes, L. monocytogenes, S. aureus, A. baumannii* and *P. aeruginosa*. The most synergistic effect was displayed by *S. integerrimum* + *Strychnos spinosa* roots having, the ΣFIC value of 0.33, against *P. aeruginosa*.

The toxicity of the plant species was evaluated using the brine shrimp lethality assay (BSLA). When plant species were evaluated individually, the aqueous extracts had five plant species showing potential toxicity (*Dichapetalum cymosum, Ekebergia capensis, Gardenia volkensii, Trichilia emetica* and *Waltheria indica*) and 12 organic extracts demonstrated toxicity. When dilutions of toxic extracts were made (1.00 mg/ml, 0.50 mg/ml, 0.25 mg/ml, 0.13 mg/ml, 0.06 mg/ml and 0.03 mg/ml) it was observed that the toxicity of extracts decreased with lower concentrations. For the evaluation of the 22

different sets of combinations tested, two combinations (*A. delagoensis* + *Pyrenacantha kaurabassana* and *D. cymosum* + *Euclea natalensis*) displayed consistent toxicity in both aqueous and organic extracts. Six combinations portrayed increased toxicity compared to when these plant species were tested individually.

Overall, the results demonstrated that the use of medicinal plant species for infectious diseases related to blood purification is somewhat valid, since 35% of the plant extracts had noteworthy activity against some of the pathogens. The plant extracts displayed high antimicrobial activity against *C. acnes*, which for 80% of the time correlates with the traditional use reported by the participants. The results also demonstrated that the combination of plant species does not always guarantee increased efficacy, and that not all medicinal plant species are safe for use. This study served to provide an informative view to the concept of blood purification. It is clear that when looking into blood purification, ailments like acne and other skin disorders should not be disregarded. The role that blood purification play in laypeoples' rituals emphasise that holistic and spiritual healings of the laypeople should also be considered.

CONFERENCE PRESENTATIONS

Oral presentation: An ethnobotanical survey of plant species used for blood purification by rural communities in northern Maputaland, South Africa. Faculty of Science and Agriculture Symposium. University of Zululand, 17 October 2019 (Appendix A for abstract).

Oral presentation: An ethnobotanical survey and toxicity study of plant species used for blood purification by rural communities in northern Maputaland, South Africa. South African Association of Botanists annual conference. University of Free State, Qwaqwa campus, 7 – 10 January 2020 (Appendix B for abstract).

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ABBREVIATIONS AND ACRONYMS

Aq	Aqueous
ATCC	American type culture collection
BSLA	Brine shrimp lethality assay
CFU	Colony forming units
CNS	Central nervous system
D:M	Dichloromethane: methanol
DCM	Dichloromethane
DMSO	Dimethyl sulfoxide
FIC	Fractional inhibitory concentrations
g	Grams
GPS	Global positioning system
Gr.	Grade
GBS	Group B *Streptococcus*
hrs	Hours
INT	*P*- iodonitrotetrazolium salt
MeOH	Methanol
mg/kg	Milligrams per kilogram
mg/ml	Milligrams per millilitre
MIC	Minimum inhibitory concentration
min	Minutes
ml	Millilitres
mm	Millimetres
N/A	No analysis
º C	Degrees Celsius
SANBI	South African National Biodiversity Institute
TSA	Tryptone Soya agar
TSB	Tryptone Soya broth
v/v	Volume per volume
µg/ml	Micrograms per millilitre

µl	Microliter
ΣFIC	Sum of the fractional inhibitory concentration index

CHAPTER 1

INTRODUCTION

1.1 BACKGROUND

The present study is one of many studies conducted under the project S378/08 registered by Professor H. de Wet. The project was designed to document indigenous knowledge that laypeople in rural communities (northern Maputaland) have about medicinal plant species used to treat various illnesses and to evaluate their possible toxicity and efficacy. The published papers under this project included a study on antidiarrheal plant species (De Wet *et al*., 2010); plant species used to treat respiratory infections (York *et al*., 2011); plant species used to treat sexually transmitted infections (De Wet *et al*., 2012); plant species used to treat various skin disorders (De Wet *et al*., 2013); herbal remedies used by women for the treatment of gynaecology and obstetrics complaints (De Wet and Ngubane, 2014) and plant species used to treat hypertension (De Wet *et al*., 2016). The idea of a study that focuses only on blood cleansing plant species came from the numerous reporting of these plant species in the previous surveys. The information gained from these studies is evidence that laypeople are still very much dependent on medicinal plant species for their primary health care. With each publication, numerous new plant species were documented for the first time to treat the above-mentioned ailments. This indicated that there is possibly still a wealth of indigenous plant knowledge not recorded in rural communities of northern Maputaland. As a biodiversity hotspot, this area has a high concentration of endemic plant species, which make the richness of vegetation in this area unique (Boon, 2010).

There has been no previous study focusing on plant species used for blood purification in northern Maputaland or KwaZulu-Natal. However, recently there has been a review of South African medicinal plant species used as blood purifiers (Van Vuuren and Frank, 2020). There is limited information about plants used for blood purification. Consequently ethnopharmacologists can not evaluate these plant species due to limited background

information. Scientific evaluations need to be done on blood purifier plant species, especially to test if they have any antimicrobial activities, which could validate their use. Concurrently with efficacy evaluation, it is also important to investigate their potential toxicity to humans.

1.2 DESCRIPTION AND HISTORY OF BLOOD PURIFICATION

It is noticeable from the surveys done on medicinal plant species used by the different cultures in South Africa that the perception of how people see and understand what a blood purification plant is, is not clear. A blood purifier, also known in traditional folk medicine as an alterative or depurative is defined as a substance that removes toxins from the bloodstream via a process recognised as detoxification (Smith, 2013). The context of blood purification often seems to be associated with allopathic medicine where specialized equipment is used to specify known hormonal, blood lipid and chemical factors (haemodialysis and haemofiltration). Even if this equipment filters the blood toxins, they cannot compare to traditional medicine since they are not designed to cover holistic healing (Akter *et al*., 2012). Blood impurities are agents that imbalance the immune system. Current medical tests cannot determine subclinical levels of blood toxin conditions. Meanwhile, Traditional Health Practitioners and herbalists diagnose blood infections by symptoms related to various skin conditions, weakened immune system, headaches, arthritis, fatigue and urinary tract infections (Chauhan, 2013). Therefore, blood purifiers have been observed as medications taken for both therapeutic purposes, as well as for general health (Akter *et al.,* 2012).

Blood purification is a practice that has been carried out traditionally for thousands of centuries, especially in the Ayurvedic health system (Akter *et al*., 2012). One of the patent medicines in the 1800s was Sarsaparilla (from the root of *Smilax* sp.), which was used as a blood purifier and was used to treat various dermatological disorders, including scrofula, tetter (impetigo, herpes zoster and herpes simplex), sores, eczema (humid tetter) and boils (Albert, 2000). In the 1900s, medicines were classified into several broad categories, which included specific cures, blood purifiers, tonics, bitters, and women's medicines

(Albert, 2000). In a textbook written by GH Fox in 1900 entitled “Photographic Atlas of the Diseases of the Skin” the author point out that many skin diseases result from “impurities of the blood”. These impurities are toxins that accumulated in the blood through improper diets, irregular bowel movements, pollution, obesity and lack of physical activity (Nisha, 2015). These toxins in the stomach may be transported to other organs of the body through blood circulation and cause blood and other infections (Akter *et al*., 2012). The toxins in the blood may be as a result of bacteria, viruses or heavy metals that may cause disorders such as paralysis, skin infections, kidney/urinary tract infections, heart disease and sepsis (Rimmelé and Kellum, 2011). The common cause of sepsis is the presence of bacteria in the blood (bacteremia), however, infections like wounds, insect bites, dental extractions and surgery may also result in sepsis (Luo, 2017).

The liver, kidneys and lymphatic system interact to purify toxins (Akter *et al*., 2012). However, there are times when external factors are accumulated in too high concentrations for purifying organs to purge the toxins, and as a result, illnesses arise (Chauhan, 2013). In such instances, blood purification methods may include blood cleansing food, tea or juices, or traditional and herbal blood purifiers to sustain and improve the functioning of the blood purification organs (Kerkar, 2018). Although the perception of “impure blood” seems to be the reason for medical consultation, there are limited studies on blood purification. One of these studies is from Bangladesh, where Akter *et al*. (2012) documented 149 medicinal plant species used as blood purifiers by folk medicinal practitioners. Chauhan (2013) documented 13 blood purifying plant species that are commonly used in different herbal formulations sold in Indian markets.

1.2.1 Bloodletting

Bloodletting is the method that has been used for many centuries by surgeons and Traditional Health Practitioners for blood purification. In some countries this method is still active. According to De Smet (1998), the indigenous people of central Africa resorted to this practice to treat headaches, fever, pneumonia, and pleurisy (inflammation of the lining

of the lungs), and even for painful ulcers. The four known forms of bloodletting are scarification, cupping, venesection, and leeching (De Smet, 1998).

During scarification the practitioner marks incisions on the skin of the area where there is an alleged problem with a razor blade or anciently the sharp edge of a stone or bone. Crushed medicine (mostly herbals) is sometimes rubbed into the incision, presumably for direct immersion of the active constituents of the drug through the blood vessels (De Smet, 1998). For cupping, horns were used which consist generally of an antelope, cow or goat horn, but in certain areas the calabash fruit is also used. The cup/horn is pressed to the surface of the affected skin by using a suction-like force. This is followed by removing the cup and creating superficial incisions by a knife. Later the cup is replaced, and the procedure is repeated until some blood is drained (Ghods *et a*l., 2016). De Smet (1998) documented that this is practiced because of a belief that the pain is caused by poisoned blood that needs to be removed from the clean blood.

Venesection (phlebotomy) is another procedure of bloodletting. Blood vessels, commonly hand veins (and sometimes elbow or knee veins) are cut with a knife for direct blood drainage (Ghods *et al*., 2016). Ancient instruments used for cutting include anything sharp, such as horns, thorns, stones, animal teeth or quills (Parapai, 2008). This method is believed to heal illnesses by eliminating impure fluids, particularly blood. Venestction is used for treating fever, eye complaints, convulsive disorders, insomnia, anxiety, headache, a pounding pulse, inflammation of lungs and heavy breathing (Parapai, 2008). This procedure is monitored by observing the rhythm of the patient's pulse, if any change occurs on pulse performance, the procedure must be stopped immediately (Parapai, 2008).

In the late 20th and 21st century leech therapy was popular and used to relieve venous congestion and to maintain blood flow (Porshinsky *et al*., 2011). Leeches are smeared in a mixture of mustard and turmeric, to disinfect and enhance its appetite for sucking blood prior to the procedure. The blood leeching procedure is performed by removing the leech from the jar with its mouth accurately projected to the affected area (wound, swelling, gout

or for blood purification) and are removed once they suck out all “toxic blood”. Leeches endorse healing by allowing fresh oxygenated blood to spread until normal circulation can be restored. This will reduce muscle swelling when blood is sucked out of the circulatory system (Kumar *et al*., 2012).

1.3 ETHNOBOTANICAL SURVEYS

When scrutinising ethnobotanical surveys done worldwide, there is often a few plant species specifically mentioned for blood purification. Some examples include surveys from India, Kerala province, where *Cassia absus* L., *Cynodon dactylon* (L.) Pers. and *Ziziphus nummularia* (Burm.f.) Wight & Arn. were mentioned for blood purification (Sureshkumar *et al.*, 2017). In Pakistan in the Karamar valley, *Bauhinia variegata* Linn., *Chenopodium album* L., *Cuscuta reflexa* Roxb., *Fagonia arabica* L. and *Fumaria parviflora* L. were documented (Khalid *et al*., 2017). In Iran, south of Kerman *Berberis integerrima* Bunge, *Cichorium pumilum* Jacq., *Sageretia thea* (Osbeck) M.C.Johnst. and *Trifolium pretense* L. were documented (Sadat-Hosseini *et al.*, 2017). Alonso-Castro *et al*. (2016) documented that plant species that were perceived to purify the blood and strengthen the body in Mexican ancient traditional medicine are now referred to as immune-stimulants. These authors documented 104 plant species that are used as immune-stimulants in Mexico, Central America and the Caribbean.

A similar trend is observed in South Africa with ethnobotanical studies. In a study done in Namaqualand on over a hundred recorded plant species, nine plant species (*Aloe ferox* Mill, *Cassytha ciliolata* Nees, *Conyza scabrida* DC., *Galenia africana* L., *Helichrysum odoratissimum* Sweet, *Limeum africanum* Zeyh. ex Moq., *Melianthus pectinatus* Harv., *Parmelia* sp. and *Pteronia camphorata* (L.) L.*)* were documented for blood purification. This area is dominated by Khoisan and Nama culture and these plant species are mostly part of their *Materia Medica* (Nortje and Van Wyk, 2015). Also in this region, a study by Van Wyk (2008) on Cape Duch *Materia Medica* five plant species were recorded for blood purification including *Arctopus echinatus* L., *Arctopus monacanthus* Carmichael ex Sond., *Bulbine alooides* (L.) Willd., *Bulbine lattfolia* (L.f.) Roem. & Scholt. and *Lobelia pinifolia* L.

In the south-eastern Karoo three (*Cissampelos capensis* L.f., *Limeum aethiopium* Burm.f. and *Sutherlandia microphylla* Burch. ex DC.) of the 86 plant species used for medicinal purpose are used specifically to cleanse the blood (Van Wyk *et al.*, 2008). In an ethnobotanical survey done in the Little Karoo of South Africa, 196 plant species were recorded and 10 of these plant species (*Aloe ferox* Mill, *A. echinatus*, *Chironica baccifera* L., *Cichorium intybus* L., *Euclea undulate* Thunb., *Kedrostis nana* Cogn., *Lessertia frutescens* (L) Goldblatt & J.C Manning, *Pegolettia baccharidifolia* Less. and *Tulbaghia capensis* L.) were reported as blood purifiers (Hulley and Van Wyk, 2017). In Venda (Limpopo Province) out of 12 plant species evaluated for antimicrobial properties, only *Bolusanthus speciosus* Harms was mentioned for blood purification purposes (Mulaudzi *et al*., 2011). De Beer and Van Wyk (2011) did an ethnobotanical survey in the Northern Cape Province and recorded 64 medicinal plant species with only *Hoodia gordonii* Sweet documented as a blood purifier plant.

Two traditional Muthi markets in Durban, KwaZulu-Natal, sell four plant species (*Aloe aristata* Haw., *A. ferox*, *Artemisia afra* Jacq. and *Haworthia limifolia* Marloth) for blood purification (Coopoosamy and Naidoo, 2012). Also in this region Ndhlala *et al*., (2011a) conducted a study on commercial herbal mixtures and the three mixtures specifically used for blood purification were *Ingungumbane mahlabizifo* (*Sutherlandia frutescens* R.Br., *Aloe ferox* Mill., *Hypoxis hemerocallidea* Fisch., C.A.Mey. & Avé-Lall. and other ingredients), *Stameta* (*H. hemerocallidea, Mentha piperita L., Pimpinella anisum* L. and unspecified *Aloe* species) and *Vuka uphile* herbal remedies with unknown ingredients.

In 1996, Anne Hutchings published a book entitled “Zulu Medicinal Plant species: An Inventory”. The book revealed 1032 plant species (representing approximately 25% of KwaZulu-Natal’s flora) of which 73 are mentioned to purify or cleanse the blood as shown in Table 1.1 (Hutchings *et al.*, 1996). The last mentioned information stresses the importance of blood purification plants in the Zulu culture of medicinal plant practice. It is important to mention that Hutchings concentrated on plant species used by Traditional Health Practitioners and not on laypeople and that she did not conduct any interviews in northern Maputaland.

De Wet and Ngubane (2014), conducted an ethnobotanical survey in northern Maputaland documenting medicinal plant species used by women for the treatment of gynaecology and obstetric complaints. They documented 32 plant species of which 15 were reported for blood purification purposes. The documented blood purifier plant species are *Bridelia cathartica* G.Bertol., *Commiphora neglecta* Verdoorn, *Crotalaria monteiroi* Taub. ex Baker f. var. *galpinii* Burtt Davy, *Euclea natalensis* A.DC., *Garcinia livingstonei* T.Anderson, *Grewia occidentalis* L., *Kigelia africana* (Lam.) Benth., *Ochna natalitia* Walp., *Opuntia stricta* (Haw.), *Ozoroa engleri* R.Fern & A., *Peltophorum africanum* Sond., *Ranunculus multifidus* Forssk., *Rhoicissus digitata* (L.f.) Gilg & M.Brandt, *Senecio serratuloides* DC. and *Trichilia emetica* Vahl.

1.4 BLOOD PURIFIER HERBS AND PRODUCTS

For blood purification most modern families don't consult Traditional Health Practitioners, but rather prefer herbs and herbal products being sold over the counter under food supplements. The common concept of blood purification world wide had made the blood purifier products to be easily accessable. Figure 1.1 illustrate some of the herbal products that are available globally for the purification of blood. The product *Purim,* from Himalaya Herbals (India), is an Ayurvedic herbal formulation which helps to sustain skin health using broad and systemic blood purification systems. *Purim* is made of herbs with powerful natural anti-oxidants that support healthy liver and blood cells, skin health and skin immunity. The herbs in *Purim* tablets are believed to eradicate toxins from the body and keep a healthy skin. Another over the counter product, *Banyan Botanicals Blood Cleanse* (Oregon, United States of America)*,* is a combination of five medicinal plant species (*Arctium lappa* L., *Azadirachta indica* A.Juss, *Curcuma longa* Linn., *Rubia tinctorum* L. and *Tinospora cordifolia* Miers) which can get rid of toxins from the blood, lymph, and liver with the aim to provide a vibrant and healthy skin. The Matla Blood Cleanser product (South Africa) assists to cleanse, detoxify and to purify the body. It contains *A. ferox*, *Sutherlandia* spp. and *Taraxacum officinale* F.H.Wigg.

Table 1.1 The list of plant species mentioned under the classification of blood purifiers in literature

Plant name	Vernacular name (IsiZulu)	Plant part used	Disease and preparation	References
Acacia caffra Willd.	Umthole/ umtholo	Bark	Bark infusion is taken as a blood cleansing emetic.	Watt and Breyer-Brandwijk, 1962; Hutchings *et al*., 1996
Acalypha peduncularis E.Mey.	Usununundu	Root	Infusion made from roots are taken as a tonic.	Watt and Breyer-Brandwijk, 1962; Hutchings *et al*., 1996
Acokanthera oppositifolia (Lam) Codd	Inhlungunyembe/ubuhlungu benyoka/ umkhwangu/ umhlagaliso	Leaves	Leaf decoction is taken for stomach-ache, snake bites, spider bites, blood poisoning and septic spots.	Mabogo, 1990; Hutchings *et al*., 1996
Aloe maculata Forssk.	Icena	Root	Root decoction are taken to treat skin disorders internally.	Watt and Breyer-Brandwijk, 1962; Hutchings *et al*., 1996
Artemisia afra Jacq.	Umhlonyane/ umhlonyane omncane	Leaves	Decoction is taken as blood purifiers for acne and boils.	Hutchings *et al*., 1996
Athrixia phylicoides DC.	Icholocholo/ itshelo/ umtshanela	Whole plant	Plant infusion is taken as blood purifier for sores and boils.	Watt and Breyer-Brandwijk, 1962; Hutchings *et al*., 1996
Brachypetala discolor DC.	Iphahla/ isisduli/ isiphanga/ umduli/ umphahla	Leaves	Leaf infusion is taken as a tonic.	Palmer and Pitman, 1972; Hutchings *et al*., 1996

Plant name	Vernacular name (IsiZulu)	Plant part used	Disease and preparation	References
Callilepis laureola DC.	Ihlamvu/ impila	Root	Roots are taken as a tonic by young girls in early stages of menstruation.	Hutchings *et al*., 1996
Capparis tomentosa Lam.	Inkunzi ebomvu/ iqwaningi/ ukokwane/ umasubane/ umqoqolo	Root bark	Root bark is an ingredient in a blood purifying decoction known as *imbiza*, a term used for enemas taken to cleanse the body.	Pujol, 1990; Hutchings *et al*., 1996
Catharanthus roseus (L.) G.Don	Ikhwinini	Flowers, root	a) Tea made from flowers is used for blood cleansing and milky sap is used for insect bites and warts. b) Roots are used for toothache, liver congestion and scurvy skin complaints. It is also used as a tonic, haemostatic and vermifuges.	Roberts, 1983; Mabogo, 1990; Hutchings *et al*., 1996
Cephalaria zeyheriana Szabó	Uzondle	Root	The decoction from roots is used as blood purifiers, possible against syphilis, swelling and pains.	Watt and Breyer-Brandwijk, 1962; Hutchings *et al*., 1996
Chenopodium album L.	Isijabane/ imbikilicane	Whole plant	a) Used by Xhosa's as blood purifier for a condition reported to be characterized by red lips. b) The Tswana traditionally eat cooked plant species once a week as blood purifier and to keep the stomach "working well". Seed	Watt and Breyer-Brandwijk, 1962; Hutchings *et al*., 1996

Plant name	Vernacular name (IsiZulu)	Plant part used	Disease and preparation	References
			paste is applied to skin diseases in India.	
Chrysanthemoides monilifera (L.) Norl.	Inkhupuyane/ itholonja/ umthombothi	Fruits	Small frequent doses of juice from fruit are reported to be administered by Zulu/Xhosa/Sotho as blood strengtheners and purifiers to men suffering from impotence or weakened by intestinal ailments.	Roberts, 1983; Hutchings *et al*., 1996
Clausena anisata Hook.f., De Wild. & Staner	Umsanka/ umnukambiba/ isifuthu	Leaves	Leaves are made into tea taken to strengthen and purify blood, for liver diseases and for bad breath.	Pujol, 1990; Hutchings *et al*., 1996
Clutia heterophylla Sond.	Ubuhlungu bedila	Leaves, root	Decoction of root and leaves with unspecified parts of an *Aloe* sp. and *Ptaeroxylon obliquun* are used for anthrax.	Gerstner, 1938; Hutchings *et al*., 1996
Clutia hirsuta Eckl. & Zeyh. ex Sond.	Ubuhlungu bedila/ ungwaleni	Leaves, root	Decoctions of roots and leaves with unspecified parts of an *Aloe* sp. and *Ptaeroxylon obliquun* are used for anthrax.	Gerstner, 1938; Hutchings *et al*., 1996
Commiphora zanzibarica Engl.	Iminyela/ umthekweni omncane	Root	Decoctions from grounded roots, mixed with those of *Cladostemon kirkii* are taken for boils.	Pooley, 1998; Hutchings *et al*., 1996

Plant name	Vernacular name (IsiZulu)	Plant part used	Disease and preparation	References
Crabbea hirsuta Harv.	Umusa	Whole plant	Plant species are used for toothache, blood-poisoning from anthrax and to disinfect anthrax-infested meat.	Watt and Breyer-Brandwijk, 1962; Hutchings *et al*., 1996
Crinum sp.	Umduze	Root	Decoction used as blood purifier for treatment of scrofula.	Bryant, 1966; Hutchings *et al*., 1996
Curtisia dentata (Burm.f.) C.A.Sm.	Igejalibomvu/ umhlibe/ umlahleni/ ijundu mhlahleni/ inkunzithwalitshe	Bark	Bark is used for stomach ailments including diarrhoea and also as blood strengthener and aphrodisiac.	Pujol, 1990; Hutchings *et al*., 1996
Cymbopogon validus Stapf ex Burtt Davy	Isicunge/ isiqunga	Root, shoots	They are used in milk decoctions, taken in doses of two small cups twice a day to strengthen the blood and nervous system.	Watt and Breyer-Brandwijk, 1962; Hutchings *et al*., 1996
Dicoma anomala Sond,	Isihlabamakhondlwane/ umuna	Root	Root decoctions are administered orally or as enemas to children believed to be suffering from blood disorders.	Watt and Breyer-Brandwijk, 1962; Hutchings *et al*., 1996
Dicoma zeyheri Sond.	Isihlabamakhondlwane	Unspecified	Decoction is administered as blood strengtheners to mothers after long and difficult births.	Roberts, 1983; Hutchings *et al*., 1996
Dioscorea sylvatica Eckl.	Ingwevu/ ufudu	Tubers	Boiled tubers are taken for blood problems.	Watt and Breyer-Brandwijk, 1962; Hutchings *et al*., 1996

Plant name	Vernacular name (IsiZulu)	Plant part used	Disease and preparation	References
Ekebergia capensis Sparrm.	Isismanaye/ umathunzini/ umnyamathi/ uvungu	Bark	Ground bark paste is used on abscesses and boils or infused in hot water to steam for pimples, taken as blood purifying emetic and a wash.	Pujol, 1990; Hutchings *et al*., 1996
Elephantorrhiza elephantina (Burch.) Skeels	Intolwane/ umdabu/ ugweje	Root	Decoction made from roots is administered in small doses three times a day to cleanse the womb after women gave birth.	Pujol, 1990; Hutchings *et al*., 1996
Embelia ruminata Mez	Ibhinini	Leaves	Leaves are chewed as bitter tonic and consumed as refreshments.	Gerstner, 1938; Hutchings *et al*., 1996
Entada rheedii Spreng.	Inkwindi/ intindili/ umbhone	Seeds	Pounded seeds are taken as blood tonics.	Pujol, 1990; Hutchings *et al*., 1996
Eragrotis plana Nees	Umtshiki/ umvithi	Root	Decoction of roots mixed with those of *Rubia cordifolia* and *Conotricha* sp. are taken for profuse menstruation.	Bryant, 1966; Hutchings *et al*., 1996
Eriospermum sp.	Insulansula/ isidwanga	Tubers	Preparations from tubers of an unidentified *Eriospermum* species are taken as emetics to “clear out the liver” and are also administered by herbalists to married couples after miscarriage.	Watt and Breyer-Brandwijk, 1962; Hutchings *et al*., 1996

Plant name	Vernacular name (IsiZulu)	Plant part used	Disease and preparation	References
Euclea natalensis A.DC.	Ichithamuzi/ idungamuzi/ inkunzane/ umshekisane/ isinzimane	Root bark	Decoction from root bark and roots of *Polygala fruticosa*, *Raphionacme* sp. and bulbous roots of *Crinum* sp. and *Cyrtanthus obliquus* and root bark of *Zanthoxylum capense*, *Capparis tomentosa* and *Rauvolfia caffra* are used for blood purification.	Gerstner, 1938; Hutchings *et al*., 1996
Ficus natalensis Hochst.	Udende/ uluzi/ umdende/ umthombe/ isihlamfane	Root	Overnight root infusion is taken orally three times a day to cleanse the blood.	Pujol, 1990; Hutchings *et al*., 1996
Gnidia cuneata Meisn.	Isidikili	Unknown	Unspecified parts are used as blood purifiers for skin eruptions.	Watt and Breyer-Brandwijk, 1962; Hutchings *et al*., 1996
Gnidia sp.	Isilenge	Root bark	Root bark decoction is taken as blood purifiers and for boils.	Watt and Breyer-Brandwijk, 1962; Hutchings *et al*., 1996
Harpephyllum caffrum Bernh. ex Krauss	Umgwenya	Bark	Bark decoction is taken in doses of one/ two glasses daily or in large doses as emetics to purify blood and skin problems like acne and eczema.	Pujol, 1990; Hutchings *et al*., 1996
Helinus integrifolius Kuntze	Ubhubhubhu	Root	Emetic from root is used for bile and blood tonics.	Pujol, 1990; Hutchings *et al*., 1996

Plant name	Vernacular name (IsiZulu)	Plant part used	Disease and preparation	References
Heteromorpha trifoliata Eckl. & Zeyh.	Umbangandlala	Root	The Xhosa's use root for short of breath, coughs, dysentery, scrofula, colic, as blood purifier and for threadworm in horses.	Watt and Breyer-Brandwijk, 1962; Hutchings *et al*., 1996
Hybanthus enneaspermus F.Muell.	Ungqengendlela	Root	Root infusion is taken as blood purifying emetics.	Hulme, 1954; Hutchings *et al*., 1996
Ipomoea albivenia Sweet	Umangfongo	Root	Root decoction is taken orally or as enemas to purify the blood.	Watt and Breyer-Brandwijk, 1962; Hutchings *et al*., 1996
Ipomoea purpurea (L.) Roth	Uboqo/ ijalambu/ ijalapha	Leaves, stem	Stems are used as purgatives for stomach disorders and leaves and stem infusions are taken as blood purifying emetics.	Bryant, 1966; Hulme, 1954; Hutchings *et al*., 1996
Jatropha zeyheri Sond.	Ugodide	Shoots, tubers	Tuber decoction is used as blood purifiers. Young shoots are rubbed on sores.	Watt and Breyer-Brandwijk, 1962; Hutchings *et al*., 1996
Lagenaria sphaerica E.Mey.	Uselwa/ iselwa-makhosi/ uthangazane olukhulu	Root	Pounded root decoction is used for treating swelling thought to be caused by blood disorders.	Hulme, 1954; Hutchings *et al*., 1996
Linum thunbergii Eckl. & Zeyh.	Ithathelimpofu	Root	The roots are soaked in cold water and administered as an emetic for blood purification.	Hulme, 1954; Hutchings *et al*., 1996
Matricaria nigellifolia DC.	Ikhambi eliphofana/ umhlonyane/ umsolo	Leaves	Used by Xhosa and Mfengu people for treating skin rashes and anthrax poisoning in humans.	Watt and Breyer-Brandwijk, 1962; Hutchings *et al*., 1996

Plant name	Vernacular name (IsiZulu)	Plant part used	Disease and preparation	References
	womlambo/ umahlababesangwini			
Melianthus comosus Vahl.	Ibonya	Poultice	Poultice from the plant is applied to sores and snakebites.	Gerstner, 1938; Hutchings *et al*., 1996
Melianthus dregeanus Sond.	Ibonya	Leaves	Decoction made from leaves, with the addition of an unspecified plant are taken at night and in early morning to cleanse blood.	Watt and Breyer-Brandwijk, 1962; Hutchings *et al*., 1996
Momordica foetida Schumach.	Intshungu	Leaves, root	Decoction made from root or leaves, with the addition of *Pittosporum viridiflorum* and *Vernonia natalensis* are taken for boils.	Watt and Breyer-Brandwijk, 1962; Hutchings *et al*., 1996
Ochna holstii Engl.	Isibhanku	Bark	Herbalists use the bark as a tonic to purify blood.	Gerstner, 1938; Hutchings *et al*., 1996
Paretta sp.	Isimunyane/ isonyane	Leaves	Leaves are chewed as bitter tonic.	Gerstner, 1938; Hutchings *et al*., 1996
Pittosporum viridiflorum Sims	Umvusamu/ umfusamvu/ umkwenkwe	Bark	Bark decoction is taken in the morning to purify blood.	Watt and Breyer-Brandwijk, 1962; Hutchings *et al*., 1996
Polygala fruticosa P.J.Bergius	Ithethe	Root	Root is an ingredient in a blood purifying decoction known as *imbiza*, taken orally for blood cleansing and also to steam patients with scrofula.	Bryant, 1966; Hutchings *et al*., 1996

Plant name	Vernacular name (IsiZulu)	Plant part used	Disease and preparation	References
Polygala virgata Vell.	Ithethe/ uju lwezinyosi/ unohlonishwayo obomvu	Root	Infusion made from roots are taken as blood purifying emetic.	Hulme, 1954; Hutchings *et al*., 1996
Ptaeroxylon obliquum Radlk.	Umbhaqa/ umthathe	Root	Root decoction is administered in half-cup doses twice a day to purify blood.	Watt and Breyer-Brandwijk, 1962; Hutchings *et al*., 1996
Pterocelastrus rostratus Walp.	Usahlulamanye	Root	Root is used as a digestive tract and blood-cleansing purgative.	Bryant, 1966; Hutchings *et al*., 1996
Pterocelastrus tricuspidatus Walp.	Usahlulamanye	Root	Root is used as digestive tract and blood cleansing purgative.	Bryant, 1966; Hutchings *et al*., 1996
Raphionacme sp.	Umathangane/ umathanjana	Root	Root of unidentified *Raphionacme* sp. is an ingredient in blood purifying decoction taken for scrofula.	Bryant, 1966; Hutchings *et al*., 1996
Rauvolfia caffra Sond.	Umhlambanasi/ umjele/ umhlambamazi/ umkhabamasi	Root bark	Decoction made from root bark of *Zanthoxylum capense*, *Capparis tementosa* and *Euclea natalensis* are used for blood purification whereas roots/bulb of *Polygala fruticosa*, *Crinum* sp., *Cyrtanthus obliquus* and *Raphionacme* sp. are used in the treatment of scrofula.	Bryant, 1966; Hutchings *et al*., 1996
Rhamnus prinoides L'Hér.	Ulunyenye/ umgwenye/ unyenya/ umhlinye/ umgilindi	Root	Debarked root decoction is taken for blood purification.	Watt and Breyer-Brandwijk, 1962; Hutchings *et al*., 1996

Plant name	Vernacular name (IsiZulu)	Plant part used	Disease and preparation	References
Rhus chirindensis Baker f.	Inhlokoshiyane enkulu	Bark	Used to strengthen the body circulation and rheumatism.	Pujol, 1990; Hutchings *et al*., 1996
Rumex lanceolatus Thunb.	Idolo lenkonyane	Whole plant	The plant is one of the ingredients used for blood purifying remedies.	Hutchings *et al*., 1996
Schotia brachypetala Sond.	Umgxamu/ uvovovo/ ihlusi/ ihluze/ umxamo	Bark	Bark infusion are taken as emetics for pimples.	Hulme, 1954; Hutchings *et al*., 1996
Sclerocarya birrea Hochst.	Umganu	Bark	Bark decoction is used as an enema for malaria and diarrhoea, and are taken as teas twice a day to strengthen the heart or blood cleansing emetics before marriage.	Gerstner, 1938; Pujol, 1990; Hutchings *et al*., 1996
Senecio serratuloides DC.	Unsukumbili/ umaphozisa/ umkulelo	Leaves	Decoction from leaves is taken as blood purifier for skin eruptions.	Watt and Breyer-Brandwijk, 1962; Hutchings *et al*., 1996
Sphenostylis angustifolia Sond.	Ithethe	Leaves	The infusions made from leaves are administered for blood and stomach cleansing emetics.	Hulme, 1954; Hutchings *et al*., 1996
Stephania abyssinica Walp.	Umthombo omthambana/ umbombo	Root	Decoction from powdered root mixed with *Momordica foetida* are taken for boils and blood purification.	Watt and Breyer-Brandwijk, 1962; Hutchings *et al*., 1996
Tacazzea apiculata Oliv.	Isimondane	Unspecified	Unspecified parts are taken as a tonic with water or milk.	Gerstner, 1938; Hutchings *et al*., 1996

Plant name	Vernacular name (IsiZulu)	Plant part used	Disease and preparation	References
Teucrium kraussii Codd	Isihlungu	Whole plant	a) Strong infusion is taken as emetics for snakebites. b) Decoction is taken as tonic by Xhosa people.	Watt and Breyer-Brandwijk, 1962; Hutchings *et al*., 1996
Vernonia colorata Drake	Ibozane	Root	Root is used as a tonic and to cure boils.	Pooley, 1998; Hutchings *et al*., 1996
Vitellariopsis marginata (N.E.Br.) Atthrev.	Amasethole/ umphumbulu/ umnqambomabele	Leaves, root	a) Root is used to treat indigestion and blood poisoning. b) Leaves and root decoction is administered orally or as an enema to purify blood, strengthens body and as sexual stimulant.	Pujol, 1990; Hutchings *et al*., 1996
Xysmalobium undulatum (L.) W.T.Aiton	Ishinga/ ishongwe	Stem, tubers	a) Grounded stem infusion is taken as emetics against poisoning. b) Tubers are also used for sores and wounds, as snuffs for headache, as poison antidote and for diarrhoea, dysentery, indigestion and fevers.	Watt and Breyer-Brandwijk, 1962; Gelfand *et al*., 1985; Hutchings *et al*., 1996
Zanthoxylum capense Harv.	Isinungwane/ amabele entontombi	Root bark	Root bark is an ingredient for a blood purifying decoction known as *imbiza*. This is taken orally and used as a steam bath by patients suffering from scrofula.	Bryant, 1966; Hutchings *et al*., 1996

Figure 1.1 Some herbal products that are sold for blood purification as an alternative instead of using the actual medicinal plant. Purim tablets (a), Benyan tablets (b), Sela syrup (c) and Sela tea (d).

In South Africa there are two other common herbal products: *Sela Blood Clean Mixture* and *Sela Tea* with the ingredient of *A*. *ferox* has been specially formulated to help clean the body of toxins, purify the blood and improve bowel movement for optimal health.The blood purifying tonics are a combination of natural herbs with nutrient-rich mineral salts with blood cleansing properties. These herbs are frequently bitter and have increased organic minerals and trace elements required by the cell and its environments like iron, potassium, silica, sodium, magnesium salts and calcium salts. Alteratives possess therapeutic action for all the cleansing and filtering organs in the body “Alterative” simply means that the blood infections are altered for a healthier immune system.

The plant species in Table 1.2 are food spices, herbs and teas sold on the commercial market for consumption purposes. They have also been considered as having blood purifying properties.

Table 1.2 Some herbs and spices that appear to have blood cleansing properties

Scientific name	Common name of herb	Part used	Function	References
Allium sativum L.	Garlic	Leaves	Chewing a few cloves of garlic every morning before breakfast filters the blood and improve the immune system to fight against diseases.	Gebreyohannes and Gebreyohannes, 2013
Arctium lappa L.	Burdock	Root, seed	Clears congestion, reduces swelling and dismisses toxins through skin, kidneys and bowels. A tonic enriching the blood and strengthening the entire system.	Bureau, 2016
Azadirachta indica A. Juss.	Neem	Bark, leaves, seeds	Has antiseptic, antifungal and antiviral properties that are vital for blood cleansing. Fight blood clots and can be used to treat skin ailments, ulcers, arthritis, and gum diseases.	Zareen *et al*., 2015
Coriandrum sativum L.	Coriander	Fleshy shoots	Detoxifying agent for indigestion, worms, a diuretic and a good laxative.	Rajeshwari and Andallu, 2011
Curcuma longa Linn.	Turmeric	Root	Treatment for fever, liver and urinary diseases, antiseptic inflammatory troubles of the joints, itching, eczema, parasitic skin diseases, coughs and colds. It has also been used for, boils, scabies, sore eyes, smallpox, chicken pox, insect bites, as a blood purifier and an anthelmintic.	Sabale *et al*., 2013

Scientific name	Common name of herb	Part used	Function	References
Cuscuta reflexa Roxb.	Cuscuta	Flower	The flower is used for blood purification and promote liver and kidney health.	Saini *et al.*, 2015; Lalchand *et al*., 2017
Echinacea angustifolia DC. and *Echinacea purpurea* (L.) Moench	Echinacea	Shoots, flowers, roots	An immune stimulant. Improve body's resistance against both bacterial and viral infections. A detoxifying agent.	Goldhaber-Fiebert and Kemper, 1999
Ocimum tenuiflorum L.	Basil	Leaves	Leaves are chewed for blood cleansing and removal of toxins from the body. It assists liver and kidneys to remove built-up toxins in the body.	Marwat *et al*., 2011
Petroselinum crispum (Mill.) Fuss	Parsley	Leaves	A blood cleansing agent that fights against aczema, oedema and UTI.	Chauhan and Aishwarya, 2018
Phyllanthus emblica L.	Indian gooseberry	Fruits, leaves, seeds, bark, root	Purifies blood, detoxifies and reinforces the immune system. It improves the performance of the liver and pancreas.	Mirunalini *et al*., 2013

Scientific name	Common name of herb	Part used	Function	References
Taraxacum officinale F.H. Wigg.	Dandelion	Root, leaves, flower	Improves blood circulation and purifies the blood.	Hoffmann, 1999
Urtica dioica L.	Nettle	Root, leaves	As a natural detoxifier, blood purifier, and diuretic. Nettles work to remove impurities such as bacteria, calcium crystal deposits, grit and kidney stones.	Ahmed and Parsuraman, 2014

Table 1.3 Some of the plant species documented for the use of blood purification, spiritual connection and other ailments by different cultural groups in South Africa

Scientific name	Plant part and its uses	Cultural group	References
Senegalia caffra (Thunb.) P.J.H. Hurter & Mabb. = (*Acacia caffra*)	Bark used for abdominal complaints, cold, fever, mouth ulcers and as a blood purifier.	Sotho	Madikizela *et al*., 2017
Acokanthera oppositifolia (Lam.) Codd	Foliage in small amounts treats constipation, clean the stomach and is a blood purifier.	Rastafarian, Xhosa, Zulu	Philander, 2011

Scientific name	Plant part and its uses	Cultural group	References
Albuca flaccida Baker	Bulbs are used for an emetic for dieting, a blood purifier, treat cholesterol, hypertension, cancer, ulcers, and cleanses the womb and blood in the intestines.	Rastafarian, Xhosa, Zulu	Philander, 2011
Aloe arborescens Mill.	The leaf is use as a blood purifier, treat gastrointestinal complaints, treat skin ailments, acne and as a skin moisturizer.	Rastafarian	Philander, 2011
Aloe aristata Haw.	Leaf sap is used as a blood purifier and cures skin rashes, sunburns and wounds.	Venda, Zulu	Coopoosamy and Naidoo, 2012
Aloe ferox Mill.	Sap from leaves treat sores, wounds, acne, burns, blisters, cold sores, cracked lips, insect bites, itchy places, immune boosters, mouth sores, sunburns, ulcers, rashes. Infusion is taken for blood purification and constipation.	Khoisan, Venda, Xhosa, Zulu	Coopoosamy and Naidoo, 2012; Nortje and Van Wyk, 2015
Ammocharis coranica Herb.	Fresh, wet scales of bulbs are cooked and used as enemas for blood cleansing or applied topically to open wounds or boils.	Unspecified group in South Africa	Louw *et al*., 2002
Artemisia afra Jacq.	Foliage used for coughs, colds, fever, loss of appetite, colic, headache, earache, intestinal worms, blood purifier.	Khoisan, Zulu	Coopoosamy and Naidoo, 2012; Nortje and Van Wyk, 2015
Asparagus capensis L.	Root cleanses blood and to treat hypertension.	Rastafarian	Philander, 2011

Scientific name	Plant part and its uses	Cultural group	References
Boophone disticha Herb.	Bulb is used as an emetic to purify bladder, uterus and blood. Treat headache, stomach pains and eye problems.	Coloured, Rastafarian	Philander, 2011
Bowiea volubilis Harv. Ex Hook.f.	Bulb used to treat syphilis, rheumatism, fever and as a blood purifier.	Rastafarian, Tswana, Zulu	Monakisi, 2007; Philander, 2011
Bridelia cathartica G. Bertol.	A handful of boiled roots is taken orally twice a day to treat dysmenorrhoea, menorrhagia, infertility, oligomenorrhoea, premature birth and to cleanse the blood when pregnant.	Zulu	De Wet and Ngubane, 2014
Bulbine foley E.Phillips	Root is used for erectile dysfunction, kidney and bladder problems, arthritis, cancer, hypertension, and as a blood purifier.	Coloured, Rastafarian	Philander, 2011
Bulbine natalensis Baker	Roots are boiled and the extract taken orally for blood purification. The treatment of various ailments such as diabetes and urinary problems.	Tswana	Monakisi, 2007
Capparis tomentosa Lam.	Leaves and root decoctions treat wounds and skin problems and a blood cleanser.	Swati, Xhosa, Zulu	Komoreng *et al*., 2017
Cissampelos capensis Thunb.	Root decoctions used for blood purification and to treat boils and diarrhoea.	Coloured, Rastafarian	Philander, 2011
Crossyne guttata (L.) D.Müll.-Doblies & U.Müll.-Doblies	Bulb is used as an emetic to purify bladder, uterus and blood.	Rastafarian	Philander, 2011

Scientific name	Plant part and its uses	Cultural group	References
Cymbopogo spp.	Whole plant is used as a love charm, protective home charm, cleanser after funerals and menstruation. Internally used for chest pains, infant abdominal pain, colds and as a blood strengthener.	Rastafarian, Zulu	Philander, 2011
Datura stramonium L.	Decoction of roots is taken orally as a blood purifier.	Swati, Xhosa	Komoreng *et al*., 2017
Drimia elata Jacq.	Bulb decoction treat arthritis, bring sensation after stroke, cleanses the body; applied for swelling; as wash of bad luck and for blood purification.	Rastafarian	Philander, 2011
Elephantorrhiza elephantina (Burch.) Skeels	Root decoctions are used for blood and womb cleansing by women after birth.	Sotho, Tswana, Xhosa, Zulu	Monakisi, 2007; Komoreng *et al*., 2017
Euclea natalensis A.DC.	Decoction of freshly cut roots is taken orally two to three times a day by a pregnant woman for blood purification.	Zulu	De Wet and Ngubane, 2014
Garcinia livingstonei T.Anderson	Roots decoction is taken orally twice a day after a meal to treat dysmenorrhoea, menorrhagia, infertility, oligomenorrhoea, premature birth and to cleanse the blood when pregnant.	Zulu	De Wet and Ngubane, 2014
Gasteria croucheri Baker	Whole plant is a protective medicine against bad spirits and lightning; as a tonic wash, purgative and blood purifier. Treat skin rash, warts and ringworm.	Rastafarian, Xhosa	Philander, 2011

Scientific name	Plant part and its uses	Cultural group	References
Harpephyllum caffrum Bernh.	Extracts from bark is used for acne, eczema and as a blood purifier.	Zulu	Coopoosamy and Naidoo, 2012
Haworthia fasciata Haw.	Whole plant is used as a protective medicine against *tokoloshe* and lightning, ingested as a tonic wash, purgative and blood purifier. Treatment for skin rash, warts, and ringworm.	Rastafarian, Xhosa	Philander, 2011
Haworthia limifolia Marloth	Foliage decoction purifies the blood and used for coughs, skin rashes, sunburns and burns.	Venda, Zulu	Coopoosamy and Naidoo, 2012
Hermania spp.	Foliage treat ringworm and other skin ailments. Chewed leaf pulp applied on sores as plaster. Taken orally as blood purifier and for stomach-ache.	Rastafarian	Philander, 2011
Hypoxis hemerocallidea Fisch., C.A. Mey. & Avé-Lall.	Bulb decoction is used to treat Human Immunodeficiency Virus (HIV) and arthritis, as an immune system booster, strengthens the blood and for purging.	Rastafarian, Tswana, Zulu	Monakisi, 2007; Philander, 2011
Hypoxis latifolia Wight	Tuber infusion is used as blood cleansing emetics.	Xhosa, Zulu	Komoreng *et al*., 2017
Inula graveolens (L.) Desf.	Tuber decoction is used to cleanse the womb, lungs and blood; general health tonic, an abortifacient.	Coloured, Rastafarian	Philander, 2011

Scientific name	Plant part and its uses	Cultural group	References
Kigelia africana (Lam.) Benth.	Bark decoction is taken orally, half a cup thrice a day for blood cleansing and pelvic pains during pregnancy.	Zulu	De Wet and Ngubane, 2014
Leonotis leonurus (L.) R.Br.	Foliage is used to treat cancer, ulcers, gout, and dandruff, also taken as a blood purifier.	Rastafarian	Philander, 2011
Limeum africanum Zeyh. ex Moq.	Leaf decoction used for infants with convulsion, epilepsy, blood impurities, used for women's ailments, for retained placenta.	Khoisan	Nortje and Van Wyk, 2015
Lobostemon fruticosus H.Buek	Foliage is used to treat ringworm and skin ailments. Chewed leaf pulp applied as plaster on sores. Is also a blood purifier and used for stomach-ache.	Rastafarian	Philander, 2011
Melianthus pectinatus Harv.	Leaves are a wash for painful legs and feet, compress on fractured legs, wounds, sores and abrasions. Leaf infusions used for influenza; root decoction used for urinary tract infections, as blood purifiers and root snuff for headache.	Khoisan	Nortje and Van Wyk , 2015
Ochna natalitia Walp.	Roots decoction is taken orally three times a day to treat menorrhagia, dysmenorrhoea and for blood purification in a pregnant woman.	Zulu	De Wet and Ngubane, 2014

Scientific name	Plant part and its uses	Cultural group	References
Ornithogalum flaccidum (Jacq.) J.C.Manning & Goldblatt	Bulbs prepared as emetics for dieting and blood purification; treats cholesterol, hypertension, cancer, ulcers and clean the womb.	Coloured, Rastafarian, Xhosa, Zulu	Philander, 2011
Parmelia spp.	Infusions made from lichen used for back pains, stomach-ache, to maintain fertility in women, as blood purifier and treatment for influenza and colds.	Khoisan	Nortje and Van Wyk, 2015
Pelargonium spp.	Foliage used for blood and liver purification. Treats women's ailments and problematic gall bladder.	Rastafarian	Philander, 2011
Pelargonium triste (L.) L'Hér.	Tuber decoction is used for haemorrhoids, internal bleeding, tuberculosis, chest ailments, dysentery, anaemia, diarrhoea and as a blood purifier.	Rastafarian	Philander. 2011
Peltophorum africanum Sond.	Bark and leaf decoction treat abdominal disorders, used for liver cleansing, painful joints and back, toothache, ascites, coughs, fever, sore throat, tuberculosis, blood detoxification, to treat infertility, skin rashes and blisters.	Sotho, Venda, Zulu	Madikizela *et al*., 2017
Pteronia camphorata (L.) L.	Leaves and twigs infused to relieve toothache, flatulence, tuberculosis, rheumatism and used for blood purification.	Khoisan	Nortje and Van Wyk, 2015

Scientific name	Plant part and its uses	Cultural group	References
Rhoicissus digitata Gilg & M.Brandt	Bulbs prepared as enema for blood purification and intestinal cleansing, to treat gastrointestinal complaints and cancer. Used as an amulet that destroys gossip.	Rastafarian, Xhosa, Zulu	Philander, 2011
Salix mucronata Thunb.	Foliage relieves water in the body and diabetes, used in blood cleansing, dieting and as diuretic.	Coloured, Rastafarian	Philander, 2011
Schotia brachypetala Sond.	Bark used for heartburn, pimples, diarrhoea and as a blood purifier.	Zulu	Coopoosamy and Naidoo, 2012

There is the famous quotation from Hippocrates', which reads “let food be your medicine and your medicine be your food” Thus, the use of food ingredients may have a holistic purpose, and possibly the use as blood purifiers may act as an overall health tonic.

1.5 MEDICINAL PLANT SPECIES USED FOR BLOOD PURIFICATION AND SPIRITUAL CONNECTION IN SOUTH AFRICA

In some South African cultures, blood is considered very sacred. It is believed that the person’s guardian angels and ancestors are found within the body, particularly in the blood. Therefore, it is perceived important that the blood should be cleansed from any impurities, including bad spirits. It has been stated that when a person’s blood is pure their souls are described as divine and the connection between them and their ancestors is strong (Mvunabandi, 2008). Medicinal plant species that are used for blood purification are used in African tradition for soul cleansing, treating ailments and preventing blood from infections. A study that was conducted by Monakisi (2007), had documented that the most used plant species in the Northern Cape Province are for blood purification, followed by plant species to eliminate evil spirits and enhance luck (charms). These findings complement those of Cocks and Møller, (2002) which investigated the use of indigenous and indigenized medicines to enhance well-being in South Africa. Table 1.3 shows various plant species that are used as blood purifiers for a spiritual connection by different cultural groups in South Africa. These plant species are also documented to treat other ailments that could have been triggered as a result of toxins in the blood (“dirty blood”). There are various spiritual reasons for people to purify blood including lack of good luck, no luck in love, cleanse body off the deceased past lover, cleanse the womb after birth and spiritual cleansing.

1.6 ANTIMICROBIAL PERSPECTIVE OF IMPURE BLOOD

Blood poisoning is a common term referring to an infection that indicates the presence of bacteria in the bloodstream. Bacteria, which normally live harmlessly on the skin, in the nose or mouth can invade the body’s bloodstream and release poisonous toxins,

especially when the patient is immuno-compromised (Chauhan, 2013). Bacteremia is a condition from the presence of bacteria in blood, and may be due to ordinary events, like forceful tooth brushing, dental or medical procedures, or arise from infections such as pneumonia or a urinary tract infection (Smith and Nehring, 2017). Blood purifiers are regarded to be the ideal fit in the treatment of these conditions, because they are said to filter toxins as well as live and dead pathogens from the blood. In this study, seven pathogens were selected to be tested against the antimicrobial activity of blood purifiers used in the study. The following bacteria are perceived to be connected with blood poisoning:

1.6.1 *Cutibacterium acnes*

Cutibacterium acnes is a slow-growing micro-aerophilic, Gram-positive anaerobic bacillus that is part of the normal flora of the oral cavity, large intestine, the conjunctiva and on the skin of humans (Jensen *et al*., 2017; Boisrenoult, 2018). This bacterium is thought to be the major cause of acne vulgaris (chronic skin diseases) due to the fact that a number of widely different antibiotics, antiseptics and bacteriocins active against *C. acnes* are also efficient treatments for acne (Lomholt *et al*., 2017). In rare cases, the bacteria cause cardiac disorder (constrictive pericarditis) (Jensen *et al*., 2017). Other ailments include septic arthritis in shoulder, hip and knee joints (Boisrenoult, 2018).

1.6.2 *Listeria monocytogenes*

Listeria monocytogenes is a Gram-positive facultative anaerobic organism. This motile, non-spore forming bacterium has a rod-shape. *Listeria monocytogenes* is commonly known as a foodborne pathogen and an agent of listeriosis. This bacteria targets and damage the liver to causes septicaemia. Patients with septicaemia are prone to brain and placenta infections (Rouquette and Berche, 1996). *Listeria monocytogenes* can be found in soil, water and food products like vegetables contaminated by the soil, food that is source of protein (contaminated dairy products, raw and/or half cooked meat products and poultry) (Schuppler and Loessner, 2010).

1.6.3 *Staphylococcus aureus*

Staphylococcus aureus is a Gram-positive commensal, round-shaped bacterium found in the upper respiratory tract, on the skin and may sometimes threaten human health (Mousavi *et al*., 2017). It cause infections ranging from pneumonia, abscesses, wounds, skin infections to bacteremia and sepsis (Kobayashi *et al*., 2015).

1.6.4 *Streptococcus agalactiae*

Streptococcus agalactiae is part of Group B *Streptococcus* (GBS). It is a Gram-positive non-sporing coccus and a facultative anaerobic organism. Group B *Streptococcus* pathogens are commonly found in the intestines and urinary tract (Hanna and Noor, 2020). *Streptococcus agalactiae* is a causative agent for pneumonia, meningitis, neonatal septicaemia, skin infections, gastrointestinal tract and bone and joints infections (Pietrocola *et al*., 2018).

1.6.5 *Streptococcus pyogenes*

Streptococcus pyogenes is a Gram-positive non-motile coccus located within the throat or skin (Reglinski and Sriskandan, 2015), where they infect the skin, rash, mouth blisters, peeling of skin of the palms and soles, necrosis, throat infection and anthrax (Walker *et al*., 2014). From the skin, *S. pyogenes* easily penetrate the outer epidermis into blood veins where it causes bacteremia and septic shock or be transfered to other organs to cause inflammation of the entire heart, infect central nervous system and cause superficial infections of fever (Terao, 2012).

1.6.6 *Acinetobacter baumannii*

Acinetobacter baumannii is a non-fermentative Gram-negative strictly aerobic cocco-bacillus that has emerged as an opportunistic nosocomial pathogen (common in the hospital environment and hospitalized patient), and is popular for its multidrug resistance

(MDR) (Breslow *et al*., 2011). Patients with the compromised immune system and surgical wounds or wounds in general are more prone to *A. baumannii* infection. *Acinetobacter baumannii* could infect the following: intravascular and urinary catheters; skin and mucous membranes; as well as gastrointestinal, urinary and respiratory tracts causing wounds, burns and bacteremia (Zarrilli *et al*., 2009). If not treated, the bacteria may cause sepsis.

1.6.7 *Pseudomonas aeruginosa*

Pseudomonas aeruginosa is an environmentally ubiquitous, flagellated, Gram-negative bacterium that quickly adapts to new environments (Mathee *et al*., 2008). The bacteria are regarded as opportunistic pathogens that cause pneumonia in immunocompromised patients (El-Mowafy *et al*., 2014; Goncalves-de-Albuquerquea *et al*., 2016). The bacteria can cause cystic fibrosis, urinary tract infections, septicaemia, endophthalmitis, ear, nose and throat infections, pneumonia and infections in burn wounds (Bodey *et al*., 1983; Banerjee *et al*., 2017).

1.7 OTHER DISORDERS REQUIRING BLOOD PURIFICATION

Blood purification (cleansing) is highly crucial for the entire body functioning as the body depends on healthy blood. The toxic substances due to alcohol, poor diet, drugs or medication, environmental toxicity and unhealthy lifestyle (Chauhan, 2013) can gradually damage the body's organs, which will begin to malfunction. The symptoms start to appear as the level of toxins in the blood increases, causing an imbalance in the system, indicating that the blood may need purification (methods vary according to cultural groups). As shown in Table 1.4, some of the symptoms include constant headaches, allergies, fatigue, low level of immunity, to name out a few. The appearance of any such symptom, which varies among cultures, is often perceived to be a sign that the blood needs to be detoxified and allow the body organs to restore their proper function (Kerkar, 2018).

Table 1.4 Some possible diseases that are regarded as symptoms of toxic blood

Disorders	References
Abscesses, acne, anthrax, eczema, **boils, pimples**, scurvy, , **skin disorders, sores**, warts, **wounds**	Hulme, 1954; Watt and Breyer-Brandwijk, 1962; Gelfand *et al*., 1985; Mabogo, 1990; Pujol, 1990; Hutchings *et al*., 1996.
Bad breath	Gerstner, 1938, Pujol, 1990.
Colic, constipation, **diarrhoea, dysentery, stomach-ache**	Gerstner, 1938; Watt and Breyer-Brandwijk, 1962; Bryant, 1966; Gelfand *et al*., 1985; Mabogo, 1990.
Cough, fever, malaria	Gerstner, 1938; Watt and Breyer-Brandwijk, 1962; Gelfand *et al*., 1985.
Headache	Watt and Breyer-Brandwijk, 1962; Gelfand *et al*., 1985.
Heart diseases, indigestion	Gerstner, 1938; Gelfand *et al*., 1985.
Infertility	Pujol, 1990.
Insect bites, snake bites, spider bites	Roberts, 1983; Mabogo, 1990.
Liver complaints, liver congestion	Watt and Breyer-Brandwijk, 1962; Mabogo, 1990.
Rheumatism	Gerstner, 1938; Pujol 1990.
Scrofula	Watt and Breyer-Brandwijk, 1962; Bryant, 1966.
Shortness of breath	Watt and Breyer-Brandwijk, 1962.
Swelling	Hulme, 1954.
Toothache	Mabogo, 1990; Watt and Breyer-Brandwijk, 1962.

*Disorders indicated in bold may be related to infections.

1.8 TOXICITY AND LINK TO BLOOD PURIFICATION PLANT SPECIES

There is the belief that medicinal plant species are harmless since they are natural. However, plants are capable of producing adverse effects (abnormal, undesirable or

harmful change following exposure to the potentially toxic substance), particularly if taken in excess doses, over long periods, or when taken with synthetic drugs (Adeneye, 2014).

Evaluation for a clear understanding of how adverse effects are activated is needed. Once understood, it becomes easy to develop reliable information on required dosages for safety administration to public health (Pleus, 2014). In many cases, plant poisoning in humans is a result of misidentification of the plant and lack of information on the plant use. It is therefore crucial that medicinal plant species are subjected to proper evaluation for their efficacy and safety (Gadaga and Tagwireyi, 2014).

Plants produce various secondary compounds as a defence against micro-organisms, as well as herbivores (Ndhlala *et al*., 2013). The complex substances include several groups of compounds of which some are deadly to humans even when consumed in small quantities. Ndhlala *et al.* (2013) reported that poisonous plants can induce adverse effects through contact with the skin, blood (wounds), eyes or when ingested. Plant toxins can affect a broad spectrum of human organs. Others may upset significant functional body systems like the central nervous system (CNS) and thereby meddling with the coordination of nerve functions of the body (Ndhlala *et al*., 2013). The level of effect may be determined by the preparation method, plant part, mode of administration, the growth stage of the plant and the dosage taken per remedy (Ndhlala *et al*., 2013).

Plant toxicity has been known for a very long time and humans had developed skills to use toxic plants for their benefit. This includes the use of poisonous plants for hunting, euthanasia, war, suicide, rituals, abortions and executions (Ndhlala *et al*., 2013). Toxic plants that act on the central nervous system cause paralysis when shot by hunters with arrow poisons for hunting and warfare. Examples are *Taxus* spp. alkaloids, atropine and toxiferine together with various cardiac glycosides. In southern Africa, the San hunters rely on rapid-acting cardiac glycosides from genera such as *Acokanthera*, *Adenium*, *Boophone* and *Strophanthus* (Ndhlala *et al*., 2013). Toxins that are described to distress the gastrointestinal tracts are the lectins. Lectins kill the cell linings of the gastrointestinal tracts. *Abrus precatorius* L. and *Jatropha curcas* Wall. are some of the plant species

reported to possess lectins. However, these medicinal plant species are also useful in traditional medicine to treat bacterial and fungal infections (Ndhlala *et al*., 2013).

A toxicity assay is one of the most important pharmacological tools to validate the safety of medicinal plant species for human and animal use. The bioassays can predict potential toxicity or mutagenic effects of plant extracts (Carballo *et al*., 2002; Kamanja *et al*., 2018). In the case of brine shrimp lethality assay (BSLA), this is done by determining the relationship between brine shrimp (larvae) mortality and toxicity of the plant (Ghuman *et al*., 2016). The BSLA provides a relatively low-cost assay that is simple and provides rapid screening of plant extracts (Hamidi *et al*., 2014).

People who rely on medicinal plant species for their healthcare believe that illnesses are caused by internal disruption or “uncleanliness” within the body. Therefore, toxic plants are believed to purify the body and blood through purging (Hutchings, 1989). For blood purification people in rural areas often use traditional and herbal medicine to induce adverse reactions on the bodily system. Adverse reactions may induce vomiting or any other type of purging to promote good health (Clement *et al*., 2017). Some plants have toxins (i.e. *Abrus precatorius* seeds secrete abrin, and *Ricinus communis* L. seeds secrete ricin) that are useful for inducing vomitting or act as a laxative for removing “excess bile” (Salgar *et al*., 2018). Sometimes poisonous plants are used to induce emesis (vomiting) in order to purge the ingested poison. For example, as a first aid, when children consume poisonous fruits the people in rural areas suggest using *ipecac* [*Carapichea ipecacuanha* (Brot.) L.Anderson] root to purge out ingested poison. Some toxic plants are used as a sole purpose of being laxatives (to relieve constipation) and as diuretics (Tessema *et al*., 2020). Also, in the case of fever children are given enemas of toxic plants to reduce body heat or given oral decoctions to stimulate sweating to cool the body (Clement *et al*., 2017). The people who use traditional plants never cease to emphasize the importance of following instructions precisely as sometimes the concentration of the plant concoction is the only way to distinguish between a plant that may be poisonous or curative (Chanda *et al*., 2015).

1.9 PROBLEM STATEMENT

The use of medicinal plant species in South Africa to treat various ailments is vast, especially in rural communities. Although there are several clinics and two hospitals in northern Maputaland, the use of medicinal plant species is still preferred above allopathic medicine. Previous studies in this region noted that the main reasons for that are; culture, the belief that it is safe, freely available in and around the homesteads for usage as well as being effective. Documentation of the use of plants by laypeople in northern Maputaland only started a decade ago (De Wet *et al.,* 2010; York *et al*., 2011; De Wet *et al*., 2013; De Wet and Ngubane, 2014; De Wet *et al.,* 2016). During these ethnobotanical surveys, 79 plant species were documented for the first time being used to treat various diseases. The studies showed the wealth of knowledge found in these rural communities, which will be lost in time if not documented. In South Africa, there is a huge market of concoctions and remedies sold on muthi markets, traditional chemists by herbalists and over-the-counter in supermarkets or pharmacies for blood purification. The blood-cleansing practice is common in a way that there are shared tips on how people make their detoxifiers at home. However, it is concerning that the antimicrobial evaluation for most of these blood purifiers or plant species used as ingredients to these blood purifiers had never been conducted. The safety of these remedies is also not clear. The information available on the toxicity of South African medicinal plant species is not extensive, potential toxicity evaluation of the blood purifying plant species will add to this knowledge. It is also important to inform the users about the negative effects of these plant species if any. There is also very little information known of any efficacy testing of known South African blood purifying plant species. Results from this study will thus enhance the knowledge in the field of blood purifying plant species.

1.10 RESEARCH QUESTIONS

i. How do laypeople of northern Maputaland diagnose blood impurities?
ii. How efficient are blood purifier plant species against blood infectious pathogens?
iii. How safe are these blood purification plant species for human consumption?

1.11 AIM OF THE STUDY

The aim of the study is to gather knowledge on the medicinal plant species used by laypersons in northern Maputaland as blood purifiers and to evaluate their antimicrobial efficacy and safety.

1.12 OBJECTIVES OF THE STUDY

For this aim to be successful, the subsequent objectives to be followed were to:

i. Conduct an ethnobotanical survey, focusing on laypeople's knowledge on blood purifying plant species.
ii. Collect and identify the plant species used as blood purifiers.
iii. Extract the plant samples as preparation for further investigations.
iv. Test for antimicrobial activity against various micro-organisms associated with blood infections, in both individual plant species and plant species combinations.
v. Perform a general toxicity study on the collected plant species and plant combinations.
vi. Compare the antimicrobial activity and toxicity between individual and plant species combinations.

1.13 HYPOTHESES

i. People in northern Maputaland use various medicinal plant species as blood purifiers.
ii. The study will document numerous vernacular names mentioned for the first time.

iii. The plant species would have an excellent antimicrobial activity towards some of pathogens that would be used in the study.
iv. Most of the plant species will show no potential toxicity effects when evaluated.
v. The plant species combinations will have an increased activity as compared to single plant species.
vi. The plant species combinations will have a reduced toxicity rate than single plant species.

1.14 SIGNIFICANCE OF THE STUDY

The review of literature on medicinal plant species used by the different cultures in South Africa does not reveal the reasoning behind blood purification application. Results from this study should clarify how the people in northern Maputaland know if a person needs to purify the blood. The ethnobotanical information is valuable to provide traditional and cultural insights into blood purification use. This information will provide a direction for scientists, by hinting on pathogens that could be used when evaluating the antimicrobial activity of blood purifies. Scientists cannot evaluate blood purification plant species if they do not understand the emic insight of plant users. That could be a possible reason for the few scientific evaluations on blood purifier plants. Results from this study will also contribute greatly towards the toxicological data and safety of South African medicinal plant species.

CHAPTER 2

ETHNOBOTANY OF PLANT SPECIES USED IN NORTHERN MAPUTALAND AS BLOOD PURIFIERS

2.1 INTRODUCTION

Medicinal plant species have been used by our ancestors for centuries as folk remedies to treat various ailments (Street and Prinsloo, 2013). Seeds, root, leaf, fruit, bark, flowers or even the whole plant can be used as a source of medicine, individually or in combination. The active compounds found in these plant parts have direct or indirect therapeutic effects and are used as medicinal agents (Jamshidi-Kai *et al*., 2018). Since plants are natural, affordable and result in comparatively fewer complications (according to laypeople) than allopathic medicines, they are preferred by many people to treat illnesses (Ahmad *et al*., 2013). Street and Prinsloo (2013) reported that the increased number of ethnobotanical and ethnopharmacological studies conducted worldwide is because the World Health Organization is encouraging the use of medicinal plant species and their assessment for safety and efficacy.

The rural communities of northern Maputaland have for a long time been using these plant species as part of their primary health care, regardless of the availability of allopathic medicine in the 21st century. The reason is that plants are easily accessible in their living area and are part of their culture (Mahomoodally, 2013; Williams *et al*., 2013; Mshengu, 2015). Medicinal plant species are also used to treat the spiritual origins of diseases, as well as physical symptoms and thus play an important role in the local communities' way of life.

2.2 METHODOLOGY

2.2.1 Study area

The survey was conducted in Mbazwane, Mseleni and Ntshongwe communities in northern Maputaland, KwaZulu-Natal (Figure 2.1). The three communities in the study are part of the Umhlabuyalingana Local Municipality, which falls under Umkhanyakude District Municipality. The homesteads visited during the survey were found between 32º21ˈ and 32º41ˈ latitudes and 27º22ˈ and 27º60ˈ longitudes with a surface area of 3 621 km^2 in northern Maputaland. The Municipality region is classified as 99% rural, with a population of 172 077. Nearly 50% of all residents are residing in traditional dwellings with very little infrastructure. It is thus a very poor region with, an unemployment rate of 47%. Life expectancy is a low 43.1 years. Despite being classified as rural, 91.6% of the households have access to health facilities and 72% of the population has access to schools (Umhlabuyalingana local municipality IDP 2020/2021). Most inhabitants of these communities reside in poorly ventilated and overcrowded living spaces and those are a risk factor for infectious diseases, according to Sanders and Bradshaw (2010). These types of living arrangements cause the rapid spread of infections, especially considering that more than 10% of the study area's population shares overcrowded living spaces (York, 2012).

2.2.2 Ethnobotanical survey and plant species collection

The ethnobotanical survey was conducted from October 2018 to January 2019 after receiving ethical clearance (UZREC 171110-030 PGM 2018/609) (Appendix C) from the University of Zululand Research Ethical Committee. The 55 homesteads visited were sampled by convenience sampling method, which allows for participants with the information required by the researcher to participate in the study (Etikan *et al*., 2016).

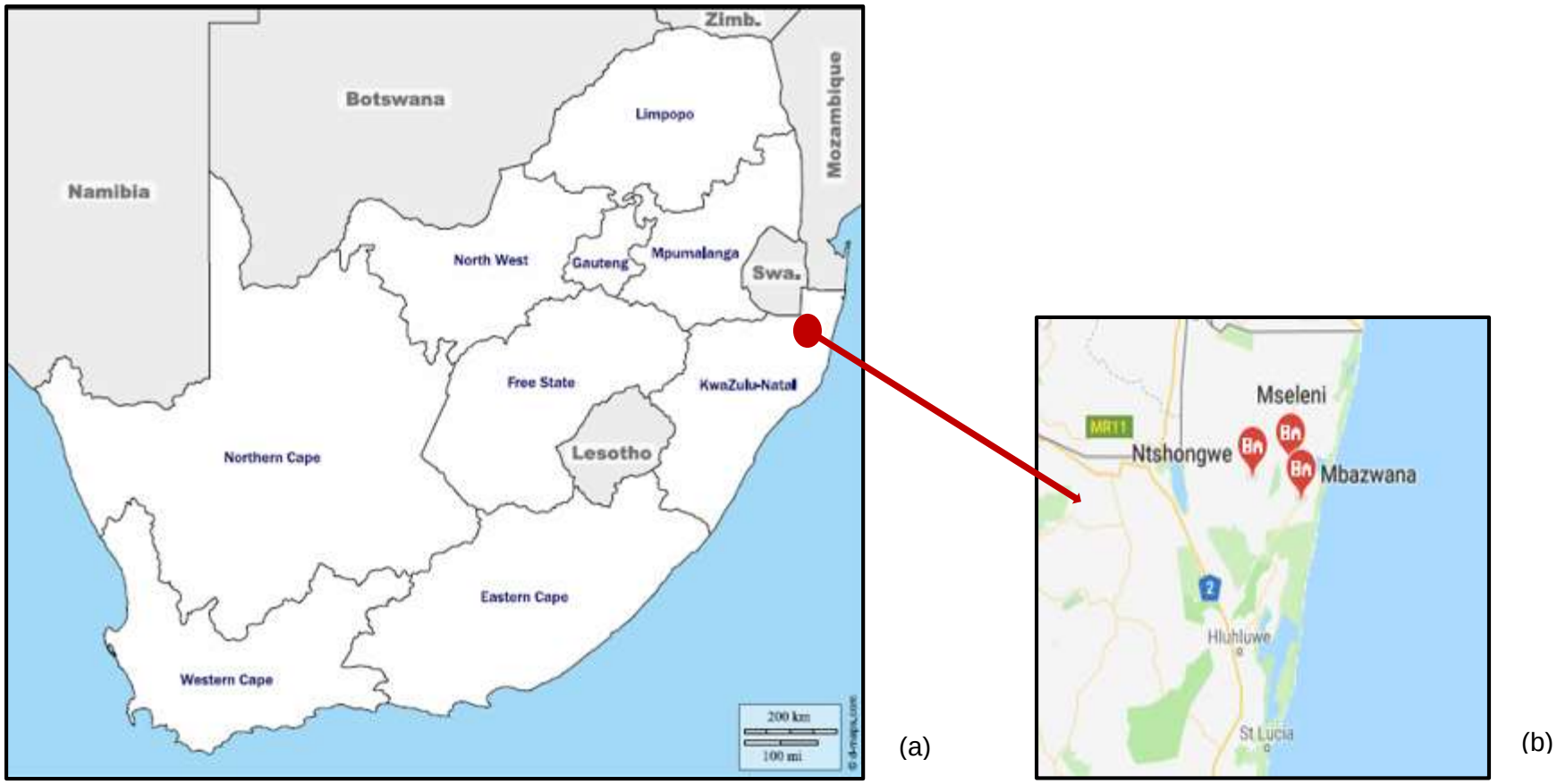

Figure 2.1 Area of the study where (a) is the map of South Africa and (b) is an insert focused on the three communities of northern Maputaland which were involved in the study. Maps from Google maps (https://d-maps.com/carte.php?num_car=4412&lang=en).

The required information being the knowledge of the concept of blood purification, the symptoms and plant species used for blood purification. Structured questionnaire (Appendix D) was used to conduct these interviews. Global Positioning System (GPS) was used to mark the specific locationof each homestead visited. The aim and objectives of the study were explained to the respondents where upon they signed the consent form (Appendix E) before the interview commenced (Figure 2.2). The interviews were conducted in IsiZulu, the dominant language of the participants. In most interviews, the researcher read the questions and wrote answers given to save time and because many of the participants could not read and write.

Figure 2.2 A typical setting for conducting interviews. Captured by H. de Wet. Consent for capturing pictures was given by the participants.

The purpose of the questionnaire was to obtain the following information: socio-demographic data (age, gender, educational background, occupation), an understanding of the term blood purification, symptoms that cause the need for blood to be purified, plant species used for blood purification (vernacular names), availability of plant species, plant

part(s) used, preparation methods, administration mode and quantity (dosage) and side effects. After interviews, the participants received two kilograms of (2 kg) rice as an incentive. Plant species mentioned were collected (Figure 2.3) as voucher specimens pressed *in situ* and as enough material for plant extractions. Doctor T.H.C. Mostert used the voucher specimens to authenticate plant species. Plant specimens were stored in the herbarium of the Department of Botany (University of Zululand). The author names were authenticated from International Plant Names Index website (IPNI).

Figure 2.3 A typical demonstration for plant collection during an ethnobotanical survey (collection of *Acanthospermum glabratum*). Captured by H. de Wet.

2.3 RESULTS AND DISCUSSION

2.3.1 Socio-demographic information

The researcher visited a total of 55 homesteads to acquire information on plant species used in Mbazwane, Mseleni and Ntshongwe for blood purification. In each family, the member that participated in the interviews was the most informative. Every visit lasted approximately twenty minutes, plus 30 minutes for plant collection at most. The survey resulted in a total of 50 females and five males being interviewed. Table 2.1 displays the socio-demographic information of the participants (age, gender, level of education), plant species used as blood purifiers, plant part use and the location of their homesteads. The majority of participants knew more than two plant species associated with blood purification. Figure 2.4 is an analysis of the socio-demographic information. This information showed that 6 % of the participants were young people (13 – 20 years) and 47% for both adults (21 – 65 years) and elders (>65 years). Twenty-seven percent of participants were illiterate, while 35% had only primary education, 36% had accomplished secondary education (with three teenagers interviewed still at school) and two percent have a tertiary education. Eighty percent of participants gathered knowledge from their parents, elders or communities and 20% received their information from their peers. Amongst the 55 participants, 85% admitted to passing the information they have to other people (especially young children) and 15% noted that young people are not interested in tradition or culture, hence there is no use transferring knowledge to them.

The survey was conducted during the week. This could be an explanation for the majority of participants being female elders and adults. This group of people do not have a formal job. They stay at home to look after their grandchildren and homesteads or stay at home due to unemployment. In rural areas, young adult males and men move to towns and cities for employment. The few males that were interviewed mentioned the plant species that are used to enhance good luck and charisma. From this observation, an explanation given was that males know only these uses because they depend mostly on good luck and charisma when seeking employment or courting.

Table 2.1 Information of participants and plant species mentioned as blood purifiers in northern Maputaland

Homestead GPS reading	Homestead number	Age of informant	Gender	Highest level of education	Blood purifier plant species	Plant part use
S27º30.509' E32º33.262'	01	71	Female	None	*Bridelia cathartica* *Abrus precatorius*	Roots Roots
S27º30.633' E32º33.220'	02	59	Female	Gr. 4	*B. cathartica* *Ochna natalitia* *Ranunculus multifidus* *Rhoicissus digitata* *Senecio serratuloides*	Leaves Roots Shoots Leaves and/or roots Shoots
S27º29.905' E32º33.949'	03	92	Female	None	*B. cathartica* *Lippia javanica* *S. serratuloides*	Leaves and/or roots Leaves Leaves
S27º29.712' E32º34.315'	04	43	Female	Gr. 6	*Canthium inerme* *Cymbopogon marginatus* *Hypoxis hemerocallidea* *Prosopis cineraria* *R. multifidus* *Scadoxus puniceus* *Sclerocarya birrea* *Sapium integerrimum* *Strychnos spinosa* *Ximenia caffra*	Roots Whole plant Corm Roots Shoots Bulb Bark Roots Roots Roots
S27º29.779' E32º34.234'	05	89	Female	None	*Aloe marlothii* *C. inerme* *Krauseola mosambicina* *S. serratuloides*	Leaves Roots Shoots Leaves and/or roots
S27º29.766' E32º34.231'	06	62	Female	Gr. 4	*P. cineraria* *S. serratuloides* *S. spinosa*	Roots Shoots Roots

Homestead GPS reading	Homestead number	Age of informant	Gender	Highest level of education	Blood purifier plant species	Plant part use
					Vangueria infausta	Roots
S27º29.902’ E32º34.141’	07	63	Female	None	*B. cathartica* *C. marginatus* *S. serratuloides*	Leaves and/or roots Leaves Whole plant
S27º26.240’ E32º21.635’	08	59 & 39	Male & Female	None & Gr. 4	*A. marlothii* *Combretum molle* *C. marginatus* *H. hemerocallidea* *R. digitata* *Schotia brachypetala* *S. birrea* *Terminalia sericea*	Leaves Roots Whole plant Corm Roots Bark Bark Roots
S27º26.272’ E32º21. 255’	09	63	Female	Gr. 1	*Euclea natalensis* *C. marginatus* *Dichapetalum cymosum*	Roots Whole plant Roots
S27º26.225’ E32.21.104’	10	42	Female	Gr. 12	*B. cathartica* *C. marginatus* *Ricinus communis* *S. serratuloides*	Leaves Whole plant Leaves Whole plant
S27º26.262’ E32º21.109’	11	57	Female	None	*Vangueria infausta* *R. communis*	Roots Leaves and/or roots
S27º26.324’ E32.21.212	12	≥ 65	Female	None	*Gymnosporia senegalensis* *Synaptolepis kirkii*	Roots Roots
S27º26.230’ E32º21.968’	13	65	Female	Gr. 4	*C. molle* *C. marginatus* *Garcinia livingstonei* *S. birrea* *S. serratuloides* *T. sericea*	Roots Shoots Bark Bark Shoots Leaves

Homestead GPS reading	Homestead number	Age of informant	Gender	Highest level of education	Blood purifier plant species	Plant part use
					Withania somnifera *Ziziphus mucronata*	Shoots Roots
S27º26.239' E32º22.518'	14	30	Female	Gr. 12	*S. birrea* *Trichilia emetica* *Melia azedarach*	Bark Bark Leaves
S27º26.378' E32º22.451'	15	≥ 65	Female	None	*Vachellia xanthophloea* *C. marginatus* *Acanthospermum glabratum*	Bark Roots Whole plant
S27º25.978' E32º22.408'	16	65	Female	Gr. 7	*C. marginatus* *Persea americana*	Shoots Seeds
S27º25.034' E32º22.652'	17	19	Female	Gr. 12	*Euphorbia ingens* *G. volkensii* *S. birrea* *Solanum panduriforme* *T. emetica*	Leaves Roots Bark Fruits Roots
S27º25.599' E32º22.711'	18	62	Female	None	*Catunaregam obovata* *O. serrulata* *S. brachypetala* *T. sericea* *Waltheria indica*	Fruits Roots Bark Leaves Roots
S27º25.542' E32º22.711'	19	65	Female	None	*B. cathartica* *C. marginatus* *Hippobromus pauciflorus* *Pyrenacantha scandens* *T. emetica* *V. infausta* *W. somnifera*	Leaves Whole plant Twigs Twigs Bark Roots Whole plant
S27º25.625' E32º22.792'	20	30	Female	Gr. 12	*C. marginatus* *Kigelia africana* *S. aculeastrum*	Whole plant Bark Fruits

Homestead GPS reading	Homestead number	Age of informant	Gender	Highest level of education	Blood purifier plant species	Plant part use
					S. spinosa *Tabernaemontana elegans*	Fruits Fruits
S27º25.550' E32º22.785'	21	42	Female	Gr. 6	*C. obovata* *H. hemerocallidea* *Hyphaene natalensis* *P. cineraria* *T. sericea*	Fruits Corm Roots Roots Roots
S27º25.441' E32º22.677'	22	61	Female	Gr. 4	*Garcinia livingstonei* *K. africana* *L. javanica* *T. sericea*	Bark Bark Shoots Roots
S27º25.209' E32º23.021'	23	30	Female	Gr. 11	*V. sieberiana* *G. senegalensis* *S. birrea* *T. sericea* *V. infausta* *X. caffra*	Bark Bark and/or leaves Bark Roots Roots Roots
S27º25.197' E32º22.991'	24	54	Female	Gr. 6	*Albertisia delagoensis* *B. cathartica* *C. molle* *H. natalensis* *P. kaurabassana* *R. digitata* *T. sericea*	Roots Leaves Roots Stem Root tuber Bark Roots
S27º25.271' E32º23.063'	25	37	Female	Gr. 12	*V. sieberiana* *A. delagoensis* *B. cathartica* *K. africana* *K. mosambicina* *Momordica balsamina*	Bark Roots Roots Bark Whole plant Leaves

Homestead GPS reading	Homestead number	Age of informant	Gender	Highest level of education	Blood purifier plant species	Plant part use
					S. birrea *S. panduriforme* *T. sericea*	Bark Fruits Leaves
S27º42.38' E32º38.475'	26	58	Female	None	*B. cathartica* *T. sericea*	Bark and/or roots Leaves and/or bark
S27º25.017' E32º23.249'	27	46	Female	Gr. 4	*V. sieberiana* *B. cathartica* *Cissampelos hirta* *C. molle* *G. senegalensis* *Landolphia kirkii* *P. kaurabassana* *T. sericea* *V. infausta*	Bark Leaves Roots Bark and/or roots Roots Roots Roots tuber Roots Roots
S27º24.645' E32º23.724'	28	48	Female	Gr. 6	*Hydnora abyssinica* *P. scandens*	Rhizome Twigs
S27º24.610' E32º23.715'	29	34	Male	Gr. 11	*B. cathartica* *C. obovata* *P. scandens* *T. sericea*	Leaves Fruits Roots Roots
S27º25.903' E32º22.965'	30	35	Female	Gr. 7	*O. serrulata* *S. integerrimum*	Roots Bark
S27º25.844' E32º23.085'	31	78	Female	Gr. 9	*G. livingstonei* *Iphamba* * *M. balsamina* *O. natalitia* *P. scandens* *S. integerrimum*	Roots Roots Leaves Roots Stem Bark

Homestead GPS reading	Homestead number	Age of informant	Gender	Highest level of education	Blood purifier plant species	Plant part use
					S. spinosa *Syzygium cordatum* *V. infausta*	Roots Bark Roots
S27º25.914' E32º23.081'	32	49	Male	Gr. 5	*B. cathartica* *C. molle* *C. marginatus* *R. multifidus* *S. serratuloides* *T. sericea*	Leaves Leaves and/or roots Whole plant Bulb Whole plant Leaves
S27º25.811' E32º23.348'	33	53	Female	Gr. 1	*A. glabratum* *C. marginatus* *K. mosambicina*	Whole plant Whole plant Whole plant
S27º25.549' E32º24.314'	34	36	Female	Gr. 12	*V. xanthophloea* *B. cathartica* *Carica papaya* *C. obovata* *C. marginatus* *T. sericea*	Bark Leaf stalk Leaves Fruits Whole plant Roots
S27º25.456' E32º24.334'	35	63	Female	None	*B. cathartica* *C. molle* *T. sericea* *W. somnifera*	Leaves Roots Roots Whole plant
S27º25.618' E32º24.313'	36	92	Female	None	*Brachylaena discolor* *B. cathartica* *C. obovata*	Leaves Leaves Fruits
S27º25.602' E32º24.434'	37	20	Female	Gr. 12	*A. delagoensis* *C. obovata* *T. sericea* *W. somnifera*	Roots Fruits Roots Whole plant

Homestead GPS reading	Homestead number	Age of informant	Gender	Highest level of education	Blood purifier plant species	Plant part use
S27º25.617' E32º24.422'	38	38	Female	Gr. 7	*B. cathartica* *M. balsamina* *T. sericea* *W. somnifera*	Roots Leaves Roots Whole plant
S27º24.824' E32º25.356'	39	21	Female	Gr. 12	*T. sericea* *A. glabratum*	Roots Whole plant
S27º25.372' E32º22.964'	40	38	Female	Gr. 10	*Elephantorrhiza elephantina* *E. natalensis* *S. birrea* *S. kirkii* *H. abyssinica*	Roots Roots Bark Roots Rhizome
S27º25.337' E32º23.061'	41	25	Female	Gr. 12	*B. cathartica* *C. marginatus* *S. birrea*	Leaves Whole plant Bark
S27º24.708' E32º23.538'	42	17	Male	Gr. 11	*P. scandens*	Stem
S27º59.855' E32º39.644'	43	65	Female	Gr. 7	*Grewia caffra* *P. cineraria* *T. sericea*	Roots Roots Roots
S27º59.663' E32º39.412'	44	36	Female	Gr. 10	*S. brachypetala* *T. sericea* *W. somnifera*	Bark and/or roots Roots Roots
S27º40.432' E32º40.305'	45	14	Female	Gr. 8	*C. roseus* *G. senegalensis*	Roots Leaves
S27º40.488' E32º40.322'	46	43	Female	Gr. 3	*C. obovata* *B. discolor* *K. mosambicina* *T. sericea*	Fruits Leaves Whole plant Roots
S27º40.145'	47	30	Female	Gr. 12	*C. obovata*	Fruits

Homestead GPS reading	Homestead number	Age of informant	Gender	Highest level of education	Blood purifier plant species	Plant part use
E32º40.410’					*C. marginatus* *K. mosambicina* *Ozoroa engleri* *S. serratuloides*	Whole plant Whole plant Roots Shoots
S27º39.678’ E32º40.798’	48	48	Female	None	*C. obovata* *Clausena anisata* *K. africana* *T. sericea* *T. emetica* *S. brachypetala*	Fruits Leaves Bark Roots Bark Bark
S27º23.690' E32º24.533'	49	58	Female	None	*B. discolor* *C. obovata* *C. anisata* *O. serrulata* *T. sericea* *X. caffra*	Leaves Fruits Leaves Roots Roots Roots
S27º22.128' E32º31.771'	50	48	Female	Gr. 9	*C. obovata* *K. africana* *S. serratuloides* *T. sericea*	Fruits Bark Shoots Leaves
S27º22.012' E32º31.787'	51	41	Male	Gr. 7	*S. birrea* *T. emetica*	Bark Bark
S27º27.008' E32º31.840'	52	23	Male	Degree	*Ekebergia capensis* *K. africana* *K. mosambicina* *Psidium guajava* *R. multifidus* *R. communis* *C. roseus* *S. cordatum* *T. sericea*	Bark Bark Leaves Leaves Leaves Leaves Roots Bark Leaves

Homestead GPS reading	Homestead number	Age of informant	Gender	Highest level of education	Blood purifier plant species	Plant part use
					W. somnifera	Shoots
S27º21.848' E32º31.931'	53	21	Female	Gr. 12	*Bidens pilosa* *C. roseus* *M. balsamina* *V. infausta* *W. somnifera*	Roots Roots Shoots Roots Shoots
S27º22.293' E32º31.996'	54	72	Female	None	*B. discolor* *B. cathartica* *K. africana*	Leaves Leaves Bark
S27º22.188' E32º31.945	55	64	Female	Gr. 7	*C. obovata* *S. integerrimum* *T. sericea*	Fruits Bark Bark

(*) = Unidentified plant(s)

Female participants were the ones with the most knowledge regarding the use of plants for treating various infections. In many cases it was reported that skin disorders and womb infections are some of the symptoms requiring blood purification. These two conditions both affect the well-being of females. As part of looking after the homesteads and children, the in-depth information of these plant species is introduced to women who seek advice for treating various ailments infecting their children. Many women in Maputaland make a living by selling medicinal plant species to a “middle men” whom are selling it to the markets in big cities such as Durban (KwaZulu-Natal) and Johannesburg (Gauteng) and that is also a reason why women are knowledgeable on medicinal plant species. It is a way to add to their subsistence income. The practice of harvesting medicinal plant species for income is not sustainable from a botanical perspective because some of the plant species in this area are at the threat of becoming extinct.

De Wet and Ngubane (2014) reported in a study from this region that the younger family members would direct them to the elders, claiming that they do not have any knowledge about medicinal plant species. However, it was interesting to find that during the current survey the majority of children were at educational facilities, the few that were available to participate on the survey, were equally informative if not more than some elders about these plants and so was the younger adults (21 – 45 years). Both the young and older informants’ interest in blood purifying plants was because they are viewed to be good for skin cleansing and their popularity for charisma effects. A couple of young females testified to have additionally used the blood purifiers as aphrodisiacs, for good luck and to seek marriage and/or popularity. For all the aforementioned reasons it was no surprise that about 30% of respondents knew more than two plant species used as a blood purifier.The recorded information displayed that interviewed elders knew fewer plant species, with unclear information about the uses of these plant species. That is probably because old age is more prone to suffer memory loss. The missing information, however, was recovered with the younger generation. This discovery counteracts previous studies from this area where the elders were more informative about the medicinal plant species and their uses (De Wet *et al*., 2010; York *et al*., 2011; De Wet *et al*., 2012; De Wet *et al*., 2013; De Wet and Ngubane 2014; De Wet *et al*., 2016).

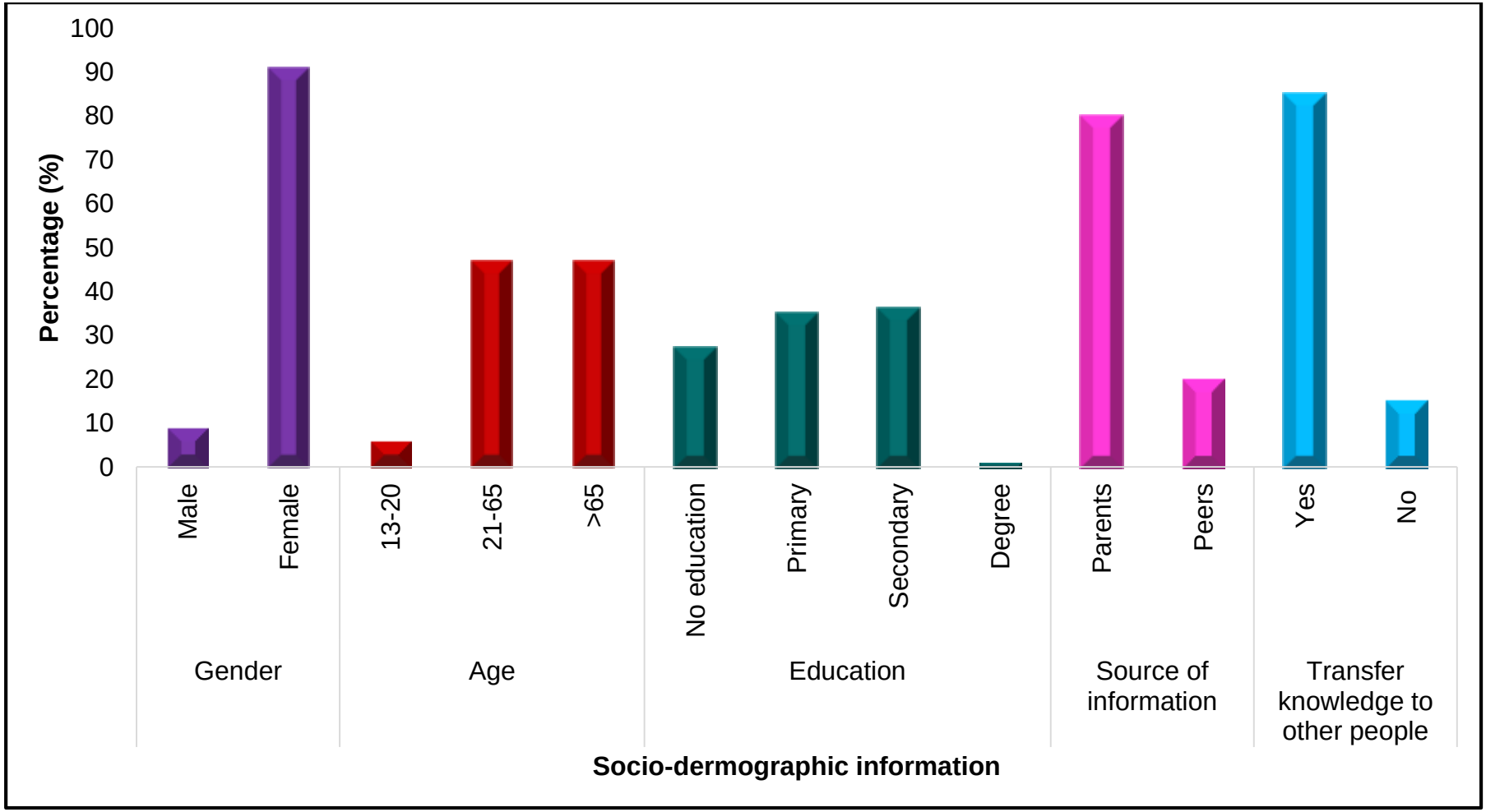

Figure 2.4 Socio-demographic information in percentage of participants interviewed in northern Maputaland.

The possible explanation for this was that, unlike thes previous studies, the current study focused on plants that intrigued the youth. Women want perfectly glowing skin, the luck, and charisma that blood purifiers supposedly provide. The youth make it their mission to get information from the elders about plants that they can use to cleanse themselves. The study also established that the level of education or the increasing availability of infrastructures in these areas over the last 10 years does not affect the way people view and use the available plants. Although the roads had improved and there is unlimited availability of transport, traditional medicine is still the number one primary health care system, compared to nearby clinics and hospitals.In the current study, a teenage girl in grade 12 was more interested and informative in the content of plant species used for blood purification than her mother. The interest of this young girl in medicinal plant species comes from her aspiration of becoming a medical doctor, with the dream of moulding a relationship between traditional medicine and western medicine. Also, a 23-year-old university graduate could identify every plant his mother mentioned and gave detailed information for each plant preparation and administration mode.

2.3.2 Blood purifier plant species used by laypeople in northern Maputaland

The people of northern Maputaland described blood purification as a mechanism used to improve their ability to prevent infections and to treat these ailments if and when infected. Also, in a literal sense blood purification is classified as '*izihlazi*', which is translated to 'cleansers'. Sixty-five plant species were recorded for blood purification and 54 plant species species were available for collection, from which 58 plant parts were collected. The information documented on Table 2.2 is for the mode of preparation, dosage and traditional use of the blood purifier plants as well as mentions of other ailments which participants linked to blood infections. Participants claimed that most of these plant species have no recognizable side effects, although *C. obovata*, *C. roseus* and *T. emetica* must be used with caution (the density of the decoction must be weak and properly filtered). These plant species are known in these communities to be fatal if administered without proper dosage.

Table 2.2 Methods of preparation and administration of plant species, as well as ailments treated by single blood purifier plant species as described by the participants

Botanical name	Zulu name	Part(s) used	Number of times mentioned	Preparation and administration mode of blood purifier remedies	Other ailments mentioned by participants to be treated by the same plant	References of the plant species used for blood purification or other ailments specifically mentioned by participants in the study
Abrus precatorius L.	Umsinsi	Roots	2	A handful of roots are ground and boiled for 30 min then cooled down. Drink daily a mug (250 ml) until sores or rash healed which is an indication of clean blood.	Boils, chest pains, tonic, worms	Githens, 1949; Abbiw, 1990; Hutchings *et al*., 1996; Chinyemba, 2012; Ndhlala *et al*., 2013; Xavier *et al*., 2014; Tugume *et al*., 2016
Acanthospermum glabratum (DC.) Wild.	Isinama	Whole plant	3	Plant is soaked in warm water and taken as an enema for children who show signs of weak immunity and have a strong fever.	Fatigue, excessive body heat	York, 2012, De Wet and Ngubane, 2014
Albertisia delagoensis (N.E.Br.) Froman	Ungandingandi	Roots	3	The roots are grinded and soaked in water overnight. Drink half a cup in the morning for blood cleansing.	Sores, boils, tapeworm	None found in literature
Aloe marlothii A. Berger	Inhlaba	Leaves	2	(i) Add few drops of aloe sap in half a glass of water and drink in the morning for blood cleansing. (ii) Leaf sap is applied topically on pimples, warts and sores for healing.	Heart diseases, high blood pressure, burns, cuts	Watt and Breyer-Brandwijk, 1962; Hutchings *et al*., 1996; Taylor *et al*, 2001; De Wet *et al*., 2012; York, 2012
Bidens pilosa L.	Uqadolo	Roots	1	A handful of ground roots are boiled in 2 L of water and strained. Half a glass of the decoction is taken orally every morning for blood cleansing.	None mentioned	Bryant, 1966; Mabogo, 1990; Von Koenen, 2001; De Wet *et al.,* 2012; De Wet *et al*., 2013; Asowata-Ayodele *et al*., 2016
Brachylaena discolor DC.	Iphahla	Leaves	3	The ground leaves are boiled, strained and allowed to cool down. The decoction is taken as an enema for cleansing and strengthening the body.	Fatigue	Githens, 1949; Watt and Breyer-Brandwijk, 1962; Mabogo, 1990; Taylor *et al*., 2001; Corrigan *et al.*, 2011; De Wet *et al*., 2013; Maroyi, 2013

Botanical name	Zulu name	Part(s) used	Number of times mentioned	Preparation and administration mode of blood purifier remedies	Other ailments mentioned by participants to be treated by the same plant	References of the plant species used for blood purification or other ailments specifically mentioned by participants in the study
Bridelia cathartica G.Bertol.	Umkhawulangazi/ ungazini	Leaves, roots	17	(i) A handful of fresh leaves is lightly grinded and boiled in 1 L of water for 30 min. The decoction is allowed to cool down, strained, and stored in a sealable bottle. A quarter of a cup (63 ml) is taken every morning with sugar to cleanse the body. (ii) A handful of leaves or roots is ground or cut and boiled in 2 L of water for 15-30 min. Once cooled down and strained, half a mug (125 ml) is taken in the morning and evening to clean the blood. (iii) Leaves or roots are boiled and strained and taken once in a while as an emetic to cleanse the body or as an enema for fatigue.	Menstrual pains, fertility	Watt and Breyer-Brandwijk, 1962
Carica papaya L.	Upopo	Leaf stalk	1	The milky sap from the leaf stalk is topically applied for various skin disorders including ringworm and boils.	None mentioned	Aravind *et al*., 2013
Canthium inerme Kuntz	Umvuthwamini	Roots	2	The ground roots are boiled for steaming that is followed by bathing in a cooled decoction for blood cleansing.	Good luck charm	None found in literature
Catharanthus roseus (L.) G.Don	Imbali	Roots	3	The roots are soaked in warm water and administered as an enema for blood cleansing and strength.	Stomach-ache, tapeworms	Ndhlala *et al.*, 2013; Rahul, 2013
Catunaregam obovata (Hochst.) A.E.Gonç.	Isikwakwane *	Fruits	10	Not more than three mature fruits are cut and soaked in a cup of warm water. The infusion must be properly strained and one cup is taken as an emetic. This cleanses the body and prevents sickness.	None mentioned	None found in literature
Clausena anisata Hook.f., De Wild. & Staner	Umsanga/umwas hampunzi/ isifudu/ umnukambiba	Leaves	5	(i) Leaves are boiled and quarter of a cup is taken for internal sores. For external sores you bath in the decoction. (ii) Boil the ground leaves and use it as an enema to treat sores and abscesses.	None mentioned	Githens, 1949; Watt and Breyer-Brandwijk, 1962; Abbiw, 1990; Pujol, 1990; Taylor *et al*., 2001; Lall and Kishore (2014)

Botanical name	Zulu name	Part(s) used	Number of times mentioned	Preparation and administration mode of blood purifier remedies	Other ailments mentioned by participants to be treated by the same plant	References of the plant species used for blood purification or other ailments specifically mentioned by participants in the study
Combretum molle R.Br. ex G. Don	Imbondo	Leaves, roots	6	Leaves or roots are grinded and soaked in 5 L of warm water overnight. Strain and drink one cup every morning. This will cause vomiting. This way it cleanses the system and function as an antidote for snake bite.	Strengthens the body, fatigue	Abbiw, 1990; Mabogo, 1990; Pooley, 1998; Corrigan *et al*., 2011; De Wet *et al*., 2012
Cymbopogon marginatus Stapf ex Burtt Davy	Isiqunga	Whole plant	15	Grind fresh or dried plant species and soak in bath water for love charms, good luck and skin problems.	None mentioned	Watt and Breyer-Brandwijk, 1962
Ekebergia capensis Sparrm.	Umnyamathi	Bark	1	Use the bark to steam for pimples and an emetic for blood purification.	Good luck charm, love charm	Gerstner, 1938; Mabogo, 1990; Pujol, 1990; Mulaudzi *et al*., 2011
Elephantorrhiza elephantina (Burch.) Skeels	Intolwane/ umdabu	Roots	2	The roots are grinded and boiled to be administered as an enema. This improves blood circulation, strengthens the body and cleanses the blood.	Stops bleeding, sores	Bryant, 1966; Gelfand *et al*., 1985; Von Koenen, 2001; Monakisi, 2007; De Wet *et al*., 2013; Mpofu *et al*., 2014
Euphorbia ingens E.Mey.	Umhlenhle	Leaves	2	The milky sap is applied topically on skin affected by warts.	Protects homesteads against lighting	Gerstner, 1938
Garcinia livingstonei T. Anderson	Ugobandlovu/ umphimbi	Bark	3	Bark scraps are soaked in water and given as an enema to children showing signs of weakening or fatigue.	None mentioned	De Wet and Ngubane, 2014
Gardenia volkensii K.Schum.	Umvalasangweni	Roots	1	Boil a handful of roots and steam for pimples. You can also boil the roots, strain it and drink for cleansing of the body and for intestinal worms.	Sore and inflamed eyes, headache	Von Koenen, 2001
Grewia caffra Meisn.	Upata*	Roots	2	Grind the dry roots then soak it in water and drink a quarter of a mug regularly to make sure your blood stays clean (prevention).	None mentioned	Gerstner, 1938; Corrigan *et al*., 2011
Gymnosporia senegalensis Loes.	Ubuhlangwe/ isihlangwane	Bark, leaves, roots	3	The roots are ground and mixed with sulphur powder and boiled for 30 min. The strained decoction is taken orally in the mornings (half a glass) for blood cleansing.	None mentioned	None found in literature

Botanical name	Zulu name	Part(s) used	Number of times mentioned	Preparation and administration mode of blood purifier remedies	Other ailments mentioned by participants to be treated by the same plant	References of the plant species used for blood purification or other ailments specifically mentioned by participants in the study
Hippobromus pauciflorus (L.f.) Radlk.	Iqhume/ umfazi othethayo	Twigs	1	The grinded twigs are soaked in warm water and administered as an enema to cleanse the body internally.	Fatigue, love charms	Pendota *et al*., 2009
Hydnora abyssinica A.Braun	Umavumbuka	Stem	2	Dry material is ground and a handful is boiled in 5 L of water for steaming and bathing. This is used for cleansing the body and skin.	Good luck charm, pimples	None found in literature
Kigelia africana (Lam.) Benth.	Umvongothi	Bark	5	The bark is ground and boiled in 1 L of water. Give one teaspoon (5 ml) to infants twice a day (or in the morning) to cleanse their bodies.	Boils, rash	Gerstner 1938; Von Koenen, 2001; Corrigan *et al*., 2011; Naidoo *et al*., 2013; De Wet and Ngubane, 2014; Lall and Kishore, (2014)
Krauseola mosambicina (Moss.) Pax & K. Hoffm.	Ikhambi	Whole plant	5	Crush one handful of the plant and soak it in 1 L of warm water and strain. It is used as an enema to cleanse the body and for fatigue.	Diarrhoea	None found in literature
Lippia javanica Spreng.	Umsuzwane	Leaves, shoots	2	(i) Leaves or shoots are boiled. Drink when cooled to strengthen the body, improve blood circulation and to get rid of dirt in the blood. (ii) Boil leaves or shoots and steam the body or used for bathing for body, relaxing and to get rid of bad spirits.	Headache, fever, chest pains	Mabogo, 1990; Pooley, 1998
Momordica balsamina L.	Umkaka	Leaves	3	Paste is made from leaves and applied on ringworm rashes and sores.	Blisters, rash, fever	Von Koenen, 2001.
Ochna serrulata Walp.	Umngenisanyosi	Roots	3	The roots are cut and boiled in 2 L of water for 30 min. The decoction is taken orally three times a day for blood and womb cleansing.	Menstrual pain, fertility	None found in literature
Ozoroa engleri R.Fern. & A.Fern.	Isifice	Roots	1	A handful of roots is grinded and soaked in warm water for few min. The infusion is taken as an enema.	Fever	De Wet *et al*., 2012; De Wet *et al*., 2013; De Wet and Ngubane, 2014
Persea americana Mill.	Ukotapheya	Seed	1	Cut seed is soaked in warm water and taken orally in the morning for cleansing the body.	Kidney complains	Asowata-Ayodele *et al*., 2016

Botanical name	Zulu name	Part(s) used	Number of times mentioned	Preparation and administration mode of blood purifier remedies	Other ailments mentioned by participants to be treated by the same plant	References of the plant species used for blood purification or other ailments specifically mentioned by participants in the study
Prosopis cineraria (L.) Druce	Ushashane	Roots	4	The roots are boiled and strained. Decoction is used as a mouthwash for toothache.	Chest complaints	Pareek *et al*., 2015
Pyrenacantha kaurabassana Baill.	Inzema	Root tuber	2	(i) A handful of ground dried root tuber is soaked in 2 L of cold water. The infusion is taken orally in the mornings for cleansing of blood. (ii) The powder of the root tuber is placed on sores.	Wounds	None found in literature
Pyrenacantha scandens (Thunb.) Planch. Ex Harv.	Unginakile/ umakhokhothwane/ inhlanhla emhlophe	Stem	4	(i) Soak a handful of stem in 5 L of cold water overnight. Strain 1 L of the infusion in the morning and drink to induce vomiting. This is done to cleanse the body. (ii) Soak a mug of ground stems in 5 L of cold water overnight with silver money coins. Use the mixture to bath at dawn without using soap for cleansing.	Good luck charm, love charm	None found in literature
Rhoicissus digitata Gilg & M.Brandt	Isinwazi	Root tuber	2	Roots are chopped and boiled in 1 L of water. This is taken orally in the morning before eating to cleanse the body.	Dysmenorrhea, kidney complaints	None found in literature
Ricinus communis L.	Umhlakuva*/ umpono	Leaves	3	(i) The leaves are boiled, strained, allow to cool and taken orally for blood cleansing and sores. (ii) Leaves are soaked in warm water. The infusion is taken orally to cleanse bladder and kidneys.	Skin rash, toothache	Von Koenen, 2001; Hulley and van Wyk, 2017
Sapium integerrimum (Hochst. ex Krauss) J. Léonard	Igowane/ umgaqampunzi	Bark, roots	4	The bark is infused in cold water and used for bathing to treat skin ailments. The bark is also used to steam for blood cleansing.	Pimples	None found in literature
Schotia brachypetala Sond.	Umgxamu/ uvovovo	Bark, roots	4	The bark and roots are ground and boiled for steaming to cleanse the body and purify the blood.	Pimples, diarrhoea	Hulme, 1954
Senecio serratuloides DC.	Unsukumbili	Leaves, shoots, whole plant	9	A handful of crushed dried or fresh plant is boiled in 1 L of water. Half a cup of the decoction is taken twice a day for adults and two spoons for children for blood cleansing and sores.	Excessive body heat, burns, sores and wounds	Watt and Breyer-Brandwijk, 1962; Pooley, 1998

Botanical name	Zulu name	Part(s) used	Number of times mentioned	Preparation and administration mode of blood purifier remedies	Other ailments mentioned by participants to be treated by the same plant	References of the plant species used for blood purification or other ailments specifically mentioned by participants in the study
Solanum aculeastrum Dunal	Intuma enkulu/ umthuma omkhulu	Fruits	1	Fruits are cut open and applied topically on warts and other skin infections.	Toothache	None found in literature
Solanum panduriforme Drège ex Duna	Umthuma/ intuma	Fruit	2	The fruit is used to treat warts. It is cut open and applied topically on warts.	Burns, toothache	None found in literature
Strychnos spinosa Lam.	Umhlala/ amahlali	Roots	4	Eating the fruits helps to get rid of the mucus that builds in the chest thus cleanse the body from unnecessary dirt.	Sore eyes	Von Koenen, 2001
Synaptolepis kirkii Oliv.	Uvuma omhlophe	Roots	2	The roots are ground and powder is snuffed to inhibit haemorrhage.	None mentioned	None found in literature
Tabernaemontana elegans Stapf	Umkhahla	Fruits	1	Few fruits are ground and soaked in 2 L of warm or cold water. Drink in the morning one cup as an emetic for blood cleansing. Not every morning but regularly for internal cleansing and preventing illnesses.	Fatigue, fever	None found in literature
Terminalia sericea Burch. ex DC.	Ikonono/ umkonono	Bark, roots	22	Ground roots are soaked in warm water. In the morning strain and drink a cup of the infusion and wait a few min before drinking a lot of warm water to induce vomiting to cleanse the blood.	None mentioned	Von Koenen, 2001
Trichilia emetica Vahl	Umkhuhlu	Bark, roots	4	(i) The roots are ground, boiled (for a long time) and strained. The decoction is used as an enema to cleanse the womb. (ii) The bark is ground and soaked in water for enemas that is used once in a while for arthritis and cleansing the body. Make sure the decoction is weak.	Diarrhoea, constipation, back pains	None found in literature
Vachellia xanthophloea Benth.	Umkhanyakude	Bark	2	(i) The bark is ground and boiled. Then it is strained and taken orally for blood cleansing or as an emetic. (ii) The bark is boiled and used to steam and bath for cleansing of blood and obtaining good luck and act as a love charms.	None mentioned	Hutchings *et al*., 1996; Corrigan *et al*., 2011; Monakisi *et al*., 2007

Botanical name	Zulu name	Part(s) used	Number of times mentioned	Preparation and administration mode of blood purifier remedies	Other ailments mentioned by participants to be treated by the same plant	References of the plant species used for blood purification or other ailments specifically mentioned by participants in the study
Vangueria infausta Burch.	Umvili/ Uvilapha	Roots	6	(i) Cut or ground roots are boiled in water and taken orally for blood cleansing. (ii) A handful of ground bark is soaked in warm water overnight. One quarter of a mug (one tablespoon for children) of the infusion is taken orally in the morning prior to breakfast as a body cleanser.	Menstrual pains	Von Koenen, 2001
Waltheria indica L.	Uvemvane*	Roots	1	Ground roots are soaked in warm water and used as a wash for sores.	None mentioned	Von Koenen, 2001
Withania somnifera (L.) Dunal	Uvimba/ ubuvimba	Whole plant, roots	8	The roots or whole plant is grinded and soaked in warm water, strained and use as an enema to cleanse the body.	Fever, tapeworms, colds, fatigue	Von Koenen, 2001; Moteetee and Kose, 2016

* New Zulu name

There were various plant preparations mentioned by the participants, however, the one that stood out was the preparation of *P. scandens*, where the participants mentioned the use of “white” (silver) coins to enhance good luck charms. This could be compared to tossing coins into a fountain for good luck. The critea that was used by the researchers to collect plant species mentioned by participants included the availability, and that the plant was not on the South African National Biodiversity Institute’s (SANBI) red list for plant species.

The Zulu names which according to their absence in literature were mentioned for the first time in this study were: *isikwakwane* (*C. obovata*); *upata* (*G. caffra*); *umhlakuva* (*R. communis*); *umbungwa* (*L. kirkii*) and *uvemvane* (*W. indica*). This did not mean that these plant species had never been documented before, but rather demonstrate the complexity of IsiZulu. IsiZulu is a very diverse language and it is quite common that the IsiZulu spoken in southern KwaZulu-Natal has different terms or phases from IsiZulu spoken in northern or eastern KwaZulu-Natal, which can also differ when compared with the same language in the other provinces (Corrigan *et al*., 2011; Mineroff *et al*., 2018). It is possible for people living in the same area to know the same plant species by different names. For example, the people of three communities in Umhlabuyalingana know *C. anisata* as *umsanga/ umwashampunzi/ umnukambimba* and *P. scandens* as *unginakile/ umakhokhothwane/ inhlanhla emhlophe*. Names sometimes arise from certain events common to a particular ethnic group and become popular to that group and region. There was more than one instance where the same Zulu name is used for different plant species. For example, *umsinsi* referred to *Abrus precatorius* in the current study, whereas in the study of De Wet and Ngubane (2014) *umsinsi* was used to refer to *Erythrina humeana* and *E. lysistemon*. This stressed the importance of voucher specimens for botanical identifications of plant names rather than only using literature.

Twenty-two plant species were recorded for the first time globally to be used for blood purification or ailments that participants associated with blood infections. These plant species are: *A. delagoensis, C. papaya, C. obovata, C. hirta, D. cymosum, G. senegalensis, H. abyssinica, H. natalensis, K. mosambicina, L. kirkii, O. serrulata, P.*

guajava, P. kaurabassana, P. scandens, R. digitata, S. integerrimum, S. puniceus, S. aculeastrum, S. panduriforme, S. kirkii, S. cordutum and *T. emetica*. In South Africa, the use of herbal medicine or traditional medicine for blood purification is common. Different formulations varying from tablets, syrups, concoctions and/ or tea are found in supermarkets and pharmacies that market purification of the blood. Furthermore, the people of northern Maputaland had varied plant species classified into a specific group (locally known as *izihlazi*), which were considered only for blood cleansing. However, this is the first study in northern Maputaland to document and evaluate blood purifier plants, and that could be the reason for documenting 22 plant species for the first time for blood purification. Furthermore, for a region that had been claimed to be a hotspot for biodiversity, there is always a high chance that new medicinal plant species could be reported.

The most frequently mentioned plant species used as blood purifiers by the local laypeople were *T. sericea* (45%), *B cathartica* (33%), *C. marginatus* (27%)*, C. obovata* (22), *S. birrea* (18%) and *S. serratuloides* (18%) (Table 2.2 and Figure 2.5). The researcher observed that these plant species (except *C. marginatus*) were abundantly available in the area of study. This explains why more than 80% of the informants knew at least one of these as blood purifier plant species.

The plant species recorded occur in 40 families. The most common families were Leguminosae consisting of five species, followed by Euphorbiaceae, Asteraceae and Rubiaceae with four species each and Apocynaceae and Solanaceae three species each (Table 2.3). Alonso-Castro *et al*. (2016) reported that Asteraceae, Leguminosae and Euphorbiaceae families are known for their ability to treat and prevent infections, which according to the participants of the study and Chauhan (2013) are one of the main functions for blood purifiers. Most species of Rubiaceae are reported to have good antibacterial activity according to Karou *et al*., (2011) and Kala, (2015). The *Ochna, Pyrenacantha, Solanum* and *Vachellia* genera had more than one species used for blood purification. There were plant species mentioned in this study (Table 2.3), but could not be collected.

Bridelia cathartica

Catunaregam obovata

Senecio serratuloides

Sclerocarya birrea

Terminalia sericea

Figure 2.5 Some of the frequently used plant species as blood purifiers. Photo credit: H. de Wet

Table 2.3 The plant families for the species mentioned for blood purification and their voucher numbers

Plant family and species name	Plant type	Voucher specimen number
Aloaceae		
Aloe marlothii	Succulent	TYORK 19*
Amaryllidaceae		
Scadoxus puniceus	Herb	ns.zwan 09
Anacardiaceae		
Ozoroa engleri	Tree	ns.zwan 46
Sclerocarya birrea.	Tree	ns.zwan 10
Apocynaceae		
Catharanthus roseus	Shrub	ns.zwan 45
Landolphia kirkii	Tree	Not available*
Tabernaemontana elegans	Tree	ns.zwan 31
Arecaceae		
Hyphaene natalensis	Tree	ns.zwan 32
Asteraceae		
Acanthospermum glabratum	Herb	ns.zwan 42
Bidens pilosa	Herb	ns.zwan 48
Brachylaena discolor	Herb	ns.zwan 43
Senecio serratuloides	Herb	ns.zwan 05
Bignoniaceae		
Kigelia africana	Tree	ns.zwan 30
Caricaceae		
Carica papaya	Tree	Not available*
Caryophyllaceae		
Krauseola mosambicina	Herb	ns.zwan 14
Celastraceae		
Gymnosporia senegalensis	Shrub	ns.zwan 22
Clusiaceae		
Garcinia livingstonei	Tree	ns.zwan 23
Combretaceae		
Combretum molle	Tree	ns.zwan 16
Terminalia sericea	Tree	ns.zwan 18
Cucurbitaceae		
Momordica balsamina	Herb	ns.zwan 35
Dichapetalaceae		
Dichapetalum cymosum	Shrub	ns.zwan 20
Ebenaceae		
Euclea natalensis	Shrub	ns.zwan 19
Euphorbiaceae		
Bridelia carthartica	Shrub	ns.zwan 01
Euphorbia ingens	Succulent	Not available*

Plant family and species name	Plant type	Voucher specimen number
Ricinus communis	Shrub	ns.zwan 21
Sapium integerrimum	Tree	ns.zwan 11
Fabaceae		
Abrus precatorius	Herb	ns.zwan 02
Hydnoraceae		
Hydnora abyssinica	Rhizome	ns.zwan 39
Hypocidaceae		
Hypoxis hemerocallidea	Herb	SC Ngubane 15
Icacinaceae		
Pyrenacantha kaurabassana Baill.	Herb	ns.zwan 38
Pyrenacantha scandens	Herb	ns.zwan 40
Lauraceae		
Persea americana	Tree	ns.zwan 27
Leguminosae		
Vachellia sieberiana	Tree	ns.zwan 33
Vachellia xanthophloea	Tree	ns.zwan 26
Elephantorrhiza elephantina	Tree	S. Nciki 12
Prosopis cineraria	Tree	ns.zwan 08
Schotia brachypetala	Tree	ns.zwan 17
Loganiaceae		
Strychnos spinosa	Tree	ns.zwan 12
Meliaceae		
Ekebergia capensis	Tree	ns.zwan 50
Trichilia emetica	Tree	ns.zwan 25
Menispermaceae		
Albertisia delagoensis	Shrub	ns.zwan 34
Cissampelos hirta	Shrub	ns.zwan 37
Myrtaceae		
Psidium guajava	Tree	ns.zwan 49
Syzygium cordatum	Tree	ns.zwan 51
Ochnaceae		
Ochna natalitia	Shrub	ns.zwan 03
Ochna serrulata	Shrub	ns.zwan 41
Olacaceae		
Ximenia caffra	Tree	ns.zwan 13
Poaceae		
Cymbopogon marginatus	Grass	Not available*
Ranunculaceae		
Ranunculus multifidus	Herb	S. Nciki 23
Rhamnaceae		
Ziziphus mucronata	Tree	S. Nciki 32
Rubiaceae		
Canthium inerme	Shrub	ns.zwan 07
Catunaregam obovata	Shrub	ns.zwan 52

Plant family and species name	Plant type	Voucher specimen number
Gardenia volkensii	Tree	ns.zwan 28
Vangueria infausta	Tree	ns.zwan 15
Rutaceae		
Clausena anisata	Shrub	ns.zwan 47
Sapindaceae		
Hippobromus pauciflorus	Tree	ns.zwan 29
Solanaceae		
Solanum aculeastrum	Shrub	Not available*
Solanum penduriforme	Shrub	S. Nciki 38
Withania somnifera	Shrub	ns.zwan 24
Sterculiaceae		
Waltheria indica	Herb	ns.zwan 36
Thymelaeaceae		
Synaptolepis kirkii	Shrub	Not available*
Tiliaceae		
Grewia caffra	Shrub	ns.zwan 44
Verbenaceae		
Lippia javanica	Herb	ns.zwan 06
Vitaceae		
Rhoicissus digitata	Shrub	ns.zwan 04

(*) = The plant species were not available for collection or identification parts (leaves, flowers or seeds) were not yet available. Identification was done using vernacular names.

The main restriction from collecting some plant species being that the plant species were not available close to the homestead ,or if the plant was available, it was reported that the roots was used but no above ground vegetative part was available for identification. In these few cases the researcher identified the plant *in situ* with the guidance of supervisor Prof de Wet. Due to sustainable harvesting practises and to protect the limited availability these plant species were not collected for experimental work.

Trees are the most mentioned blood purifier growth form (40%), followed by shrubs (32%), herbs (22%), succulents (3%), grass and rhizomes with 1% respectively. It was noted that if the plant mentioned was a herb, the whole plant or the shoots were used for preparing remedies.From a shrub or tree the commonly used parts were roots, leaves and bark. Sometimes the fruits, bulbs, corms and seeds were also mentioned as blood purifiers. The roots (although not sustainable) and bark were the commonly used parts to

prepare remedies for blood purification. The participants believe that the red colour of the bark or roots (e.g. *S. birrea, R. digitata* or from the rhizome of *H. abyssinica*) show that the plant could be used for blood related ailments. The review conducted by Van Vuuren and Frank, (2020) stated that the people of San and Sotho cultures also uses colour red in the plant species to associate the plant to blood related infections. The whiteness of some bark or roots (*E. capensis, G. volkensii, S. cordatum* or *V*. *infausta*) indicates that the plant be used for cleansing. According to Van Wyk *et al*. (2009), both bark and roots plant parts contain highly concentrated active therapeutic ingredients. These could be the reasons for the popularity of these two plant parts.

The blood purification remedies were prepared by soaking (in cold or warm water) or boiling the appropriate plant part in water. The remedies prepared by soaking in water are referred to as infusions, while boiled remedies are decoctions. In Figure 2.6, it is shown that 52% of plant species were boiled while 35% was infused in water. Some plant species were prepared for an application as poultice or ash from the burned plant species.

Plant infusions and decoctions were recorded to be administered either orally, anally, by steaming (using a blanket to cover the body under boiled decoctions to open skin pores for blood cleansing and acne) or used in bathwater. Nine percent of cut or crushed plant species (bulb or leaves) were topically applied on the skin and 4% of burned plant species were administered through inhaling the smoke (Figure 2.7).

According to the participants, it is important to precisely follow the instructions of the amount of material needed, the volume of solvent to be added for each preparation and following the time specified for boiling or simple soaking plant material. These procedures are believed to guarantee suppression of the toxic compounds in a plant or to enhance their efficacy. Therefore, dosages along with the suitable mode to administer the remedy must be given with clear instructions to patients (Van Wyk *et al*., 2009).

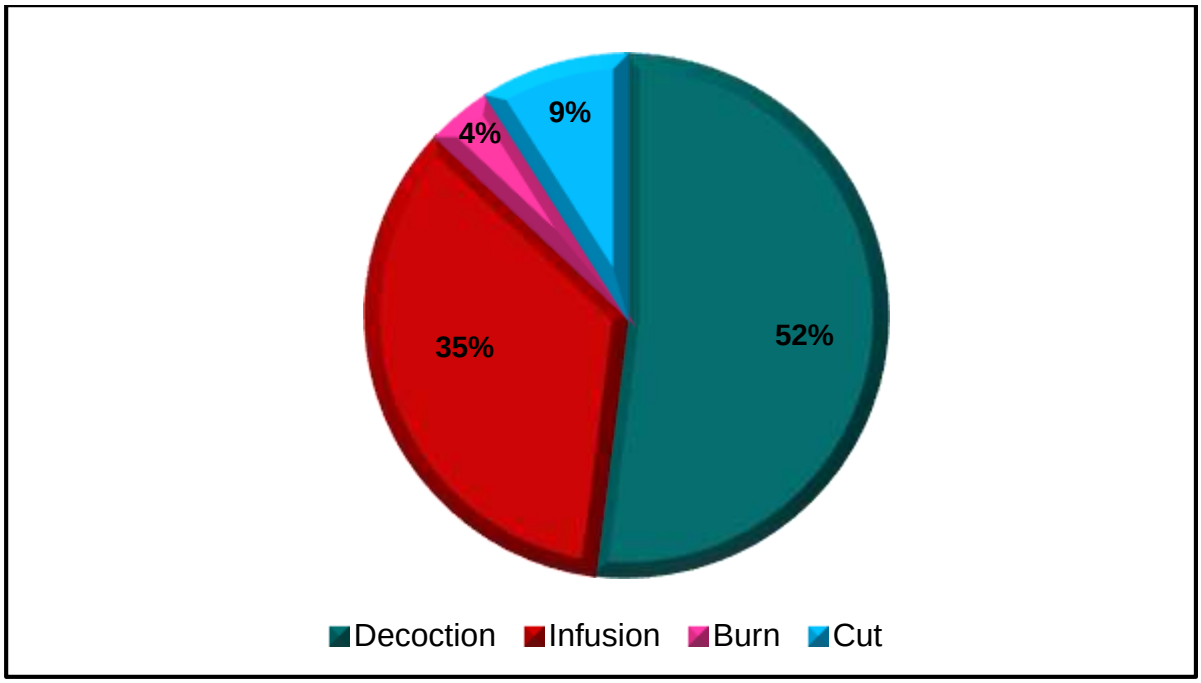

Figure 2.6 Methods used to prepare blood purifying remedies.

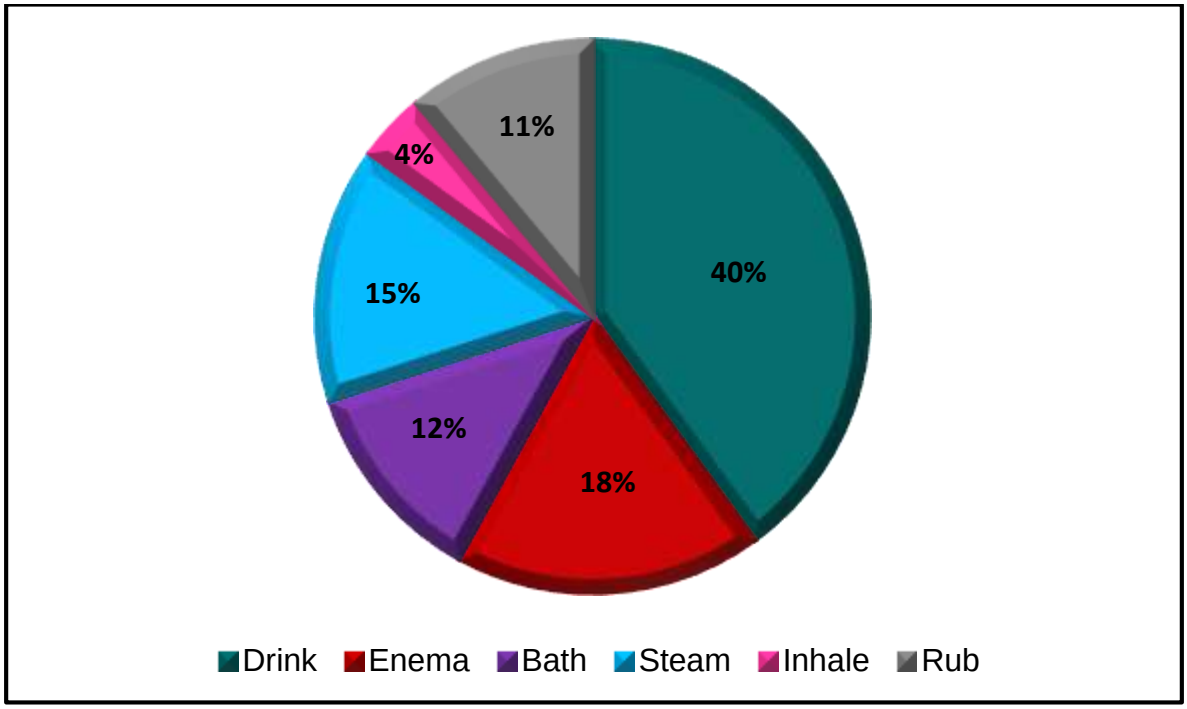

Figure 2.7 Different modes of administration reported in the study.

Most of the remedies prepared for blood purification in northern Maputaland are administered orally, as an emetic [drinking large content (5 litres) of a weak, lukewarm herbal infusion, or decoction, before consuming anything to induce vomiting] or drinking half a glass in the morning before breakfast, or before sleep. In some cases, both these directives were indicated. The participants reported that this is done to ensure that the remedy can flush the system from impurities that may have accumulated. This has previously been noted in other ethnobotanical reports (Van Wyk *et al*., 2009). The other common alternative was an enema administration. Similar to an emetic, this mode is used to flush all diseases from the system; but the ingredients of this remedy may be bitter or poisonous if taken orally and hence the rectal route is preferred. This mode is commonly used for infants and children. The use of an enema method is also popular for relieving constipation. The other most frequent administration mode was steaming. The medicinal herbs are boiled in water and patients are covered with a blanket over the steaming basin to allow perspiration. This mode is used for respiratory problems and skin problems. Herbal steaming help improves skin complexion by opening pores and stimulating cutaneous circulation. This is believed to exfoliate all dirt acquired by the skin. It is believed that perspiration helps dispel blood infections (Van Wyk *et al*., 2009). Steaming is believed to open the nasal and chest cavities and is also used to treat fever. In addition to steaming, a patient also uses these remedies for bathing to heal the sores. A poultice is prepared and pasted or rubbed on boils, burns or blisters to speed up the recovery.

Since the women in northern Maputaland also sustain their living by selling medicinal plant species, Figures 2.8 and 2.9 show some homesteads where the women were preparing medicinal plant species for selling. Although the community do have meetings where people are educated about sustainable harvesting, there are still individuals who do not partake in these meetings. This may be one of the reasons why some plant species were no longer available for collection. However, there are women who are professional harvester with an issued permit. Although it may seem unsustainable to cut down the big amarula tree (*S. birrea*), the tree was a threat to the nearby homesteads if collapsing during the cases of strong wind and storms (Figure 2.9). The harvesters attend workshops

organised by community leaders where they are taught about sustainable harvesting, they also renew their permits every two years.

Figure 2.8 Women cutting some medicinal plant material in preparation for selling.

According to the participants, blood purification improves the functioning of the immune system (weak body) or is associated with spiritual connections. The symptoms when blood purifying is needed included visible signs of weakness, smelly urine and breath, sores in children, regular boils, pimples, red eyes, bladder and kidney complaints, burning of the body and bad luck (Figure 2.10). The study also discovered that the interviewees associate most skin and urinary tract infection (including infertility) to blood impurity symptoms. That is because laypeople do not know that not all skin infections are due to bacterial pathogens, but can also be caused by fungal pathogens such as ringworm.The participants also believe that tapeworms develop in person's intestine because you don't regularly cleanse your body and you have some blood toxins that produce tapeworms in the digestive system. In traditional medicine, blood purification is a systemic treatment, hence the numerous mention of using plant species for both cultural and spiritual purposes.

Figure 2.9 A big *Sclerocarya birrea* tree felled for its bark to be sold to muthi market buyers.

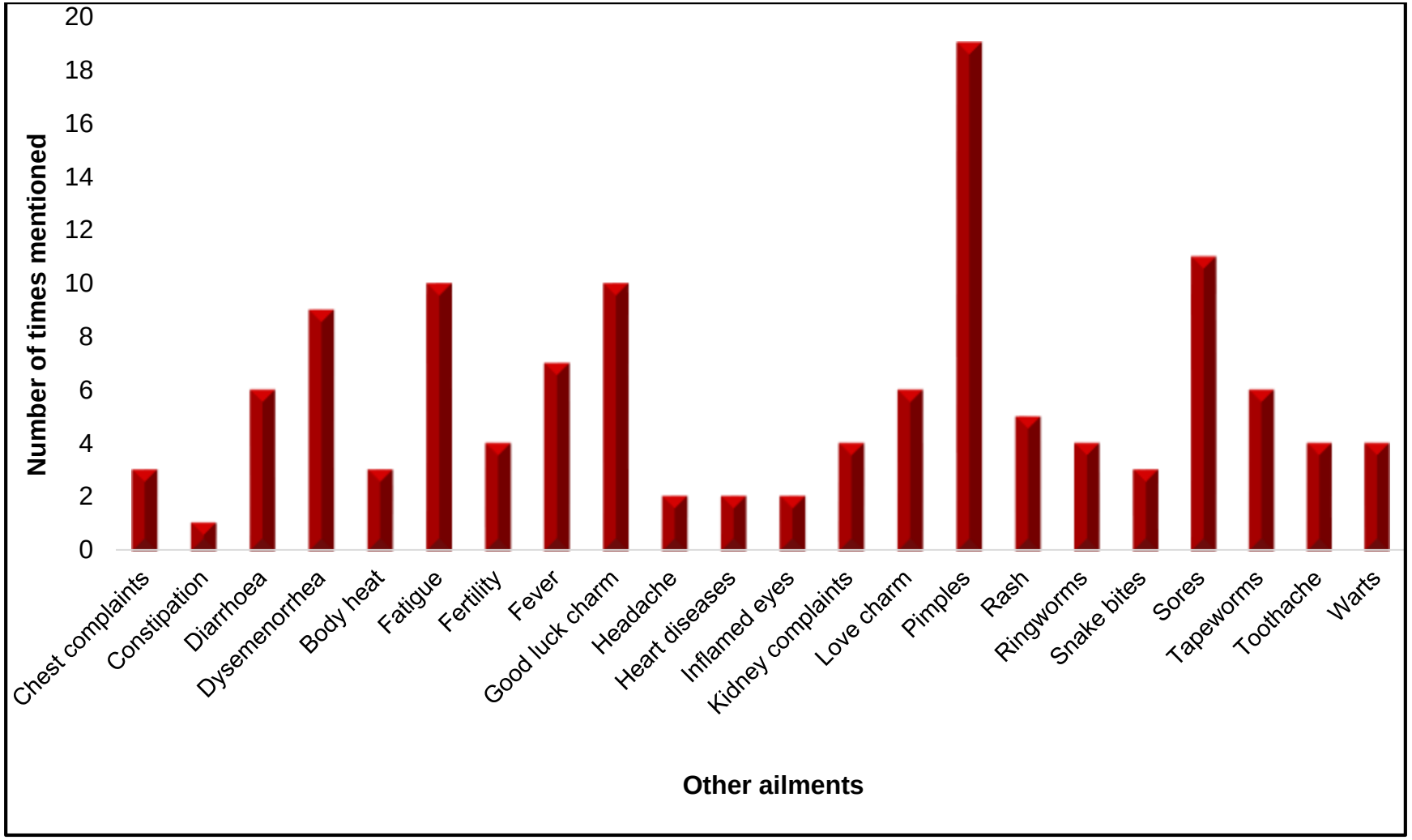

Figure 2.10 Ailments that are used as signs and symptoms of toxic blood and treated by the mentioned blood purifier plant species.

2.3.3 Plant species used in combination for blood purification

The 32 different sets of plant combinations used for blood purification were also mentioned for the first time by this study (Table 2.4). This could be as a result of an abundance of plant diversity in this area (Boon, 2010). It is easy for each participant to formulate their combination when trying to increase the efficacy of remedies. The combinations in this study were all mentioned for the first time.

It was noted that there were four plant species that were commonly used in combinations. These plant species being *V. infausta* infused into four different combinations and *S. birrea, S. spinosa* and *T. sericea* infused in three different combinations respectively. Although it was the first documentation of plant species used for blood purification, several studies had been done in northern Maputaland where combinations were used for other ailments (diarrhoea, respiratory diseases, sexually transmitted infections, skin disorders, hypertension and gynaecological complaints) (De Wet *et al*., 2010; York *et al*., 2011; De Wet *et al*., 2012; De Wet *et al*., 2013; De Wet and Ngubane, 2014; De Wet *et al*., 2016).

Blood purification is associated with various fungal, viral and bacterial infections as well as, cultural and spiritual beliefs, hence multiple plant species are needed against different symptoms caused by different pathogens (Williams, 2010). These combinations, used as blood purifiers, consisted of anything from two to four plant species in one combination. The combinations are recommended by these communities because they are believed to enhance the efficacy of plant species, counteract or to reduce the toxicity of harmful plant species and increase the life span of the remedy, according to the laypeople. This practice is common amongst rural communities. Tugume *et al.* (2016) documented that the people from some rural communities in Uganda apply this technique for similar reasons as the current study.

Table 2.4 Methods of preparation, administration and ailments treated by blood purifier plant combinations as described by the interviewees

Botanical name	Zulu name	Part(s) used	Number of times mentioned	Method of preparation and mode of administration*	Other ailments mentioned to be treated by the same plant	References of the plant species used for blood purification and other related symptoms
Abrus precatorius L.	Umsinsi	Roots	1	A handful of roots is grinded and mixed with a handful of ***B. cathartica*** roots. The mixture is boiled for 30 min and allow to cool down. A mug (250 ml) is taken orally every morning until sores or rash healed which is an indication of clean blood.	Boils, chest pains, tonic, worms	Githens, 1949; Abbiw, 1990; Hutchings *et al*., 1996; Chinyemba, 2012; Ndhlala *et al*., 2013; Xavier *et al*., 2014; Tugume *et al*., 2016.
Albertisia delagoensis (N.E.Br.) Forman	Ungandingandi	Roots	1	Few strands of roots are grinded and mixed with a teaspoon (5 ml) powder of ***P. kaurabassana*** root tuber and boiled in 2 L of water. Drink a quarter of a cup two to three times a day for blood and body cleansing.	Sores, boils	None found in literature.
Bridelia cathartica G.Bertol.	Umkhawulangazi/ ungazini	Leaves, roots	2	(i) It is used in combination with ***A. precatorius*** as described previously (*A. precatorius*) for sores, rash and blood cleansing. (ii) Roots are grinded and mixed with grinded roots of ***R. digitata***, then boiled in 2 L of water and cool down and strained. A quarter of a cup (63 ml) is taken in the morning for blood cleansing.	Menstrual pains, fertility	Watt and Breyer-Brandwijk, 1962.
*Cissampelos hirta*** Klotzsch	Umanyokana	Roots	1	The roots are mixed with the roots of ***G. senegalensis*** and ***L. kirkii***. The mixture is boiled in 2 L of water. Adults take half a mug orally and children 1-2 spoons three times a day for body cleansing and strengthening.	Diarrhoea	None found in literature.
Combretum molle R.Br. ex G. Don	Imbondo	Leaves, roots	2	(i) Roots are grinded and mixed with the grinded roots of ***T. sericea*** and soaked in 5 L of warm water overnight. In the morning use the mixture to induce vomiting for body cleansing. (ii) Mix the roots with the roots of ***T. sericea*** and ***Z. mucronata***, soak in warm water for a short time and use as an enema for internal body cleansing.	Strengthens the body, fatigue	Abbiw, 1990; Mabogo, 1990; Pooley, 1998; Corrigan *et al*., 2011; De Wet *et al*., 2012.

Botanical name	Zulu name	Part(s) used	Number of times mentioned	Method of preparation and mode of administration*	Other ailments mentioned to be treated by the same plant	References of the plant species used for blood purification and other related symptoms
Cymbopogon marginatus Stapf ex Burtt Davy	Isiqunga	Whole plant	2	(i) Mix the plant with the roots of ***P. cineraria*** and ***S. integerrimum*** and boil in water, use the steam for pimples, good luck charm and to cleanse the blood. (ii) The grinded plant is mixed with the bark of ***S. brachypetala*** and boiled, the steam is used for pimples and body cleansing.	Skin disorders, pimples, good luck charm	Watt and Breyer-Brandwijk, 1962.
*Dichapetalum cymosum*** Engl.	Ubangalala	Roots	1	Roots are grinded with the roots of ***E. natalensis*** and soaked in cold water overnight. In the morning you drink the infusion to induce vomiting for cleansing or to bath in for love charm and good luck.	None mentioned	None found in literature.
*Euclea natalensis*** A.DC.	Udelunina	Bark, roots	2	(i) A handful of bark or roots is mixed with a handful of ***S. birrea*** bark and boiled to steam for cleansing blood, get rid of pimples and bad luck. (ii) It is used in combination with ***D. cymnosum*** as described previously (*D. cymnosum*) for good luck, love charm and to cleanse the body.	Snake bite, toothache	Gerstner, 1938; Corrigan *et al*., 2011; De Wet and Ngubane, 2014.
Garcinia livingstonei T. Anderson	Ugobandlovu/ umphimbi	Bark	2	(i) The bark is mixed with that of ***S. birrea*** and boiled. After proper straining it is taken orally to treat diarrhoea. (ii) The roots are mixed with the roots of *iphamba*****, *O. natalitia* and *V. infausta* and soaked in cold water overnight. Use the infusion to bath in for seven days in a row for body cleansing to prevent bad luck.	None mentioned	De Wet and Ngubane, 2014.
Gymnosporia senegalensis Loes.	Ubuhlangwe/ isihlangwane	Bark, leaves, roots	2	(i) The roots are grinded and mixed with sulphur powder and boil for 30 min. The strained decoction is taken orally in the mornings (half a glass) for blood cleansing. (ii) The roots are mixed with the roots of ***C. hirta*** and ***L. kirkii*** and boiled. Adults drink half a mug and children 1-2 spoons (15 ml) three times a day for cleaning the body and to strengthen it.	None mentioned	None found in literature.
*Hyphaene natalensis*** Kunze	Ilala	Stem	1	Chopped stem is mixed with grinded roots of ***R. digitata*** and boiled in 1 L of water. The decoction is taken orally in the morning for body cleansing.	Dysmenorrhea	None found in literature.
*Hypoxis hemerocallidea***	Inkomfe	Corm	1	It is mixed with the crushed shoots of ***S. serratuloides*** and boiled for 30 min in 2 L of water.	None mentioned	Monakisi, 2007; Ndhlala *et al*., 2011a; De Wet *et*

Botanical name	Zulu name	Part(s) used	Number of times mentioned	Method of preparation and mode of administration*	Other ailments mentioned to be treated by the same plant	References of the plant species used for blood purification and other related symptoms
Fisch., C.A.Mey. & Avé-Lall.				Drink half of a glass two times a day (few spoons for children) to cleanse blood, treat rash and sores.		*al*., 2012; Street and Prinsloo, 2013; De Wet and Ngubane, 2014; Hulley and van Wyk, 2017.
Krauseola mosambicina (Moss.) Pax & K. Hoffm.	Ikhambi	Whole plant	1	The leaves are mixed with those of ***P. guajava*** and ***M. azedarach***, boiled and administered orally in the morning for blood purification.	Diarrhoea	None found in literature.
*Landolphia kirkii*** Dyer	Umbungwa***	Roots	1	The roots are mixed with those of ***C. hirta*** and ***G. senegalensis*** and boiled. The decoction is taken orally three times a day using half a mug for adults and 1-2 spoons for children.	None mentioned	None found in literature.
Ochna natalitia Walp.	Isizemane	Roots	2	(i) A handful of cut or grinded roots is mixed with the roots of grinded ***B. cathartica*** and boiled in 2 L of water. One cup is diluted in 5 L of water and used as an emetic every morning for blood cleansing. (ii) It is used in combination with ***G. livingstonei***, ***iphamba***** and ***V. infausta*** as described previously (*G. livingstonei*) to cleanse the body and prevent bad luck.	Wounds	De Wet and Ngubane, 2014.
Prosopis cineraria (L.) Druce	Ushashane	Roots	2	(i) Mix with the roots of ***S. spinosa*** and ***V. infausta*** and boil in 2 L of water. Take two plastic bottle caps (10 ml) twice a day to cleanse blood and ease menstrual pain. (ii) It is used in combination with ***C. marginatus*** and ***S. integerrimum*** as described previously (*C. marginatus*) to treat pimples and cleanse blood.	Chest complaints	Pareek *et al*., 2015.
*Psidium guajava*** L.	Amagwava/ ugwava	Leaves	1	The leaves are boiled with ***K. mosambicina*** and ***M. azedarach*** leaves and taken orally in the mornings for blood cleansing.	Stomach-ache, diarrhoea	None found in literature.
Pyrenacantha kaurabassana Baill.	Inzema	Root tuber	1	It is used in combination with ***A. delagoensis*** as described previously (*A. delagoensis*) to cleanse the blood and the body.	Wounds	None found in literature.
*Ranunculus multifidus***Forssk.	Ixhaphozi	Shoots	3	A handful of shoots is mixed with a handful of ***S. serratuloides*** shoots and crushed. Then boiled in 2	Pimples	Von Koenen, 2001; Van Wyk, 2008.

Botanical name	Zulu name	Part(s) used	Number of times mentioned	Method of preparation and mode of administration*	Other ailments mentioned to be treated by the same plant	References of the plant species used for blood purification and other related symptoms
				L of water. Strain the decoction and pour in a sealable bottle. Drink daily in the morning until your skin is clean or you no longer feel pain during menstruation.		
Rhoicissus digitata Gilg & M.Brandt	Isinwazi	Root tuber	1	Roots are chopped and mixed with the stem of ***H. natalensis*** to be boiled in 1 L of water. It is taken orally in the morning before eating to cleanse the body.	Dysmenorrhea, kidney complaint	None found in literature.
Sapium integerrimum Hochst. ex Krauss) J. Léonard	Igowane/ umgaqampunzi/ umhlepha	Bark, roots	2	(i) It is used in combination with ***C. marginatus*** as described previously (*C. marginatus*) to treat pimples, charm and to cleanse the blood. (ii) The bark is mixed with the grinded roots of ***S. spinosa*** to steam for love charms.	None mentioned	None found in literature.
*Scadoxus puniceus*** (L.) Friis & Nordal	Idumbe lehlathi	Bulb	1	Grinded material is mixed with a handful of freshly grinded roots of ***X. caffra*****, *H. hemerocallidea*****, *R. multifidus*** and brimstone (sulphur) and boiled in 5 L of water. Take half a glass orally three times a day to drain discharge, get rid of smell in urine, dirt in the blood, and for pimples.	None mentioned	None found in literature.
Schotia brachypetala Sond.	Umgxamu/ uvovovo	Bark, roots	1	It is used in combination with ***C. marginatus*** as described previously (*C. marginatus*) to treat pimples and body cleansing	Diarrhoea	Hulme, 1954.
*Sclerocarya birrea*** Hochst.	Umganu	Bark	10	(i) A handful of fresh bark is mixed with bark or roots of ***E. natalensis*** and is used for steaming to clean the blood, get rid of pimples and bad luck. (ii) It is used in combination with ***G. livingstonei*** as described previously (*G. livingstonei*) to treat diarrhoea. (iii) It is used in combination with ***V. sieberiana*** as described previously (*V. sieberiana*) for internal cleansing of infants.	Diarrhoea	Gerstner, 1938; Pujol, 1990; Von Koenen, 2001.
Senecio serratuloides DC.	Unsukumbili	Leaves, shoots, whole plant	2	(i) It is used in combination with ***R. multifidus*** as described previously (*R. multifidus*) to treat skin and dysmenorrhea. (ii) A handful of crushed shoots is mixed with ***H. hemerocallidea*** **corm** and boiled for 30 min in 2 L of water. Half a glass is taken twice a day by adults and	Excessive body heat	Watt and Breyer-Brandwijk, 1962; Pooley, 1998.

Botanical name	Zulu name	Part(s) used	Number of times mentioned	Method of preparation and mode of administration*	Other ailments mentioned to be treated by the same plant	References of the plant species used for blood purification and other related symptoms
				a few spoons by children for blood cleansing and to treat rash and sores.		
Strychnos spinosa Lam.	Umhlala/ amahlali	Roots	3	(i) A handful of roots is mixed with roots of ***C. inerme*** and grinded. Soak in water and used for bathing. This is done for good luck, love charm and body cleansing. (ii) It is used in combination with ***P. cineraria*** and ***V. infausta*** as described previously (*P. cineraria*) to treat dysmenorrhea and cleanse the blood. (iii) Grinded roots are mixed with the bark of ***S. integerrimum*** to steam for love charm.	Sore eyes	Von Koenen, 2001.
*Syzygium cordatum*** Hochst.	Umdoni	Bark	2	The bark is mixed with the bark of *T. sericea* and soaked in warm water over night. The infusion is used as an emetic for body cleansing.	Diarrhoea, stomach-ache	None found in literature.
Terminalia sericea Burch. ex DC.	Ikonono/ umkonono	Bark, roots	3	(i) It is used in combination with ***C. molle*** as described previously (*C. molle*) to cleanse blood. (ii) It is used in combination with ***C. molle*** and ***Z. mucronata*** as described previously (*C. molle*) to cleanse the body. (iii) The bark is used in combination with ***S. cordatum*** as previously described (*S. cordatum*) to cleanse the body.	None mentioned	Von Koenen, 2001.
Vachellia sieberiana DC.	Umkhaya	Bark, roots	4	(i) The grinded roots of ***V. sieberiana***, ***V. infausta*** and ***X. caffra*** are mixed together and soak in warm water. The infusion half a cup (125 ml) is taken orally or as an enema once in a while for blood cleansing. (ii) The bark is mixed with the bark of ***S. birrea*** and boiled. It is used as an enema for internal cleansing for a baby.	Extreme body heat, fatigue	Abbiw, 1990; Hutchings *et al*., 1996.
Vangueria infausta Burch.	Umvili/ uvilapha	Roots	3	(i) It is used in combination with ***V. sieberiana*** and ***X. caffra*** as described previously (*V. sieberiana*) for blood cleansing. (ii) It is used in combination with ***P. cineraria*** and ***S. spinosa*** as described previously (*P. cineraria, S. spinosa*) to treat dysmenorrhea and cleanse blood.	Menstrual pains	Von Koenen, 2001.

Botanical name	Zulu name	Part(s) used	Number of times mentioned	Method of preparation and mode of administration*	Other ailments mentioned to be treated by the same plant	References of the plant species used for blood purification and other related symptoms
				(iii) It is used in combination with ***G. livingstonei***, ***O. natalitia*** and ***iphamba******* as described previously (*G. livingstonei*) to cleanse the body and prevent bad luck.		
*Ximenia caffra*** Sond.	Umthunduluka	Roots	4	(i) A handful of freshly ground roots is mixed with ***H. hemerocallidea***, ***S. puniceus***, ***R. multifidus*** and brimstone (sulphur) and boiled to 5 L of water. Take half a glass orally three times a day to drain discharge, get rid of smell in urine, dirt in the blood, and pimples. (ii) It is used in combination with ***V. sieberiana*** and ***V. infausta*** as described previously in *V. sieberiana* to cleanse blood.	Piles, abdominal pain	Von Koenen, 2001.
*Ziziphus mucronata*** Willd.	Umphafa, umlahlankosi	Roots	1	Mix the roots with the roots of ***T. sericea*** and ***C. molle*** and soak in warm water for few min. It is used as an enema for internal body cleansing.	Fever	Von Koenen, 2001.

*Plant species in bold are combined with the ones mentioned in first column **Plant species species only used in combination;*** New Zulu name;

**** Unidentified plant as it was not available for collection

2.4 SUMMARY

- Fifty-five participants were interviewed for this study (five male participants and 50 female participants).
- The study recorded a total of 65 plant species that were mentioned for blood purification of which 22 (32%) were recorded for the first time as blood purifiers. There were five plant species which had their vernacular names (Zulu names) recorded for the first time by this study.
- The six most frequently mentioned plant species in the study were *B. cathartica* (33%), *C. obovata* (22%), *C. marginatus* (27%), *S. birrea* (18%), *S. serratuloides* (18%) and *T. sericea* (45%).
- The roots were mentioned by 23 participants as a plant part recommended for blood purification remedies, of which 52% of those remedies were decoctions.
- Out of 20 infectious disorders mentioned as symptoms for blood infection, pimples were the most associated with blood infections as it was mentioned by 19 participants.
- Amongst the 65 plant species mentioned, some were reported to be used in combinations to increase plant activity. There were 32 different sets of combinations reported in this study for blood purification.
- There were four plant species that were involved in most combinations: *S. birrea* (infused with *E. natalensis, G. livingstonei* and *V. sieberiana*), *S. spinosa* (with *C. inerme, P. cineraria* + *V. infausta*, and *S. integerrimum*), *T. sericea* (with *C. molle, C. molle* + *Z. mucronata*, and *S. cordatum*) and *V. infausta* (with *P. cineraria* + *S. spinosa, V. sieberiana* + *X. caffra*, and *G. livingstonei* + *O. natalitia* + *iphamba*).

CHAPTER 3

ANTIMICROBIAL ACTIVITY OF BLOOD PURIFIER PLANT SPECIES

3.1 INTRODUCTION

In Chapter 2, the study documented how laypeople in northern Maputaland perceive blood purification and what ailments are affiliated to the use of a blood purifier and which plant species (plant parts) are used. The participants mentioned 65 plant species of which 54 specimens were available for collection and 58 plant parts collected. They also mentioned combining different plant species to increase the efficacy of the plants. Consequentially, this Chapter was then designed to evaluate the antimicrobial activity of these plant species and their combinations to correlate the traditional use to a scientific rationale, whereby the link between blood purification and infections may be validated. The main aim of the Chapter was to determine if the plant species selected for blood infections hold any antimicrobial properties when tested against indicator pathogens that are associated with blood infections.

3.2 METHODOLOGY

3.2.1 Sample preparation

Fifty-four plant species were collected *in situ* (58 plant samples) and transported to the Department of Botany, University of Zululand. In the department, plant material was air-dried in an aerated room. The bark, roots, corm, bulbs and fruits were chopped into smaller pieces to speed up the drying process and to prevent decay. The samples were not oven-dried to avoid disruption of chemical components. Once the material was dried, it was milled into a fine powder using a Polychem Supplies cc hammer mill. The plant material (in the form of powder) was then stored in sealed containers in a cool dry place until it was used to prepare plant extracts. From the information acquired during the

survey (Chapter 2), the study found that the traditional ways to prepare blood purifying remedies consist of water as a solvent. Therefore, the study used the same methods to prepare extracts and organic solvents were also used for comparison.

3.2.1.1 Aqueous extracts

The aqueous extracts were prepared as decoctions or infusions. For decoctions, 10 g (equivalent to approximately a handful of fresh plant material) of powdered plant material was added to 200 ml of distilled water and boiled for 10 min. For infusions, 10 g of powdered plant material was soaked in cold or warm distilled water (as per participant's methods) and thereafter placed in a Merck shaker at 130r/min at 37 °C overnight. Thereafter, each water extract was filtered through a Whatman no. 1 filter paper through a Buchner funnel with the aid of the vacuum pump (Merck). The filtrate was stored at -80 °C in a Snijders Labs freezer for 24 hrs. The frozen filtrate was placed in a freeze drier (Lasec) for lyophilisation. The resulting dried solid matter (extract) was stored in labelled plastic containers in a cool dry place until further analysed.

3.2.1.2 Organic extracts

Ten grams of ground plant material was added to 200 ml of 1: 1 dichloromethane and methanol (DCM: MeOH) solvent. After handshaking, the mixture was placed on a platform shaker (Merck) at 37 °C overnight for thorough mixing. The mixture was filtered using a Merck vacuum pump, Buchner funnel, Whatman no. 1 filter paper and a Buchner filter flask. The filtrate was placed in a fume hood for solvent evaporation. The solid remnants (extract) were collected into labelled containers and stored in a cool dry place.

After extraction and distillation of the samples, the aqueous and dichloromethane: methanol extracts were measured to calculate their percentage yield. A total of 116 plant sample extracts were prepared (58 aqueous and 58 organic extracts). The percentage yield was calculated as per Equation 3.1

$$\% \text{ yield} = \frac{\text{dry weight of extract}}{\text{dry weight of plant material}} \times 100$$

Equation 3.1

3.2.2 Preparation of cultures

American Type Culture Collection (ATCC) bacterial strains of organisms associated with blood infections were selected as indicators of the extract's activity. The bacterial pathogens included Gram-positive bacteria; *Cutibacterium acnes* ATCC 11827, *Listeria monocytogenes* ATCC 19111, *Staphylococcus aureus* ATCC 25923, *Streptococcus agalactiae* ATCC 1236, *Streptococcus pyogenes* ATCC 127831, and Gram-negative bacteria; *Acinetobacter baumannii* ATCC 19606 and *Pseudomonas aeruginosa* ATCC 27853 (Table 3.1). All strains were obtained from Davies Diagnostics (South Africa).

An inoculum of 1 ml from a freeze-dried stock culture (-20 °C) was transferred into Tryptone Soya broth (TSB) (Oxoid) and incubated at 37 °C for 24 hrs. Thioglycolate broth (Oxoid) was used for *C. acnes* and was incubated at 37 °C for seven days anaerobically in a candle jar.

3.2.3 Minimum inhibitory concentration assay

The micro-dilution method described by Eloff (1998) was adapted in the present study with minor modifications. This technique is sensitive as it evaluates the inhibition of microbial growth of lower concentrations. For the antimicrobial analysis, sterile 96-well microtitre plates were aseptically prepared under a laminar flow cabinet unit (Labcon) by adding 100 µl of TSB into each well A volume of 100 µl of each plant extract (32 mg/ml) and the controls [ciprofloxacin antibiotic (0.01 µg/ml) and acetone (32 mg/ml)] respectively were transferred into the wells in the first row of the microtitre plates. Ciprofloxacin was selected as the positive control because it is known for the broad-spectrum activity against bacteria and to confirm the antimicrobial susceptibility of the test pathogens.

Table 3.1 Pathogenic organisms selected for the study

Pathogen name and ATCC number	Microbial classification	Ailments associated with blood impurities	Growth medium used	Reference
Cutibacterium acnes ATCC 11827	Gram-positive	Acne vulgaris	Thioglycolate	Jensen *et al*., 2017; Boisrenoult, 2018
Listeria monocytogenes ATCC 19111	Gram-positive	Listeriosis, nausea, diarrhoea	TSB	Ferber and Peterkin, 1991
Staphylococcus aureus ATCC 25923	Gram-positive	Wounds, skin ailments	TSB	Kobayashi *et al*., 2015
Streptococcus agalactiae ATCC 1236	Gram-positive	UTI, endocarditis, neonatal septicaemia, bacteremia	TSB	Pietrocola *et al.*, 2018
Streptococcus pyogenes ATCC 127831	Gram-positive	Impetigo, rash, mouth blisters, palms and soles desquamation, necrosis, oedema, septic shock	TSB	Walker *et al*., 2014
Acinetobacter baumannii ATCC 19606	Gram-negative	Wounds, burns, UTI, surgical infections, bacteremia, skin infections, sepsis	TSB	Zarrilli *et al*., 2009
Pseudomonas aeruginosa ATCC 27853	Gram-negative	UTI, skin infections, CNS, bacteremia	TSB	Juayang *et al*., 2017

ATCC = American Type Culture Collection; TSB = Tryptone Soya broth; CNS = central nervous system; UTI = urinary tract infection

The acetone was used as a negative control to indicate whether the solvent has any inhibitory activity on its own. Serial dilutions were performed longitudinally by transferring 100 µl starting from first row to the last one and in so doing, diluting the extracts and controls by 50% each time. The final concentrations of the extracts thus ranged from 8.00 mg/ml in the first row (A) to 0.06 mg/ml in the last row (H) of a microtitre plate. Outside the laminar flow unit, sub-cultured bacteria (100 µl) was added to all the wells of each microtitre plate. Before use, the culture was first diluted in broth to a just-turbid consistency (0.5 McFarland turbidity standard) and diluted 1:100 with fresh sterile broth to give a density of approximately 1×10^6 colony forming units/ml (CFUs/ml). Each microtitre plate was sealed with a sterile adhesive seal to prevent evaporation of the extracts. The plates were then incubated at 37 °C for 24 hrs. To confirm that the culture were not contaminated, each diluted pathogen-broth mixture was also streaked onto a Tryptone Soya agar plate (TSA) and incubated overnight. For *C. acnes*, the microtitre plates were incubated at 37 °C for seven days under anaerobic conditions using a candle gas jar. Since *C. acnes* does not grow readily on agar, the contamination of the culture was determined by streaking the Thioglycolate broth culture onto a TSA plate, which was then incubated under aerobic conditions. If growth was evident, then the test was discarded.

After incubation, 40 µl of 0.40 mg/ml of *p*-iodonitrotetrazolium (INT) dissolved in sterile water was added to all microtitre plate wells. *P*-iodonitrotetrazolium was used as an indicator of microbial growth, as it changes from colourless to red in the presence of biological active micro-organisms (Eloff, 1998). After the addition of INT, the plates were left at room temperature and assessed regularly for the development of red colour in the culture control columns. Each pathogen responds differently to INT, therefore if the culture control change to red that signified that the pathogen had responded and therefore the plate was ready for results to be captured. The MIC was reported as the lowest concentration of the extract that resulted in inhibition of microbial growth, represented by a clear colour in the wells. The MIC values ≤ 0.16 mg/ml were considered to have noteworthy activity. Values >0.16 <1.00 mg/ml were considered moderate in activity (Van Vuuren and Holl, 2017). In cases where the MIC of certain plant extracts was not detected

(i.e. MIC < 0.06 mg/ml), the extracts were diluted 10-fold to yield a starting concentration of 0.32 mg/ml and MIC assays were repeated. Tests were performed in duplicate (or triplicate when inconsistent results were obtained) for each pathogen.

For the combination studies, microtitre plates were aseptically prepared by adding 100 µl of the growth medium in all wells. For combinations of two plant species, 50 µl from each plant extract at a starting concentration of 32 mg/ml were combined adding up to 100 µl in the first row of the microtitre plate to make a 1:1 combination. Where four plant extracts were combined, 25 µl from each extract, were combined to make up a volume of 100 µl in each well in a 1:1:1:1 combination. Serial dilutions were performed on the extracts as earlier mentioned for individual plant species. Tests were undertaken in duplicate or triplicate with positive (ciprofloxacin 0.01 µg/ml) and negative (acetone and sterile medium) controls included in each assay. The MIC values were determined for the plant combinations and the Fractional Inhibitory Concentration (FIC) was calculated for each combination (Equation 3.2). The ΣFIC (FIC index) was then calculated by adding together the FIC values.

$$FICa = \frac{MIC(a) \text{ in combination with (b)}}{MIC(a) \text{independently}} \qquad FICb = \frac{MIC(b) \text{ in combination with (a)}}{MIC(b) \text{independently}}$$

Equation 3.2

In the equation, the (a) and (b) represent different plant species in combination. Where more than two plant species were combined, the equation also brought into account the number of plant samples forming part of each combination. The value of the ΣFIC was used to determine if the plant species in a combination are synergistic, additive, non-interactive or antagonistic. The plant species were regarded as synergistic if their ΣFIC value ≤ 0.50. The additive effect was observed when the ΣFIC value was 0.50 ≤ 1.00. For the non-interactive interaction, the ΣFIC value was 1.00 ≤ 4.00 while antagonism occurred when the ΣFIC value > 4.00 (Van Vuuren and Viljoen, 2011a). Tests were performed in duplicate (or triplicate when inconsistent results were obtained) against each pathogen.

3.3 RESULTS AND DISCUSSION

3.3.1 Antimicrobial activity of plant species used individually for blood purification

There were a number of plant species that demonstrated promising results in terms of moderate and noteworthy antimicrobial activity. The highest extract yield of 28% was observed for aqueous extract of *H. abyssinca* and *T. elegans* had the highest yield of 18% amongst the organic extracts. Nineteen plant extracts displayed noteworthy effects against the growth of at least one pathogen (Table 3.2). There were two plant species while not demonstrating any noteworthy activity that did have broad-spectrum moderate efficacy with the organic extracts of *E. natalensis* and *O. serrulata*. The roots of *E. natalensis* organic extract moderately inhibited the Gram-positive bacterial strains at a MIC value of 0.25 mg/ml and *P. aeruginosa* at a MIC value of 0.50 mg/ml. The use of *E. natalensis* was mentioned twice in Chapter 2 (Table 2.2) to treat acne and for blood cleansing. The organic extract of *O. serrulata* roots moderately inhibited *C. acnes* at an MIC value of 0.19 mg/ml; *L. monocytogenes*, and *S. aureus* at a MIC value of 0.50 mg/ml in both cases and *P. aeruginosa* at an MIC value of 0.38 mg/ml. The plant species was mentioned three times during surveys for blood and womb cleansing. The aqueous extracts were less active than the organic extracts, except *A. delagoensis,* which was the only aqueous extract that demonstrated noteworthy activity (0.13 mg/ml) against *C. acnes*. There were three other plant extracts that had a narrow spectrum of inhibition, but their aqueous extracts had successfully inhibited bacterial strains: *H. hemerocallidea, R. multifidus* and *S. serratuloides. Hypoxis hemerocallidea* extract showed moderate inhibition against *S. pyogenes* for both aqueous and organic extracts with MIC values of 0.50 mg/ml each. The plant is known to be used for rashes, sores and blood cleansing. The aqueous extract of *R. multifidus* shoots suppressed the growth of *C. acnes* at 0.50 mg/ml MIC value, whereas the organic extract inhibited the pathogen at an MIC value of 0.25 mg/ml. The organic extracts also moderately suppressed *S. aureus* at an MIC value of 0.50 mg/ml.

Table 3.2 The MIC values (mg/ml) for plant species that were specified as blood purifiers

Plant species	% Yield		*C.acnes* ATCC 11827		*L. monocytogenes* ATCC 19111		*S. aureus* ATCC 25923		*S. agalactiae* ATCC 1236		*S. pyogenes* ATCC 127831		*A. baumannii* ATCC 19606		*P. aeruginosa* ATCC 27853	
	Aq	**D: M**	**Aq**	**D: M**	**Aq**	**D: M**	**Aq**	**D: M**	**Aq**	**D: M**	**Aq**	**D: M**	**Aq**	**D: M**	**Aq**	**D: M**
A. precatorius R	8.70	9.50	1.50	**0.03**	8.00	**0.38**	8.00	**0.50**	8.00	**0.50**	8.00	1.00	8.00	8.00	>8.00	1.50
A. glabratum Wp	12.40	11.80	3.00	**0.50**	8.00	2.00	>8.00	2.00	>8.00	2.00	>8.00	3.00	>8.00	2.00	2.00	1.00
A. delagoensis R	25.40	15.30	**0.13**	**0.05**	**0.50**	**0.50**	**0.50**	1.00	1.00	1.00	1.50	1.00	2.00	1.00	>8.00	1.00
B. pilosa R	6.10	6.90	8.00	**0.50**	6.00	1.00	3.00	1.00	>8.00	1.00	6.00	2.00	8.00	2.00	8.00	0.75
B. discolor Sh	8.40	12.90	>8.00	0.75	8.00	1.00	8.00	1.00	8.00	1.00	>8.00	2.00	>8.00	2.00	4.00	**0.50**
B. cathartica L	12.40	8.70	8.00	1.00	4.00	2.00	8.00	2.00	8.00	2.00	8.00	4.00	>8.00	4.00	4.00	1.00
B. cathartica R	10.80	8.60	3.00	0.75	4.00	2.00	>8.00	2.00	>8.00	1.50	4.00	4.00	>8.00	4.00	8.00	**0.50**
C. inerme R	3.30	12.20	1.00	**0.13**	6.00	**0.25**	6.00	1.00	>8.00	1.00	2.00	2.00	>8.00	4.00	8.00	**0.50**
C. roseus R	12.10	10.30	8.00	**0.75**	>8.00	6.00	>8.00	8.00	>8.00	4.00	>8.00	4.00	>8.00	4.00	8.00	1.00
C. hirta R	5.40	4.70	**0.50**	**0.06**	8.00	1.00	8.00	2.00	8.00	2.00	4.00	2.00	8.00	2.00	>8.00	1.00
C. anasita L	18.60	13.60	>8.00	**0.50**	>8.00	2.00	>8.00	2.00	>8.00	2.00	>8.00	4.00	>8.00	2.00	>8.00	1.00
C. molle L	12.10	12.30	**0.25**	**0.13**	4.00	2.00	2.00	2.00	2.00	2.00	2.00	1.50	>8.00	2.00	4.00	**0.38**
C. molle R	12.90	7.90	**0.19**	**0.09**	2.00	2.00	4.00	1.00	4.00	1.00	1.00	**0.50**	8.00	2.00	>8.00	**0.50**
D. cymosum R	7.60	14.10	6.00	1.00	>8.00	3.00	>8.00	2.00	>8.00	2.00	>8.00	4.00	>8.00	1.00	>8.00	**0.50**
E. capensis B	13.30	12.70	0.75	**0.06**	3.00	1.00	1.50	2.00	2.00	2.00	2.00	0.75	>8.00	2.00	>8.00	**0.50**
E. natalensis R	11.40	7.90	>8.00	**0.25**	>8.00	**0.25**	>8.00	**0.25**	>8.00	**0.25**	>8.00	**0.25**	>8.00	1.00	>8.00	**0.50**
G. livingstonei B	10.10	12.30	**0.38**	**0.38**	2.00	1.00	1.00	**0.50**	1.00	2.00	1.50	2.00	>8.00	2.00	>8.00	**0.50**
G. volkensii R	8.20	7.50	>8.00	>8.00	>8.00	1.00	>8.00	2.00	>8.00	2.00	>8.00	2.00	>8.00	2.00	>8.00	**0.50**

Plant species	% Yield		*C.acnes* ATCC 11827		*L. monocytogenes* ATCC 19111		*S. aureus* ATCC 25923		*S. agalactiae* ATCC 1236		*S. pyogenes* ATCC 127831		*A. baumannii* ATCC 19606		*P. aeruginosa* ATCC 27853	
	Aq	**D: M**	**Aq**	**D: M**	**Aq**	**D: M**	**Aq**	**D: M**	**Aq**	**D: M**	**Aq**	**D: M**	**Aq**	**D: M**	**Aq**	**D: M**
G. caffra R	9.40	7.10	>8.00	1.00	>8.00	2.00	>8.00	2.00	>8.00	2.00	>8.00	4.00	>8.00	4.00	>8.00	1.00
G. senegalensis L	13.15	11.95	2.00	1.00	8.00	2.00	8.00	2.00	8.00	2.00	8.00	4.00	>8.00	4.00	2.00	**0.50**
G. senegalensis R	17.10	12.10	2.00	**0.06**	8.00	**0.13**	>8.00	**0.13**	8.00	**0.13**	4.00	**0.25**	>8.00	1.00	>8.00	**0.50**
H. pauciflorus T	6.20	5.50	4.00	**0.50**	>8.00	1.50	>8.00	2.00	>8.00	3.00	>8.00	3.00	>8.00	4.00	>8.00	**0.50**
H. abyssinica Rh	28.20	15.10	1.00	0.75	4.00	2.00	>8.00	2.00	>8.00	2.00	4.00	4.00	>8.00	4.00	>8.00	0.75
H. hemerocallidea C	23.10	13.70	2.00	1.00	2.00	1.50	2.00	2.00	2.00	2.00	**0.50**	**0.50**	>8.00	2.00	>8.00	2.00
H. natalensis R	12.70	8.20	1.00	**0.50**	4.00	1.00	8.00	1.00	8.00	3.00	8.00	4.00	>8.00	4.00	8.00	**0.50**
K. africana B	14.80	11.70	2.00	1.00	>8.00	2.00	8.00	2.00	>8.00	2.00	3.00	4.00	>8.00	4.00	>8.00	1.00
K. mosambicina Sh	9.60	12.00	2.00	1.00	>8.00	2.00	>8.00	2.00	>8.00	2.00	4.00	4.00	>8.00	4.00	>8.00	1.00
L. javanica L	13.00	10.30	8.00	**0.50**	>8.00	2.00	>8.00	2.00	4.00	3.00	3.00	2.00	>8.00	2.00	>8.00	**0.50**
M. azedarach L	10.80	8.00	1.00	**0.13**	8.00	**0.50**	8.00	**0.50**	>8.00	4.00	8.00	3.00	6.00	2.00	>8.00	**0.50**
M. balsamina L	11.40	9.10	1.00	**0.25**	>8.00	3.00	>8.00	2.00	8.00	3.00	4.00	1.50	>8.00	2.00	>8.00	**0.50**
O. natalitia R	21.30	7.20	4.00	**0.25**	>8.00	1.50	>8.00	1.00	>8.00	2.00	8.00	1.00	>8.00	1.00	>8.00	**0.50**
O. serrulata R	9.70	8.10	3.00	**0.19**	>8.00	**0.50**	4.00	**0.50**	>8.00	1.00	4.00	**0.38**	>8.00	1.00	>8.00	1.00
O. engleri R	27.90	12.20	**0.50**	**0.004**	4.00	**0.02**	2.00	**0.02**	4.00	**0.01**	2.00	**0.02**	4.00	**0.50**	>8.00	**0.50**
P. americana Sd	7.10	5.60	4.00	**0.05**	8.00	1.00	8.00	**0.50**	8.00	1.00	6.00	2.00	8.00	2.00	8.00	2.00
P. cineraria R	7.30	7.10	8.00	**0.13**	>8.00	2.00	>8.00	2.00	>8.00	2.00	>8.00	4.00	>8.00	4.00	>8.00	1.00
P. guajava L	13.00	14.70	**0.38**	**0.06**	3.00	2.00	4.00	3.00	4.00	2.00	1.00	2.00	>8.00	2.00	>8.00	**0.50**
P. kaurabassana Rt	9.60	11.20	2.00	**0.50**	>8.00	8.00	>8.00	8.00	>8.00	2.00	>8.00	4.00	>8.00	3.00	>8.00	1.00

Plant species	% Yield		*C.acnes* ATCC 11827		*L. monocytogenes* ATCC 19111		*S. aureus* ATCC 25923		*S. agalactiae* ATCC 1236		*S. pyogenes* ATCC 127831		*A. baumannii* ATCC 19606		*P. aeruginosa* ATCC 27853	
	Aq	**D: M**	**Aq**	**D: M**	**Aq**	**D: M**	**Aq**	**D: M**	**Aq**	**D: M**	**Aq**	**D: M**	**Aq**	**D: M**	**Aq**	**D: M**
P. scandens T	12.30	12.40	6.00	**0.50**	8.00	1.00	8.00	8.00	>8.00	2.00	>8.00	2.00	>8.00	4.00	>8.00	3.00
R. multifidus Sh	12.40	11.80	**0.50**	**0.25**	4.00	1.00	>8.00	**0.50**	>8.00	2.00	>8.00	6.00	>8.00	4.00	8.00	2.00
R. digitata R	9.90	8.30	8.00	**0.50**	>8.00	2.00	>8.00	4.00	>8.00	2.00	>8.00	2.00	>8.00	2.00	>8.00	4.00
R. communis L	16.40	10.70	1.00	1.00	>8.00	2.00	>8.00	8.00	8.00	2.00	4.00	2.00	>8.00	4.00	8.00	1.00
S. integerrimum R	7.40	9.90	1.00	**0.13**	>8.00	1.00	2.00	3.00	2.00	2.00	2.00	1.00	>8.00	4.00	>8.00	1.00
S. puniceus Bb	4.40	12.60	1.00	0.75	8.00	1.00	8.00	3.00	>8.00	2.00	8.00	4.00	>8.00	3.00	>8.00	**0.50**
S. brachypetala B	11.60	18.40	1.50	**0.50**	4.00	2.00	4.00	4.00	8.00	2.00	2.00	2.00	8.00	4.00	>8.00	1.00
S. birrea B	15.90	13.00	**0.25**	**0.09**	2.00	1.00	4.00	**0.25**	4.00	1.00	2.00	1.00	>8.00	2.00	>8.00	**0.19**
S. serratuloides Sh	12.80	11.20	**0.50**	**0.25**	4.00	**0.50**	8.00	2.00	>8.00	3.00	>8.00	8.00	>8.00	2.00	>8.00	1.00
S. spinosa R	8.80	10.80	1.00	1.00	>8.00	2.00	>8.00	8.00	>8.00	2.00	>8.00	4.00	>8.00	4.00	>8.00	3.00
S. cordatum B	10.10	8.20	**0.25**	**0.13**	4.00	2.00	4.00	2.00	>8.00	2.00	0.75	2.00	>8.00	4.00	>8.00	1.00
T. elegans F	10.40	18.50	3.00	**0.50**	6.00	1.00	8.00	1.00	8.00	2.00	8.00	2.00	>8.00	4.00	8.00	1.00
T. sericea R	19.80	13.20	**0.25**	**0.05**	2.00	**0.50**	1.00	0.75	2.00	2.00	1.00	2.00	>8.00	1.00	1.00	**0.13**
T. emetica B	17.30	15.30	4.00	2.00	4.00	2.00	4.00	3.00	>8.00	2.00	4.00	4.00	>8.00	2.00	>8.00	2.00
T. emetica R	9.50	7.00	>8.00	**0.50**	>8.00	8.00	>8.00	8.00	>8.00	2.00	>8.00	4.00	>8.00	3.00	>8.00	1.00
V. sieberiana B	6.30	8.20	1.00	**0.50**	8.00	1.00	>8.00	2.00	>8.00	3.00	6.00	3.00	>8.00	4.00	>8.00	1.00
V. xanthophloea B	7.30	9.40	2.00	**0.50**	>8.00	1.00	>8.00	2.00	>8.00	3.00	>8.00	4.00	>8.00	4.00	>8.00	1.00
V. infausta R	7.70	6.10	2.00	**0.50**	>8.00	2.00	>8.00	8.00	>8.00	2.00	>8.00	4.00	>8.00	4.00	>8.00	2.00
W. indica R	6.90	10.00	2.00	1.00	>8.00	2.00	8.00	2.00	>8.00	2.00	2.00	2.00	8.00	2.00	>8.00	2.00

Plant species	**% Yield**		***C.acnes* ATCC 11827**		***L. monocytogenes* ATCC 19111**		***S. aureus* ATCC 25923**		***S. agalactiae* ATCC 1236**		***S. pyogenes* ATCC 127831**		***A. baumannii* ATCC 19606**		***P. aeruginosa* ATCC 27853**	
	Aq	**D: M**	**Aq**	**D: M**	**Aq**	**D: M**	**Aq**	**D: M**	**Aq**	**D: M**	**Aq**	**D: M**	**Aq**	**D: M**	**Aq**	**D: M**
W. somnifera Wp	14.00	9.60	8.00	**0.05**	8.00	**0.50**	>8.00	**0.50**	>8.00	**0.50**	3.00	1.00	>8.00	1.00	>8.00	1.00
X. caffra R	12.50	10.00	1.00	**0.06**	2.00	0.75	4.00	2.00	4.00	1.00	4.00	2.00	>8.00	4.00	>8.00	1.00
Culture control (growth medium)	NA		>8.00	>8.00	>8.00	8.00	>8.00	>8.00	>8.00	>8.00	>8.00	>8.00	>8.00	>8.00	>8.00	>8.00
Negative control (acetone)			>8.00	>8.00	>8.00	>8.00	>8.00	>8.00	>8.00	>8.00	>8.00	>8.00	>8.00	>8.00	>8.00	>8.00
Positive control (0.01 µg/ml Ciprofloxacin)			0.04		0.08		0.01		0.01		0.04		0.02		0.02	0.01

ATCC = American type culture collection; Aq = aqueous; D: M = dichloromethane: methane; B = bark; Bb = bulb; C = corm; F = fruits; L = leaves; Rh = rhizome; R = roots; Rt = Root tuber; Sd = seeds; S = stem; Sh = shoots; T = twigs; Wp = whole plant; NA = not applicable; Numbers highlighted in black-bold had moderate activity; Numbers highlighted in red-bold had noteworthy activity.

The aqueous extract of *S. serratuloides* inhibited the growth of *C. acnes* at an MIC value of 0.50 mg/ml and the organic extract at an MIC value of 0.25 mg/ml. The most susceptible bacterial strains were the Gram-positive *C. acnes* and Gram-negative *P. aeruginosa*. *Cutibacterium acnes* was inhibited by 12 aqueous extracts of which one extract had noteworthy activity and 42 organic extracts with 19 of them displaying noteworthy activity. The least susceptible bacterial strain in the study was *A. baumannii* (Gram-negative bacteria). From a total of 54 plant species (58 samples) tested in the study, only the organic root extract of *O. engleri* was able to moderately inhibit the growth of *A. baumannii* at the MIC value of 0.50 mg/ml.

3.3.2 The plant extracts that displayed noteworthy antimicrobial activity

Noteworthy activity of *A. precatorius* roots against *C. acnes* was noted, where the growth of the bacteria was inhibited at a MIC value of 0.03 mg/ml with the organic extract. The activity of *A. precatorius* organic extract against *C. acnes* was one of the most effective that was evaluated. The extract moderately inhibited the growth of *L. monocytogenes* at the MIC value 0.38 mg/ml, and of *S. aureus* and *S. agalactiae* at 0.50 mg/ml MIC values each. *Abrus precatorius* roots was mentioned once in this study for skin ailments, chest pains and blood cleansing. According to the findings of the study, the reported traditional use of *A. precatorius* for blood infections caused by *C. acnes* (skin diseases) was validated. In a study that was designed to evaluate the activity of *A. precatorius* seeds using a disc-diffusion method, the ethanol extract was reported to have a noteworthy effect against *S. aureus* (Sreeramulu *et al*., 2009). Adelowotan *et al*. (2008) documented that the aqueous extracts of leaves, stem and seeds obtained a MIC value of 0.26 mg/ml, 0.26 mg/ml and 0.06 mg/ml against *S. aureus,* respectively. Only the leaf extract suppressed the growth of *P. aeruginosa* at MIC values of 0.51 mg/ml and 0.26 mg/ml in aqueous and methanol extracts, respectively. In another study, the aqueous extract of *A. precatorius* seeds was highly active against the growth of *S. aureus* with a growth inhibition zone of 15.20 mm (Karamoko *et al*., 2012). These findings are not in-line with those of the present study where the aqueous extract of *A. precatorius* had weak activity

against *S. aureus*. However, it was noted that the plant parts used in these two studies are different.

A previous disc-diffusion antimicrobial study was undertaken on *A. precatorius* leaves and roots where they both showed moderate activity (Yusufu *et al*., 2017). In a study that tested the methanol: water (3:2) leaf extract of *A. precatorius*, the MIC values against *S. aureus* and *P. aeruginosa* strains were both 3.13 mg/ml (weak activity) (Alayande *et al*., 2017). In a study that assessed antibacterial activity of *A. precatorius*, the ethanol leaves extract demonstrated poor activity against *S. aureus* and *P. aeruginosa* (Oka and Nweze, 2020). A study done by Madikizela *et al*. (2013) evaluated the antimicrobial efficacy of four extracts of *A. precatorius* leaves and seeds against *S. aureus*. Both plant parts were considered non-active with the MIC value of 1.56 mg/ml each in 80% ethanol extract and 12.50 mg/ml for leaf extracts in petroleum ether and dichloromethane solvents. The present study investigated the roots of *A. precatorius* which showed a strong activity against *C*. *acnes*. In the studies that evaluated the leaves, most of the results demonstrated weak antimicrobial activity, except for the study of Madikizela *et al*. (2013), where the seeds of *A. precatorius* had a strong activity against *S. aureus*. The availability of seeds for any plant is seasonal, hence that could be the reason people also use the roots of the plant.

The roots of *A. delagoensis* portrayed noteworthy suppression on the growth of *C. acnes* in both aqueous (MIC value of 0.13 mg/ml) and organic (MIC value of 0.05 mg/ml) extracts. The aqueous and organic extracts also moderately inhibited the growth of *L. monocytogenes* at a MIC value of 0.50 mg/ml. There was also moderate inhibition (MIC value of 0.50 mg/ml) observed against the *S. aureus* strain where the aqueous extract was tested. This plant species was reported in Chapter 2 as a good blood purifier and its good activity against *C. acnes* validates the use of the plant since skin infections are quite often associated with the need for blood purification. *Albertisia delagoensis* has to the best of our knowledge not been studied previously for antimicrobial activity.

The organic roots extract of *C. inerme* showed noteworthy activity against *C. acnes, L. monocytogenes* and *P. aeruginosa* with MIC values of 0.13 mg/ml, 0.25 mg/ml and 0.50 mg/ml respectively. The findings of the study validate the use of *C. inerme*, especially for *C. acnes* infections. Although demonstrating moderate activity, the plant species has a potential efficacy against sore eyes if the pain stems from endophthalmitis caused by *P. aeruginosa*. In that case, there is validated correlation between the traditional use of *C. inerme* in Chapter 2 and antimicrobial effect in this study. A study was conducted in 2016 which documented that the dichloromethane: methanol extract of *C. inerme* twigs which was moderately active against *P. aeruginosa* (MIC value of 0.25 mg/ml) and had a weak activity of 2.00 mg/ml and 4.00 mg/ml against *C. acnes* and *S. aureus* respectively (Nciki *et al*., 2016). The present study and that by Nciki *et al*. (2016), tested the same bacterial strains (*C. acnes* ATCC 11827, *S. aureus* ATCC 25923 and *P. aeruginosa* ATCC 27853). The findings of the current and previous study demonstrated that different parts of a plant have different components and therefore cannot be compared in terms of efficacy.

The aqueous extract of *C. hirta* roots had moderate activity against *C. acnes* (MIC value of 0.50 mg/ml) and the organic extract portrayed a noteworthy effect with a MIC value of 0.06 mg/ml inhibition against the same bacterial strain. The plant species was mentioned once in the study for blood cleansing, stomach-ache and to treat a weak immune system. The antimicrobial activity only validate the use of *C. hirta* for skin related ailments, regarding they are associated with blood purification. There is a lack of antimicrobial evaluation studies for *C. hirta,* but one previous MIC study (Van Vuuren *et al*., 2015), tested the extracts of *C. hirta* on *S. aureus* and it was found to have poor activity. Da Silva Mendes *et al*. (2020), reviewed the pharmacology of five *Cissampelos* species (including *C. hirta*) and the only antimicrobial activity documented was for *C. pareira*. The authors also reported that *C. mucronata, C. pareira* and *C. sympodialis* were the most studied species within the *Cissampelos* genus.

The roots and leaf extracts of *C. molle* inhibited the growth of *C. acnes* in both the aqueous extracts (MIC values of 0.19 mg/ml and 0.25 mg/ml respectively) and organic extracts (MIC values of 0.09 mg/ml and 0.13 mg/ml respectively). The organic extract of

both plant parts inhibited the growth of *P. aeruginosa* at a MIC value of 0.50 mg/ml for roots and 0.38 mg/ml for leaves. The plant species was mentioned once in the study as a blood cleanser and strengthener. Both the pathogens that responded positively to the extract were reported as the main pathogens that cause skin infections, hence the reported traditional use of *C. molle* is valid based on the *in vitro* results obtained in the study. The leaves and roots of *C. molle* in the present study yielded similar results, with the leaves yielding even lower MIC values. According to these findings, users should be advised to replace the use of the roots with the leaves because of sustainable harvesting factors. A study that was published in 2012 reported that the leaves of *C. molle* had a weak activity of 1.33 mg/ml and 2.67 mg/ml in aqueous and dichloromethane: methanol extracts respectively against the *S. aureus* strain (York *et al*., 2012). This previous study and the present study were both conducted in the same geographical area, used the same parts and solvents, therefore the difference between these two studies could be due to using different strains of *S. aureus*. In another study using the disc-diffusion assay, the acetone bark extract of *C. molle* had weak activity against *S. aureus* (Asres *et al*., 2006).

The organic extract of *E. capensis* bark had noteworthy activity against *C. acnes* with a MIC value of 0.06 mg/ml and *P. aeruginosa* with 0.50 mg/ml. The use of *E. capensis* bark was mentioned once in the study for pimples, blood cleansing or love charms. The findings of the present study validates the traditional use since *C. acnes* and *P. aeruginosa* are known to affect the skin, however, the spiritual use cannot be related to the results projected in the study. Oyedeji–Amusa *et al*. (2020) documented that the dichloromethane bark extract of *E. capensis* had noteworthy activity against *P. aeruginosa* (MIC value of 0.13 mg/ml) whereas in the leaf extraction it had moderate activity (MIC value of 0.19 mg/ml). A study by York *et al*. (2012) found that the aqueous and dichloromethane: methanol leaf extracts for *E. capensis* were inactive against *S. aureus* with the MIC values of. 10.67 mg/ml and 13.33 mg/ml respectively. The results from these three studies suggest that the bark is more effective against *S. aureus* than the leaves.

The organic extract of *G. senegalensis* roots demonstrated broad-spectrum antimicrobial activity in the current study. The extract had noteworthy inhibition against four bacterial strains. *Cutibacterium acnes* was inhibited at a MIC value of 0.06 mg/ml and *L. monocytogenes*, *S. aureus* and *S. agalactiae* were all inhibited at a MIC value of 0.13 mg/ml. The roots of *G. senegalensis* were mentioned by two participants to be used for blood purification (Chapter 2) and the antimicrobial activity validates its use against pathogens that may cause skin and bloodstream infections. In a previous antimicrobial study, the roots of *G. senegalensis* were active against the strains of *S. aureus* when tested with several solvents. For the methanol and ethanol extracts against *S. aureus,* growth was inhibited at 0.16 mg/ml (Mathabe *et al*., 2006). The results of this study demonstrate congruency except for the aqueous extracts which in the current study did not demonstrate any activity. The plant species in the present study were from KwaZulu-Natal and this geographical location differed from the previously mentioned study where the plant samples were sourced from Limpopo Province, a different province in South Africa. In another study that used the disc-diffusion assay, the water and methanol extracts of the stem bark and root bark were active against *S. aureus,* whereas resistance was observed against other types of extracts tested within the study (Matu and Van Staden, 2003). Mulaudzi *et al*. (2012) noted that the root of *G. senegalensis* showed no positive activity against *S. aureus* for both aqueous and different organic extracts in the study. The obtained MIC values were 3.13 mg/ml, 6.25 mg/ml, 0.78 mg/ml and 0.78 mg/ml for aqueous, petroleum ether, dichloromethane and ethanol respectively. In the previous studies reviewed for *G. senegalensis,* either different techniques or different solvent extractions were used and that may have resulted in the differences observed. It is important to also note that the present study evaluated both the leaves and roots of *G. senegalensis*, however, the leaves showed weak activity against all bacterial strains used in the study.

The organic extract of *M. azedarach* (leaves) was antimicrobially active against *C. acnes* at 0.13 mg/ml. The plant species was mentioned once in the ethnobotanical survey (Chapter 2) to treat pimples. Therefore, noteworthy performance of *M. azedarach* against *C. acnes* support the traditional use of the plant. In a study that focused on the essential

oils from aerial parts of *M. azedarach*, high activity (inhibition zones ranging from 19 mm – 30 mm) was observed for *S. aureus* (Meziane and Goumri, 2014). The results are somewhat congruent (taking into account the different methods used) to those of the present study where the organic extract was active against *S. aureus* at a MIC value of 0.50 mg/ml.

The organic roots extract for *O. engleri* demonstrated the most noteworthy broad-spectrum activity against all Gram-positive strains or moderate activity against the Gram-negative bacterial strains. *Ozoroa engleri* inhibited the growth of *C. acnes* at the MIC value of 0.004 mg/ml which was the most active inhibition observed in the study. Even though *O. engleri* was the most antimicrobially effective plant extract in the study, the use of the plant species was only mentioned once for blood cleansing. The efficiency displayed by *O. engleri* against pathogens associated with blood infections validate the reported traditional use in Chapter 2. The results from this study correlated to those of the study that was conducted from northern Maputaland by Nciki *et al*. (2016). In their study, the organic bark extract of *O. engleri* inhibited the growth of *C. acnes, S. aureus* and *P. aeruginosa* at MIC values of 0.13 mg/ml, 0.75 mg/ml and 0.38 mg/ml respectively along with other bacteria that were used in the study. The organic leaf extract inhibited the growth of *C.* acnes and *P. aeruginosa* at MIC values of 0.50 mg/ml and 0.38 mg/ml respectively (Nciki *et al*., 2016). The values were lower in the present study and the study used a different plant part (roots). Although the leaves have shown strong activity, they are not available during winter time, therefore people could substitute the leaves with the roots for that period once possible toxicity is evaluated. Another study reported that *O. engleri* organic extract had moderate activity against *Candida albicans* at a MIC value of 0.75 mg/ml and *Oligella ureolytica* at 0.50 mg/ml (Naidoo *et al*., 2013). *Candida alibicans* is associated with skin conditions and *O. ureolytica* is associated with sexually transmitted infections and interestingly, these two infections were mostly indicated for ‘dirty blood’ by laypeople in northern Maputaland.

The organic extract of *P. americana* seed had notable suppression of *C. acnes* (MIC value of 0.05 mg/ml) and moderate activity (MIC value of 0.50 mg/ml) against *S. aureus.*

Persea americana was mentioned once for kidney complaints and blood cleansing. It has been established that the kidneys are part of the blood purification system that occur naturally in the body. Therefore, the use of *P. americana* to assist the blood purification process is comprehensible. The use of *P. americana* for kidney infections from *C. acnes* is therefore validated according to the results of the current study. Although *C. acnes* is commonly known for acne and/or pimples, it may cause different infections including kidney infections, oral cavity infection, eye infection and the infection of joints (Perry and Lambert, 2011). A study was conducted where a low concentration of 0.4 mg/ml ethanolic extract of *P. americana* peel had noteworthy activity (16.40 mm and 16.26 mm inhibition zones) against *S. aureus* and *P. aeruginosa* respectively (Lubis *et al*., 2017).

Prosopis cineraria roots (organic extract) showed noteworthy activity only against *C. acnes* (MIC value of 0.13 mg/ml). The plant species was reported once as a treatment for toothache and blood cleansing. The results of the current study support the traditional use of *P. cineraria* roots for treating skin complaints which is very often linked to blood cleansing. A disc-diffusion assay was performed where the leaves and seed pods of a methanolic *P. cineraria* extract portrayed moderate activity against *S. aureus,* whereas the stem extract had weak activity (Preeti *et al*., 2017). In another study, *S*. *aureus* was not inhibited in the disc diffusion assay of *P. cineraria* leaves (Alkaabi *et al*., 2020).

Cutibacterium acnes was susceptible to both aqueous and organic leaf extracts of *P. guajava*. This bacterial strain was inhibited at the MIC value of 0.38 mg/ml for the aqueous extract, and 0.06 mg/ml for the organic extract. The organic extract also suppressed the growth of *P. aeruginosa* at an MIC value of 0.50 mg/ml. In Chapter 2, this plant species was mentioned once for the treatment of stomach-ache, diarrhoea and blood cleansing. Although *C. acnes* could cause other diseases besides skin infections, stomach-ache or diarrhoea are not one of those infections. Therefore, *P. guajava* could only be validated for blood cleansing. In a parallel study from northern Maputaland, the results displayed that the aqueous extract of the leaves had a moderate activity of 0.50 mg/ml value whilst the dichloromethane: methanol extract had less activity (MIC value 0.93) against *S. aureus* (Van Vuuren *et al*., 2015). The other study found that both the aqueous and

dichloromethane: methanol leaf extracts had weak activity. The aqueous extract obtained an MIC value of 2.00 mg/ml and the organic extract displayed an MIC value of 0.63 mg/ml against *S. aureus* (York *et al*., 2012). Results of these two studies were congruent with the current study where *P. guajava* had poor inhibition at MIC values of 4.00 mg/ml and 3.00 mg/ml against *S. aureus* with aqueous and organic extracts respectively.

The organic extract of *S. integerrimum* roots had noteworthy inhibition of *C. acnes* with a MIC value of 0.13 mg/ml. *Sapium integerrimum* was mentioned by four participants as a good remedy for pimples and blood cleansing. Van Vuuren and Frank (2020) reported that pimples are used as the main indicator for blood infection. Therefore, according to the result of this study, the traditional use of *S. integerrimum* for the reported ailments was validated, especially since *C. acnes* is the main cause of pimples. No literature could be found with any information regarding the antimicrobial activity of *S. integerrimum*.

The present study recorded that *S. birrea* bark inhibited the growth of *C. acnes.* The aqueous extract had a moderate effect (MIC value 0.25 mg/ml) and the organic extract had noteworthy inhibition (MIC value 0.09 mg/ml). The organic extract also inhibited the growth of *S. aureus* and *P. aeruginosa* at the minimum concentrations of 0.25 mg/ml and 0.19 mg/ml respectively. *Sclerocarya birrea* was one of the more frequently mentioned plant species, being reported by 10 participants for blood and body cleansing, bad luck, pimples and diarrhoea. The results in this study validate the traditional use of *S. birrea* for blood cleansing and pimples. In 2012, a study documented that the young bark of *S. birrea* had moderate activity against a *S. aureus* strain with an MIC value of 0.25 mg/ml for both aqueous and organic extracts (York *et al*., 2012). Then in 2015, another study documented similar results for *S. birrea* of moderately suppressing the growth of *S. aureus* in aqueous (MIC value of 0.50 mg/ml) and organic (MIC value of 0.35 mg/ml) extracts (Van Vuuren *et al.*, 2015). In 2016, Nciki *et al*.'s findings revealed that *C*. *acnes, S. aureus* and *P. aeruginosa* were all susceptible to the bark (organic extract) of *S. birrea* with MIC values of 0.02 mg/ml, 0.50 mg/ml and 0.19 mg/ml respectively. The aqueous extract was only effective against *C. acnes* with a MIC value of 0.50 mg/ml. All four studies support the use of *S. birrea* bark against *S. aureus* related infections.

Syzygium cordatum bark displayed effective results against one bacterial strain, *C. acnes*. The aqueous extract was moderately active at an MIC value of 0.25 mg/ml, whereas the organic extract had noteworthy activity at 0.13 mg/ml. The plant species was reported as a traditional treatment for stomach-ache, diarrhoea and for body cleansing. The results in the present study suggest that *S. cordatum* is a possible remedy for *C. acnes* infections rather than the reported traditional uses. Therefore, the use of *S. cordatum* for stomach-ache and diarrhoea is not validated. A study that was designed to evaluate pharmacological properties of medicinal plant species found that the ethanol extract of *S. cordatum* leaf had a noteworthy activity with an MIC value of 0.01 mg/ml against a *S. aureus* strain. The dichloromethane extract moderately inhibited *S. aureus* at the minimum concentration of 0.20 mg/ml (Mulaudzi *et al*., 2012). Another study was conducted where the methanolic extract of *S. cordatum* fruit and seed had poor inhibition of *S. aureus* and *P. aeruginosa* which were the only strains relevant to the present study (Maliehe *et al*., 2015).

The aqueous root extract of *T. sericea* showed efficacy by inhibiting *C. acnes* at an MIC value of 0.25 mg/ml. The organic extract had noteworthy suppression against the growth of *C. acnes* at an MIC value of 0.05 mg/ml and *P. aeruginosa* at an MIC value of 0.13 mg/ml. Moderate activity was observed against *L. monocytogenes* (MIC value of 0.50 mg/ml). *Terminalia sericea* was the most mentioned plant species in the study. It was reported 22 times as a blood cleanser (Chapter 2). Both *C. acnes* and *P. aeruginosa* causes skin infections, and more often the skin infections are associated with blood purification (Van Vuuren and Frank, 2020). Therefore, the findings of this study validate the traditional use of *T. sericea*. Although the plant species did not have the broadest spectrum antimicrobial activity, it was one of the three extracts that had noteworthy inhibition against more than one bacterial strain. York *et al*. (2012) did a study on medicinal plant species used for respiratory infections in which the authors documented that the bark of *T. sericea* displayed moderate effect (MIC values of 0.50 mg/ml each) against *S. aureus* in both aqueous and organic extracts. Another study supported these results by recording that the aqueous and dichloromethane: methanol leaf extracts had weak inhibition of *S. aureus*. The results of this study also documented that the organic

leaf extract of *T. sericea* was moderately active towards *C. acnes* (MIC value of 0.25 mg/ml) and less active against *P. aeruginosa* (MIC value of 0.75 mg/ml) (Nciki *et al*., 2016). The variation of results between the different studies could be explained by the fact that the three studies tested different plant parts. Also, the results of these studies suggested that the roots of *T. sericea* had the most effective antimicrobial activity compared to its leaves and bark.

The organic extract of *W. somnifera* had noteworthy activity against the growth of *C. acnes*, with a MIC value of 0.05 mg/ml. The bacterial strains of *L. monocytogenes, S. aureus* and *S. agalactiae* were moderately suppressed, each at a MIC value of 0.50 mg/ml. The plant species was mentioned by six participants as a blood cleanser and for other medical conditions such as fever, fatigue and for tapeworms. This plant could only be validated for blood cleansing since only *C. acnes* was susceptible. *Withania somnifera* extracts (ethyl acetate, ethanol, dichloromethane and hexane) were previously evaluated using the disc-diffusion method and have shown noteworthy activity against *S. aureus.* These previous findings also demonstrated that at the same concentrations, ethyl acetate extract had noteworthy inhibition of *P. aeruginosa*; whereas the ethanol and dichloromethane had moderate activity, and hexane extract had no activity against *P. aeruginosa* (Sundaram *et al*., 2011). Another disc-diffusion study reported that *W. somnifera* root extracts (acetone, ethyl acetate and ethanol) had moderate activity against *S. aureus* and noteworthy activity against *P. aeruginosa* (Swaminathan and Santhi, 2019). Rizwana *et al*, (2016) also conducted a study that used the disc-diffusion assay to investigate the antimicrobial activity of different parts of *W. somnifera.* That study found that the acetone and methanol stem extracts had excellent activity against methicillin-resistant *S. aureus, S. pyogenes* and *P. aeruginosa,* whereas the ethanol and chloroform extracts were weakly active. The different leaf extracts of the plant were active with inhibition zones in the range of 16 mm – 28 mm when tested against methicillin-resistant *S. aureus,* 14 – 34 mm against *S. pyogenes* and 19 – 35 mm against *P. aeruginosa*. The root extracts, although least active compared to other tested plant parts, had moderate activity against *S. pyogenes* and *P. aeruginosa* and no activity against methicillin resistant *S. aureus* (Rizwana *et al*., 2012). Nciki *et al*. (2016) recorded that *C. acnes* and *S. aureus*

both were resistant to *W. somnifera* (both aqueous and organic root extracts). The present study had moderate inhibition of *S. aureus* and weak inhibition of *S. pyogenes* and *P. aeruginosa,* whereas in the reviewed literature, most studies of *W. somnifera* demonstrated excellent activity. Inversely, where *W. somnifera* had excellent activity against *C. acnes* in the present study, Nciki *et al*. (2016), found that the roots had weak activity against *C. acnes*. These differences are a result of different plant parts evaluated by the different studies.

In the current study, *X. caffra* organic extract for roots had successfully suppressed the growth of *C. acnes* (MIC value of 0.06 mg/ml). The plant species was mentioned by four participants as a remedy for pimples, abdominal pain and blood cleansing. The results obtained validated the reported traditional use of *X. caffra* except against abdominal pain. A study that evaluated the antimicrobial activity of *X. caffra's* twigs recorded that the organic extract had moderate inhibition against *C. acnes* (MIC value of 0.50 mg/ml) and *P. aeruginosa* (MIC value of 0.25 mg/ml) (Nciki *et al*., 2016). The root extract had greater inhibition against *C. acnes* compared to the twigs even though the harvesting is not sustainable. In another study, the ethanol extract of *X. caffra* roots had weak activity against *C. acnes* (MIC value > 0.50 mg/ml) (Lall *et al*., 2018). The slight differences within these studies could be due to different plant parts investigated. It is known that the distribution of compounds throughout the plant vary and some compounds may be present in one part of the plant and yet be absent in the other part of the same plant (Patel *et al*., 2018).

The study noted that extracts of *B. cathartica* leaves and roots had no efficacy when evaluated against indicator bacterial strains of the study. *Bridelia cathartica* was one of the more frequently mentioned (33%) plant species in the study and was reported to be used for body and blood cleansing, fatigue, menstrual pains and infertility. However, after evaluating it antimicrobial activity the study does not validate traditional use of *B. cathartica*. York *et al*. (2012) also found little correlation between antimicrobial efficacies and traditional use. Another study (Madureira *et al*., 2012), reported that the organic root extract inhibited *P. aeruginosa* growth and promoted noteworthy inhibition of *S. aureus*

(MIC values between 0.13 mg/ml – 0.008 mg/ml). The extraction used in these studies involved different solvents, and this could possibly account for the differences observed as plant compounds extracted are dependent on the solvents used (Abdulkadir *et al*., 2015).

3.3.3 Overall microbial susceptibility

Cutibacterium acnes was the most susceptible bacterial strain in the study. The pathogen was susceptible to 81% of the plant extracts (aqueous and organic), of which 47% demonstrated noteworthy activity. Pimples were mentioned as the number one indicator for blood infection (‘dirty blood’). *Cutibacterium acnes* is known to cause skin infections, to which pimples or acne is one of those infections. The efficacy of 81% of plant species mentioned in the study towards *C. acnes* provides their credibility to traditionally treat acne-related conditions. The susceptibility of the pathogen was not surprising since Gram-positive bacteria are known for their cell wall permeability towards antimicrobials (Lambert, 2002; Jones, 2017).

The other susceptible bacterial strain in the study was Gram-negative *P. aeruginosa*. *Pseudomonas aeruginosa* is known for causing, eye infections, skin infections, urinary tract infections, and septicaemia amongst other disorders. These diseases had an indirect link to blood purification need (except for septicaemia, which is a bloodstream infection). Chauhan (2013) had reported that eye infection is associated with infections of the blood. Although this is a Gram-negative bacteria the walls of this pathogen has proven to be breached easily by the medicinal plant species.

3.3.4 The antimicrobial activity of plant species combinations

The antimicrobial activity of plant species combinations used in northern Maputaland to increase plant efficacy was recorded in Table 3.3 along with the FIC index (ΣFIC). Where plant extracts demonstrated MIC values of > 8.00 mg/ml, the ΣFIC could not be calculated. In such cases, a tentative interpretation of the combination was provided

based on examination and comparison with the MIC values of the plant species when tested on their own. For example, if the plant species had MIC values > 8.00 mg/ml when tested independently and a MIC value of 2.00 mg/ml when tested in combination, the combination would be considered synergistic. If the combination had a MIC value of > 8.00 mg/ml, the combination would be considered non-interactive. The tentative interpretations are either synergy, additive, non-interactive or antagonistic and were used to indicate if the combination had increased or decreased antimicrobial activity compared to the MIC values of the plant species when investigated individually. A total of 308 plant combinations (each with at least two plant species samples) were evaluated for interactive activity and 20 synergistic interactions were detected. There was a total of 88 combinations that presented antagonistic effects when evaluated against pathogens of the current study (Table 3.3).

The combination of *A. precatorius* + *B. cathartica* roots was the most effective combination demonstrating broad-spectrum (against five pathogens) synergy. There was 36% synergy detected with the combinations; aqueous extract for *C. acnes* (ΣFIC value 0.13); with organic extracts against *L. monocytogenes* (ΣFIC value 0.40)*, S. aureus* (ΣFIC value 0.31), *A. baumannii* (ΣFIC value 0.38) and *P. aeruginosa* (ΣFIC value 0.50). There were also additive effects that were detected in the aqueous extracts against *S. aureus* and *A. baumannii*. The interaction between the aqueous extracts of *A. precatorious* + *B. cathartica* (roots) was antagonistic against *L. monocytogenes, S. agalactiae* and *P. aeruginosa*, and the organic extract was antagonistic against *S. agalactiae*. This blood purifying combination was mentioned in Table 2.2 to be also used for the treatment of boils and sores and the use of the combination is validated since these ailments are caused by *S. aureus*. When these plant species were evaluated individually, *A. precatorius* was only effective against *C. acnes* while *B. cathartica* demonstrated a poor antimicrobial response. The interaction between these two plant species hence increased the antimicrobial activity.

Table 3.3 The MIC values (mg/ml) and ΣFIC of plant species used in combinations for blood purification against seven indicator pathogens

Plant combinations	Aqueous (MIC value mg/ml)	ΣFIC	Description	DCM: MeOH (MIC value mg/ml)	ΣFIC	Description	Plant name**	Aqueous (MIC value mg/ml)	DCM: MeOH (MIC value mg/ml)
***Cutibacterium acnes* ATCC 11827**									
A. precatorius R + *B. cathartica* R	**0.25**	**0.13**	**Synergistic**	**0.13**	2.10	Non-interactive	*A. precatorius*	1.50	**0.03**
							B. cathartica R	3.00	0.75
A. delagoensis R + *P. kaurabassana* Rt	1.50	6.38	Antagonistic	**0.50**	5.82	Antagonistic	*A. delagoensis*	**0.13**	**0.05**
							P. kaurabassana	2.00	**0.50**
B. cathartica R + *R. digitata* R	2.00	**0.46**	**Synergistic**	**0.50**	**0.83**	Additive	*B. cathartica* R	3.00	0.75
							R. ditata	8.00	**0.50**
C. hirta R + *G. senegalensis* R	1.00	1.25	Non-interactive	**0.06**	**1.00**	Additive	*C. hirta*	**0.50**	**0.06**
							G. senegalensis R	2.00	**0.06**
C. molle R + *T. sericea* R	0.75	3.49	Non-interactive	**0.50**	7.80	Antagonistic	*C. molle* R	**0.19**	**0.09**
							T. sericea	**0.25**	**0.05**
D. cymosum R + *E. natalensis* R	2.00	ND*	**Synergistic**	1.00	2.50	Non-interactive	*D.cymosum*	6.00	1.00
							E. natalensis	>8.00	**0.25**
E. natalensis R + *S. birrea* B	1.50	ND*	Non-interactive	0.75	5.49	Antagonistic	*E. natalensis*	>8.00	**0.25**
							S. birrea	**0.25**	**0.09**
G. livingstonei R + *O. natalitia* R + *V. infausta* R	2.00	2.28	Non-interactive	**0.50**	1.44	Non-interactive	*G. livingstonei*	**0.38**	**0.38**
							O. natalitia	4.00	**0.25**
							V. infausta	2.00	**0.50**
H. natalensis R + *R. digitata* R	4.00	2.25	Non-interactive	**0.50**	**1.00**	Additive	*H. natalensis*	1.00	**0.50**
							R. ditata	8.00	**0.50**
H. hemerocallidea C + *S. serratuloides* Sh	1.00	1.25	Non-interactive	**0.38**	1.01	Non-interactive	*H. hemerocallidea*	2.00	1.00
							S. serratuloides	**0.50**	**0.25**
K. mosambicina L + *P. guajava* L+ *M. azedarach* L	0.75	1.04	Non-interactive	**0.50**	4.15	Antagonistic	*K. mosambicina*	2.00	1.00
							P. guajava	**0.38**	**0.06**
							M. azedarach	1.00	**0.13**
O. natalitia R + *B. cathartica* R	0.75	**0.22**	**Synergistic**	**0.38**	1.01	Non-interactive	*O. natalitia*	4.00	**0.25**
							B. cathartica R	3.00	0.75
P. cineraria R +	2.00	1.13		0.75	6.00	Antagonistic	*P. cineraria*	8.00	**0.13**

Plant combinations	Aqueous (MIC value mg/ml)	ΣFIC	Description	DCM: MeOH (MIC value mg/ml)	ΣFIC	Description	Plant name**	Aqueous (MIC value mg/ml)	DCM: MeOH (MIC value mg/ml)
S. integerrimum R			Non-interactive				*S. integerrimum*	1.00	**0.13**
P. cineraria R + *S. spinosa* R + *V. infausta* R	2.00	1.08	Non-interactive	0.75	2.75	Non-interactive	*P. cineraria*	8.00	**0.13**
							S. spinosa	1.00	1.00
							V. infausta	2.00	**0.50**
R. multifidus Sh + *S. serratuloides* Sh	**0.50**	**1.00**	Additive	**0.50**	2.00	Non-interactive	*R. multifidus*	**0.50**	**0.25**
							S. serratuloides	**0.50**	**0.25**
S. integerrimum R + *S. spinosa* R	4.00	4.00	Non-interactive	**0.38**	1.71	Non-interactive	*S. integerrimum*	1.00	**0.13**
							S. spinosa	1.00	1.00
S. puniceus Bb + *H. hemerocallidea* C + *R. multifidus* Sh	1.00	1.17	Non-interactive	**0.50**	1.06	Non-interactive	*S. puniceus*	1.00	0.75
							H. hemerocallidea	2.00	1.00
							R. multifidus	**0.50**	**0.25**
S. spinosa R + *C. inerme* R	**0.09**	**0.09**	**Synergistic**	**0.38**	1.71	Non-interactive	*S. spinosa*	1.00	1.00
							C. inerme	1.00	**0.13**
T. sericea R + *S. cordatum* B	1.00	4.00	Non-interactive	**0.38**	5.56	Antagonistic	*T. sericea*	**0.25**	**0.05**
							S. cordatum	**0.25**	**0.13**
V. sieberiana B + *V. infausta* R + *X. caffra* R	**050**	**0.42**	**Synergistic**	**0.19**	1.24	Non-interactive	*V. sieberiana*	1.00	**0.50**
							V. infausta	2.00	**0.50**
							X. caffra	1.00	**0.06**
V. sieberiana B + *S. birrea* B	2.00	5.00	Antagonistic	**0.25**	1.58	Non-interactive	*V. sieberiana*	1.00	**0.50**
							S. birrea	**0.25**	**0.09**
X. caffra R + *H. hemerocallidea* C + *R. multifidus* Sh + *S. puniceus* Bb	0.75	**0.84**	Additive	**0.13**	**0.69**	Additive	*X. caffra*	1.00	**0.06**
							H. hemerocallidea	2.00	1.00
							R. multifidus	**0.50**	**0.25**
							S. puniceus	1.00	0.75
***Listeria monocytogenes* ATCC 19111**									
A. precatorius R + *B. cathartica* R	>8.00	ND*	Antagonistic	**0.25**	**0.40**	**Synergistic**	*A. precatorius*	8.00	**0.38**
							B. cathartica R	4.00	2.00
A. delagoensis R + *P. kaurabassana* Rt	2.00	ND*	Additive	2.00	1.13	Non-interactive	*A. delagoensis*	**0.50**	**0.50**
							P. kaurabassana	>8.00	8.00
B. cathartica R + *R. digitata* R	>8.00	ND*	Antagonistic	2.00	**1.00**	Additive	*B. cathartica* R	4.00	2.00
							R. digitata	>8.00	2.00
C. hirta R +	>8.00	ND*	Antagonistic	**0.50**	2.25		*C. hirta*	8.00	1.00

Plant combinations	Aqueous (MIC value mg/ml)	ΣFIC	Description	DCM: MeOH (MIC value mg/ml)	ΣFIC	Description	Plant name**	Aqueous (MIC value mg/ml)	DCM: MeOH (MIC value mg/ml)
G. senegalensis R						Non-interactive	*G. seneralensis* R	8.00	**0.13**
C. molle R + *T. sericea* R	4.00	2.00	Non-interactive	1.00	1.25	Non-interactive	*C. molle* R	2.00	2.00
							T. sericea	2.00	**0.50**
D. cymosum R + *E. natalensis* R	>8.00	ND*	Non-interactive	1.00	2.17	Non-interactive	*D. cymosum*	>8.00	3.00
							E. natalensis	>8.00	**0.25**
E. natalensis R + *S. birrea* B	>8.00	ND*	Antagonistic	1.00	4.00	Non-interactive	*E. natalensis*	>8.00	**0.25**
							S. birrea	2.00	**0.25**
G. livingstonei R + *O. natalitia* R + *V. infausta* R	>8.00	ND*	Antagonistic	2.00	1.44	Non-interactive	*G. livingstonei*	2.00	1.00
							O. natalitia	>8.00	1.50
							V. caffra	>8.00	2.00
H. natalensis R + *R. digitata* R	>8.00	ND*	Antagonistic	4.00	3.00	Non-interactive	*H. natalensis*	4.00	1.00
							R. digitata	>8.00	2.00
H. hemerocallidea C + *S. serratuloides* Sh	>8.00	ND*	Antagonistic	4.00	5.33	Antagonistic	*H. hemerocallidea*	2.00	1.50
							S. serratuloides	4.00	**0.50**
K. mosambicina L + *P. guajava* L+ *M. azedarach* L	>8.00	ND*	Antagonistic	4.00	4.00	Non-interactive	*K. mosambicina*	>8.00	2.00
							P. guajava	3.00	2.00
							M. azedarach	8.00	**0.50**
O. natalitia R + *B. cathartica* R	>8.00	ND*	Antagonistic	2.00	1.17	Non-interactive	*O. natalitia*	>8.00	1.50
							B. cathartica R	4.00	2.00
P. cineraria R + *S. integerrimum* R	8.00	ND*	Additive	2.00	1.50	Non-interactive	*P. cineraria*	>8.00	2.00
							S. integerrimum	>8.00	1.00
P. cineraria R + *S. spinosa* R + *V. infausta* R	>8.00	ND*	Non-interactive	2.00	**1.00**	Additive	*P. cineraria*	>8.00	2.00
							S. spinosa	>8.00	2.00
							V. infausta	>8.00	2.00
R. multifidus Sh + *S. serratuloides* Sh	>8.00	ND*	Antagonistic	2.00	3.00	Non-interactive	*R. multifidus*	4.00	1.00
							S. serratuloides	4.00	**0.50**
S. integerrimum R + *S. spinosa* R	>8.00	ND*	Non-interactive	3.00	2.25	Non-interactive	*S. integerrimum*	>8.00	1.00
							S. spinosa	>8.00	2.00
S. puniceus Bb + *H. hemerocallidea* C + *R. multifidus* Sh	>8.00	ND*	Antagonistic	4.00	3.56	Non-interactive	*S. puniceus*	8.00	1.00
							H. hemerocallidea	2.00	1.50
							R. multifidus	4.00	1.00
S. spinosa R +	>8.00	ND*	Antagonistic	1.00	2.25		*S. spinosa*	>8.00	2.00

Plant combinations	Aqueous (MIC value mg/ml)	ΣFIC	Description	DCM: MeOH (MIC value mg/ml)	ΣFIC	Description	Plant name**	Aqueous (MIC value mg/ml)	DCM: MeOH (MIC value mg/ml)
C. inerme R						Non-interactive	*C. inerme*	6.00	**0.25**
T. sericea R + *S. cordatum* B	>8.00	ND*	Antagonistic	2.00	2.25	Non-interactive	*T. sericea*	2.00	**0.50**
							S. cordatum	4.00	2.00
V. sieberiana B + *V. infausta* R + *X. caffra* R	>8.00	ND*	Antagonistic	1.00	1.28	Non-interactive	*V. sieberiana*	8.00	1.00
							V. caffra	>8.00	2.00
							X. caffra	2.00	0.75
V. sieberiana B + *S. birrea* B	>8.00	ND*	Antagonistic	2.00	6.00	Antagonistic	*V. sieberiana*	8.00	1.00
							S. birrea	2.00	**0.25**
X. caffra R + *H. hemerocallidea* C + *R. multifidus* Sh + *S. puniceus* Bb	>8.00	ND*	Antagonistic	4.00	4.00	Non-interactive	*X. caffra*	2.00	0.75
							H. hemerocallidea	2.00	1.50
							R. multifidus	4.00	1.00
							S. puniceus	8.00	1.00
***Staphylococcus aureus* ATCC 25923**									
A. precatorius R + *B. cathartica* R	8.00	ND*	Additive	**0.25**	**0.31**	**Synergistic**	*A. precatorius*	8.00	**0.50**
							B. catharica R	>8.00	2.00
A. delagoensis R + *P. kaurabassana* Rt	1.00	ND*	Non-interactive	1.00	**0.56**	Additive	*A. delagoensis*	**0.50**	1.00
							P. kaurabassana	>8.00	8.00
B. cathartica R + *R. digitata* R	>8.00	ND*	Non-interactive	3.00	1.13	Non-interactive	*B. catharica* R	>8.00	2.00
							R. digitata	>8.00	4.00
C. hirta R + *G. senegalensis* R	>8.00	ND*	Antagonistic	**0.25**	1.06	Non-interactive	*C. hirta*	8.00	2.00
							G. senegalensis R	>8.00	**0.13**
C. molle R + *T. sericea* R	8.00	5.00	Antagonistic	**0.50**	**0.58**	Additive	*C. molle* R	4.00	1.00
							T. sericea	1.00	0.75
D. cymosum R + *E. natalensis* R	>8.00	ND*	Non-interactive	1.00	2.25	Non-interactive	*D. cymosum*	>8.00	2.00
							E. natalensis	>8.00	**0.25**
E. natalensis R + *S. birrea* B	>8.00	ND*	Antagonistic	**0.50**	1.25	Non-interactive	*E. natalensis*	>8.00	**0.25**
							S. birrea	4.00	1.00
G. livingstonei R + *O. natalitia* R + *V. infausta* R	>8.00	ND*	Antagonistic	2.00	2.08	Non-interactive	*G. livingstonei*	1.00	**0.50**
							O. natalitia	>8.00	1.00
							V. infausta	>8.00	8.00
H. natalensis R + *R. digitata* R	>8.00	ND*	Antagonistic	4.00	2.50	Non-interactive	*H. natalensis*	8.00	1.00
							R. digitata	>8.00	4.00

Plant combinations	Aqueous (MIC value mg/ml)	ΣFIC	Description	DCM: MeOH (MIC value mg/ml)	ΣFIC	Description	Plant name**	Aqueous (MIC value mg/ml)	DCM: MeOH (MIC value mg/ml)
H. hemerocallidea C + *S. serratuloides* Sh	4.00	1.25	Non-interactive	4.00	2.00	Non-interactive	*H. hemerocallidea*	2.00	2.00
							S. serratuloides	8.00	2.00
K. mosambicina L + *P. guajava* L+ *M. azedarach* L	>8.00	ND*	Antagonistic	4.00	3.78	Non-interactive	*K. mosambicina*	>8.00	2.00
							P. guajava	4.00	3.00
							M. azedarach	>8.00	**0.50**
O. natalitia R + *B. cathartica* R	>8.00	ND*	Antagonistic	2.00	1.50	Non-interactive	*O. natalitia*	>8.00	1.00
							B. catharica R	>8.00	2.00
P. cineraria R + *S. integerrimum* R	>8.00	ND*	Antagonistic	4.00	1.67	Non-interactive	*P. cineraria*	>8.00	2.00
							S. integerrimum	2.00	3.00
P. cineraria R + *S. spinosa* R + *V. infausta* R	>8.00	ND*	Non-interactive	4.00	**1.00**	Additive	*P. cineraria*	>8.00	2.00
							S. spinosa	>8.00	8.00
							V. infausta	>8.00	8.00
R. multifidus Sh + *S. serratuloides* Sh	>8.00	ND*	Antagonistic	4.00	5.00	Antagonistic	*R. multifidus*	>8.00	**0.50**
							S. serratuloides	8.00	2.00
S. integerrimum R + *S. spinosa* R	>8.00	ND*	Antagonistic	4.00	**0.92**	Additive	*S. integerrimum*	2.00	3.00
							S. spinosa	>8.00	8.00
S. puniceus Bb + *H. hemerocallidea* C + *R. multifidus* Sh	>8.00	ND*	Antagonistic	4.00	3.67	Non-interactive	*S. puniceus*	8.00	3.00
							H. hemerocallidea	2.00	2.00
							R. multifidus	>8.00	**0.50**
S. spinosa R + *C. inerme* R	>8.00	ND*	Antagonistic	8.00	4.50	Antagonistic	*S. spinosa*	>8.00	8.00
							C. inerme	6.00	1.00
T. sericea R + *S. cordatum* B	>8.00	ND*	Antagonistic	1.00	**0.92**	Additive	*T. sericea*	1.00	0.75
							S. cordatum	4.00	2.00
V. sieberiana B + *V. infausta* R+ *X. caffra* R	>8.00	ND*	Antagonistic	4.00	1.50	Non-interactive	*V. sieberiana*	>8.00	2.00
							V. infausta	>8.00	8.00
							X. caffra	4.00	2.00
V. sieberiana B + *S. birrea* B	>8.00	ND*	Antagonistic	2.00	1.56	Non-interactive	*V. sieberiana*	>8.00	2.00
							S. birrea	4.00	1.00
X. caffra R + *H. hemerocallidea* C + *R. multifidus* Sh + *S. puniceus* Bb	>8.00	ND*	Antagonistic	4.00	3.25	Non-interactive	*X. caffra*	4.00	2.00
							H. hemerocallidea	2.00	2.00
							R. multifidus	>8.00	**0.50**
							S. puniceus	8.00	3.00

Plant combinations	Aqueous (MIC value mg/ml)	ΣFIC	Description	DCM: MeOH (MIC value mg/ml)	ΣFIC	Description	Plant name**	Aqueous (MIC value mg/ml)	DCM: MeOH (MIC value mg/ml)
***Streptococcus agalactiae* ATCC 1236**									
A. precatorius R + *B. cathartica* R	>8.00	ND*	Antagonistic	4.00	8.00	Antagonistic	*A. precatorius*	8.00	**0.50**
							B. cathartica R	>8.00	1.50
A. delagoensis R + *P. kaurabassana* Rt	>8.00	ND*	Antagonistic	2.00	1.50	Non-interactive	*A. delagoensis*	1.00	1.00
							P. kaurabassana	>8.00	2.00
B. cathartica R + *R. digitata* R	>8.00	ND*	Non-interactive	2.00	2.50	Non-interactive	*B. cathartica* R	>8.00	1.50
							R. digitata	>8.00	2.00
C. hirta R + *G. senegalensis* R	>8.00	ND*	Antagonistic	2.00	8.50	Antagonistic	*C. hirta*	8.00	2.00
							G. senegalensis R	8.00	**0.13**
C. molle R + *T. sericea* R	8.00	3.00	Non-interactive	2.00	1.50	Non-interactive	*C. molle* R	4.00	2.00
							T. sericea	2.00	2.00
D. cymosum R + *E. natalensis* R	>8.00	ND*	Non-interactive	2.00	4.50	Antagonistic	*D. cymosum*	>8.00	2.00
							E. natalensis	>8.00	**0.25**
E. natalensis R + *S. birrea* B	>8.00	ND*	Antagonistic	2.00	5.00	Antagonistic	*E. natalensis*	>8.00	**0.25**
							S. birrea	4.00	1.00
G. livingstonei R + *O. natalitia* R + *V. infausta* R	>8.00	ND*	Antagonistic	2.00	**1.00**	Additive	*G. livingstonei*	1.00	2.00
							O. natalitia	>8.00	2.00
							V. infausta	>8.00	2.00
H. natalensis R + *R. digitata* R	4.00	ND*	**Synergistic**	2.00	**0.83**	Additive	*H. natalensis*	8.00	3.00
							R. digitata	>8.00	2.00
H. hemerocallidea C + *S. serratuloides* Sh	>8.00	ND*	Antagonistic	4.00	1.67	Non-interactive	*H. hemerocallidea*	2.00	2.00
							S. serratuloides	>8.00	3.00
K. mosambicina L + *P. guajava* L+ *M. azedarach* L	>8.00	ND*	Antagonistic	2.00	**0.83**	Additive	*K. mosambicina*	>8.00	2.00
							P. guajava	4.00	2.00
							M. azedarach	>8.00	4.00
O. natalitia R + *B. cathartica* R	>8.00	ND*	Non-interactive	1.00	1.25	Non-interactive	*O. natalitia*	>8.00	2.00
							B. cathartica R	>8.00	1.50
P. cineraria R + *S. integerrimum* R	>8.00	ND*	Antagonistic	2.00	**1.00**	Additive	*P. cineraria*	>8.00	2.00
							S. integerrimum	2.00	2.00
P. cineraria R + *S. spinosa* R + *V. infausta* R	>8.00	ND*	Non-interactive	2.00	**1.00**	Additive	*P. cineraria*	>8.00	2.00
							S. spinosa	>8.00	2.00
							V. infausta	>8.00	2.00

Plant combinations	Aqueous (MIC value mg/ml)	ΣFIC	Description	DCM: MeOH (MIC value mg/ml)	ΣFIC	Description	Plant name**	Aqueous (MIC value mg/ml)	DCM: MeOH (MIC value mg/ml)
R. multifidus Sh + *S. serratuloides* Sh	>8.00	ND*	Non-interactive	3.00	2.00	Non-interactive	*R. multifidus*	>8.00	2.00
							S. serratuloides	>8.00	3.00
S. integerrimum R + *S. spinosa* R	>8.00	ND*	Antagonistic	2.00	**1.00**	Additive	*S. integerrimum*	2.00	2.00
							S. spinosa	>8.00	2.00
S. puniceus Bb + *H. hemerocallidea* C + *R. multifidus* Sh	8.00	ND*	Additive	3.00	2.00	Non-interactive	*S. puniceus*	>8.00	2.00
							H. hemerocallidea	2.00	2.00
							R. multifidus	>8.00	2.00
S. spinosa R + *C. inerme* R	8.00	ND*	Additive	1.00	**0.75**	Additive	*S. spinosa*	>8.00	2.00
							C. inerme	>8.00	1.00
T. sericea R + *S. cordatum* B	8.00	ND*	Non-interactive	2.00	**1.00**	Additive	*T. sericea*	2.00	2.00
							S. cordatum	>8.00	2.00
V. sieberiana B + *V. infausta* R + *X. caffra* R	>8.00	ND*	Antagonistic	2.00	1.22	Non-interactive	*V. sieberiana*	>8.00	3.00
							V. infausta	>8.00	2.00
							X. caffra	4.00	1.00
V. sieberiana B + *S. birrea* B	>8.00	ND*	Antagonistic	2.00	1.33	Non-interactive	*V. sieberiana*	>8.00	3.00
							S. birrea	4.00	1.00
X. caffra R + *H. hemerocallidea* C + *R. multifidus* Sh + *S. puniceus* Bb	8.00	ND*	Non-interactive	2.00	**1.00**	Additive	*X. caffra*	4.00	1.00
							H. hemerocallidea	2.00	2.00
							R. multifidus	>8.00	2.00
							S. puniceus	>8.00	2.00
***Streptococcus pyogenes* ATCC 12783**									
A. precatorius R + *B. cathartica* R	8.00	1.50	Non-interactive	4.00	2.50	Non-interactive	*A. precatorius*	8.00	1.00
							B. cathartica R	4.00	4.00
A. delagoensis R + *P. kaurabassana* Rt	8.00	ND*	Non-interactive	2.00	1.25	Non-interactive	*A. delagoensis*	1.50	1.00
							P. kaurabassana	>8.00	4.00
B. cathartica R + *R. digitata* R	8.00	ND*	Non-interactive	2.00	**0.75**	Additive	*B. cathartica* R	4.00	4.00
							R. digitata	>8.00	2.00
C. hirta R + *G. senegalensis* R	8.00	1.80	Non-interactive	2.00	4.50	Antagonistic	*C. hirta*	4.00	2.00
							G. senegalensis R	4.00	**0.25**
C. molle R + *T. sericea* R	6.00	6.00	Antagonistic	8.00	10.00	Antagonistic	*C. molle* R	1.00	**0.50**
							T. sericea	1.00	2.00
D. cymosum R +	8.00	ND*	Additive	4.00	8.50	Antagonistic	*D. cymosum*	>8.00	4.00

Plant combinations	Aqueous (MIC value mg/ml)	ΣFIC	Description	DCM: MeOH (MIC value mg/ml)	ΣFIC	Description	Plant name**	Aqueous (MIC value mg/ml)	DCM: MeOH (MIC value mg/ml)
E. natalensis R							*E. natalensis*	>8.00	**0.25**
E. natalensis R + *S. birrea* B	8.00	2.50	Non-interactive	4.00	10.00	Antagonistic	*E. natalensis*	>8.00	**0.25**
							S. birrea	2.00	1.00
G. livingstonei R + *O. natalitia* R + *V. infausta* R	8.00	ND*	Non-interactive	4.00	2.33	Non-interactive	*G. livingstonei*	1.50	2.00
							O. natalitia	8.00	1.00
							V. infausta	8.00	4.00
H. natalensis R + *R. digitata* R	4.00	ND*	Additive	4.00	1.50	Non-interactive	*H. natalensis*	3.00	4.00
							R. digitata	>8.00	2.00
H. hemerocallidea C + *S. serratuloides* Sh	8.00	ND*	Non-interactive	2.00	2.13	Non-interactive	*H. hemerocallidea*	**0.50**	**0.50**
							S. serratuloides	>8.00	8.00
K. mosambicina L + *P. guajava* L+ *M. azedarach* L	8.00	2.33	Non-interactive	4.00	2.11	Non-interactive	*K. mosambicina*	4.00	4.00
							P. guajava	1.00	2.00
							M. azedarach	8.00	3.00
O. natalitia R + *B. cathartica* R	8.00	1.50	Non-interactive	1.00	**0.63**	Additive	*O. natalitia*	8.00	1.00
							B. cathartica R	4.00	4.00
P. cineraria R + *S. integerrimum* R	6.00	ND*	Non-interactive	6.00	2.25	Non-interactive	*P. cineraria*	>8.00	4.00
							S. integerrimum	2.00	1.00
P. cineraria R + *S. spinosa* R + *V. infausta* R	>8.00	ND*	Antagonistic	2.00	**0.50**	Additive	*P. cineraria*	>8.00	4.00
							S. spinosa	>8.00	4.00
							V. infausta	8.00	4.00
R. multifidus Sh + *S. serratuloides* Sh	>8.00	ND*	Antagonistic	4.00	**0.58**	Additive	*R. multifidus*	>8.00	6.00
							S. serratuloides	>8.00	8.00
S. integerrimum R + *S. spinosa* R	4.00	ND*	Non-interactive	8.00	3.00	Non-interactive	*S. integerrimum*	2.00	1.00
							S. spinosa	>8.00	4.00
S. puniceus Bb + *H. hemerocallidea* C + *R. multifidus* Sh	>8.00	ND*	Antagonistic	8.00	6.44	Antagonistic	*S. puniceus*	8.00	4.00
							H. hemerocallidea	**0.50**	**0.50**
							R. multifidus	>8.00	6.00
S. spinosa R + *C. inerme* R	>8.00	ND*	Antagonistic	3.00	1.13	Non-interactive	*S. spinosa*	>8.00	4.00
							C. inerme	2.00	2.00
T. sericea R + *S. cordatum* B	2.00	2.33	Non-interactive	4.00	2.00	Non-interactive	*T. sericea*	1.00	2.00
							S. cordatum	0.75	2.00
V. sieberiana B + *V. infausta* R+ *X. caffra* R	4.00	ND*	Additive	4.00	1.11	Non-interactive	*V. sieberiana*	6.00	3.00
							V. infausta	8.00	4.00
							X. caffra	4.00	2.00

Plant combinations	Aqueous (MIC value mg/ml)	ΣFIC	Description	DCM: MeOH (MIC value mg/ml)	ΣFIC	Description	Plant name**	Aqueous (MIC value mg/ml)	DCM: MeOH (MIC value mg/ml)
V. sieberiana B + *S. birrea* B	8.00	2.67	Non-interactive	2.00	1.33	Non-interactive	*V. sieberiana*	>8.00	4.00
							S. birrea	6.00	3.00
X. caffra R + *H. hemerocallidea* C + *R. multifidus* Sh + *S. puniceus* Bb	>8.00	ND*	Antagonistic	1.00	**0.67**	Additive	*X. caffra*	4.00	2.00
							H. hemerocallidea	**0.50**	**0.50**
							R. multifidus	>8.00	6.00
							S. puniceus	8.00	4.00
***Acinetobacter baumannii* ATCC 19606**									
A. precatorius R + *B. cathartica* R	8.00	ND*	Additive	2.00	**0.38**	**Synergistic**	*A. precatorius*	8.00	8.00
							B. cathartica R	>8.00	4.00
A. delagoensis R + *P. kaurabassana* Rt	>8.00	ND*	Antagonistic	4.00	3.33	Non-interactive	*A. delagoensis*	2.00	1.00
							P. kaurabassana	>8.00	3.00
B. cathartica R + *R. digitata* R	8.00	ND*	Additive	4.00	1.50	Non-interactive	*B. cathartica* R	>8.00	4.00
							R. digitata	>8.00	2.00
C. hirta R + *G. senegalensis* R	>8.00	ND*	Antagonistic	2.00	1.50	Non-interactive	*C. hirta*	8.00	2.00
							G. senegalensis ***R***	>8.00	1.00
C. molle R + *T. sericea* R	>8.00	ND*	Antagonistic	2.00	1.50	Non-interactive	*C. molle* R	8.00	2.00
							T. sericea	>8.00	1.00
D. cymosum R + *E. natalensis* R	>8.00	ND*	Non-interactive	1.00	**0.75**	Additive	*D. cymosum*	>8.00	1.00
							E. natalensis	>8.00	1.00
E. natalensis R + *S. birrea* B	>8.00	ND*	Non-interactive	1.00	**0.50**	**Synergistic**	*E. natalensis*	>8.00	1.00
							S. birrea	>8.00	2.00
G. livingstonei R + *O. natalitia* R + *V. infausta* R	>8.00	ND*	Non-interactive	2.00	1.17	Non-interactive	*G. livingstonei*	>8.00	2.00
							O. natalitia	>8.00	1.00
							V. infausta	>8.00	3.00
H. natalensis R + *R. digitata* R	>8.00	ND*	Non-interactive	4.00	1.50	Non-interactive	*H. natalensis*	>8.00	4.00
							R. digitata	>8.00	2.00
H. hemerocallidea C + *S. serratuloides* Sh	6.00	ND*	**Synergistic**	4.00	2.00	Non-interactive	*H. hemerocallidea*	>8.00	2.00
							S. serratuloides	>8.00	2.00
K. mosambicina L + *P. guajava* L+ *M. azedarach* L	>8.00	ND*	Antagonistic	2.00	**0.83**	Additive	*K. mosambicina*	>8.00	4.00
							P. guajava	>8.00	2.00
							M. azeradach	6.00	2.00
O. natalitia R + *B. cathartica* R	>8.00	ND*	Non-interactive	4.00	2.50	Non-interactive	*O. natalitia*	>8.00	1.00
							B. cathartica R	>8.00	4.00

Plant combinations	Aqueous (MIC value mg/ml)	ΣFIC	Description	DCM: MeOH (MIC value mg/ml)	ΣFIC	Description	Plant name**	Aqueous (MIC value mg/ml)	DCM: MeOH (MIC value mg/ml)
P. cineraria R + *S. integerrimum* R	8.00	ND*	Additive	2.00	**0.50**	**Synergistic**	*P. cineraria*	>8.00	4.00
							S. integerrimum	>8.00	4.00
P. cineraria R + *S. spinosa* R + *V. infausta* R	>8.00	ND*	Non-interactive	4.00	**1.00**	Additive	*P. cineraria*	>8.00	4.00
							S. spinosa	>8.00	4.00
							V. infausta	>8.00	3.00
R. multifidus Sh + *S. serratuloides* Sh	>8.00	ND*	Non-interactive	4.00	1.50	Non-interactive	*R. multifidus*	>8.00	4.00
							S. serratuloides	>8.00	2.00
S. integerrimum R + *S. spinosa* R	>8.00	ND*	Non-interactive	4.00	**1.00**	Additive	*S. integerrimum*	>8.00	4.00
							S. spinosa	>8.00	4.00
S. puniceus Bb + *H. hemerocallidea* C + *R. multifidus* Sh	>8.00	ND*	Non-interactive	4.00	1.33	Non-interactive	*S. puniceus*	>8.00	3.00
							H. hemerocallidea	>8.00	2.00
							R. multifidus	>8.00	4.00
S. spinosa R + *C. inerme* R	>8.00	ND*	Non-interactive	>8.00	ND*	Antagonistic	*S. spinosa*	>8.00	4.00
							C. inerme	>8.00	4.00
T. sericea R + *S. cordatum* B	4.00	ND*	**Synergistic**	2.00	2.25	Non-interactive	*T. sericea*	>8.00	1.00
							S. cordatum	>8.00	4.00
V. sieberiana B + *V. infausta* R+ *X. caffra* R	8.00	ND*	Additive	2.00	**0.35**	**Synergistic**	*V. sieberiana*	>8.00	4.00
							V. infausta	>8.00	3.00
							X. caffra	>8.00	4.00
V. sieberiana B + *S. birrea* B	8.00	ND*	Additive	2.00	**0.75**	Additive	*V. sieberiana*	>8.00	4.00
							S. birrea	>8.00	2.00
X. caffra R + *H. hemerocallidea* C + *R. multifidus* Sh + *S. puniceus* Bb	4.00	ND*	**Synergistic**	4.00	1.02	Non-interactive	*X. caffra*	>8.00	4.00
							H. hemerocallidea	>8.00	2.00
							R. multifidus	>8.00	4.00
							S. puniceus	>8.00	3.00
***Pseudomonas aeruginosa* ATCC 27853**									
A. precatorius R + *B. cathartica* R	>8.00	ND*	Antagonistic	**0.38**	**0.50**	**Synergistic**	*A. precatorius*	>8.00	1.50
							B. cathartica R	8.00	**0.50**
A. delagoensis R + *P. kaurabassana* Rt	>8.00	ND*	Non-interactive	1.00	1.50	Non-interactive	*A. delagoensis*	>8.00	1.00
							P. kaurabassana	>8.00	1.00
B. cathartica R + *R. digitata* R	>8.00	ND*	Antagonistic	2.00	2.25	Non-interactive	*B. cathartica* R	8.00	**0.50**
							R. digitata	>8.00	4.00
C. hirta R +	>8.00	ND*		**0.50**	**0.75**	Additive	*C. hirta*	>8.00	1.00

Plant combinations	Aqueous (MIC value mg/ml)	ΣFIC	Description	DCM: MeOH (MIC value mg/ml)	ΣFIC	Description	Plant name**	Aqueous (MIC value mg/ml)	DCM: MeOH (MIC value mg/ml)
G. senegalensis R			Non-interactive				*G. senegalensis* R	>8.00	**0.50**
C. molle R + *T. sericea* R	>8.00	ND*	Antagonistic	1.00	4.85	Antagonistic	*C. molle* R	>8.00	**0.50**
							T. sericea	1.00	**0.13**
D. cymosum R + *E. natalensis* R	>8.00	ND*	Non-interactive	**0.50**	**1.00**	Additive	*D. cymosum*	>8.00	**0.50**
							E. natalensis	>8.00	**0.50**
E. natalensis R + *S. birrea* B	>8.00	ND*	Non-interactive	**0.50**	1.36	Non-interactive	*E. natalensis*	>8.00	**0.50**
							S. birrea	>8.00	**0.19**
G. livingstonei R + *O. natalitia* R + *V. infausta* R	>8.00	ND*	Non-interactive	1.50	2.25	Non-interactive	*G. livingstonei*	>8.00	**0.50**
							O. natalitia	>8.00	**0.50**
							V. infausta	>8.00	2.00
H. natalensis R + *R. digitata* R	>8.00	ND*	Antagonistic	4.00	4.50	Antagonistic	*H. natalensis*	8.00	**0.50**
							R. digitata	>8.00	4.00
H. hemerocallidea C + *S. serratuloides* Sh	>8.00	ND*	Non-interactive	**0.50**	**0.38**	**Synergistic**	*H. hemerocallidea*	>8.00	2.00
							S. serratuloides	>8.00	1.00
K. mosambicina L + *P. guajava* L+ *M. azedarach* L	>8.00	ND*	Non-interactive	2.00	3.33	Non-interactive	*K. mosambicina*	>8.00	1.00
							P. guajava	>8.00	**0.50**
							M. azedarach	>8.00	**0.50**
O. natalitia R + *B. cathartica* R	>8.00	ND*	Antagonistic	2.00	4.00	Non-interactive	*O. natalitia*	>8.00	**0.50**
							B. cathartica R	8.00	**0.50**
P. cineraria R + *S. integerrimum* R	>8.00	ND*	Non-interactive	2.00	2.00	Non-interactive	*P. cineraria*	>8.00	1.00
							S. integerrimum	>8.00	1.00
P. cineraria R + *S. spinosa* R + *V. infausta* R	>8.00	ND*	Non-interactive	1.50	**0.92**	Additive	*P. cineraria*	>8.00	1.00
							S. spinosa	>8.00	3.00
							V. infausta	>8.00	2.00
R. multifidus Sh + *S. serratuloides* Sh	>8.00	ND*	Antagonistic	1.00	**0.75**	Additive	*R. multifidus*	8.00	2.00
							S. serratuloides	>8.00	1.00
S. integerrimum R + *S. spinosa* R	>8.00	ND*	Non-interactive	**0.50**	**0.33**	**Synergistic**	*S. integerrimum*	>8.00	1.00
							S. spinosa	>8.00	3.00
S. puniceus Bb + *H. hemerocallidea* C + *R. multifidus* Sh	>8.00	ND*	Non-interactive	2.00	2.00	Non-interactive	*S. puniceus*	>8.00	**0.50**
							H. hemerocallidea	>8.00	2.00
							R. multifidus	8.00	2.00
S. spinosa R +	>8.00	ND*		3.00	3.50		*S. spinosa*	>8.00	3.00

Plant combinations	Aqueous (MIC value mg/ml)	ΣFIC	Description	DCM: MeOH (MIC value mg/ml)	ΣFIC	Description	Plant name**	Aqueous (MIC value mg/ml)	DCM: MeOH (MIC value mg/ml)
C. inerme R			Antagonistic			Non-interactive	*C. inerme*	8.00	**0.50**
T. sericea R + *S. cordatum* B	>8.00	ND*	Antagonistic	2.00	8.69	Antagonistic	*T. sericea*	1.00	**1.13**
							S. cordatum	>8.00	1.00
V. sieberiana B + *V. infausta* R+ *X. caffra* R	>8.00	ND*	Non-interactive	**0.50**	**0.42**	**Synergistic**	*V. sieberiana*	>8.00	1.00
							V. infausta	>8.00	2.00
							X. caffra	>8.00	1.00
V. sieberiana B + *S. birrea* B	>8.00	ND*	Non-interactive	2.00	4.45	Antagonistic	*V. sieberiana*	>8.00	1.00
							S. birrea	>8.00	**0.19**
X. caffra R + *H. hemerocallidea* C + *R. multifidus* Sh + *S. puniceus* Bb	>8.00	ND*	Antagonistic	4.00	4.00	Non-interactive	*X. caffra*	>8.00	1.00
							H. hemerocallidea	>8.00	2.00
							R. multifidus	8.00	2.00
							S. spinosa	>8.00	3.00

ATCC = American type culture collection; DCM: MeOH = dichloromethane: methane; B = bark; Bb = bulb; C = corm; F = fruits; L = leaves; Rh = rhizome; R = roots; Rt = Root tuber; Sd = seeds; S = stem; Sh = shoots; T = twigs; Wp = whole plant; ND* = not detected due to MIC value >8 mg/ml and thus interpretation is tentative; Numbers highlighted in black-bold had moderate activity in MIC or additive interactive effects; Numbers highlighted in red-bold had noteworthy antimicrobial activity or synergistic interactive effects; (**) = The grey area represent MIC values of plant species when evaluated individually and is included for comparison.

The aqueous extract of *B. cathartica* + *R. digitata* roots demonstrated synergy against *C. acnes* with an ΣFIC value of 0.46. The organic extract of the combination portrayed additive effects against *C. acnes, L. monocytogenes* and *S. pyogenes.* Other than the antagonism that was detected against the aqueous extracts of *L. monocytogenes, A. baumannii* and *P. aeruginosa*, the combination of *B. cathartica* + *R. digitata* roots was non-interactive to the remainder of pathogens tested. The combination was mentioned once in Chapater 2 for dysmenorrhea, fertility and blood purification. According to the results of the study, the traditional use of this combination was valid only against *C. acnes* infections. The evaluation of both *B. carthatica* and *R. digitata* roots as single plant species displayed that these plant extracts had weak activity. Applying these plant species as a combination against traditional uses was validated as their activity was enhanced.

The aqueous extracts of *D. cymosum* + *E. natalensis* combination demonstrated synergy against *C. acnes*. This combination also displayed additive effects against *S. pyogenes* and the organic extracts against *A. baumannii* and *P. aeruginosa*. The organic extract had antagonistic effects against *S. agalactiae* and *S. pyogenes*. This combination was mentioned as a cleanser for bad luck. As the traditional use is for spiritual purposes, it validation based on antimicrobial activities is not feasible. However, it could be said that the use of this combination is effective against acne due to the positive results for the aqueous extracts obtained against *C. acnes*. Combining *D. cymosum* with *E. natalensis* did not affect the results of this plant; both individually and in combination only *C. acnes* was susceptible.

Besides the 7% synergy (ΣFIC value 0.50) shown by the organic extract against *A. baumannii*, the combination of *E. natalensis* roots + *S. birrea* bark was ineffective. Seven extracts were non-interactive and six were antagonistic. The combination was reported for blood cleansing, pimples, toothache, snake poison and bad luck (Chapter 2). The results support the traditional use of this combination for pimples, but not toothache. *Sclerocarya birrea* inhibited the growth of *C. acnes*, however, the interaction with *E. natalensis* was synergistic towards *A. baumannii*. The possible interpretation for this

would be that interactions of *E. natalensis* + *S. birrea* supressed the anti-acne components and promoted synergistic effect against *A. baumannii*.

Hyphanae natalensis + *R. digitata* roots (aqueous extract) displayed synergy against *S. agalactiae* with an ΣFIC value of 0.38. The combination's aqueous extract was additive towards *S*. *pyogenes* (tentative value for ΣFIC) and organic extract against *C. acnes* (ΣFIC value 1.00) and *S. agalactiae* (ΣFIC value 0.83). Antagonistic effects were detected against *P. aeruginosa* as well as in the aqueous extracts against *L. monocytogenes* and *S. aureus*. The combination was mentioned in Chapter 2 as a blood purifier and used for dysmenorrhea. According to the results obtained here, *H. natalensis* + *R. digitata* roots would only be effective against infections caused by *S. agalactiae* (wounds, burns, surgical and skin infections). The interaction of *H. natalensis* + *R. digitata* increased the efficiency of these plant species; when evaluated individually they were both ineffective.

Interestingly, the combination of *H. hemerocallidea* corm + *S. serratuloides* shoots (organic extract) was effective enough to permeate the complex walls of *P. aeruginosa* (ΣFIC value of 0.38) and *A. baumannii* with an aqueous extract (tentative ΣFIC value). The extract was either non-interactive (64%) or antagonistic (22%) against the Gram-positive bacteria. The combination was mentioned to purify the blood and to treat rashes and sores. The results showed that this combination could be used for skin ailments or other ailments linked to *A. baumannii* and *P. aeruginosa*. Both *H. hemerocallidea* and *S. serratuloides* lacked efficiency when evaluated individually; hence interaction between the two plant species induced synergy.

The aqueous extract of *O. natalitia* + *B. cathartica* roots exhibited synergy against *C. acnes* (ΣFIC value 0.22). The organic extract possessed an additive effect on *S. pyogenes*. The aqueous extract had antagonistic effects against *L. monocytogenes, S. aureus* and *P. aeruginosa*. The combination was mentioned once in the study for pimples and blood cleansing. The traditional use of this combination for blood cleansing and skin disorders (pimples) is supported by the results of the study since the aqueous extract had achieved synergistic effects. The combination of *O. natalitia* and *B. cathartica*

roots had increased efficiency compared to the individual evaluation of these plant species.

In a combination of *P. cineraria* + *S. integerrimum* roots, the organic extract was synergistic against *A. baumannii* with an ΣFIC value of 0.50. The aqueous extract of the combination was additive to *L. monocytogenes* and *A. baumannii* (tentative ΣFIC values). The organic extract also had an additive effect against *S. agalactiae* (ΣFIC value 1.00). The combination of *P. cineraria* + *S. integerrimum* was non-interactive to 50% of its interactions. This combination was reported as a treatment for pimples and blood diseases.The obtained results support the use of this combination only blood infections caused by *A. baumannii* (wounds, urinary tract infections). It is important to note that individually *P. cineraria* and *S. integerrimum* were effective *C. acnes* inhibitors.

The interaction of *S. integerrimum* + *S. spinosa* roots (organic extract) was synergistic against *P. aeruginosa* (ΣFIC value 0.33). The organic extract also produced the additive effects noted against *S. aureus* (ΣFIC value 0.92)*, S. agalactiae* (ΣFIC value 1.00) and *A. baumannii* (ΣFIC value 1.00). The aqueous extract had an antagonistic effect against *S. aureus* and *S. agalactiae*. The combination was mentioned to be used for blood cleansing and bringing love. While the spiritual traditional use cannot be validated, the study showed that the *S. integerrimum* + *S. spinosa* root combination is effective against *P. aeruginosa*. Individually, *S. spinosa* roots did not inhibit the growth of any indicator pathogens used in the study.

The combination of *S. spinosa* + *C. inerme* roots (aqueous extract) showed synergy when evaluated against *C. acnes* (ΣFIC value 0.09). The aqueous and organic extracts were additive against *S. agalactiae* with tentative ΣFIC evaluation for the former and an ΣFIC value of 0.75 for the latter. The combination was reported as a body cleanser, as well as a good luck and love charm. Infusing *C. inerme* and *S. spinosa* increased the antimicrobial efficacy of the aqueous extract. Therefore, the findings support the traditional use of the combination for blood cleansing as it is often linked to acne.

The aqueous extract of *T. sericea* + *S. cordatum* roots was synergistic towards *A*. *baumannii* only (tentative ΣFIC). The combination showed additive properties against *S. aureus* (ΣFIC value 0.92) and *S. agalactiae* (ΣFIC value 1.00) in the organic extract. The combination was mentioned once to treat diarrhoea and body cleansing. According to this study, this combination could be recommended against *A. baumannii* but not for other pathogens because it demonstrated some antagonism. It was interesting for an aqueous extract to have synergy against this bacterial strain when the individual plant species were not effective against both the aqueous and organic extracts.

The aqueous extract of *V. sieberiana* + *V. infausta* + *X. caffra* roots demonstrated synergism against *C. acnes* with an ΣFIC value of 0.42 and the organic extract was synergistic against Gram-negative strains *A. baumannii* and *P. aeruginosa* with 0.35 and 0.42 ΣFIC values respectively. The aqueous extract was additive to *S. pyogenes* and *A. baumannii*, (tentative ΣFIC evaluation). The aqueous extract was found to be antagonistic against *L. monocytogenes, S. aureus* and *S. agalactiae*. The combination was mentioned once (Chapter 2) as effective remedy for blood cleansing, fatigue and fever. *Vachellia sieberiana* and *V. infausta* roots were poorly active when evaluated individually, hence this combination increased the efficiency.

The combination of *X. caffra* roots + *H. hemerocallidea* corm + *R. multifidus* shoots + *S*. *puniceus* bulb (aqueous extract) was synergistic against *A. baumannii*, and additive against *C. acnes.* The organic extract had additive effects against *C. acnes* (ΣFIC value 0.69), *S. agalactiae* (ΣFIC values 1.00) and *S. pyogenes* (ΣFIC values 0.67). The combination was mentioned once for vaginal complaints, pimples and blood cleansing. The correlation between the traditional use of the combination and these ailments is feasible as the responsible organisms for some of these diseases demonstrate susceptibility to the combinations. The combination of these plant species showed increased activity as when they were investigated individually where only *X. caffra* extract had noteworthy activity.

It is important to note that there were nine combinations that had no synergistic interaction with any of the pathogens of the study, regardless of the solvent used. These combinations were either dominantly non-interactive or had antagonistic reactions. The root combination of *A. delagoensis* + *P. kaurabassana* was antagonistic against *C. acnes* in both aqueous and organic extracts. In the combination of *C. hirta* + *G. senegalensis* (roots), the aqueous extract showed mostly antagonism against *L. monocytogenes, S. aureus, S. agalactiae* and *A. baumannii*. The organic extract had antagonistic reactions against *S. agalactiae* and *S. pyogenes*. The combination of *C. molle* + *T. sericea* (roots) displayed antagonism against five pathogens (*S. aureus, S. pyogenes* and *A. baumannii* for the aqueous extracts and *C. acnes* and *P. aeruginosa* for the organic extracts). The aqueous extract of *G. livingstonei* + *O. natalitia* + *V. infausta* was antagonistic against *L. monocytogenes, S. aureus* and *S. agalactiae*. The aqueous extract of *K. mosambicina* + *P. guajava* + *M. azedarach* leaf combination had antagonistic interactions against *L. monocytogenes, S. aureus, S. agalactiae* and *A. baumannii*. Although it had no synergistic interactions, the combination of *P. cineraria* + *S. spinosa* + *V. infausta* had only one antagonistic reaction which was against *S. pygenes* (aqueous extract). For the combination of *R. multifidus* + *S. serratuloides* shoots, antagonistic interactions were found against five pathogens; *L. monocytogenes, S. aureus, S. pyogenes* and *P. aeruginosa* with aqueous axtracts and *S. aureus* when the organic extract was tested. The combination of *S. puniceus* + *H. hemerocallidea* + *R. multifidus* (aqueous extract) displayed antagonistic effects against *L. moncytogenes, S. aureus* and *S. pyogenes*. Lastly, the combination of *V. sieberiana* + *S. birrea* bark aqueous extract was antagonistic against *C. acnes, L. monocytogenes, S. aureus* and *S. agalactiae*. The organic extract showed antagonism against *L. monocytogenes* and *P. aeruginosa*. It was noted that most of these combinations were reported to treat acne, and very few combinations displayed antagonistic reactions against *C. acnes* in this study.

In general, the analysis of the combinations displayed that the interaction of most plant extracts did not increase the antimicrobial efficacy of the plant species. The study found that out of 308 combinations evaluated in the study only 6% had synergistic effects (Figure 3.1). One needs to note here that antimicrobial efficacy may not be the only

reason why plant species are combined and other pharmacological factors such as reduction of toxicity or nutritional tonic-like factors may have played a role in the combination selection.

The analysis of results also suggested that *X. caffra* could be inducing the synergy in combinations since it was mostly paired with plant species that were initially non-active and synergistic when combined. The other plant species that possibly induced improved efficiency was the roots of *R. digitata*. The study also noted that *A. baumannii* was the most susceptible pathogen when subjected to combinations, although it was resistant when exposed to plant samples independently. *Acinetobacter baumannii* is a Gram-negative bacteria commonly known for infecting surgical wounds, burns, skin infection, urinary tract infection, bacteremia and sepsis (Zarrilli *et al*., 2009). Bacteremia and sepsis are known to be blood infections (Zarrilli *et al*., 2009) whilst burns, skin infections and some urinary infection had often indirect association with blood (Akter *et al*., 2012). For instance, in the present study, these ailments had been labelled as symptoms of blood infection.

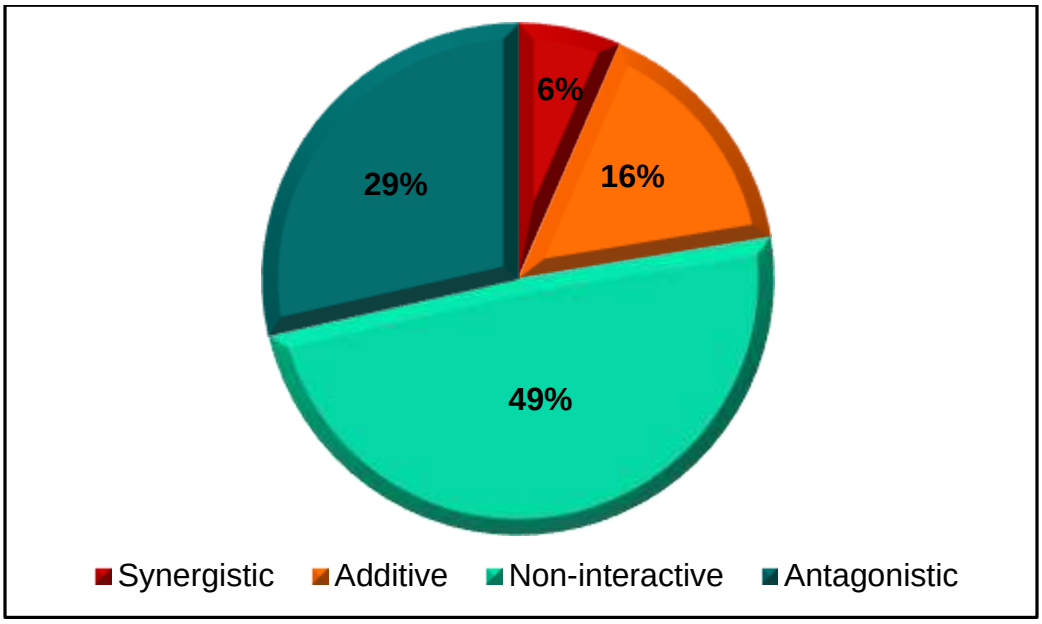

Figure 3.1 An overview for the interaction of combinations used in the study against blood pathogens.

There are limited studies that evaluated plant combinations on bacteria that instigate blood associated disorders (organisms tested in the current study). The one study that evaluated plant combinations and used some of the bacterial strains similar to those of the current study was done by Nciki *et al*. (2016). In that study, some combinations involved at least one plant species which was common in both studies (plant species highlighted in bold). For the combination of *B. discolour* + *Euphorbia tirucalli* + ***H. hemerocallidea*** + *O. engleri* + ***S. serratuloides,*** the extracts were synergistic against *S. aureus* and the aqueous extract was additive against *P. aeruginosa,* whereas the organic extract demonstrated non-interactive effects. The other combination was of ***H. hemerocallidea*** + *Solanum rigescens* (organic extract) which had synergistic effects against *S. aureus*, but additive effects against *S. aureus* and *P. aeruginosa* when the aqueous extract was tested. In the current study, the combinations that included *H. hemerocallidea* were either additive or non-interactive against *S. aureus* and *P. aeruginosa*, except for the combination that involved *S. serratuloides*, which was synergistic interactions against *P. aeruginosa* (organic extract). The other combination that included *S. serratuloides* (aqueous extract) was additive and non-additive when the organic extract was tested against both *S. aureus* and *P*. *aeruginosa*. The combination of ***C. inerme*** + *Dichrostachys cineraria* showed antagonism against *S. aureus* and *P. aeruginosa* whereas in the current study the aqueous extract of *S. spinosa* + *C. inerme* was non-interactive and the organic extract was antagonistic towards *S*. *aureus*. Both extracts were non-interactive against *P. aeruginosa*. Another combination from this study was *L. javanica* + *O. engleri* + ***S. birrea*** + ***S. cordatum*** + *T. elegans* + *V. burkei* which was either additive or non-interactive against the strains of *S. aureus* and *P. aeruginosa*. ***Sclerocarya birrea*** + ***S. cordatum*** extract was synergistic against both *S. aureus* and *P. aeruginosa* (aqueous extract), displaying an ΣFIC value of 0.13 and 0.19 respectively. The organic extract was additive (0.63 ΣFIC value) against *S. aureus* and non-interactive (1.16 ΣFIC value) against *P. aeruginosa*. The aqueous extract of the *S. brachypetala* + ***S. birrea*** combination was additive whilst the organic extract was non-interactive against *S. aureus*. The aqueous extract was synergistic against *P. aeruginosa* and the organic extract was non-interactive. In the current study, the combination of *T. sericea* + *S. cordatum* showed antagonism and additive effects against *S. aureus* in aqueous and

organic extract respectively and complete antagonistic against *P. aeruginosa* regardless of the extract. The combinations of *E. natalensis* + *S. birrea* and *V. sieberiana* + *S. birrea* were non-interactive against *S. aureus* in both the aqueous and organic extract. However, *E. natalensis* + *S. birrea* aqueous extract was additive and organic extract was non-interactive whilst *S. birrea* + *V. sieberiana* extract was additive in aqueous and antagonistic in organic extract. Lastly, the combination of *S. madagascariensis* + *S. spinosa* displayed an antagonistic effect against both *S. aureus* and *P. aeruginosa* in aqueous extract. The organic extract was synergistic (0.38 ΣFIC value) against *S. aureus* and non-interactive (1.50 ΣFIC value) against *P. aeruginosa*. In the current study, the *S. spinosa* combinations were antagonistic against *S. aureus* and *P. aeruginosa*.

3.4 SUMMARY

- *Ozoroa engleri* (organic extract) demonstrated the broadest spectrum of antimicrobial activity as the extract managed to inhibit 100% bacterial growth (71% noteworthy inhibition; 29% moderate inhibition).
- *Ozoroa engleri* also had the most activity as the organic extracts had noteworthy inhibition of *C. acnes* (0.004 mg/ml), *L*. *monocytogenes* (0.02 mg/ml), *S. aureus* (0.02 mg/ml), *S. agalactiae* (0.01 mg/ml) and *S. pyogenes* (0.02 mg/ml) and also had moderate inhibition of *C. acnes* growth in the aqueous extract.
- From the aqueous extracts studied, only *A. delagoensis* roots demonstrated noteworthy inhibition (*C. acnes* at a MIC value of 0.13 mg/ml).
- The antimicrobial activity of *A. delagoensis* and *S. integerrimum* was evaluated for the first time globally in the current study.
- *Albertisia delagoensis* and *S. integerrimum* had noteworthy activity against *C. acnes* with the former presenting a mean MIC value of 0.13 mg/ml and 0.05 mg/ml for the aqueous and organic extracts respectively and the latter inhibiting at 0.13 mg/ml with organic extract.
- *Cutibacterium acnes* was the most susceptible pathogen in the study.

- There were 10 aqueous combinations that displayed synergistic interactions and also 10 organic extracts that were synergistic.
- The antimicrobial evaluation of all combinations were reported here for the first time.
- There was a 74% correlation between the traditional use of plant species and the antimicrobial activity of aqueous and organic extracts.
- Twenty nine percent of the combinations in the study interacted antagonistically.
- Some plant species combinations do enhance the efficacy against pathogens. However, it is important to note that not all plant species combinations produce synergy, some causes adverse reactions. Therefore, the emphasis of using plant species with caution still remains.

CHAPTER 4

TOXICITY EVALUATION OF BLOOD PURIFIER PLANT SPECIES

4.1 INTRODUCTION

According to the results obtained in Chapter 2 of the study, the main reason for the participants to use blood purifiers was to generate a reaction to stimulate the removal of impurities in the body through either purging, steaming, sneezing or enemas. According to the literature, an adverse effect from taking the wrong dose (not necessarily limited to dose, however, the study focused on plant doses) of a plant concoction could be vomiting which possibly can be a result of the toxicity of that plant (Gupta and Raina, 1998). Therefore, this Chapter examines not only testing the toxicity of the plant species used for blood purification but also assessing the safe dosage for patients. Marles and Farnsworth (1995) reported that about one-third of medicinal plant species used for the treatment of diabetes are considered toxic, however, people in rural areas are still insisting on using these plants because they do not view them as toxic. Possibly the toxicity of these plants is not evident on immediate consumption. The objectives of this Chapter was to perform a brine shrimp lethality assay (BSLA) to evaluate the toxicity of the plant species (singularly or when used in combination) that are used for blood purification in northern Maputaland. Furthermore, where toxicity was apparent the dose-response was performed to determine safe and lethal doses.

4.2 METHODOLOGY

4.2.1 Preparation of plant samples for the brine shrimp lethality assay

Each dried plant extract was prepared to a final concentration of 2.00 mg/ml. Dimethyl sulfoxide (2%) was then used as the solvent to dissolve the dried crude organic extracts due to its aprotic solvent properties to dissolve both polar and non-polar compounds (Clark *et al*., 2008). Aqueous extracts were dissolved in autoclaved distilled water. The

extracts that resist dissolving in these solvents were then placed into a sonicator bath (Labcon) until they were completely dissolved. All prepared extracts were stored at 4 ºC prior to analysis.

4.2.2 Brine shrimp lethality assay

The modified version of Cock and Van Vuuren (2015) was applied for the brine shrimp lethality assay **(**BSLA). Growth medium was prepared by dissolving 16 g of Tropic Marine® Sea Salt in 500 ml of distilled water to make artificial saltwater. The prepared medium was then dispensed into an inverted, receptacle, into which 0.5 g of dried cysts of brine shrimp (*Artemia franciscana*) (Ocean Nutrition ^{TM}) were added. To promote a high hatch rate, the brine shrimp medium was bubbled with a gyratory Kiho pump at a constant room temperature under a light source of 220–240V. The brine shrimp cysts were incubated for 18–24 hrs at 25 ºC. Conducting the toxicity assay of the extracts (Figure 4.1), 400 µl of medium (containing > 40 live larvae) was added alongside 400 µl of the plant extract into a 48-well microtiter plate. Two negative controls (non-lethal) were included. These comprised of artificial seawater and 2% DMSO (organic solvent). The positive control (lethal) was 1.6 mg/ml of potassium dichromate.

A stereo microscope (Olympus) was used to count the number of dead shrimp per well after 24 and 48 hrs. The brine shrimp was declared dead once it had no motion when observed under a microscope. After the 48-well plates were read after 48 hrs, 50 µl of acetic acid was added into each well of the plates to kill all the larvae that are still alive at this point. This allowed for the total number of shrimps to be counted in each well. A 48-well plate template was used to record all the data obtained immediately after adding extract into wells containing brine shrimps after 24 and 48 hrs. The plant extract was considered toxic if it caused a percentage mortality that is above 50%.

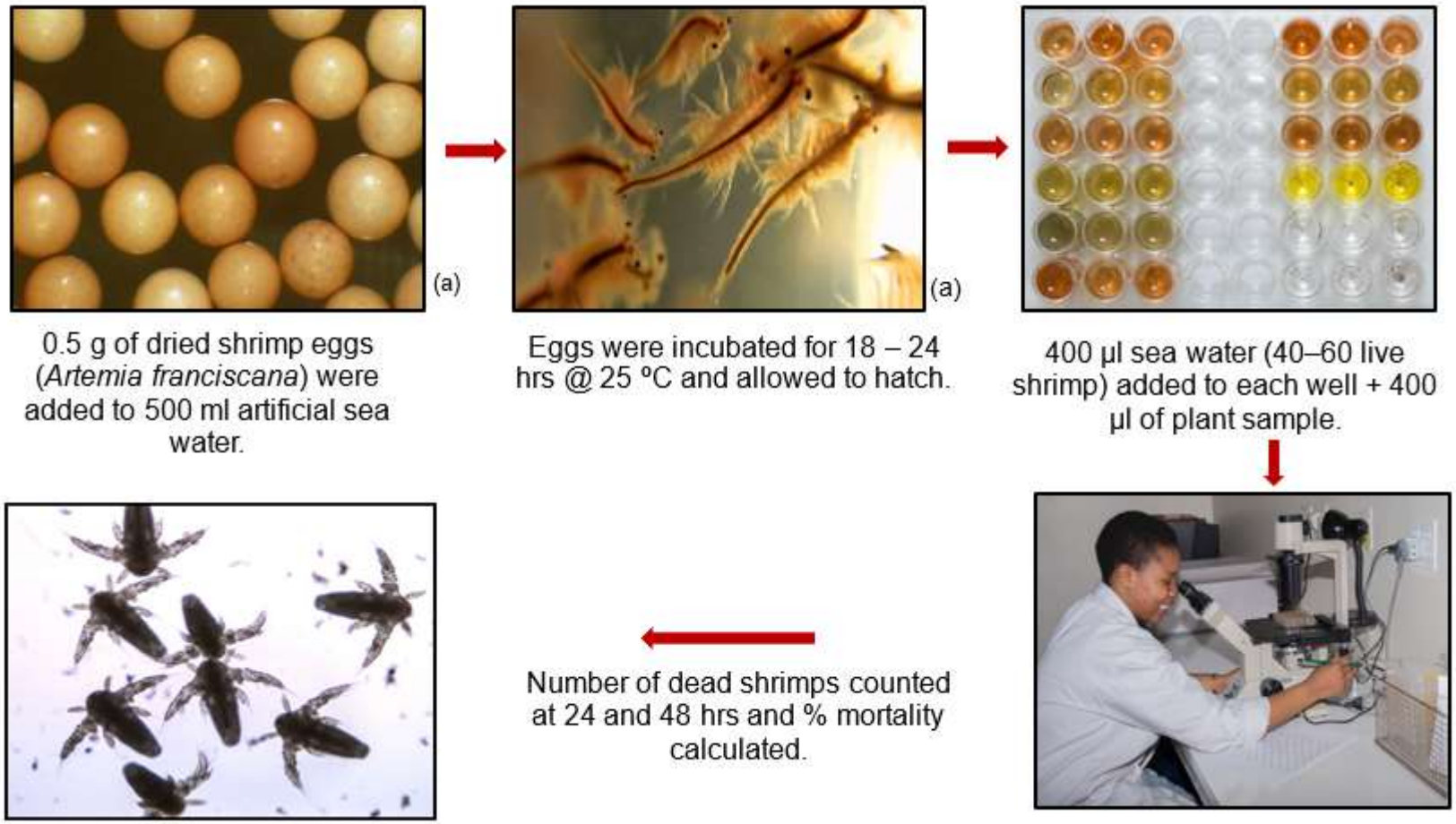

Figure 4.1 A graphical methodology for performing brine shrimp lethality assay. (a) Google images

Percentage mortality was calculated (Equation 4.1) by dividing the number of dead shrimps by the total number of shrimps in a well and multiply by a hundred.

% Mortality = (no. of dead shrimps/ total no. of shrimps) x 100 **Equation 4.1**

For all extracts that indicated toxicity further dilutions were performed. For each extract, six concentrations (0.03, 0.06, 0.13, 0.25, 0.50 and 1.00 mg/ml) were made following the same method mentioned previously. These results were recorded into a log-sigmoid dosage response curve to determine the concentration that will result in 50% (LC_{50}) death of the larvae. A straight line was drawn at 50% of the mortality rate to determine LC_{50}.

The toxicity of plant species combinations was also evaluated, if the combination consists of two plant species the plant extract was prepared in a ratio of 1:1 (200 µl for each extract to make a total of 400 µl). For three plant species, the plant sample was prepared in a ratio of 1:1:1 (133 µl of each extract to make a total 400 µl) and for a combination of four plant species the plant sample volume was prepared according to a ratio of 1:1:1:1 (100 µl for each plant extract to make a total of 400 µl). The assay was then performed in the same manner as previously described. The tests were done in triplicate. The results obtained were calculated and presented as mean ± standard deviation.

4.3 RESULTS AND DISCUSSION

4.3.1 Analysis of toxic plant species used individually to purify blood

Out of 58 extracts that were screened only 12 (21%) showed potent toxicity (>50% mortality) (Table 4.1). When examining in detail, toxicity was observed for the aqueous and organic extracts at both 24 and 48 hrs. The aqueous extracts displayed less toxicity when compared to the dichloromethane: methanol (1:1 DCM: MeOH) extracts, with only four plant species (*D. cymosum*, *E. capensis*, *T. emetica* (roots) and *W. indica*) showing toxicity at both 24 and 48 hrs.

Table 4.1 The toxicity of 58 plant extracts that were screened against brine shrimp bioassay at a concentration of 2.00 mg/ml

Plant extract	Plant part	Aqueous extract percentage mortality (%)		1:1 DCM: MeOH percentage mortality (%)	
		24 hrs	48 hrs	24 hrs	48 hrs
A. precatorious	Roots	0.00 ± 0.00	0.00 ± 0.00	3.00 ± 0.90	34.00 ± 13.22
A. glabratum	Whole plant	0.00 ± 0.00	1.00 ± 0.94	5.00 ± 3.20	7.00 ± 4.47
A. delagoensis	Roots	3.00 ± 1.25	17.00 ± 6.93	46.00 ± 5.21	**96.00 ± 6.51**
B. pilosa	Roots	1.00 ± 1.23	1.00 ± 1.23	1.00 ± 1.27	1.00 ± 1.27
B. discolor	Leaves	0.00 ± 0.78	0.00 ± 0.78	0.00 ± 0.86	0.00 ± 0.86
B. cathartica	Leaves	0.00 ± 0.61	1.00 ± 0.93	0.00 ± 0.82	17.00 ± 4.42
B. cathartica	Roots	1.00 ± 1.25	1.00 ± 1.25	1.00 ± 1.73	31.00 ± 2.61
C. inerme	Roots	3.00 ± 5.66	9.00 ± 13.00	5.00 ± 4.20	33.00 ± 15.95
C. roseus	Roots	0.00 ± 0.00	1.00 ± 0.92	4.00 ± 3.87	14.00 ± 9.05
C. hirta	Roots	3.00 ± 2.73	19.00 ± 6.78	1.00 ± 1.02	31.00 ±10.38
C. anasita	Leaves	1.00 ± 1.18	1.00 ± 1.18	1.00 ± 1.15	1.00 ± 1.15
C. molle	Leaves	1.00 ± 1.34	2.00 ± 2.18	1.00 ± 1.03	1.00 ± 2.06
C. molle	Roots	1.00 ± 0.96	1.00 ± 0.96	0.00 ± 0.00	0.00 ± 0.00
D. cymosum	Roots	**100.00 ± 0.00**	**100.00± 0.00**	**100.00 ± 0.00**	**100.00 ± 0.00**
E. capensis	Bark	**76.00 ± 2.33**	**98.00 ± 3.55**	**78.00 ± 5.06**	**98.00 ± 2.88**
E. natalensis	Roots	1.00 ± 0.87	3.00 ± 1.04	1.00 ± 1.86	9.00 ± 4.66
G. livingstonei	Bark	1.00 ± 1.72	9.00 ± 4.71	0.00 ± 0.00	36.00 ± 7.83
G. volkensii	Roots	24.00 ±12.03	**97.00 ± 5.66**	47.00 ± 3.17	**97.00 ± 2.72**
G. caffra	Roots	1.00 ± 1.09	1.00 ± 1.09	0.00 ± 0.00	3.00 ± 1.44
G. senegalensis	Leaves	1.00 ± 0.92	9.00 ± 1.65	0.00 ± 0.40	3.00 ± 0.98
G. senegalensis	Roots	6.00 ± 5.26	24.00 ± 2.36	3.00 ± 1.96	8.00 ± 5.91
H. pauciflorus	Twigs	11.00 ± 5.22	22.00 ± 7.03	1.00 ± 1.23	9.00 ± 1.39
H. abyssinica	Rhizome	1.00 ± 1.23	1.00 ± 1.23	1.00 ± 1.00	1.00 ± 1.00
H. hemerocallidea	Corm	0.00 ± 0.48	0.00 ± 0.48	12.00 ± 1.83	**77.00** ± **8.38**
H. natalensis	Roots	1.00 ± 1.23	1.00 ± 1.23	3.00 ± 1.88	22.00 ± 11.49
K. africana	Bark	0.00 ± 0.61	3.00 ± 4.42	1.00 ± 0.30	4.00 ± 2.43
K. mosambicina	Shoots	0.00 ± 0.00	1.00 ± 0.14	**56.00 ± 16.07**	**75.00 ± 14.64**
L. javanica	Leaves	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
M. azedarach	Leaves	0.00 ± 0.00	2.00 ± 3.77	0.00 ± 0.00	9.00 ± 5.25
M. balsamina	Leaves	0.00 ± 0.00	1.00 ± 1.18	1.00 ± 1.05	4.00 ± 1.06
O. natalitia	Roots	0.00 ± 0.00	3.00 ± 0.95	0.00 ± 0.81	**68.00 ± 16.39**
O. serrulata	Roots	1.00 ± 0.92	6.00 ± 7.27	1.00 ± 1.23	7.00 ± 6.36
O. engleri	Roots	1.00 ± 1.09	1.00 ± 1.03	6.00 ± 3.88	**89.00 ± 6.95**
P. americana	Seed	3.00 ± 0.42	7.00 ± 3.09	2.00 ± 4.12	5.00 ± 6.64
P. cineraria	Roots	0.00 ± 0.00	0.00 ±0.00	0.00 ± 0.00	0.00 ± 0.00
P. guajava	Leaves	7.00 ± 3.55	11.00 ± 7.02	2.00 ± 2.27	4.00 ± 1.98
P. kaurabassana	Root tuber	2.00 ± 2.39	5.00 ± 3.07	0.00 ± 0.00	1.00 ± 1.09
P. scandens	Twigs	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
R. multifidus	Shoots	9.00 ± 8.78	13.00 ± 6.90	4.00 ± 3.21	17.00 ± 8.11
R. digitata	Roots	0.00 ± 0.62	0.00 ± 0.62	38.00 ± 4.24	**81.00 ± 4.48**
R. communis	Leaves	5.00 ± 3.13	6.00 ± 3.53	2.00 ± 1.46	5.00 ± 4.04
S. integerrimum	Roots	3.00 ± 2.09	3.00 ± 1.85	1.00 ± 1.04	3.00 ± 3.87
S. puniceus	Bulb	0.00 ± 0.00	6.00 ± 5.79	1.00 ± 0.69	9.00 ± 6.18

Plant extract	Plant part	Aqueous extract percentage mortality (%)		1:1 DCM: MeOH percentage mortality (%)	
		24 hrs	48 hrs	24 hrs	48 hrs
S. brachypetala	Bark	1.00 ± 1.09	9.00 ± 5.49	2.00 ± 2.24	4.00 ± 1.91
S. birrea	Bark	3.00 ± 3.00	27.00 ± 0.76	**63.00 ± 6.40**	**91.00 ± 6.07**
S. serratuloides	Shoots	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
S. spinosa	Roots	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.75	4.00 ± 4.69
S. cordatum	Bark	0.00 ± 0.00	3.00 ± 5.85	1.00 ± 0.96	6.00 ± 7.93
T. elegans	Fruits	2.00 ± 1.33	4.00 ± 1.27	0.00 ± 0.00	2.00 ± 2.36
T. sericea	Roots	0.00 ± 0.00	4.00 ± 3.64	1.00 ± 2.14	4.00 ± 3.66
T. emetica	Roots	**99.00 ± 0.89**	**100.00 ± 0.00**	**100.00 ± 0.00**	**100.00 ± 0.00**
T. emetica	Bark	1.00 ± 2.46	7.00 ± 6.64	1.00 ± 1.06	14.00 ± 6.18
V. sieberiana	Bark	0.00 ± 0.00	4.00 ± 6.97	2.00 ± 2.12	28.00 ± 18.03
V. xanthophloea	Bark	1.00 ± 1.13	2.00 ± 2.18	1.00 ± 0.95	2.00 ± 2.25
V. infausta	Roots	1.00 ± 1.31	1.00 ± 1.31	1.00 ± 1.50	1.00 ± 1.50
W. indica	Roots	**53.00 ± 4.48**	**100.00 ± 0.84**	**100.00 ± 0.00**	**100.00 ± 0.00**
W. somnifera	Whole plant	1.00 ± 0.98	1.00 ± 0.98	0.00 ± 0.00	0.00 ± 0.00
X. caffra	Roots	2.00 ± 1.59	31.00 ± 8.06	1.00 ± 1.13	2.00 ± 2.15
Sea water		4.00 ± 0.99	4.00 ± 0.99	0.00 ± 0.00	0.00 ± 0.00
DMSO		0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Potassium dichromate		**100.00 ± 0.00**	**100.00 ± 0.00**	**100.00 ± 0.00**	**100.00 ± 0.00**

DCM = dichloromethane; MeOH = methanol; DMSO = dimethyl sulfoxide; negative controls = sea water and DMSO; positive control = potassium dichromate; the numbers that are highlighted in bold are the extracts that displayed active toxicity. The results are presented as % mortality ± standard error.

Gardenia volkensii demonstrated toxicity only after 48 hrs (Table 4.1). The level of toxicity for *D. cymosum* was as high as that of the positive control (potassium dichromate). The plant extract and the positive control obtained 100% larvae mortality through all the tests performed. These were followed by *T. emetica* and *W. indica* (aqueous extracts), which resulted in 99% and 53% deaths at 24 hrs respectively. They both killed 100% of larvae after 48 hrs exposure. *Ekebergia capensis* displayed high toxicity with 76% and 98% for the aqueous extracts; and 78% and 98% for the organic extracts after 24 and 48 hrs respectively. *Gardenia volkensii* was non-toxic when evaluated after 24 hrs (24% and 47% mortality in aqueous and organic extracts respectively). This plant extract displayed toxicity after 48 hrs in both the aqueous and organic extractions where it was recorded as 97% mortality for each. *Krauseola mosambicina* and *S. birrea* (aqueous extracts) were both non-toxic but demonstrated toxicity with the organic extracts at both 24 and 48 hrs. Then, last in this toxic category were *A. delagoensis*, *H. hemerocallidea*, *O. natalitia*,

O. engleri and *R. digitata* (organic extracts) which showed some toxicity only after 48 hrs. The increased toxicity observed with the DCM: MeOH extracts was possible since both polar and non-polar compounds of the plant species were extracted, hence providing a wider range of compounds present to be tested. In the cases of *K. mosambicina* and *S. birrea* these plant species portrayed low or no toxicity after 24 hrs of being exposed to the larvae, but after 48 hrs they displayed a sharp increase in larvae death. This pattern may be the result that the larvae only start feeding on the extract after 24 hrs because within the first 24 hrs they are believed to feed on their yolk (Sarah *et al*., 2017; Ramulondi *et al*., 2019).

Dichapetalum cymosum displayed a percentage mortality of 100% with both aqueous and organic extracts at both 24 and 48 hrs. The use of *D. cymosum* which is administered orally as a blood purifier by the people of northern Maputaland was alarming based on these results. Fortunately, the plant species was only mentioned in one household from 55 that were visited. The 2.00 mg/ml concentration of *D. cymosum* was discovered to be highly toxic and therefore the concentration was further diluted to determine the toxicity level of the extract at different concentrations. The aqueous *D. cymosum* extract showed toxicity at concentrations above 0.15 mg/ml and was non-toxic from the lowest concentration of 0.15 mg/ml after 24 hrs. After 48 hrs the plant extract displayed the first signs of non-toxicity at the lowest concentration of 0.07 mg/ml. The *D. cymosum* organic extract was toxic at all concentrations tested (1 – 0.03 mg/ml) after 24 and 48 hrs (Figure 4.2). *Dichapetalum cymosum* has been reported numerous times as a poisonous plant (“gifblaar” in Afrikaans, which is directly translated to “poisonous leaf”) to livestock. However, there were no cases reported about the toxicity or use of this plant species by humans in the literature. One of the chemical components extracted from *D. cymosum* consist mainly of fluoroacetate, which is a chemical compound that is commonly used as a metabolic poison (Peters, 1957; Egyed and Schultz, 1986; Proudfoot *et al*., 2006; Spiteller, 2008; Eason *et al*., 2011). Botha and Venter (2002), reported that the plant affects the cardiovascular system in cattle. This results in the death of animals after 4 –24 hrs of administration especially if the cattle drank water or was used for excessive work activities (like ploughing).

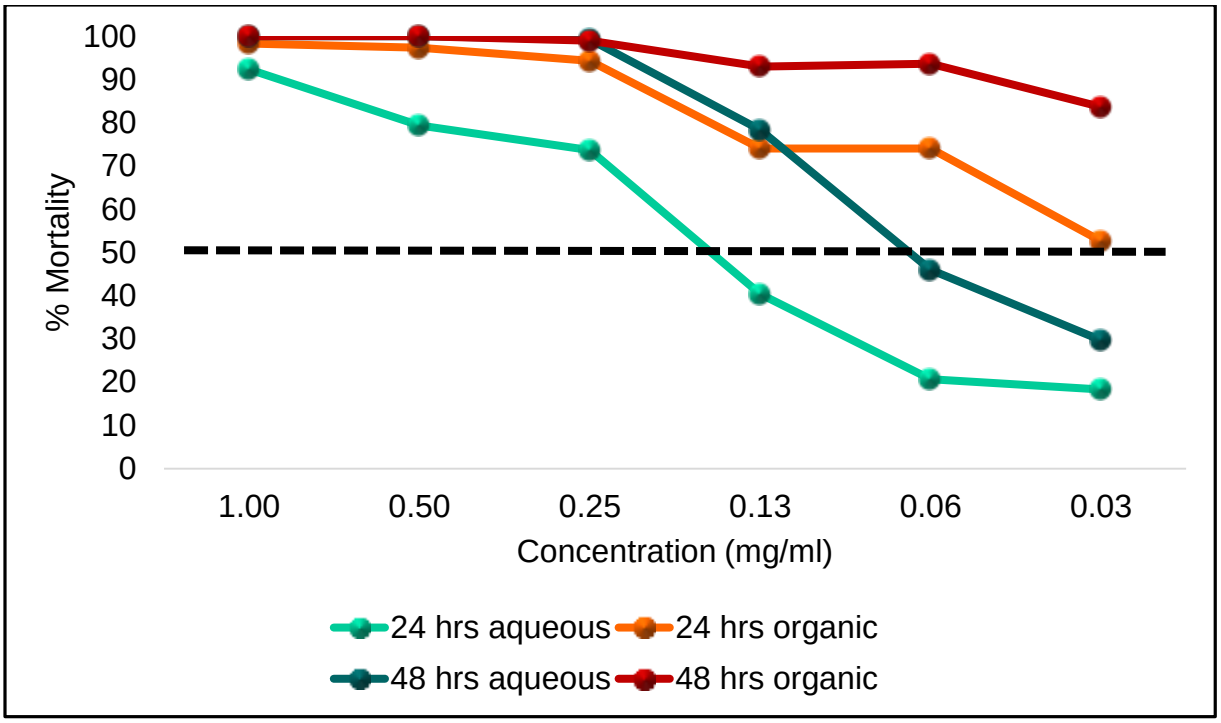

Figure 4.2 The dose-responses of *D. cymosum* roots after 24 and 48 hrs of exposure in aqueous and organic extracts. Jagged black line delineates toxicity (>50%) with non-toxicity (<50%).

The alarming toxicity of *D. cymosum* has led the current study to believe that this plant was possibly misidentified by the participant, especially since only one participant mentioned this plant. If that is the case, this could be a good example of plant toxicity by misidentification.

Ekebergia capensis bark was tested as highly toxic at the concentration of 2.00 mg/ml in the current study. The aqueous extract caused 76% death of larvae after 24 hrs of exposure. The longer periods (48 hrs) of the larvae being exposed in this aqueous extract resulted in a 98% death rate. No significant difference was observed in comparison with the organic extract as it recorded mortality of 78% and 98% after 48 hrs. The ethnobotanical survey results (Chapter 2) demonstrated that this plant species was not frequently used, however, where mentioned the plant was reported to be used for steaming and bathing but no oral ingestion. Although highly toxic, it may be used with caution since it was reported to be only used externally. At the highest concentration of

1.00 mg/ml the extract of *E. capensis* was highly toxic (71.67% mortality after 24 hrs for aqueous extract) (Figure 4.3). The aqueous extract displayed non-toxicity at the lowest concentration of 0.52 mg/ml after 24 hrs and 0.25 mg/ml after 48 hrs. In the organic extract the lowest concentration (to demonstrate non-toxicity was 0.20 mg/ml after 24 hrs and at 0.16 mg/ml after 48 hrs. From these findings, it can be deduced that *E. capensis* prepared by aqueous extraction is the safest method of preparation. In another study where an Ames test was performed to test the mutagenetic activity (Mulaudzi *et al*., 2013), the stem bark of *E. capensis* exhibited mutagenicity towards *Salmonella typhimurium* strain TA 98 at a concentration of 50 µg/ml. The bark contains the toxic compound 8-methoxy 4-methyl coumarin (Mairura, 2017). In 2014, Irungu *et al*. evaluated the cytotoxicity of *E. capensis* leaves and root bark on human larynx carcinoma (HEp2) cells and mammalian African monkey kidney (Vero) cells. The study found that the leaves contained large quantities of flavonoids and exhibited low toxicity, whereas the root bark consisted of triterpenoids which resulted in high toxicity. Triterpenoids had cytotoxic effects when screened on HCT-15 human tumour cell lines (Kim *et al*., 2012). These results from the root bark can be related to reactions occurring to the stem bark since the compounds found in the root bark are similar to those of the stem bark (Murata *et al*., 2008). Nonetheless, where *E. capensis* stem bark was tested against Vero cell lines using the MTT assay, the plant extract displayed no toxicity with CC_{50} > 100 µg/ml (Ngeny *et al*., 2013). When *E. capensis* stem bark was screened for genotoxic effects with strains TA 98 and TA 100 no genotoxic effects were found (Elgorashi *et al*., 2013). The different results observed may be due to different bioassays and solvents to extract the plant compounds (Irungu *et al*., 2014).

The roots of *Trichilia emetica* have been recorded in the current study as one of the highest toxic plant extracts, having toxic effects of 99% in brine shrimp lethality at 24 hrs and 100% lethality after 48 hrs when screened using aqueous extracts and with organic extract both after 24 and 48 hrs. The results obtained in this study validates the participant's concern for stressing the importance to use *T. emetica* only as an enema and not for oral consumption.

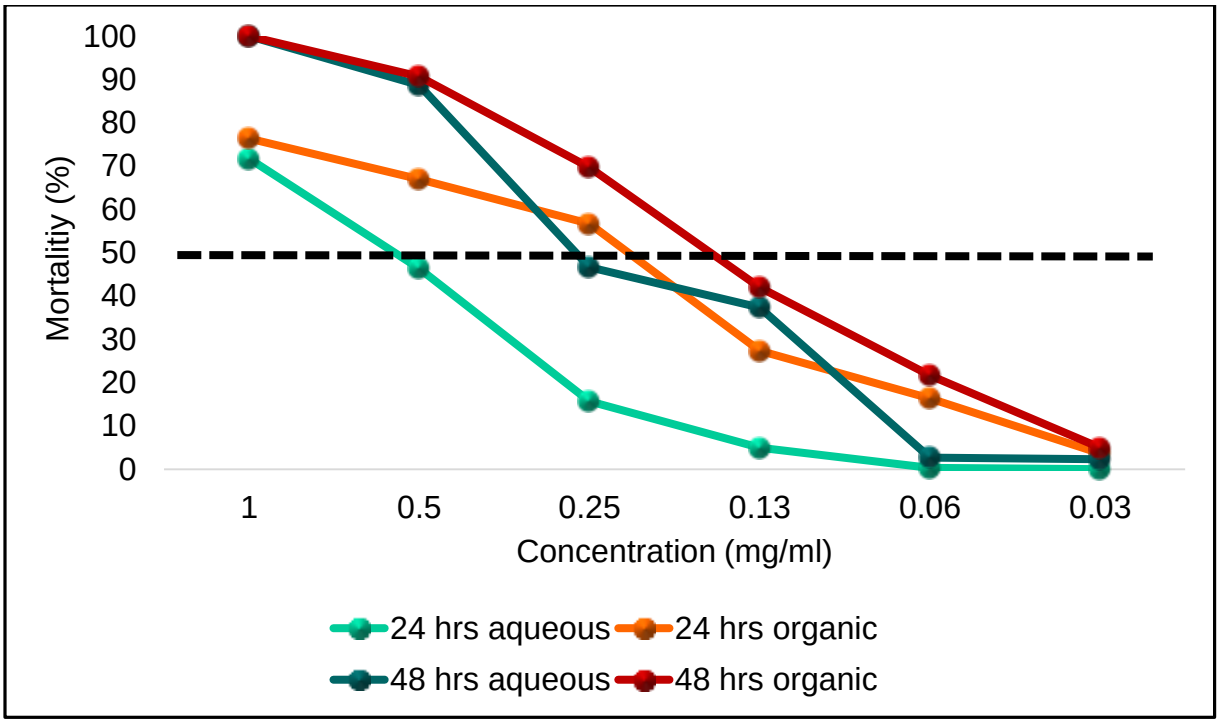

Figure 4.3 The dose-responses of *E. capensis* bark after 24 and 48 hrs of exposure in both aqueous and organic extracts. Jagged black line delineates toxicity (>50%) with non-toxicity (<50%).

The participants highlighted the importance of using very low concentrations which after the evaluation of toxicity was found to be 0.13 mg/ml considering both aqueous and organic extracts after 24 and 48 hrs when preparing remedies from *T. emetica*. The plant species is known in northern Maputaland to induce abortion when administered by pregnant women, and may sometimes be fatal when a strong concentration (presumably 2.00 mg/ml rendering the results obtained) of the plant is administered. The dose-response of *T. emetica* with the aqueous and organic extracts were toxic at the highest concentration of 1.00 mg/ml. The aqueous extract displayed non-toxicity at 0.68 mg/ml after 24 hrs and with further exposure (48 hrs) the highest concentration demonstrating non-toxicity was 0.25 mg/ml. For the organic extract, the safest concentration determined in the BSLA to use *T. emetica* was at the concentration of 0.39 mg/ml and 0.13 mg/ml after 24 and 48 hrs respectively (Figure 4.4). Although the roots of *T. emetica* have shown high toxicity, it was interesting to note that the bark of the plant tested non-toxic for both aqueous and DCM: MeOH extracts and it was the commonly mentioned plant part.

Prozesky *et al*. (2001), reported that the *Trichilia* species exhibit toxicological activities when being tested against monkey kidney cells. Bero *et al*. (2009) recorded that the leaves of *T. emetica* (dichloromethane extract) have low cytotoxicity when evaluated in the MTT assay on both J774 macrophage-like murine cells and WI38 human normal fibroblasts. Germanò *et al*. (2005) found that the roots of *T. emetica* (aqueous and ethyl ether extracts) were non-toxic in the brine shrimp bioassay. The variances in the current study and some of the other studies may be that the plant species that are used in northern Maputaland are frequently wounded by herbivores or insects and that has alerted the plant to release complex secondary metabolites to try and protect themselves from the predators and therefore secreted more active toxic compounds (Spiteller, 2008). Differences in solvents used to extract the compounds of the plant may also affect the outcome in each study. Germanò *et al*. (2005) and Ramulondi *et al*. (2019) reported that the aqueous and DCM: MeOH extracts of *T. emetica* leaf exhibited low cytotoxicity when tested with the brine shrimp assay. The plant material for both the current study and the study conducted by Ramulondi *et al*. (2019) were of different plant parts. The method of preparation is also a factor in contradictory reports of these studies. The bark in the current study was boiled and that may perhaps have activated more chemical components of the plant as compared to the leaves of Ramulondi *et al*. (2019), which were prepared by soaking in cold water (infusion) and resulted in fewer chemical components extracted (Spiteller, 2008).

Waltharia indica root extract demonstrated larvae death after 24 and 48 hrs exposure (53% and 100% respectively) to the aqueous plant extract. The plant's organic extract at 2.00 mg/ml killed 100% of the larvae after 24 and 48 hrs. Only one participant reported that the plant spesiec is taken orally at least twice a week to purify the blood. Once dilutions were made, at the highest concentration of 1.00 mg/ml all four extracts of *W. indica* roots killed 100% of larvae (Figure 4.5). At the lowest concentration of 0.17 mg/ml, the aqueous extract was non-toxic after 24 hrs and after 48 hrs it was non-toxic at the lowest concentration of 0.046 mg/ml. The concentrations in organic extracts were all toxic.

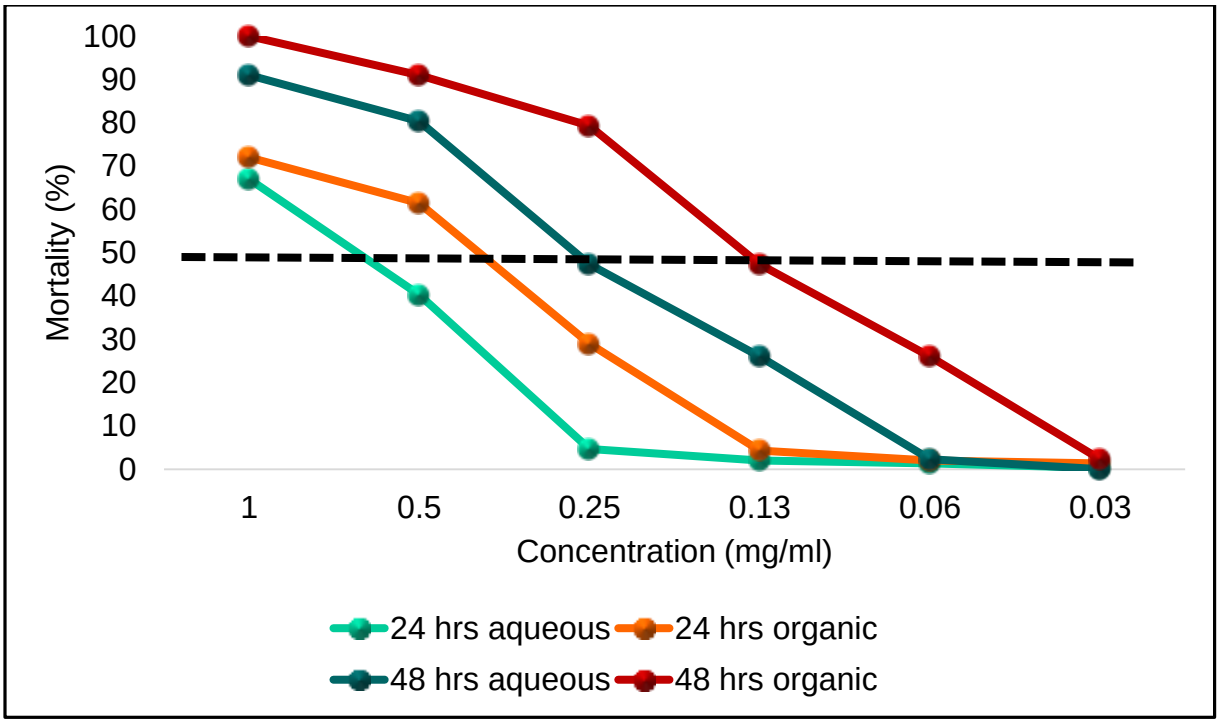

Figure 4.4 The dose-responses of *T. emetica* roots after 24 and 48 hrs of exposure in both aqueous and organic extracts. Jagged black line delineates toxicity (>50%) with non-toxicity (<50%).

In a different study, Hamidu *et al*. (2018) reported that *W. indica* has no toxicity on the liver and the kidney functioning of rats when ingested (LD_{50} of the extract was 875 mg/kg). When converted and correlated to 2.00 mg/ml which was the standard concentration in the current study, it was approximately 0.25 mg/ml. These comparisons show that the concentration ratios in the current study were higher than in Hamidu *et al*'s study since the first non-toxicity was detected at 0.17 mg/ml in the current study. An *in vivo* study by Basiru and Olayemi (2014), found that the aqueous leaves extract of *W. indica* was non-toxic at lower concentrations of 200 mg/kg – 1600 mg/kg (≈ 0.06 mg/ml – 0.47 mg/ml when correlated to concentrations of the current study). Then it was slightly toxic when the concentration of extracts increases (2000 mg/kg ≈ 0.58 mg/ml) as they were administered in rats. In the current study, *W. indica* extract was non-toxic from 0.03 mg/ml to 0.17 mg/ml, then was highly toxic at 0.58 mg/ml. The pharmacological study of Yougbare-Ziebrou *et al*. (2016) reported there was no toxicity detected in the shoots of *W. indica*. The reason for these differences could be due to that the assays conducted in

these two studies were different (*in vitro* and *in vivo*). It is known that the *in vitro* assay does not always predict the events that will unfold with the *in vivo* tests (Mulaudzi *et al*., 2013; Ngeny *et al*., 2013; Irungu *et al*., 2014; Said *et al*., 2014). When a plant extract has been ingested it is introduced to other metabolic reactions that are occurring within the gastrointestinal tract and the enzymes that are involved in those metabolic reactions could decrease or increase the toxicity level of the plant extracts. Although in the current study *W. indica* tested highly toxic, it appeared to be used with caution as the participants reported that the plant is only used as a wash for sores and should under no circumstances be ingested.

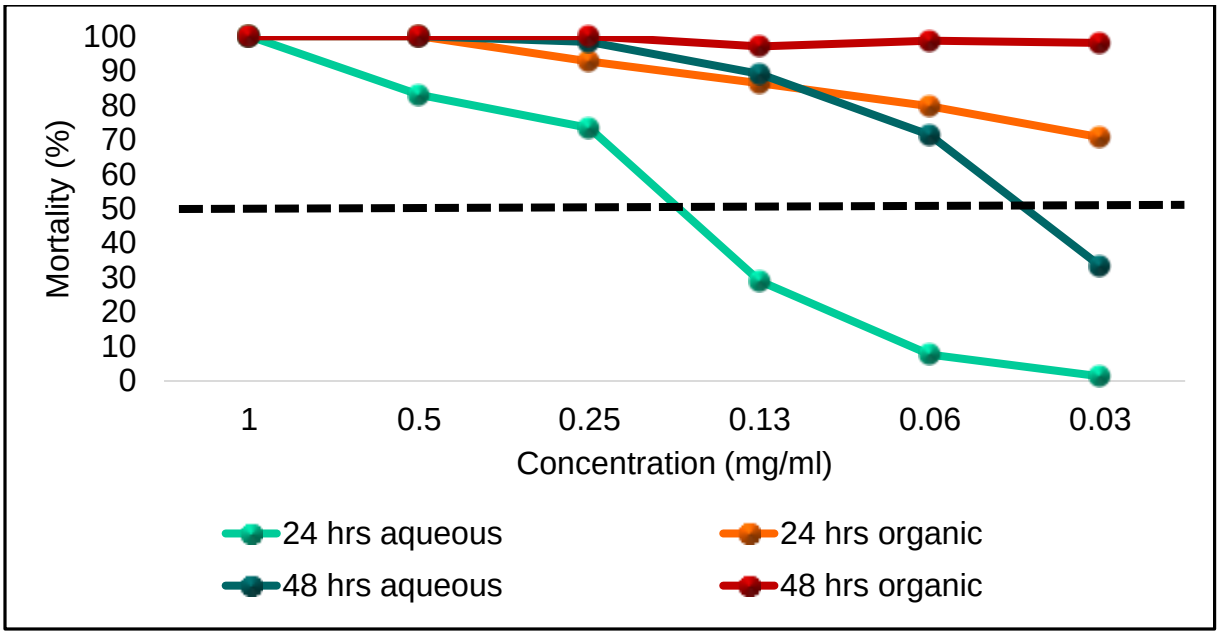

Figure 4.5 The dose-responses of *W. indica* roots after 24 and 48 hrs of exposure in both aqueous and organic extracts. Jagged black line delineates toxicity (>50%) with non-toxicity (<50%).

Gardenia volkensii roots showed an interesting turn of events where at 24 hrs of exposure the death percentage of larvae was 24% and at 48 hrs it increases up to 97% in aqueous extract. This was also observed in organic extract where at 24 hrs the death percentage was 47% and after 48 hrs increases to 97%. These findings hint that the plant species may have low toxicity, but the continuous administration of the plant for a long period may pose some health risks. At the highest concentration of 1.00 mg/ml, both aqueous and

organic extracts were non-toxic after 24 hrs (Figure 4.6). When the plant extracts were exposed to larvae for 48 hrs they released toxic substances towards the larvae. However, at the concentration of 0.40 mg/ml, the aqueous extract was non-toxic and organic extract at the concentration of 0.19 mg/ml. For both 24 and 48 hrs dilution concentrations the plant extracts displayed signs of dosage dependant toxicity. The plant species was reported once to be used either by steaming or drinking the decoction “once in a while” as a blood cleanser. The current study validate the traditional use of *G. volkensii* since they only used water as a solvent. In a study conducted to investigate antimutagenic effects of medicinal plant species, the bark of *G. volkensii* displayed potent genotoxic effects in a micronucleus test (Verschaeve *et al*., 2004). In another study, the fruits and roots of *G. volkensii* were also tested positive for toxicity when tested in the alkaline comet assay (Taylor *et al*., 2003). *Gardenia volkensii* was listed by Kuete (2014) as a toxic plant. Both these studies had evaluated different plant parts of *G. volkensii* (bark) than in the current study (roots).

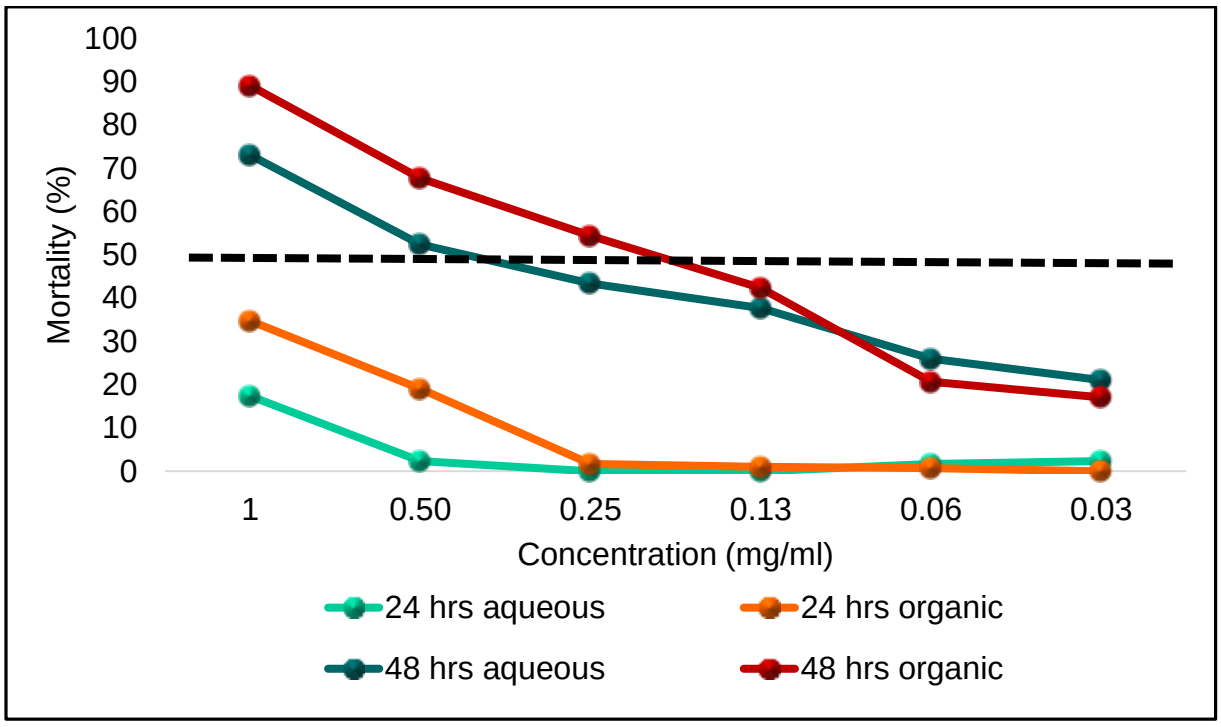

Figure 4.6 The dose-responses of *G. volkensii* roots after 24 and 48 hrs of exposure in both aqueous and organic extracts. Jagged black line delineates toxicity (>50%) with non-toxicity (<50%).

The chemical composition is different in each part of the plant; therefore, these results are hardly a contradiction but rather an emphasis on other studies that have been conducted to evaluate the toxicity of *G. volkensii*.

4.3.2 Other dose toxicity profiles

There were some plant species (*A. delagoensis, H. hemerocallidea, K. mosambicina, O. natalitia, O. engleri, R. digitata* and *S. birrea*) that only demonstrated toxicity for the organic extracts. The details for these plant species were reviewed and discussed here in-depth. The organic extracts dose-responses were given after 24 hrs (Figure 4.7) and (Figure 4.8) 48 hrs (2.00 mg/ml). All extracts studied except *S. birrea* (63%) demonstrated non-toxicity at 1.00 mg/ml. *Albertisia delagoensis* roots resulted in a 46% death of larvae after 24 hrs and 96% mortality after 48 hrs. After 48 hrs *A. delagoensis* organic extract was non-toxic at 0.048 mg/ml (Figure 4.8). The plant species is used as a tonic taken daily, however, this plant was mentioned by only one participant. The results in these study showed that the traditional used of *A. delagoensis* is supported since water is used as a solvent. These findings correlate to those of Ramulondi *et al*. (2019); where the results of the roots (aqueous extract) displayed low toxicity and an increased mortality rate with the organic extract using the same bioassay as the current study. De Wet *et al*. (2007) recorded that the leaves of *A. delagoensis* have low toxicity when tested against the Graham cell line. The alkaloids extracted from *A. delagoensis* leaves were found to be cytotoxic on the Vero cell lines. Furthermore, the alkaloids of *A. delagoensis* leaves portrayed high levels of cytotoxicity against breast, melanoma and renal cancer cell lines (Hawkes *et al*., 2011).

The findings of the current study recorded that the organic extract of *H. hemerocallidea* corm was non-toxic with no death of larvae recorded after 24 hrs. After 48 hrs the extract was toxic at the highest concentration of 1.00 mg/ml (83%) tested and non-toxic at the lowest concentration of 0.50 mg/ml (Figure 4.8). In Chapter 2 it is documented that this plant species was only mentioned once to cleanse the blood. The results in this study displayed that the traditional used of *H. hemerocallidea* is supported since water is used

as a solvent. *Hypoxis hemerocallidea* corm is very popular for its ability to treat ailments like cancer, diabetes and heart diseases (Boukes *et al*., 2008). However, the safety for long term use of the plant is yet to be tested. In a study done by Ramulondi *et al*. (2019) the same trend was found as was recorded in the current study where the aqueous extract of the corm of *H. hemerocallidea* had 5% larvae death after 48 hrs and DCM: MeOH extract resulted in 54% death of larvae after 48 hrs. A study was performed where the MTT assay was used to evaluate the cytotoxicity of four *Hypoxis* species on Vero and Hela cells. The corm extract was found to be non-toxic to Vero cells but moderate cytotoxic activity was seen on Hela cells with the highest concentration of 400 µg/ml (Sathekge, 2010). In another study, an investigation for genotoxicity of the aqueous extracts from *Hypoxis* species was conducted. The investigation included three tests: Neutral red uptake test, the alkaline and comet test assay. *Hypoxis hemerocallidea* extract demonstrated slight DNA damage in all three assays although with a negligible difference when compared to other species. All *Hypoxis* species were found to be non-genotoxic (Verschaeve *et al*., 2013). An overview by Mills *et al*. (2005) reported that in 2003 there was once a suggestion to discourage patients with HIV/AIDS to stop the use of *H. hemerocallidea* since it was hypothesized to suppress bone marrow synthesis in these patients. Chemical components of this plant such as rooperol displayed signs of affecting the cardiovascular system (Mills *et al*., 2005; Owira and Ojewole, 2009).

The organic extract of *K. mosambicina* shoots demonstrated a 56% mortality rate after 24 hrs and 75% after 48 hrs. After 24 hrs the organic extract of the plant was non-toxic once the study evaluated the dose-responses. After 48 hrs toxicity was detected at the highest concentration of 1.00 mg/ml where 51% of larvae died, and at the concentration of 0.50 mg/ml, the extract was safe (Figure 4.8). The use of *K. mosambicina* for blood purification was mentioned by only one participant in the study. The aqueous extract was non-toxic, therefore, the traditional use of the plant was validated. Unfortunately, there was no success in finding data relating to plant toxicology. According to York *et al*., (2011) this plant was recorded for the first time during their study for any medicinal use. This claim was supported by De Wet *et al*., who in 2012 reported that *K. mosambicina* has

been poorly studied pharmacologically. This study is thus the first to report on the toxicity of the plant.

Another organic plant extract that displayed toxic activity against the larvae at 48 hrs was *Ochna natalitia* root, with the death rate of 68%. The lowest concentration in which the extract showed non-toxicity was at 1.50 mg/ml (Figure 4.8). Two of the participants mentioned that the roots of the plant are used as an emetic for blood cleansing. Since the remedies were reported to be prepared using water, this study validate the safety of *O. natalitia*. Previously, the leaf extract of *O. natalitia* was tested on Vero cells using MTT assay and the cytotoxicity of the extract proved to be dosage-dependent. The lower concentration exhibited weak toxicity and with elevated concentration levels, the extract completely lysed the cells (Suleiman *et al*., 2010). The toxicity in the present study was dependent on the time of larvae exposure. The leaves of five *Ochna* species (*O. natalitia*, *Ochna pretoriensis* E. Phillips*, Ochna, pulchra* Hook. *Ochna gamostigmata* du Toit and *O. serrulata*) were evaluated for *in vitro* mutagenicity against *S. typhimurium* test strain T98 and T100. The cytotoxic activity was tested using the MTT assay on Vero cells, human hepatocellular carcinoma (C3A) cells and bovine dermis cells. The authors found that all were non-mutagenic; however, cytotoxic activity was seen on all three cell lines studied with *O. natalitia* being the less cytotoxic and *O. serrulata* highly cytotoxic (Makhafola *et al*., 2014). Interestingly, these findings do not correlate with those of the present study where *O. serrulata* was recorded as a non-toxic plant and *O. natalitia* as a potentially toxic plant. The possible explanation for these reactions can be that the two studies in comparison used different assays to evaluate toxicity and different plant parts were tested (leaves versus roots). The occurrence of toxic compounds in plant species may depend on the plant part that is used. For instance, it is common to have plant species with edible fruits but poisonous leaves or poisonous peel/shell but edible pulp in some fruits (Salgueiro *et al*., 2010).

Ozoroa engleri root extract was non-toxic for the aqueous extracts and after 24 hrs in the organic extract at the concentration of 2.00 mg/ml used in the current study. The organic extract was found to be highly toxic once exposed for 48 hrs resulting in 89% brine shrimp

death. After 48 hrs, the plant displayed 70% mortality at the highest concentration of 1.00 mg/ml and no toxicity at the concentration of 0.30 mg/ml (Figure 4.8). The traditional use of this plant species was to treat fever by using the plant as an enema (Chapter 2). The results in this study portrayed that the aqueous extract had no potential toxicity, therefore validates it safety in traditional use. Prozesky *et al*. (2001) reported that there was also a lack of information regarding medicinal activity and safety of *O. engleri*. Seemingly, this plant has been understudied up until recently.

The organic extract of *R. digitata* roots was non-toxic after 24 hrs and shown toxicity after 48 hrs with 81% mortality. Similar to other plant species that were toxic with organic extraction, the roots extract of *R digitata* was not toxic after 24 hrs but after 48 hrs it was toxic at the highest concentration of 1.00 mg/ml (66%) and non-toxic at 0.66 mg/ml (Figure 4.8). *Rhoicissus digitata* was mentioned several times as a blood purifier and used frequently as a tonic taken every morning. This study validates traditional use of *R. digitata* in lower dose concentrations (≤ 0.50 mg/ml). There were no studies found in the literature regarding the toxicity of this plant. The only other information that could be found relating to this plant was that Steenkamp (2003) listed a different species (*R. tridentate)* as potentially toxic. This inclusion thus adds new scientific data.

The bark organic extract of *S. birrea* toxicity increased from 63% to 91% after 24 and 48 hrs respectively. During the dose-response evaluation the extract was toxic at 24 hrs at the highest concentration of 1.00 mg/ml (55% larvae mortality) and non-toxic at the concentration of 0.80 mg/ml (Figure 4.7). This pattern was also evident after 48 hrs where *S. birrea* extract was highly toxic at the highest concentration of 1.00 mg/ml (98%) but non-toxic at a much lower concentration of 0.13 mg/ml (Figure 4.8). The plant was one of the most frequently used plant species in preparing blood purifying remedies (Chapter 2).The traditional use of this plant is validated as safe, especially if not administered regularly. A toxicity study was conducted with both the leaves and bark extracts which exhibited an increased mortality rate when exposed to weevils over time. On the fifth day (last day of the experiment) the mortality percentage recorded was 100% for the leaf petroleum ether extract and 93% with bark acetone extract (Babarinde *et al*., 2017). In

another study, the stem bark extract was orally given to rats at different concentrations to test for acute (24 hrs) and chronic (28 days) toxicity. After 24 hrs no death was recorded and none-to-slight behavioural changes were observed even with the highest dosage of 2000 mg/ml.

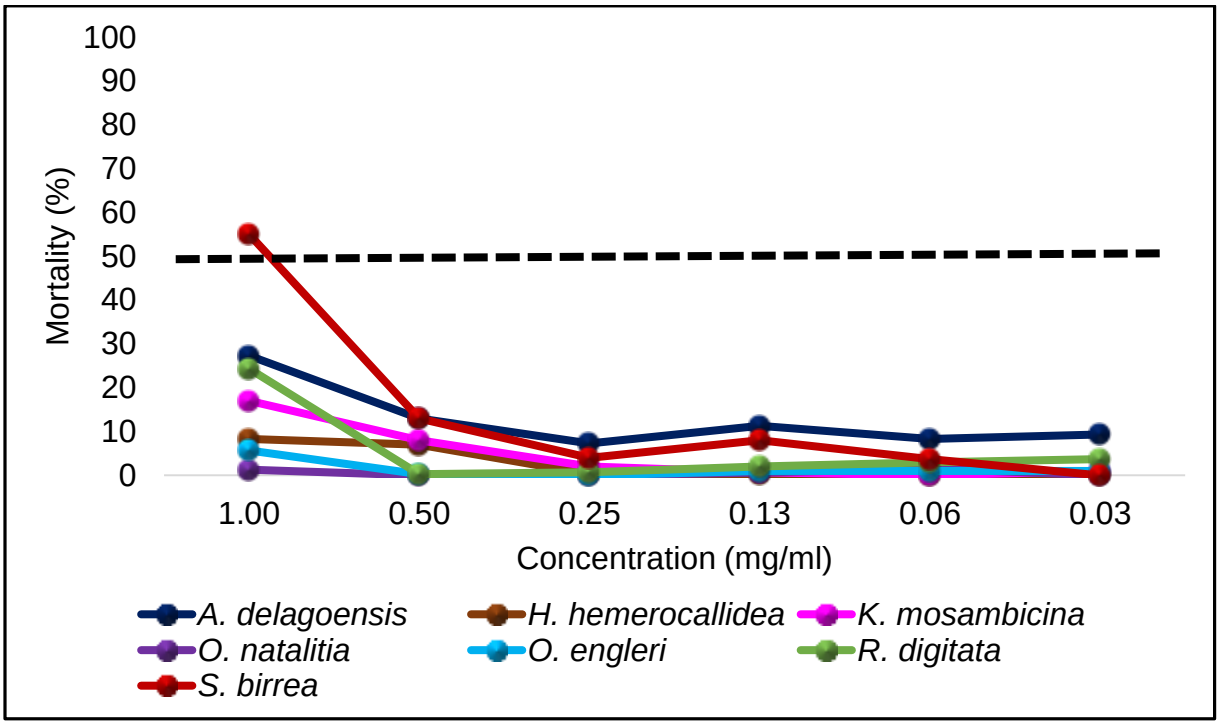

Figure 4.7 The dose-response of organic extracts after exposure to larvae for 24 hrs. Jagged red line delineates toxicity (>50%) from non-toxicity (<50%).

Whereas potent toxicity (growth inhibition, liver and kidneys malfunction) was evident with the rats that were given *S. birrea* extract for 28 days (Mawoza *et al*., 2016). The findings of the previous study are not entirely in line with the results displayed in the current study where the toxicity of the bark of *S. birrea* was measured within 48 hrs with brine shrimp. However, both studies show that *S. birrea* bark was dose-dependent and when taken frequently it induced toxicity. The contradiction in these studies may be due to the fact that one is an *in vivo* study and the other an *in vitro*, hence the reaction of extracts may not be similar. In another study of toxicity on the kernel extract of *S. birrea* fruit, a dose of 3000 mg/kg was investigated, and no toxicity detected (Muhammad *et al*., 2011).

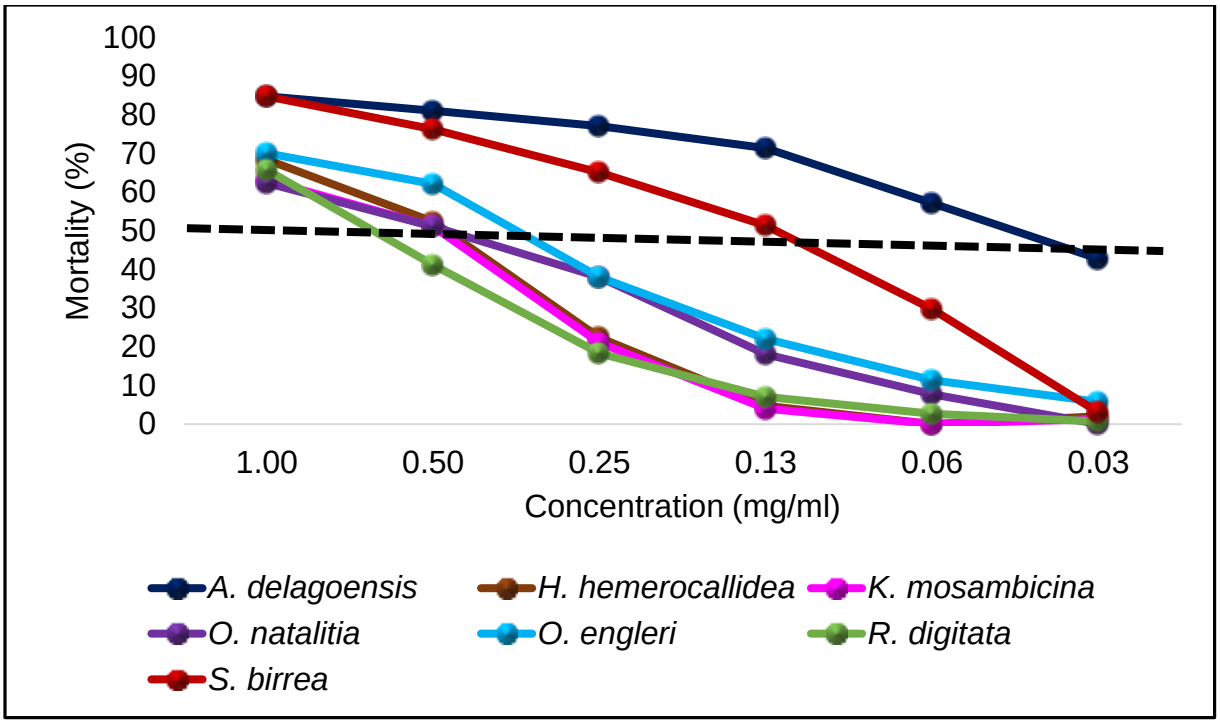

Figure 4.8 The dose-response of organic extracts after exposure to larvae for 48 hrs. Jagged red line delineates toxicity (>50%) from non-toxicity (<50%).

4.3.3 Plant extracts demonstrating non-toxicity

It must be noted that the majority (79%) of the plant extracts for both aqueous and organic extracts were considered non-toxic. Of these, the study recorded 19% of plant species extracts (*B. pilosa, B. discolour, C. anisata, C. molle leaves, H. abbysinica, L. javanica, P. cineraria, P. scadens, S. serratiloides, V. infausta* and *W. somnifera*) that had no larvae deaths at either aqueous or organic extracts when tested at a concentration of 2.00 mg/ml. Although the extracts of *B. pilosa, C. anisata, C. molle* roots, *H. abyssinica, V. infausta* and *W. somnifera* displayed 1% larvae death, this was disregarded because these extracts displayed some mortality (1%) immediately after larvae were added in wells. In this case, the larvae were dead before the extracts were even introduced and the same was observed with negative control (seawater). Some aqueous extracts (*A. precatorious, H. hemerocallidea, H. natalensis* and *R. digitata*) demonstrated no mortality but some mortality (toxicity) with the organic extract. The plant aqueous extracts that had no death after 24 hrs were *A. glabratum, C. roseus, K. africana, K. mosambicina,*

M. balsamina, S. puniceus, S. cordatum, *T. sericea* and *V. sieberiana. Bridelia cathartica* (leaves), *M. azedarach* and *V. xanthophloea* displayed no larvae deaths at 24 hrs in both aqueous and organic extracts. Plant species that had mortality only after 24 hrs of organic extraction were *G. livingstonei, G. caffra, G. senegalensis leaves, P. kaurabassana* and *T. elegans* (Table 4.1). The plant species that showed non-toxicity when evaluated were all validated as safe for traditional use.

Clausena anisata was rarely mentioned in the study with only two of the participants making use of the plant for blood cleansing. *Clausena anisata* is known to be a great insect repellent and the oil from the leaves have been reported to be toxic to insects (Okunade and Olaifa, 1987; Mukandiwa *et al*., 2016; Guo *et al*., 2018). The ethyl acetate extract of *C. anisata* twigs was tested in the brine shrimp bioassay and presented toxicity at a LC_{50} value of 6.21 µg/ml (Makirita *et al*., 2016). When tested on human leukaemic monocyte lymphone (U937) cells, the plant revealed low toxicity with LC_{50} of 74.46 µg/ml and a selective index of 2.38 (De Canha *et al*., 2018). The opposing results demonstrated by both these studies towards those of the present study could be because of the different solvents used in each study. For example, in a study where *K. africana* bark was tested positive and negative depending on the solvent that was used for extraction. When tested on LLC/MK2 monkey kidney cells the ethyl acetate bark extract was non-cytotoxic and when tested on n-hexane bark extract it displayed cytotoxicity (Zofou *et al*., 2011).

Hydnora abyssinica rhizome was mentioned twice during steaming for acne. The aqueous rhizome extract of *H. abyssinica* was given to rats in two clinical experiments and displayed no signs of toxicity or discomfort. The flowers of the plant demonstrated non-cytotoxicity against FL cells (Al-Fatimi *et al*., 2016).

Similarly, to the present study where *P. cineraria* roots indicated non-toxicity, the plant indicated non-toxicity when orally given to Wistar mice (Robertson *et al.,* 2012). Furthermore, different solvent extracts (methanol, chloroform, hexane, ethyl acetate and water) for leaves, stem, flower and roots of *P. cineraria* were tested against the THP-1 cell line for cytotoxicity and were all found to be non-toxic (Sharifi-Rad *et al*., 2019).

Senecio serratuloides is one of the more popular medicinal plant species in northern Maputaland where it is used to treat sores. In Chapter 1 sores have been indicated as one of the diagnoses for blood purification. In a recently conducted study, a hydro-ethanol extract of *S. serratuloides* was reported to be non-toxic *in vivo* (Tata *et al*., 2019). Also, the stem of *S. serratuloides* was considered relatively safe after both the aqueous and methanol extracts were tested using breast cancer (WI38) cell lines (Mariri, 2017).

The use of the whole plant of *W. somnifera* was mentioned six times in the current study as a blood purifier. Patel *et al.* (2016) conducted a safety assessment of *W. somnifera* extract on Wistar rats and found that there was no potent toxicity towards the rats. In another study, the roots of *W. somnifera* was administered daily for 21 days with a dose of up to 2000 mg/kg to rats and no adverse effects were found (Prabu *et al*., 2013).

4.3.4 Toxic evaluation of plant species used in combination

The current study recorded 32 different plant species combinations that are used for blood purification and 22 are evaluated. The combinations that were screened were subject to the availability of all plant material within the said combination. Forty-five percent of the combinations were toxic against larvae in a brine shrimp bioassay. Four plant species combinations were toxic in both the aqueous and organic extract. The two combinations (*A. delagoensis* + *P. kaurabassana* and *D. cymosum* + *E. natalensis*) were highly toxic for both aqueous and organic (DCM: MeOH) extracts at 24 and 48 hrs (Table 4.2). The combination of *S. spinosa* + *C. inerme* (aqueous extract) was non-toxic after 24 hrs and toxic after 48 hrs. Organic extracts of this combination were both toxic. Another aqueous solvent combination that displayed toxicity was that of the roots of *C. hirta* + *G. senegalensis*. Toxicity of this combination was only detected after 48 hrs in both aqueous and organic extracts.

Table 4.2 Toxicity of plant species used in combinations as blood purifiers at a concentration of 2.00 mg/ml

Plant species combinations	**Percentage mortality of aqueous extracts (%)**		**Percentage mortality of** DCM: M**eOH extracts (%)**		**Single plant species used in combinations**	**Percentage mortality of aqueous extracts (%)**		**Percentage mortality of** DCM: MeOH **extracts (%)**	
	24 hrs	**48 hrs**	**24 hrs**	**48 hrs**		**24 hrs**	**48 hrs**	**24 hrs**	**48 hrs**
A. precatorius R + *B. cathartica* R	15.00 ± 5.88	34.00 ± 4.71	2.00 ± 2.13	**75.00 ± 5.87**	*A. precatorius* R	0.00 ± 0.00	0.00 ± 0.00	3.00 ± 0.90	34.00 ± 13.22
					B. cathartica R	1.00 ± 1.25	1.00 ± 1.25	1.00 ± 1.73	31.00 ± 2.61
A. delagoensis R + *P. kaurabassana* Rt	**94.00 ± 1.27**	**100.00 ± 0.00**	**91.00 ± 6.07**	**99.00 ± 2.51**	*A. delagoensis* R	3.00 ± 1.25	17.00 ± 6.93	46.00 ± 5.21	**96.00 ± 6.51**
					P. kaurabassana Rt	2.00 ± 2.39	5.00 ± 3.07	0.00 ± 0.00	1.00 ± 1.09
B. cathartica R + *R. digitata* R	2.00 ± 2.44	30.00 ± 3.93	20.00 ± 2.77	43.00 ± 1.93	*B. cathartica* R	1.00 ± 1.25	1.00 ± 1.25	1.00 ± 1.73	31.00 ± 2.61
					R. digitata R	0.00 ± 0.62	0.00 ± 0.62	38.00 ± 4.24	**81.00 ± 4.48**
C. hirta R + *G. senegalensis* R	48.00 ± 6.30	**63.00 ± 5.68**	15.00 ± 5.59	**86.00 ± 8.02**	*C. hirta* R	3.00 ± 2.73	19.00 ± 6.78	1.00 ± 1.02	31.00 ±10.38
					G. senegelansis R	6.00 ± 5.26	24.00 ± 2.36	3.00 ± 1.96	8.00 ± 5.91
C. molle R + *T. sericea* R	3.00 ± 3.76	21.00 ± 12.04	0.00 ± 0.00	1.00 ± 0.93	*C. molle* R	1.00 ± 0.96	1.00 ± 0.96	0.00 ± 0.00	1.00 ± 0.00
					T. sericea R	0.00 ± 0.00	4.00 ± 3.64	1.00 ± 2.14	4.00 ± 3.66
D. cymosum R + *E. natalensis* R	**100.00 ± 0.00**	**100.00 ± 0.00**	**99.00 ± 1.43**	**100.00 ± 0.00**	*D. cymosum* R	**100.00 ± 0.00**	**100.00 ± 0.00**	**100.00 ± 0.00**	**100.00 ± 0.00**
					E. natalensis R	1.00 ± 0.87	3.00 ± 1.04	1.00 ± 1.86	9.00 ± 4.66
E. natalensis R + *S. birrea* B	15.00 ± 8.34	39.00 ± 6.15	0.00 ± 0.00	1.00 ± 0.92	*E. natalensis* R	1.00 ± 0.87	3.00 ± 1.04	1.00 ± 1.86	9.00 ± 4.66
					S. birrea B	3.00 ± 3.00	27.00 ± 0.76	**63.00 ± 6.40**	**91.00 ± 6.07**
G. livingstonei R + *O. natalitia* R + *V. infausta* R	19.00 ± 6.17	41.00 ± 1.45	1.00 ± 1.18	1.00 ± 1.18	*G. livingstonei* R	1.00 ± 1.72	9.00 ± 4.71	0.00 ± 0.00	36.00 ± 7.83
					O. natalitia R	0.00 ± 0.00	3.00 ± 0.95	0.00 ± 0.81	**68.00 ± 16.39**
					V. infausta R	1.00 ± 1.31	1.00 ± 1.31	1.00 ± 1.50	1.00 ± 1.50
H. natalensis R + *R. digitata* R	4.00 ± 5.45	16.00 ± 6.73	1.00 ± 1.34	2.00 ± 2.18	*H. natalensis* R	1.00 ± 1.23	1.00 ± 1.23	3.00 ± 1.88	22.00 ± 11.49
					R. digitata R	0.00 ± 0.62	0.00 ± 0.62	38.00 ± 4.24	**81.00 ± 4.48**
H. hemerocallidea C + *S. serratuloides* Sh	1.00 ± 1.28	15.00 ± 2.96	1.00 ± 0.96	1.00 ± 1.92	H. *hemerocallidea* C	0.00 ± 0.48	0.00 ± 0.48	12.00 ± 1.83	**77.00** ± **8.38**
					S. serratuloides Sh	0.00 ± 0.00	1.00 ± 1.00	0.00 ± 0.00	0.00 ± 0.00

Plant species combinations	**Percentage mortality of aqueous extracts (%)**		**Percentage mortality of** DCM: M**eOH extracts (%)**		**Single plant species used in combinations**	**Percentage mortality of aqueous extracts (%)**		**Percentage mortality of** DCM: MeOH **extracts (%)**	
	24 hrs	**48 hrs**	**24 hrs**	**48 hrs**		**24 hrs**	**48 hrs**	**24 hrs**	**48 hrs**
K. mosambicina L + *P. guajava* L + *M. azedarach* L	2.00 ± 2.62	5.00 ± 2.69	**56.00 ± 16.08**	**97.00 ± 5.87**	*K. mosambicina* L	0.00 ± 0.00	1.00 ± 0.14	**56.00 ± 16.07**	**75.00 ± 14.64**
					P. guajava L	7.00 ± 3.55	11.00 ± 7.02	2.00 ± 2.27	4.00 ± 1.98
					M. azedarach L	0.00 ± 0.00	2.00 ± 3.77	0.00 ± 0.00	9.00 ± 5.25
O. natalitia R + *B. cathartica* R	0.00 ± 0.00	11.00 ± 5.06	**77.00 ± 3.44**	**98.00 ± 3.24**	*B. cathartica* R	1.00 ± 1.43	1.00 ± 1.25	1.00 ± 1.73	31.00 ± 2.61
					O. natalitia R	0.00 ± 0.00	3.00 ± 0.95	0.00 ± 0.81	**68.00 ± 16.39**
P. cineraria R + *S. integerrimum* R	0.00 ± 0.00	2.00 ± 2.62	1.00 ± 0.87	3.00 ± 1.04	*P. cineraria* R	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
					S. integerrimum R	3.00 ± 2.09	3.00 ± 1.85	1.00 ± 1.04	3.00 ± 3.87
P. cineraria R + *S. spinosa R* + *V. infausta* R	2.00 ± 2.75	2.00 ± 2.75	1.00 ± 1.72	9.00 ± 4.71	*P. cineraria* R	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
					S. spinosa R	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.75	4.00 ± 4.69
					V. infausta R	1.00 ± 1.31	1.00 ± 1.31	1.00 ± 1.50	1.00 ± 1.50
R. multifidus Sh + *S. serratuloides* Sh	1.00 ± 0.92	15.00 ± 3.97	24.00 ± 12.03	**97.00 ± 5.66**	*R. multifidus* Sh	9.00 ± 8.78	13.00 ± 6.90	4.00 ± 3.21	17.00 ± 8.11
					S. serratuloides Sh	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
S. integerrimum R + *S. spinosa* R	2.00 ± 2.00	10.00 ± 2.40	21.00 ± 5.39	**78.00 ± 6.57**	*S. integerrimum R*	3.00 ± 2.09	3.00 ± 1.85	1.00 ± 1.04	3.00 ± 3.87
					S. spinosa R	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.75	4.00 ± 4.69
S. puniceus Bb + H. *hemerocallidea* C + *R. multifidus* Sh	7.00 ± 8.74	28.00 ± 2.59	1.00 ± 0.92	9.00 ± 1.65	*S. puniceus* Bb	0.00 ± 0.00	6.00 ± 5.79	1.00 ± 0.69	9.00 ± 6.18
					H. hemerocallidea	0.00 ± 0.48	0.00 ± 0.48	12.00 ± 1.83	**77.00** ± **8.38**
					R. multifidus Sh	9.00 ± 8.78	13.00 ± 6.90	4.00 ± 3.21	17.00 ± 8.11
S. spinosa R + *C. inerme* R	41.00 ± 9.05	**71.00 ± 9.85**	**54.00 ± 3.18**	**80.00 ± 6.84**	*S. spinosa R*	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.75	4 ± 4.69
					C. inerme R	3.00 ± 5.66	9.00 ± 13.00	5.00 ± 4.20	33.00 ±15.95
T. sericea R + *S. cordatum* B	3.00 ± 2.78	41.00 ± 1.85	6.00 ± 5.26	24.00 ± 2.36	*T. sericea* R	0.00 ± 0.00	4.00 ± 3.64	1.00 ± 2.14	4.00 ± 3.66
					S. cordatum B	0.00 ± 0.00	3.00 ± 5.85	1.00 ± 0.96	6.00 ± 7.93
V. sieberiana B + *V. infausta* R + *X. caffra* R	0.00 ± 0.00	13.00 ± 6.19	8.00 ± 2.41	**70.00 ± 5.92**	*V. sieberiana* B	0.00 ± 0.00	4.00 ± 6.97	2.00 ± 2.12	28.00 ± 18.03
					V. infausta R	1.00 ± 1.31	1.00 ± 1.31	1.00 ± 1.50	1.00 ± 1.50
					X. caffra R	2.00 ± 1.59	31.00 ± 8.06	1.00 ± 1.13	2.00 ± 2.15
V. sieberiana B +	24.00 ± 3.26	32.00 ± 5.86	5.00 ± 5.92	25.00 ± 4.00	*V. sieberiana* B	0.00 ± 0.00	4.00 ± 6.97	2.00 ± 2.12	28.00 ± 18.03

Plant species combinations	**Percentage mortality of aqueous extracts (%)**		**Percentage mortality of** DCM: M**eOH extracts (%)**		**Single plant species used in combinations**	**Percentage mortality of aqueous extracts (%)**		**Percentage mortality of** DCM: MeOH **extracts (%)**	
	24 hrs	**48 hrs**	**24 hrs**	**48 hrs**		**24 hrs**	**48 hrs**	**24 hrs**	**48 hrs**
S. birrea B					*S. birrea* B	3.00 ± 3.00	27.00 ± 0.76	**63.00 ± 6.40**	**91.00 ± 6.07**
X. caffra R + *H. hemerocallidea* C + *S. puniceus* C + *R. multifidus* Sh	18.00 +2.94	41.00 ± 1.85	11.00 ± 5.22	22.00 ± 7.03	*X. caffra* R	2.00 ± 1.59	31.00 ± 8.06	1.00 ± 1.13	2.00 ± 2.15
					H. hemerocallidea	0.00 ± 0.48	0.00 ± 0.48	12.00 ± 1.83	**77.00** ± **8.38**
					S. puniceus Bb	0.00 ± 0.00	6.00 ± 5.79	1.00 ± 0.69	9.00 ± 6.18
					R. multifidus Sh	9.00 ± 8.78	13.00 ± 6.90	4.00 ± 3.21	17.00 ± 8.11
Sea water	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	Sea water	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
DMSO	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	DMSO	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Potassium dichromate	**100.00 ± 0.00**	**100.00 ± 0.00**	**100.00 ± 0.00**	**100.00 ± 0.00**	Potassium dichromate	**100.00 ± 0.00**	**100.00 ± 0.00**	**100.00 ± 0.00**	**100.00 ± 0.00**

DCM = dichloromethane; MeOH = methanol; DMSO = dimethyl sulfoxide; B = bark; Bb = bulb; C = corm; L = leaves; R = roots; Rt = root tuber; Sh = shoot; Wp = whole plant; negative controls = sea water and DMSO; positive control = potassium dichromate; the numbers that are highlighted in bold are the extracts that displayed toxicity. The results are presented as average % mortality ± standard deviation. Shaded area, although previously presented is included here for comparison.

The next set was two combinations which were toxic at both 24 hrs and 48 hrs of organic extraction (*K. mosambicina* + *P. guajava* + *M. azedarach* and *O. natalitia* + *B. cathartica*). The combination of *A. precatorius* + *B. cathartica, V. sieberiana* + *V. infausta* + *X. caffra, R. multifidus* + *S. serratuloides* and *S. integerrimum* + *S. spinosa* (organic extracts) showed toxicity only after 48 hrs. These extracts were non-toxic when tested individually (shaded area, Table 4.2), they were still safe for use in combination since participants reported to use only water for preparation of remedies. However, when testing the organic extracts, these combinations caused toxicity when exposed to the brine shrimp for 48 hrs.

The combinations of *A. precatorius* + *B. cathartica* roots, *C. hirta* + *G. senegalensis* roots, shoots of *R. multifidus* + *S. serratuloides*, *S. integerrimum* + *S. spinosa* roots, *S. spinosa* + *C. inerme* roots and *V. sieberiana* + *V. infausta* + *X. caffra* roots had increased toxicity in combination when compared to the plant species where used individually. Although some combinations showed increased toxicity, 55% of the plant extract combinations were non-toxic. The study also recorded 36% of plant combinations (*B. cathartica* + *R. digitata, E. natalensis* + *S. birrea, G. livingstonei* + *O. natalitia* + *V. infausta, H. natalensis* + *R. digitata, H. hemerocallidea* + *S. serratuloides, S. puniceus* + *H. hemerocallidea* + *R. multifidus*, *V. sieberiana* + *S. birrea* and *X. caffra* + *H. hemerocallidea* + *S. puniceus* + *R. multifidus*) that reduced the toxicity of the plant species when tested in combination (Figure 4.9). Interestingly, *H. hemerocallidea, O. natalitia and S. birrea* were formerly mentioned (Chapter 2) to be used in combinations but individually. Hence the participants possibly knew about the adverse effects (toxicity) and use these plant species rather in combinations in the belief that combining them will make them more effective and less toxic. The toxicity of the combinations documented in this study has not been previously investigated.

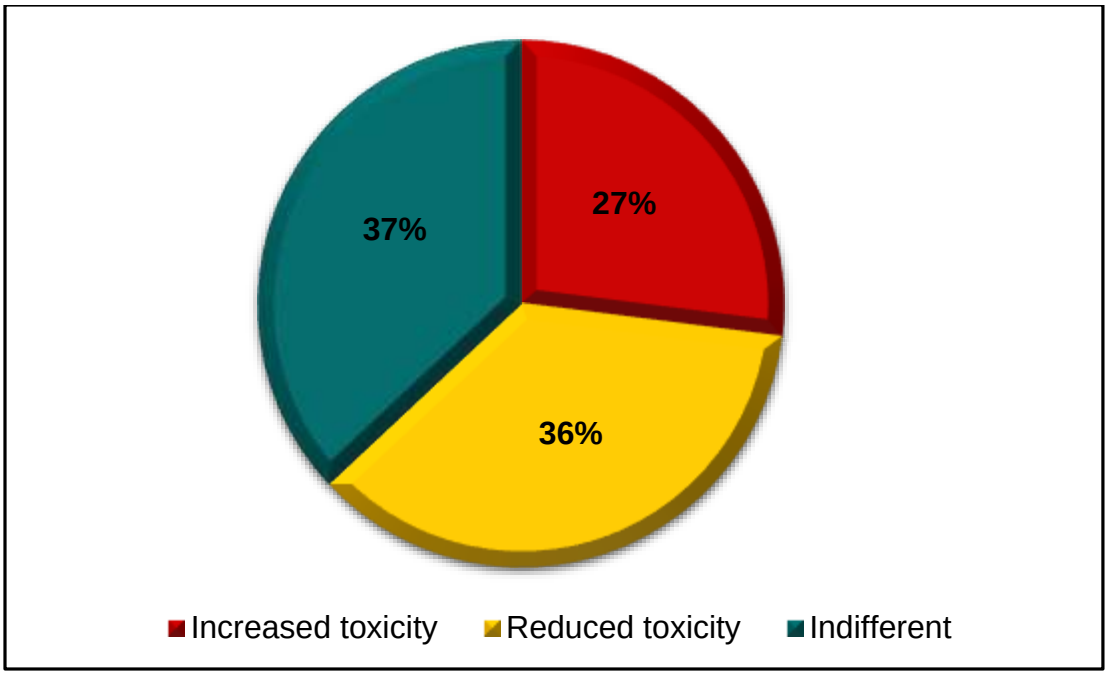

Figure 4.9 The effect of plant combination extracts toxicity on brine shrimps as compared to evaluation of individual plant extracts.

Polyherbals are rarely explored by ethnopharmacologists (Naidoo *et al*., 2013). While such studies are scarce, there has been a combination study where unlike the current study, the combination of *H. hemerocallidea* corm + *S. serratuloides* shoots was modified by adding *Musa acuminata* Colla (Ramulondi *et al*., 2019). These combination displayed reduced toxicity in the previous and current study whereas when used individually *H. hemerocallidea* showed toxicity after long period exposure in organic extract.

For the combinations that demonstrated toxicity at both 24 and 48 hrs, a dose-response effect was examined. The combination of *A. delagoensis* root + *P. kaurabassana* root tuber were highly toxic when evaluated at the standard concentration of 2.00 mg/ml. The plant displayed 94% and 100% larvae mortality in aqueous extracts after 24 and 48 hrs respectively. Organic extracts displayed larvae percentage mortality of 91% and 99% after 24 and 48 hrs respectively. This combination was only mentioned once and the results displayed that the traditional use could be validated when administered in doses ≤0.50 mg/ml. *Albertisia delagoensis* + *P. kaurabassana* were non-toxic when tested individually within the first 24 hrs of both extractions. Once the dose-response was

evaluated, after the first 24 and 48 hrs the aqueous extracts in the combination were non-toxic at the concentration of 0.86 mg/ml and 0.72 mg/ml respectively. The organic extracts first showed non-toxicity at the concentrations of 0.38 mg/ml and 0.27 mg/ml after 24 and 48 hrs respectively (Figure 4.10).

Dichapetalum cymosum + *E. natalensis* root combination killed 100% brine shrimps in each of the evaluated extracts at the concentration of the study (2.00 mg/ml). The use of this combination was mentioned by one participant and the results do not encourage it traditional use. Considering this combination, *D. cymosum* demonstrated one of the highest toxicity when tested singularly, hence the highly toxic state of this combination was anticipated. At the concentration of 1.00 mg/ml, the combination of *D. cymosum* + *E. natalensis* killed 78% of larvae with the aqueous extract and showed non-toxicity at the concentration of 0.30 mg/ml after 24 hrs (Figure 4.11). After the exposure period of 48 hrs at the highest concentration, the extract killed 96% larvae and non-toxic at the concentration of 0.16 mg/ml.

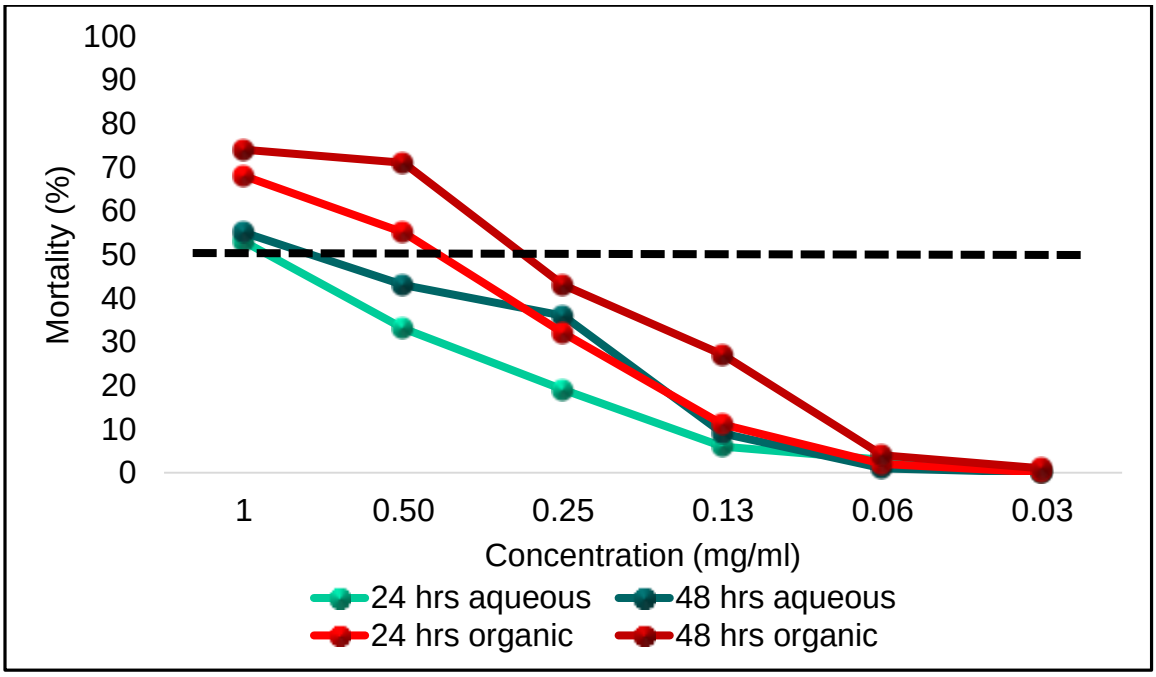

Figure 4.10 The dose-response of *A. delagoensis* root + *P. kaurabassana* root-tuber after 24 and 48 hrs of exposure in both aqueous and organic extracts. Jagged black line delineates toxicity (>50%) with non-toxicity (<50%).

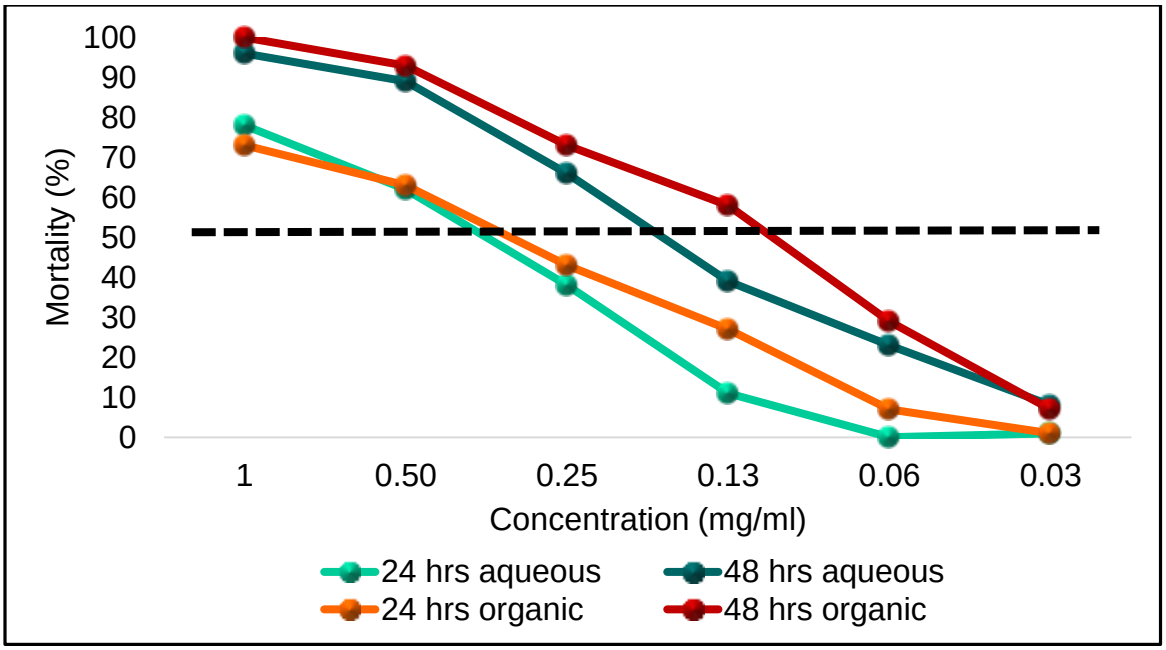

Figure 4.11 The dose-response of *D. cymosum* + *E. natalensis* roots after 24 and 48 hrs of exposure in both aqueous and organic extracts. Jagged black line delineates toxicity (>50%) with non-toxicity (<50%).

At the concentration of 1.00 mg/ml after 24 hrs the organic extract of *D. cymosum* + *E. natalensis* displayed 73% larvae mortality and non-toxicity at 0.28 mg/ml. After 48 hrs the combination killed 100% larvae at 1.00 mg/ml and was non-toxic at the concentration of 0.09 mg/ml.

Other dose-responses were also undertaken where toxicity was observed in more than one-time frame or more than one type of extract. The toxicity of *S. spinosa* + *C. inerme* (aqueous extract) demonstrated toxicity after 48 hrs exposure and was toxic at both 24 and 48 hrs with the organic extract. Similar to other combinations, this combination was mentioned once (Chapter 2). The traditional use of the combination is supported if the dose has a concentration of ≤ 0.50 mg/ml. The two plant species showed increased toxicity when combined since both *S. spinosa* and *C. inerme* extracts were non-toxic when evaluated individually. When a dose-response was evaluated of the aqueous extracts, after 24 hrs and 48 hrs in an aqueous solvent the combination was safe for use

at 0.5 mg/ml. The organic solvent extract showed that the combination was toxic at the concentration of 1.00 mg/ml and non-toxic at the concentration of 0.95 mg/ml and 0.48 mg/ml after 24 hrs and 48 hrs respectively (Figure 4.12).

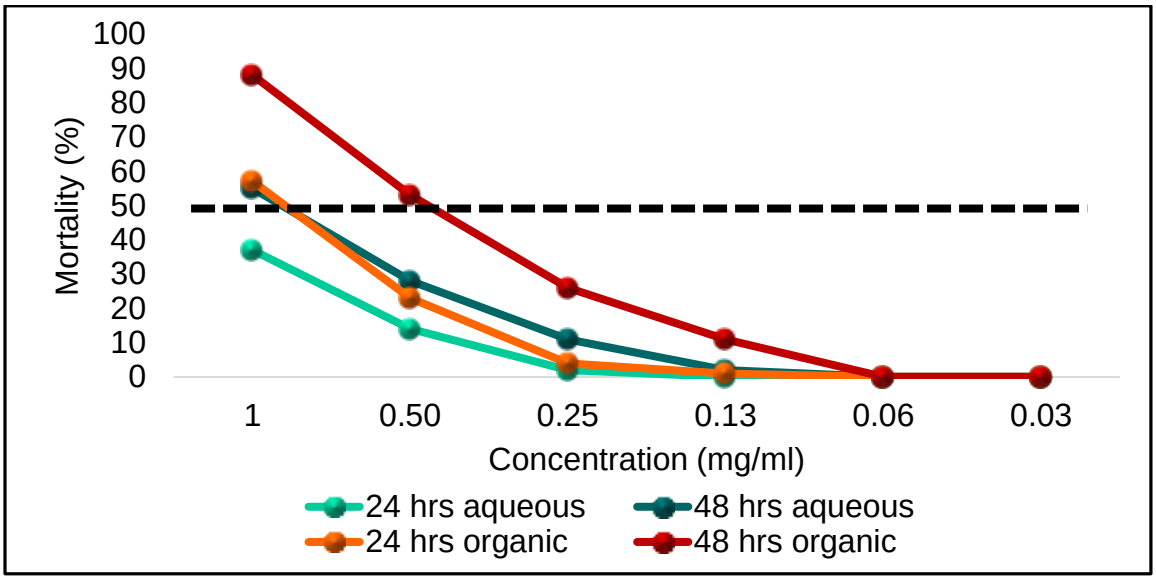

Figure 4.12 The dose-response of *S. spinosa* + *C. inerme* roots after 24 and 48 hrs of exposure in both aqueous and organic extracts. Jagged black line delineates toxicity (>50%) with non-toxicity (<50%).

Cissampelos hirta + *G. senegalensis* (roots) when tested in the aqueous combination was non-toxic after 24 hrs but toxic after 48 hrs. The organic extracts of this combination only showed toxicity after 48 hrs. The use of this combination was mentioned once in the study. When these two plant species were evaluated individually for toxicity they were safe for use. When examining the dose toxicity (Figure 4.13), the aqueous extract were non-toxicity at a concentration of 0.50 mg/ml. The organic extract was non-toxic at 0.50 mg/ml after 24 hrs and at 0.35 mg/ml after 48 hrs. At ≤ 0.50 mg/ml concentrations, the combination is considered safe for use.

For ease of comparison and to consolidate data a few other dose-responses were recorded for 24 hrs (Figure 4.14) and 48 hrs (Figure 4.15). *Krauseola mosambicina* + *P. guajava* + *M. azedarach* leaves had 56% and 97% mortality after 24 and 48 hrs respectively. This combination was mentioned once throughout the survey.

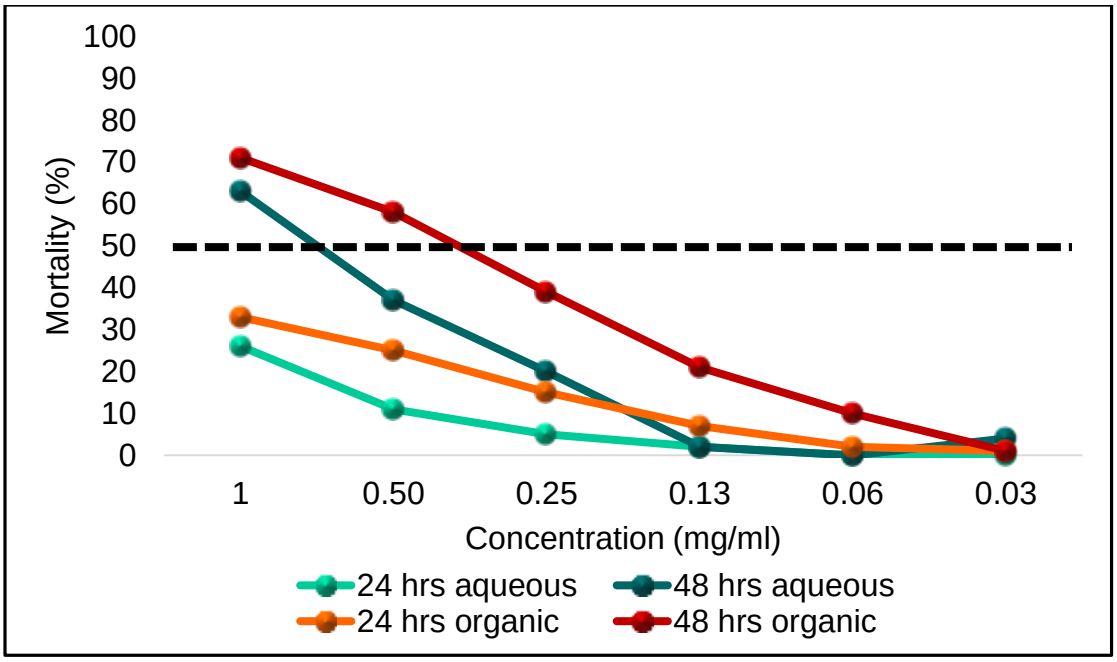

Figure 4.13 The dose-response of *C. hirta* + *G. senegalensis* roots after 24 and 48 hrs of exposure in both aqueous and organic extracts. Jagged black line delineates toxicity (>50%) with non-toxicity (<50%).

Krauseola mosambicina was the only plant in this combination of *K. mosambicina* + *P. guajava* + *M. azedarach* leaves that was toxic when evaluated individually and the toxicity had the pattern similar to that of the combination. The combination was toxic at the concentration of 1.00 mg/ml and had the non-toxicity that starts at 0.84 mg/ml after 24 hrs (Figure 4.14). After 48 hrs non-toxicity was first detected at the concentration of 0.23 mg/ml (Figure 4.15).

Ochna natalitia + *B. cathartica* roots showed 77% and 98% mortality at 24 and 48 hrs respectively. Similar to other combinations in this study, this combination was also mentioned once. When evaluated individually, toxicity was detected in *O. nataliatia* roots after 48 hrs in organic extract and *B. cathartica* was non-toxic. The results in the study showed that *O. natalitia* organic extract resulted in increased toxicity in the combination. At the concentration of 1.00 mg/ml, the *O. natalitia* + *B. cathartica* root combination was toxic, however, there was no toxicity at concentrations ≤0.55 mg/ml after 24 hrs

(Figure 4.14). After 48 hrs the combination showed non-toxicity at 0.18 mg/ml (Figure 4.15).

Ninety seven percent toxicity was detected with organic extract combination of the shoots of *R. multifidus* + *S. serratuloides* at 2.00 mg/ml. The use of this combination was mentioned once in the study. Individual evaluation of these two plant species showed that these plant species were non-toxic, therefore this combination induced toxicity. At the concentration of 1.00 mg/ml, the combination extract was toxic (93% mortality) and indicated non-toxicity at the concentration of 0.40 mg/ml (Figure 4.15).

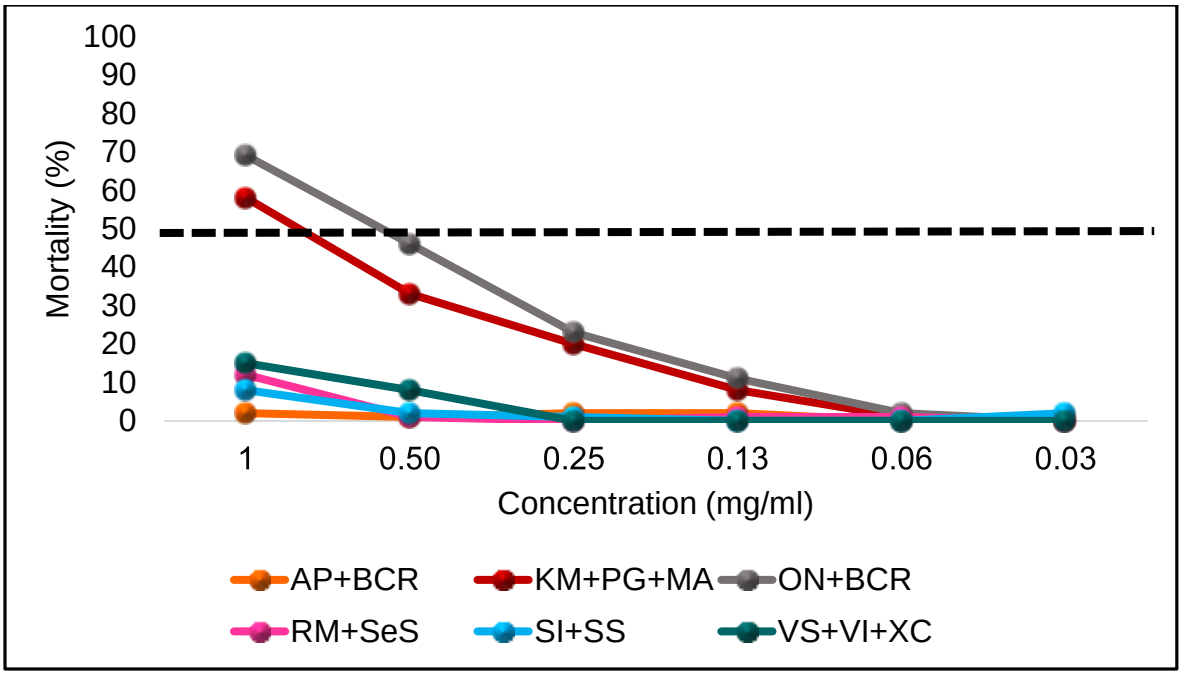

Figure 4.14 The dose-response for organic combination extracts after they had been exposed to larvae for 24 hrs. Jagged black line delineates toxicity (>50%) from non-toxicity (<50%). AP = *A. precatorius*; BCR = *B. cathartica* root; KM = *K. mosambicina*; MA = *M. azedarach*; ON = *O. natalitia*; PG = *P. guajava*; RM = *R. multifidus*; SI = *S. integerrimum*; SeS = *S. serratuloides*; SS = *S. spinosa*; VS = *V. sieberiana*; VI = *V. infausta*; XC = *X. caffra*.

The roots of *S. integerrimum* + *S. spinosa* were found to be toxic (97%) at the concentration of 2:00 mg/ml after 48 hrs in the organic extract. This combination had been

mentioned only once as a blood purifier. This combination increased in toxicity as individually *S. integerrumum* and *S. spinosa* were non-toxic. The lowest concentration considered non-toxic for the combination was detected at 0.55 mg/ml (Figure 4.15).

Toxicity (70%) for the *V. sieberiana* + *V. infausta* + *X. caffra* organic extract combination was observed only after 48 hrs. This combination was only mentioned once in the study. None of the plant species included in this combination was toxic when evaluated individually. After 24 hrs the extract was non-toxic at all concentrations evaluated in the study (Figure 4.14). After 48 hrs the extract was toxic at the concentration of 1.00 mg/ml and non-toxic at 0.50 mg/ml (Figure 4.15).

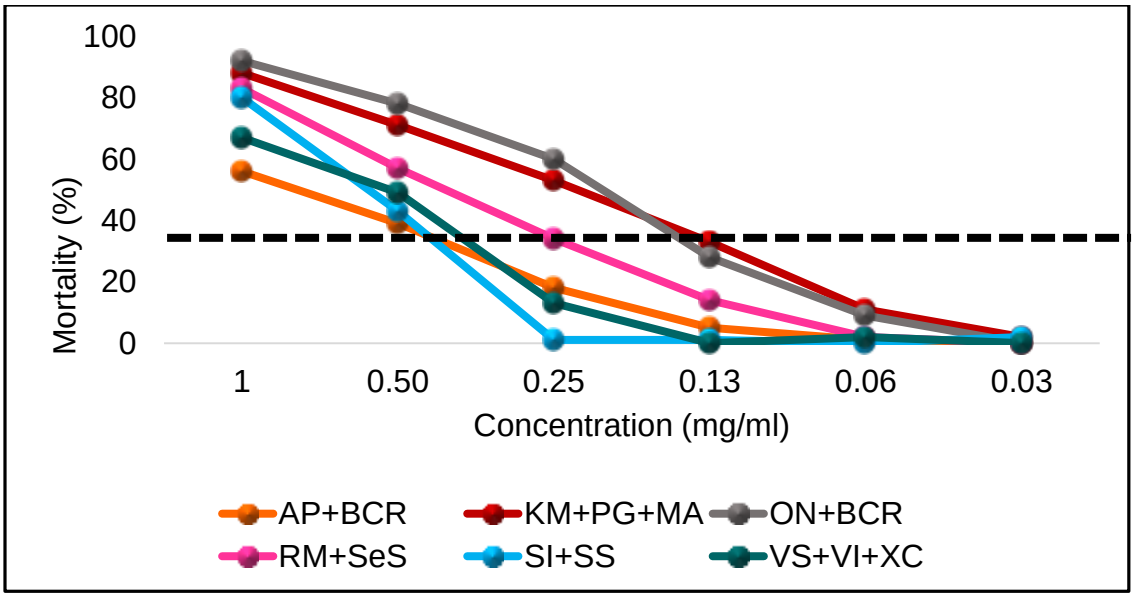

Figure 4.15 The dose-response for organic combination extracts after they had been exposed to larvae for 48 hrs. Jagged black line delineates toxicity (>50%) from non-toxicity (<50%). AP = *A. precatorius*; BCR = *B. cathartica* root; KM = *K. mosambicina*; MA = *M. azedarach*; ON = *O. natalitia*; PG = *P. guajava*; RM = *R. multifidus*; SI = *S. integerrimum*; SeS = *S. serratuloides*; SS = *S. spinosa*; VS =*V. sieberiana*; VI = *V. infausta*; XC = *X. caffra*.

There have been a number of studies conducted to test possible enhancement of antimicrobial activity by combination of southern African medicinal plant species (Mabona

et al., 2013; Zonyane *et al*. 2013; Nciki *et al*., 2016; Kudumela *et al*., 2018); however, the studies done to evaluate possible toxicity of these plant combinations are few (Sibandze *et al*., 2010; Naidoo *et al*., 2013, Ramulondi *et al*., 2019). Two other studies (Naidoo *et al*., 2013; Ramulondi *et al*., 2019) evaluated the plant combinations used in northern Maputaland and although no combination fully corresponded to the current study some combinations included plant species mentioned in the current study.

In a study by Naidoo *et al*. (2013), the *in vitro* MTT assay was used against human kidney epithelial (Graham, HEK-293) cells. Naidoo's study had 17 combinations that included some of the plant species which were used in combinations for the current study. Those plant species are highlighted in bold.

The combination of ***S. birrea*** + ***S. cordatum*** was highly cytotoxic in both aqueous and organic extracts and therefore other ratios were tested and found to be toxic. A combination of *B. pilosa* + ***R. multifidus*** + *Sarcophyte sanguinea* Forssk + *Clematis brachiata* Thunb was non-toxic against human kidney epithelial cells. ***Hypoxis hemerocallidea*** was included in eight combinations (*A. marlothii* + *H. hemerocallidea; C. papaya* + *S. serratuloides* + *H. hemerocallidea; Cassipourea malosana* (Baker) Alston + *H. hemerocallidea; Euphorbia hypericifolia* L. + *S. serratuloides* + *H. hemerocallidea* + *O. engleri; Kigelia africana* + *H. hemerocallidea; H. hemerocallidea* + *S. serratuloides* + *S. cordatum* + *S. birrea* + *A. marlothii; M. acuminata* + *S. serratuloides* + *H. hemerocallidea* and *Peltophorum africanum* Sond. + *S. serratuloides* + *H. hemerocallidea* + *K. africana*) which were all non-cytotoxic. Six combinations included ***S. serratuloides*** (*A. marlothii* + *S. serratuloides*; *C. papaya* + *S. serratuloides* + *H. hemerocallidea; E. hypericifolia* + *S. serratuloides* + *H. hemerocallidea* + *O. engleri; H. hemerocallidea* + *S. serratuloides* + *S. cordatum* + *S. birrea* + *A. marlothii; M. acuminata* + *S. serratuloides* + *H. hemerocallidea and Peltophorum africanum* Sond. + *S. serratuloides* + *H. hemerocallidea* + *K. africana*) and were non-cytotoxic. Another combination that included a plant species used in combination in the current study was ***X. caffra*** + *Tabernaemontana elegans* and was found to be non-cytotoxic in combination (Naidoo *et al*., 2013). In a study done by Ramulondi *et al*. (2019), 10 combinations

involved plant species that correspondingly were used in combinations of the current study (highlighted in bold). ***Hypoxis hemerocallidea*** was involved in five combinations (*A. marlothii* + *H. hemerocallidea; A. marlothii* + *H. hemerocallidea* + *M. balsamina; H. hemerocallidea* + *C. roseus; H. hemerocallidea* + *S. serratuloides* and *S. serratuloides* + *M. acuminata*+ *A. marlothii* + *H. hemerocallidea*) and only *H. hemerocallidea* + *C. roseus* extract was mutagenic in both TA 98 and TA 100 strains and toxic (66% shrimp mortality) in an aqueous extract with the increased concentration of 4.00 mg/ml. ***Senecio serratuloides*** was included in three combinations (*A. delagoensis* + *S. serratuloides*; *H. hemerocallidea* + *S. serratuloides* and *S. serratuloides* + *M. acuminata*+ *A. marlothii* + *H. hemerocallidea*) of which only *A. delagoensis* + *S. serratuloides* organic extract was toxic (64%) at 2.00 mg/ml and 100% and 57% respectively in both organic and aqueous solvents at 4.00 mg/ml when tested in the BLSA. ***Trichilia emetica*** + *A. marlothii* + *Hyphaene coriacea* Gaertn. extract was non-mutagenic and non-toxic at a concentration of 2.00 mg/ml, however, once the concentration increased to 4.00 mg/ml the combination was toxic in both organic (72%) and aqueous (50%) solvents (Ramulondi *et al*., 2019). The combination of ***Psidium guajava*** + *Carpobrotus dimidiatus* L. was non-toxic and non-mutagenic. Both the studies of Naidoo *et al*. (2013) and Ramulondi *et al*. (2019) demonstrated that the combination of *Aloe marlothii* + ***H hemerocallidea*** is safe for use. In a study that was conducted to determine the effect of *H. hemerocallidea* in a herb-drug combination with the transportation and regulation of drug metabolism it was reported that *H. hemerocallidea* extracts did not affect the pregnane X receptor (PXR) but inhibit the catalytic activity of cytochrome P450 enzymes (Owira and Ojewole, 2009; Fasinu *et al*., 2013; Haron *et al*., 2019). Three combinations (14%) in the current study included *H. hemerocallidea* corm. According to the results of the previous studies and the current study, *H. hemerocallidea* is safer when used in combination than when used individually.

Although in the literature there are no combinations to match those of the present study there were a few other combinations used for blood cleansing or similar effects that have been evaluated for toxicity. For instance, a case was reported in Nigeria where the 'Yoyo cleanser', a bitter blend of plant species (*Aloe vera* L., *Cinnamomum aromaticum* Nees, *Citrus aurantifolia* L., *Acinos arvensis* (Lam.) Dandy and *Chenopodium murale* L.) was

used as a blood cleanser and the patient that was regularly using it was unexpectedly admitted to the hospital and was later diagnosed with liver failure. Ekor (2014) evaluated the toxicity of this remedy and found that after 30 days of administration to rats the combination was detected to enhance levels of liver enzymes and hypokalaemia. Another study to test the toxicity of 'SuperB blood purifier' was also conducted. This herbal mixture was made from *Entandrophragma utile* Sprague and *Anocardium occidentalis* L. and when tested for acute toxicity the mixture had no potent toxicity, whereas when tested in chronic studies the mixture was distinguished to stimulate splenic enlargement and lung tumours (Ekor, 2014). The increased toxicity with plant combinations may be due to elevated toxic chemical components which create easy reactions with macromolecules like DNA and results in cell toxicity or genotoxicity (Ndhlala *et al*., 2011b). A mixture of *Cichorium intybus* L. seed, *Cassia occidentalis* hort. ex Steud. seed, whole plant of *Tamarix gallica* L., whole-plant of *Solanum nigrum* L., *Capparis spinosa* L. root, *Terminalia arjuna* (Roxb. ex DC.) Wight & Arn. bark and aerial parts of *Achillea millefolium* L. portrayed no toxic activities in patients when tested clinically (Mensah *et al*., 2019). Even though these plant species are not part of the current study, similarities are apparent with the genus *Solanum* and *Terminali*a for blood purification (Mensah *et al*., 2019). Also in Ghana, a well-known remedy concocted from a plant combination (*Anthocleista nobilis* G. Don, *Phyllanthus fraternus* G L Webster and *Vitex grandifolia* Gurke) was studied clinically for safety and efficiency and was found to be non-toxic when given to patients (Tetteh *et al*., 2017). Although the plant species tested in these studies are not similar to the plant species studied in the current study in northern Maputaland, these plant combinations were used specifically for blood cleansing. This shows that the use of plant combinations for blood purification is a common occurrence in Africa and that caution is warranted with selected combination therapy.

4.4 SUMMARY

- The toxicity of *K. mosambicina, O. engleri* and *R. digitata* was recorded and evaluated for the first time in the current study.
- The toxicity evaluation indicated that medicinal plant species prepared using water (9.3% toxicity) were much safer than using dichloromethane and methanol (22% toxicity).
- The roots of *D. cymosum, E. capensis, T. emetica* and *W. indica* were the most toxic plant extracts in this study with the mortality rate of 100%, 76%, 99% and 100% respectively with aqueous extractions after 24 hrs.
- The combinations of *A. delagoensis* + *P. kaurabassana* and *D. cymosum* + *E. natalensis* were the most toxic combinations with the mortality rates of 94% and 100% after 24 hrs in aqueous extraction. *Dichapetalum cymosum* and *E. natalensis* interaction remain very toxic even when diluted, whereas *A. delagoensis* and *P. kaurabassana* proved to be dosage dependant.
- *Prosopis cineraria* + *S. integerrimum* was the least toxic plant combination with ≤ 5% mortality.
- Six combinations (*A. precatorius* + *B. cathartica; C. hirta* + *G. senegalensis; R. multifidus* + *S. serratuloides; S. integerrimum* + *S. spinosa; S. spinosa* + *C. inerme; V. sieberiana* + *V. infausta* + *X. caffra*) showed increased toxicity when compared to the plant species tested individually.
- Thirty-six percent of plant combinations had reduced toxicity when compared to toxicity of the plant species tested independently.
- *Sclerocarya birrea* was the only plant species frequently used and detected as toxic.

CHAPTER 5

FINAL CONCLUSIONS AND RECOMMENDATIONS FOR FUTURE RESEARCH

5.1 ETHNOBOTANICAL SURVEY

The study addressed all the objectives that were specified in Chapter 1. The survey was focused on gathering indigenous knowledge of medicinal plant species used to purify blood. The first objective of this study was to collect information based on laypeople's perspective, and therefore, Traditional Health Practitioners were excluded. Fifty five people were interviewed and 65 plant species were documented for blood purification. For the second and third objectives, 54 specimens were successfully collected and identified and 58 plant parts were collected for preparing extracts. It was found that participants relate skin ailments, fever, abdominal infections, eye infections, kidney complaints and infertility to blood impurities. The importance of this component of the study signifies the documentation of 22 newly recorded plant species for the use of blood purification or related ailments. The most frequently mentioned plant species were: *B. cathartica, C. obovata, C. marginatus, S. birrea, S. serratuloides* and *T. sericea*. There were five vernacular plant names that were recorded for the first time by this study, namely; *isikwakwane (C. obovata), upata (G. caffra), umhlakuva (R. communis), umbungwa* (*L. kirkii*) and *uvemvane* (*W. indica*). The 32 different sets of plant combinations mentioned in the study were all recorded for the first time.

5.2 ANTIMICROBIAL VALIDATION OF THE PLANT SPECIES

The forth objective of the study was to investigate antimicrobial activity against various micro-organisms associated with blood infections. The study prioritised the aqueous extracts since it was the reported solvent used to prepare traditional remedies. However, the study also focused on organic extracts with the aim of obtaining the best potential activity from the plant species (there is a possibility to extract more active components

with organic solvents). The study recorded 84% antimicrobial active extracts (both moderate and noteworthy activity), where; 72% had pathogen-specific activity against *C. acnes*. The roots of *G. senegalensis* and *O. engleri* were the most antimicrobially active extracts against at least four pathogens, with a broad-spectrum activity ranging between 0.06 mg/ml – 0.50 mg/ml and 0.004 mg/ml – 0.50 mg/ml respectively. For the aqueous extract, only the roots of *A*. *delagoensis* demonstrated noteworthy effects. Four frequently mentioned plant species that were available for collection (*B. cathartica*, *S. birrea*, *S. serratuloides* and *T. sericea*) had shown some potential for antimicrobial activity.

For plant combinations, 20 extracts (6%) demonstrated synergistic effects against different pathogens with *A. baumannii* being the most susceptible pathogen. There were also 49 plant extracts (16%) that had additive effects against the test pathogens in this study. The broadest spectrum activity was observed for the combination *A. precatorius* + *B. cathartica* (roots). The combination displayed synergy against *C. acnes* (ΣFIC value of 0.13), *L. monocytogenes* (ΣFIC value of 0.40), *S. aureus* (ΣFIC value of 0.31), *A. baumannii* (ΣFIC value of 0.38) and *P. aeruginosa* (ΣFIC value of 0.50). The most antimicrobially effective interaction was between the roots of *S. spinosa* + *C. inerme*, demonstrating noteworthy activity (MIC value of 0.09 mg/ml) and the ΣFIC value of 0.09 in the aqueous extract.

5.3 THE TOXICITY OF BLOOD PURIFIER PLANT SPECIES

The fifth objective of the study was to use the BSLA to assess the toxicity of plant species collected. There were a total of 12 plant extracts that demonstrated toxicity in the study (five aqueous and 12 organic extracts). When analysing the toxicity of the most frequently used plant species (*B. cathartica, S. birrea, S. serratuloides* and *T. sericea*), the BSLA results exhibited that three of the four plant species were non-toxic at the concentration tested. However, toxicity of *S. birrea* was observed at both 24 hrs (63% mortality) and 48 hrs (91% mortality). This was evident for the dichloromethane: methanol extract, but the aqueous extracts which the participants used was found to be non-toxic. Where plant species were found to be toxic, reduced concentrations (1.00, 0.50, 0.25, 0.13, 0.63 and

0.03 mg/ml) were tested and the results displayed that toxicity decreased with the decrease in concentrations. Except for the most toxic plant species (*D. cymosum* and *W. indica*), the majority of plant extracts displayed non-toxic effects at and lower than 0.13 mg/ml.

Twenty-two different sets of plant combinations were evaluated for toxicity in equal ratios of 1:1, 1:1:1 or 1:1:1:1 depending on the number of plant species involved in a combination. The overall toxicity for aqueous extracts was 18% and that of organic extracts was 45%. When toxic combinations were investigated at varied lower concentrations, the results displayed that toxicity decreased with lower concentrations, as observed with the individual plant species tested. The toxicity of the combinations depended on the solvent used (aqueous extracts were less toxic compared to the dichloromethane: methanol extracts); time of exposure (the larvae that were subjected into extracts for 24 hrs had a higher survival rate than the larvae that was subjected on the extracts for 48 hrs) and dosage (the combinations that were toxic at the initial concentration of 2.00 mg/ml displayed decreased toxicity when the concentrations were lowered to 1.00, 0.50, 0.25, 0.13, 0.63 and 0.03 mg/ml). The majority of combinations displayed non-toxicity at concentrations lower than 1.00 mg/ml.

5.4 THE CORRELATION BETWEEN ANTIMICROBIAL ACTIVITY AND TOXICITY OF INDIVIDUAL PLANT SPECIES AND COMBINATIONS

The last objective of the study was to compare the performance between individual plant species and plant species combinations. The combinations had shown increased activity of aqueous extracts. During the antimicrobial activity of individual plant species and only the roots of *A. delagoensis* had shown noteworthy potential whilst the combinations demonstrated that 10 aqueous extracts had synergistic potential.

The combinations displayed mostly reduced toxicity when compared to studies of plant species when evaluated individually. The combinations of *A. precatorius* + *B. cathartica* roots, *C. hirta* + *G. senegalensis* roots, *S. spinosa* + *C. inerme* roots and *V. sieberiana* +

V. infausta + *X. caffra* roots were shown to have increased toxicity compared with the individual plant species within the mixture. The study found eight combinations (*B. cathartica* + *R. digitata* roots, *E. natalensis* roots + *S. birrea* bark, *G. livingstonei* + *O. natalitia* + *V. infausta* roots, *H. natalensis* + *R. digitata* roots, *H. hemerocallidea* corm + *S. serratuloides* shoots, *S. puniceus* bulb + *H. hemerocallidea* corm + *R. multifidus* shoots, *V. sieberiana* + *S. birrea* bark and *X. caffra* roots + *H. hemerocallidea* corm + *S. puniceus* bulb + *R. multifidus* shoots) to have reduced toxicity and five (*B. cathartica* + *R. digitata* roots, *E. natalensis* roots + *S. birrea* bark, *H. natalensis* + *R. digitata* roots, *H. hemerocallidea* corm + *S. serratuloides* shoots and *X. caffra* roots + *H. hemerocallidea* corm + *S. puniceus* bulb + *R. multifidus* shoots) acted synergistically.

5.5 THE CORRELATION OF TRADITIONAL USE, ANTIMICROBIAL ACTIVITY AND TOXICITY OF PLANT SPECIES USED INDIVIDUALLY FOR BLOOD PURIFICATION

When blood purifier plant species were tested against bacteria related to blood infection, some exhibited excellent activity and safety. The activity of these plant species was then correlated with reported traditional use to determine if there was a link between traditional use and associated infection (Table 5.1). The study found that 72% of plant extracts were active against *C. acnes* (33% noteworthy and 39% moderate activity) (Table 5.1). From the literature review and interviews conducted, the study found that skin disorders (especially acne) are often linked to blood purification, therefore in this instance there was a strong correlation of antimicrobial activity with traditional use. For example, *S. integerrimum* was reported to treat acne and out of seven pathogens, it only had the best noteworthy activity against *C. acnes* (0.13 mg/ml), a pathogen responsible for acne. The MIC results also displayed that the three frequently used plant species (*S. birrea, S. serratuloides* and *T. sericea* that were available for collection displayed either moderate (≤ 0.65 mg/ml) or noteworthy (≤ 0.16 mg/ml) activity against *C. acnes*. The results of study showed that 74% of the antimicrobial activity correlated to the reported traditional used of plant species.

The 19 plant extracts that exhibited some notable antimicrobial activity were analysed with the toxicity and frequency of use. Seventy-nine percent of the extracts that had noteworthy activity against pathogens had no evidence of toxicity when tested in the BSLA. The potential toxicity of *E. capensis* was very alarming.

Ekebergia capensis was highly toxic for both aqueous and dichloromethane: methanol extracts after both 24 and 48 hrs. Although the organic extracts displayed potential toxicity, the aqueous extracts of *A*. *delagoensis* and *O. engleri* were non-toxic. It is important to note that these plant extracts were non-toxic at the lower concentration tested. *Albertsia delagoensis* organic extract was non-toxic at 0.03 mg/ml. *Ekebergia capensis* aqueous extract was non-toxic at 0.52 and 0.25 mg/ml after 24 and 48 hrs, respectively. The organic extract was non-toxic at 0.20 and 0.16 mg/ml after 24 and 48 hrs, respectively. The organic extract of *O. engleri* displayed non-toxicity at 0.30 mg/ml. *Sclerocarya birrea* was non-toxic at 0.80 mg/ml for the aqueous, and 0.13 mg/ml for organic extract respectively.

Ozoria engleri was the most antimicrobially active plant extract (0.004 mg/ml against *C. acnes*) and had the broadest spectrum of activity. After 48 hrs of exposure to 2.00 mg/ml organic extract, the plant was potentially toxic, however, after dilutions, this extract was not toxic at a lower concentration of 0.30 mg/ml which is higher than the MIC value. In other words the plant can be used at a lower concentration which is not toxic and still be effective as an antimicrobial. Similarly, when evaluating *A. delagoensis, E. capensis* and *S. birrea,* lower concentrations would be effective in reducing toxicity and retaining an inhibitory response antimicrobially. The extracts of *T. emetica* roots and *W. indica* roots were highly toxic for both aqueous and organic extracts, yet they both displayed poor activity against all pathogens in the study. The results suggest that the extended use of these plant species for disorders associated with bacterial blood infections, especially against pathogens tested in the current study, should be discouraged.

Table 5.1 The frequency, activity and toxicity of plant species used individually in the study as blood purifiers

Plant species	Times mentioned (%)	Extract	Antimicrobial activity summary	Toxicity
A. precatorius R	4	Aqueous	Poor activity	Non-toxic
		DCM: MeOH	Noteworthy against *C. acnes*; Moderate against *L. monocytogenes, S. aureus* and *S. agalactiae*.	Non-toxic
A. glabratum Wp	5	Aqueous	Poor activity	Non-toxic
		DCM: MeOH	Moderate activity of *C. acnes*	Non-toxic
A. delagoensis R	5	Aqueous	Noteworthy against *C. acnes*; Moderate against *L. monocytogenes.*	Non-toxic
		DCM: MeOH	Noteworthy against *C. acnes*; Moderate against *L. monocytogenes, S. aureus.*	Toxic (at 48 hrs)
B. pilosa R	2	Aqueous	Poor activity	Non-toxic
		DCM: MeOH	Moderate against *C. acnes.*	Non-toxic
B. discolour Sh	5	Aqueous	Poor activity	Non-toxic
		DCM: MeOH	Moderate against *P. aeruginosa*.	Non-toxic
B. cathartica L	25	Aqueous	Poor activity	Non-toxic
		DCM: MeOH	Poor activity	Non-toxic
B. cathartica R	11	Aqueous	Poor activity	Non-toxic
		DCM: MeOH	Moderate against *P. aeruginosa*.	Non-toxic
C. inerme R	5	Aqueous	Poor activity	Non-toxic
		DCM: MeOH	Noteworthy against *C. acnes*; Moderate against *L. monocytogenes* and *P. aeruginosa*.	Non-toxic
C. roseus R	5	Aqueous	Poor activity	Non-toxic
		DCM: MeOH	Moderate against *C. acnes*.	Non-toxic
C. hirta R	2	Aqueous	Moderate against *C. acnes.*	Non-toxic
		DCM: MeOH	Noteworthy against *C. acnes*.	Non-toxic
C. anisata L	4	Aqueous	Poor activity	Non-toxic
		DCM: MeOH	Moderate against *C. acnes*.	Non-toxic
C. molle L	2	Aqueous	Moderate against *C. acnes*.	Non-toxic
		DCM: MeOH	Noteworthy against *C. acnes*; Moderate against *P. aeruginosa*.	Non-toxic

Plant species	Times mentioned (%)	Extract	Antimicrobial activity summary	Toxicity
C. molle R	11	Aqueous	Moderate against *C. acnes*.	Non-toxic
		DCM: MeOH	Noteworthy against *C. acnes*; Moderate against *S. pyogenes, P. aeruginosa*.	Non-toxic
D. cymosum R	2	Aqueous	Poor activity	Toxic
		DCM: MeOH	Moderate against *P. aeruginosa.*	Toxic
E. capensis B	2	Aqueous	Poor activity	Toxic
		DCM: MeOH	Noteworthy against *C. acnes*; Moderate against *P. aeruginosa*.	Toxic
E. natalensis R	5	Aqueous	Poor activity	Non-toxic
		DCM: MeOH	Moderate against *C. acnes, L. monocytogenes, S. aureus, S. agalactiae, S. pyogenes* and *P. aeruginosa.*	Non-toxic
G. livingstonei B	5	Aqueous	Moderate against *C. acnes*.	Non-toxic
		DCM: MeOH	Moderate against *C. acnes, S. aureus* and *P. aeruginosa.*	Non-toxic
G. volkensii R	2	Aqueous	Poor activity	Toxic (at 48 hrs)
		DCM: MeOH	Moderate against *P. aeruginosa.*	Toxic (at 48 hrs)
G. caffra R	2	Aqueous	Poor activity	Non-toxic
		DCM: MeOH	Poor activity	Non-toxic
G. senegalensis L	4	Aqueous	Poor activity	Non-toxic
		DCM: MeOH	Moderate against *P. aeruginosa*.	Non-toxic
G. senegalensis R	4	Aqueous	Poor activity	Non-toxic
		DCM: MeOH	Noteworthy against *C. acnes, L. monocytogenes, S. aureus* and *S. agalactiae*; Moderate against *S. pyogenes* and *P. aeruginosa*.	Non-toxic
H. pauciflorus T	2	Aqueous	Poor activity	Non-toxic
		DCM: MeOH	Moderate against *C. acnes* and *P. aeruginosa*.	Non-toxic
H. abyssinica Rh	4	Aqueous	Poor activity	Non-toxic
		DCM: MeOH	Poor activity	Non-toxic
H. natalensis R	4	Aqueous	Poor activity	Non-toxic
		DCM: MeOH	Moderate against *C. acnes* and *P. aeruginosa*.	Non-toxic

Plant species	Times mentioned (%)	Extract	Antimicrobial activity summary	Toxicity
H. hemerocallidea C	5	Aqueous	Moderate *S. pyogenes*.	Non-toxic
		DCM: MeOH	Moderate *S. pyogenes*.	Toxic (at 48 hrs)
K. africana B	13	Aqueous	Poor activity	Non-toxic
		DCM: MeOH	Poor activity	Non-toxic
K. mosambicina Sh	11	Aqueous	Poor activity	Non-toxic
		DCM: MeOH	Poor activity	Toxic
L. javanica L	4	Aqueous	Poor activity	Non-toxic
		DCM: MeOH	Moderate against *C. acnes* and *P. aeruginosa.*	Non-toxic
M. azedarach L	2	Aqueous	Poor activity	Non-toxic
		DCM: MeOH	Noteworthy against *C. acnes*; Moderate against *L. monocytogenes, S. aureus* and *P. aeruginosa.*	Non-toxic
M. balsamina L	7	Aqueous	Poor activity	Non-toxic
		DCM: MeOH	Moderate against *C. acnes* and *P. aeruginosa*.	Non-toxic
O. natalitia R	4	Aqueous	Poor activity	Non-toxic
		DCM: MeOH	Moderate against *C. acnes* and *P. aeruginosa*.	Toxic (at 48 hrs)
O. serrulata R	5	Aqueous	Poor activity	Non-toxic
		DCM: MeOH	Moderate against *C. acnes, L. monocytogenes, S. aureus* and *S. pyogenes*.	Non-toxic
O. engleri R	2	Aqueous	Moderate against C. acnes.	Non-toxic
		DCM: MeOH	Noteworthy against *C. acnes, L. monocytogenes, S. aureus, S. agalactiae* and *S. pyogenes*. Moderate against *A. baumannii* and *P. aeruginosa.*	Toxic (48 hrs)
P. americana Sd	2	Aqueous	Poor activity	Non-toxic
		DCM: MeOH	Noteworthy against *C. acnes*; Moderate against *S. aureus*.	Non-toxic
P. cineraria R	7	Aqueous	Poor activity	Non-toxic
		DCM: MeOH	Noteworthy against *C. acnes*.	Non-toxic
P. guajava L	4	Aqueous	Moderate against *C. acnes*.	Non-toxic
		DCM: MeOH	Noteworthy against *C. acnes*; Moderate against *P. aeruginosa*.	Non-toxic

Plant species	Times mentioned (%)	Extract	Antimicrobial activity summary	Toxicity
P. kaurabassana Rt	4	Aqueous	Poor activity	Non-toxic
		DCM: MeOH	Moderate against *C. acnes*.	Non-toxic
P. scandens T	9	Aqueous	Poor activity	Non-toxic
		DCM: MeOH	Moderate against *C. acnes*.	Non-toxic
R. multifidus Sh	7	Aqueous	Moderate against *C. acnes*.	Non-toxic
		DCM: MeOH	Moderate against *C. acnes* and *S. aureus*.	Non-toxic
R. digitata R	5	Aqueous	Poor activity	Non-toxic
		DCM: MeOH	Moderate against *C. acnes*.	Toxic (at 48 hrs)
R. communis L	5	Aqueous	Poor activity	Non-toxic
		DCM: MeOH	Poor activity	Non-toxic
S. integerrimum R	7	Aqueous	Poor activity	Non-toxic
		DCM: MeOH	Noteworthy against *C. acnes*.	Non-toxic
S. puniceus Bb	2	Aqueous	Poor activity	Non-toxic
		DCM: MeOH	Moderate against *P. aeruginosa*.	Non-toxic
S. brachypetala R	7	Aqueous	Poor activity	Non-toxic
		DCM: MeOH	Moderate against *C. acnes*.	Non-toxic
S. birrea B	18	Aqueous	Moderate against *C. acnes*.	Non-toxic
		DCM: MeOH	Noteworthy against *C. acnes*; Moderate against *S. aureus* and *P. aeruginosa*.	Toxic
S. serratuloides Sh	18	Aqueous	Moderate against *C. acnes*.	Non-toxic
		DCM: MeOH	Moderate against *C. acnes* and *L. monocytogenes*.	Non-toxic
S. spinosa R	7	Aqueous	Poor activity	Non-toxic
		DCM: MeOH	Poor activity	Non-toxic
S. cordatum B	4	Aqueous	Moderate against *C. acnes*.	Non-toxic
		DCM: MeOH	Noteworthy against *C. acnes*.	Non-toxic
T. elegans F	2	Aqueous	Poor activity	Non-toxic
		DCM: MeOH	Moderate against *C. acnes*.	Non-toxic
T. sericea R	40	Aqueous	Moderate against C. acnes.	Non-toxic
		DCM: MeOH	Noteworthy against *C. acnes* and *P. aeruginosa* Moderate against *L. monocytogenes*.	Non-toxic
T. emetica B	7	Aqueous	Poor activity	Non-toxic

Plant species	Times mentioned (%)	Extract	Antimicrobial activity summary	Toxicity
		DCM: MeOH	Poor activity	Non-toxic
T. emetica R	2	Aqueous	Poor activity	Toxic
		DCM: MeOH	Moderate against *C. acnes*.	Toxic
V. sieberiana B	7	Aqueous	Poor activity	Non-toxic
		DCM: MeOH	Moderate *C. acnes*.	Non-toxic
V. xanthophloea B	4	Aqueous	Poor activity	Non-toxic
		DCM: MeOH	Moderate against *C. acnes*.	Non-toxic
V. infausta R	11	Aqueous	Poor activity	Non-toxic
		DCM: MeOH	Moderate against *C. acnes*.	Non-toxic
W. indica R	2	Aqueous	Poor activity	Toxic
		DCM: MeOH	Poor activity	Toxic
W. somnifera Wp	15	Aqueous	Poor activity	Non-toxic
		DCM: MeOH	Noteworthy against *C. acnes*, Moderate against *L. monocytogenes, S. aureus* and *S. agalactiae*.	Non-toxic
X. caffra R	7	Aqueous	Poor activity	Non-toxic
		DCM: MeOH	Noteworthy against *C. acnes*.	Non-toxic

B = bark, Bb = bulb, C = corm, F = fruit, L = leaves, R = roots, Rt = root tuber, Sd = seeds, Sh = shoots, T = twigs, Wp = whole plant; DCM: MeOH = dichloromethane: methanol.

5.6 THE CORRELATION OF TRADITIONAL USE, ANTIMICROBIAL ACTIVITY AND TOXICITY OF PLANT SPECIES USED IN COMBINATIONS

It is important to note that all combinations recorded and evaluated in this study were only mentioned once. For the 22 combinations tested, 13 of them had a synergistic effect against the bacterial pathogens (Table 5.2). The results showed that 62% of the combinations were non-toxic.

The combination of *D. cymosum* + *E. natalensis* root extracts demonstrated synergy when tested against *C. acnes* and had the highest potential toxicity. The use of *D*. *cymosum* + *E. natalensis* combination is discouraged for use because even though it had one synergistic interaction, the combination was highly toxic with or without dilutions. The use of *A. precatorius* + *B. cathartica* root combination was showing the most synergistic activity against *C. acnes, L. monocytogenes, S. aureus, A. baumannii* and *P. aeruginosa*. It was noted that *B. carthartica* had poor activity when used individually, hence this is a good example of a combination of increasing efficacy of the plant. This combination displayed potential toxicity only after 48 hrs of exposure in organic extract. The combination of *A. precatorius* + *B. cathartica* root displayed an increased toxicity compared to when these plant species were used individually. However, the study did show that this combination is non-toxic at concentrations below 0.50 mg/ml. The combination of *V. sieberiana* bark + *V. infausta* root + *X. caffra* root also displayed the most potential synergistic effects against *C. acnes, A. baumannii* and *P. aeruginosa* and had no potential toxicity.

Table 5.2 The overall performance of plant species used in combinations to purify blood

Plant species	Times mentioned (%)	Extract	Antimicrobial activity	Toxicity
A. precatorius R + *B. cathartica* R	2	Aqueous	Synergistic against *C. acnes*; Additive against *S. aureus*	Non-toxic
		DCM: MeOH	Synergistic against *L. monocytogenes, S. aureus, A. baumannii* and *P. aeruginosa*.	Toxic (at 48 hrs)
A. delagoensis R + *P. kaurabassana* Rt	2	Aqueous	Additive against *L. monocytogenes*.	Toxic
		DCM: MeOH	Additive against *S. aureus.*	Toxic
B. cathartica R + *R. digitata* R	2	Aqueous	Synergistic against *C. acnes*.	Non-toxic
		DCM: MeOH	Additive against *C. acnes, L. monocytogenes* and *S. pyogenes*	Non-toxic
C. hirta R + *G. senegalensis* R	2	Aqueous	Antagonistic and non-interactive effects.	Toxic (at 48 hrs)
		DCM: MeOH	Additive against *C. acnes* and *A. baumannii*.	Toxic (at 48 hrs)
C. molle R + *T. sericea* R	2	Aqueous	Antagonistic and non-interactive effects.	Non-toxic
		DCM: MeOH	Additive against *S. aureus*.	Non-toxic
D. cymosum R + *E. natalensis* R	2	Aqueous	Synergistic against *C. acnes*; Additive against *S. pyogenes*.	Toxic
		DCM: MeOH	Additive against *A. baumannii* and *P. aeruginosa.*	Toxic
E. natalensis R + *S. birrea* B	2	Aqueous	Antagonistic and non-interactive effects	Non-toxic
		DCM: MeOH	Synergistic against *A. baumannii*.	Non-toxic
G. livingstonei R + *O. natalitia* R + *V. infausta* R	2	Aqueous	Antagonistic and non-interactive effects.	Non-toxic
		DCM: MeOH	Additive against *S. agalactiae*.	Non-toxic
H. natalensis R + *R. digitata* R	2	Aqueous	Synergistic against *S. agalactiae*; Additive against *S. pyogenes*.	Non-toxic
		DCM: MeOH	Additive against *C. acnes* and *S. agalactiae*.	Non-toxic
H. hemerocallidea C + *S. serratuloides* Sh	2	Aqueous	Synergistic against *A. baumannii*.	Non-toxic
		DCM: MeOH	Synergistic against *P. aeruginosa*.	Non-toxic
K. mosambicina L + *P. guajava* L + *M. azedarach* L	2	Aqueous	Antagonistic and non-interactive effects.	Non-toxic
		DCM: MeOH	Additive against *S. agalactiae* and *A. baumannii*.	Toxic
O. natalitia R + *B. cathartica* R	2	Aqueous	Synergistic against *C. acnes*.	Non-toxic
		DCM: MeOH	Additive against *S. pyogenes*.	Toxic
P. cineraria R + *S. integerrimum* R	2	Aqueous	Additive against *L. monocytogenes* and *A. baumannii*.	Non-toxic
		DCM: MeOH	Synergistic against *A. baumannii;* Additive against *S. agalactiae.*	Non-toxic

Plant species	Times mentioned (%)	Extract	Antimicrobial activity	Toxicity
P. cineraria R + *S. spinosa* R + *V. infausta* R	2	Aqueous	Antagonistic and non-interactive effects.	Non-toxic
		DCM: MeOH	Additive effects against *L. monocytogenes, S. aureus, S. agalactiae, S. pyogenes, A. baumannii* and *P. aeruginosa*.	Non-toxic
R. multifidus Sh + *S. serratuloides* Sh	2	Aqueous	Additive against *C. acnes*.	Non-toxic
		DCM: MeOH	Additive against *S. pyogenes* and *P. aeruginosa.*	Toxic (at 48 hrs)
S. integerrimum R + *S. spinosa* R	2	Aqueous	Antagonistic and non-interactive effects.	Non-toxic
		DCM: MeOH	Synergistic against *P. aeruginosa*. Additive against *S. aureus, S. agalactiae* and *A. baumannii*.	Toxic (at 48 hrs)
S. puniceus Bb + *H. hemerocallidea* C + *R. multifidus* Sh	2	Aqueous	Additive against *S. agalactiae.*	Non-toxic
		DCM: MeOH	Antagonistic and non-interactive effects.	Non-toxic
S. spinosa R + *C. inerme* R	2	Aqueous	Synergistic against *C. acnes*. Additive against *S. agalactiae*.	Toxic (at 48 hrs)
		DCM: MeOH	Additive against *S. agalactiae*.	Toxic
T. sericea R + *S. cordatum* B	2	Aqueous	Synergistic against *C. acnes*.	Non-toxic
		DCM: MeOH	Additive against *S. aureus* and *S. agalactiae*	Non-toxic
V. sieberiana B + *S. birrea* B	2	Aqueous	Additive against *A. baumannii*	Non-toxic
		DCM: MeOH	Additive against *A. baumannii*.	Non-toxic
V. sieberiana B + *V. infausta* R + *X. caffra* R	2	Aqueous	Synergistic against *C. acnes*; Additive against *S. pyogenes* and *A. baumannii*.	Non-toxic
		DCM: MeOH	Synergistic against *A. baumannii* and *P. aeruginosa.*	Toxic (at 48 hrs)
X. caffra R + *H. hemerocallidea* C + *S. puniceus* Bb + *R. multifidus* Sh	2	Aqueous	Synergistic against *A. baumannii;* Additive against *C. acnes.*	Non-toxic
		DCM: MeOH	Additive against *C. acnes, S. agalactiae* and *S. pyogenes*.	Non-toxic

B = bark, Bb = bulb, C = corm, R = roots, Rt = root tuber, Sh = shoots, T = twigs; DCM: MeOH = dichloromethane: methanol.

5.7 CONCLUSION

According to the ethnobotanical survey, blood purification is used as a systemic treatment for various illnesses such as skin problems, problems associated with the urinary tract, abdominal infections and as immune system stimulators. The antimicrobial effect of some plant species against the selected bacterial pathogens justify their traditional use for blood cleansing and as possible anti-infectives. Out of 19 plant extracts that showed noteworthy activity, only four plant species demonstrated toxicity in the BSLA. However, the study also highlighted the importance of preparing some remedies in low concentrations since they were toxic at higher concentrations. To conduct this study, severals hypotheses were formulated to bring advanced methods to which the study implemented to approach the problem statement. Six formulated hypotheses: Various medicinal plant species are used in northern Maputaland for blood purification to which; several of them had unpublished vernacular names; thus the plant species mentioned would have an excellent antimicrobial activity; and be safe for use. The last two hypotheses predicted that plant combinations will result in increased antimicrobial activity of some plant species and reduced toxicity to others. The results of the current study had supported all six hypotheses proposed. While the use of blood purifiers remains a largely unexplored area, this study provides some context as to the possible link between traditional use and efficacy as a blood purifier. There is no doubt that when examining African traditional medicine, holistic terms such as “blood cleansing” cannot be ignored and providing some scientific rational may forge a link between western and African medical systems.

5.8 RECOMMENDATIONS FOR FUTURE STUDIES

Most of the plant species that had noteworthy activity owe their active performance to the study’s choice of using dichloromethane: methanol extracts. Future studies could explore other solvent extractions to maximise number of compounds for biological testing. This may increase the chances for other phytochemical groups in plant species to be extracted as well. The study revealed that the use of blood purification plant species in rural areas

is often linked to participant's use for stomach-ache, urinary tract infections, infertility, kidney complaints and ringworm. This study focused on *A. baumannii, C. acnes, L. monocytogenes, P. aeruginosa, S. agalactiae, S. aureus and S. pyogenes*. Therefore, further studies could be broadened to evaluate other pathogens such as *Enterococcus faecalis*, *Escherichia coli*, *Klebsiella pneumonia, Salmonella gastroenteritis* and *Streptococcus faecalis* as well as fungi and yeast pathogens (Flores-Mireles *et al*., 2015; Ruggeri *et al*., 2016). Immunomodulators are known to sustain health by counteracting pathogens or enhancing body immunity. These are similar to the uses reported by participants for blood purifiers. Hence the immunomodulatory effects of these plant species should be considered in future studies, especially with the extracts like *B. cathartica* which was frequently mentioned as a splendid blood purifier, but exhibited weak antimicrobial activities when tested. Similarly, some remedies had been reported as tonics. Therefore, studies should look into the biological and nutritional potential of these plant species as tonics. The study also recommends that anti-biofilm effects of pathogens should be evaluated as antimicrobial effects herein were only investigated on planktonic cells and antibiofilm activity may also be apparent. It is also important to note that the study focused on *in vitro* assays, hence more in-depth *in vivo* investigations could be done in future to assess the performance of blood purifier plant species within a living model.

As a systemic treatment, the follow-up study should consider evaluating other biological activities. The plant species reported in the study are blood purifiers and most of them were reported for fatigue and skin complaints. In this instance, it is no surprise that some plant species displayed poor antimicrobial activity. Goode (1993), reported that the free radicals could lead to sepsis, therefore future studies could evaluate the anti-oxidant activity of some of these plant species. Furthermore there have been reports that there is a high correlation between blood coagulation and some blood infections (e.g. sepsis) hence the possibility of these plant species to act as anticoagulants is a distinct possibility (Allen *et al*., 2015; Meziani *et al*., 2017).

For plant combinations, it is recommended that future research analyses the interaction of plant species at different ratios. This analysis could also help to identify where one plant is having a more dominant effect than the other in the mixture (Van Vuuren and Viljoen, 2011b). The combinations of *A. precatorius* + *B. cathartica* roots and *V. sieberiana* + *V. infausta* + *X. caffra* roots were the most synergistic combinations in the study. A varied ratio analysis could be done on this combination to determine if different concentrations can be used to maximise the effect without compromising on toxicity. Where combinations were involved the bioactive compounds isolated could be combined to investigate their interactions. The interaction of these compounds could inhibit or enhance their antimicrobial effect (Van Vuuren and Viljoen, 2011b). Phytochemical screening is important for predicting and/or supporting the biological activity of plant species, therefore isolating compounds could lead to possible drug leads (Al Habsi and Hossain, 2018).

The study noted that most of the interviewees are fully aware of the plant's adverse effects, and those affects are desired to flush out the toxins in the system (for example through vomit). For future research, it is recommended that the plant species that projected potential toxicity (*A. delagoensis, D. cymosum, E. natalensis, G. volkensii, H. hemerocallidea, K. mosambicina, O. natalitia, O. engleri, R. digitata, S. birrea, T. emetica* and *W. indica*) in the present study should be investigated further for other toxicological effects such as haematotoxicity, mutagenicity, dermal toxicity, hepatotoxicity, cardiotoxicity, neurotoxicity and cytotoxicity. It is important to monitor that the plant species used for blood purification do not cause damage to blood cells or other blood associated organs whilst excreting toxins (Bloom, 1993). Some of these plant species may work as liver stimulators to assist in improving blood purification; hence it is important also to investigate their safety against the liver (hepatotoxicity).

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APPENDICES

APPENDIX A: Abstract for oral presentation at the Science Symposium, 2019.

An ethnobotanical survey of plant species used for blood purification by rural communities in northern Maputaland, South Africa.

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Abstract

There is a lack of in-depth ethnobotanical studies done on medicinal plant species that are used for blood purification. The aim of this study is to document the information of laypeople on the plant species that they use as blood purifiers. The study seeks how much knowledge do laymen have about blood impurities, symptoms and causes of blood impurities as well as plant species used as blood purifiers. A total of 55 participants residing in Mbazwana, Mseleni and Ntshongwe were interviewed depending on whether they know any plant species used for blood purification. All information discovered during the survey including plant vernacular names, plant parts used, how remedies are prepared and administered was recorded for documentation. Voucher specimen were collected for each plant to help with scientific identification and for documenting purposes. Sixty nine plant species were recorded to be used individually for blood purification and 32 plant combinations were recorded to be used as blood purifiers. The mostly mentioned blood purifier plant species were *Terminalia sericea* (x25), *Bridelia cathartica* (x18), *Cymbopogon marginatus* (x15) and *Catunaregam obovata* (x12). According to survey the common symptoms for blood impurities are skin disorders, dysmenorrhea, infertility and

constant bad luck. Most blood purification remedies are prepared as decoctions and taken orally prior to breakfast. The study discovered five new vernacular names and 22 plant species that were recorded for the first time to be used for blood purification. Blood purifies are used as both treatments and preventives. The wide variety of plant species used for blood purification is the proof that people in the northern Maputaland value traditional medicine for primary health care. It is important that the traditional knowledge of plant species is documented as preservative measure for future generations.

KEYWORDS: Blood purifier plant species, Indigenous plant knowledge, Northern Maputaland, Laypeople, Ethnobotany

APPENDIX B: Abstract for oral presentation at the SAAB annual conference, 2020.

An ethnobotanical survey and toxicity study of plant species used for blood purification by rural communities in northern Maputaland, South Africa.

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Blood purification practice, is an ancient concept which is widespread amongst African traditional medicine, but for which no modern scientific basis exists. The aim of this study was to document and evaluate the toxicity of medicinal plant species used as blood purifiers in northern Maputaland. A total of 55 laypeople with knowledge on blood purifier plant species were interviewed using structured questionnaire. Sixty-nine plant species were documented which were used singularly (in decoctions), or in combinations (with 25 other plant species) for blood purification. Plant species such as *Bridelia carthartica* G.Bertol., *Catunaregam obovata* (Hochst.) A.E.Gonç., *Cymbopogon marginatus* Stapf ex Burtt Davy, *Sclerocarya birrea* Hochst., *Senecio serratuloides* DC. and *Terminalia sericea* Burch. ex DC. were the most frequently used. Twenty-two plant species were recorded for the first time globally as blood purifiers. Amongst the forty plant families documented, Apocynaceae, Asteraceae, Euphorbiaceae, Leguminoseae, Rubiaceae and Solanaceae were dominant. The brine shrimp lethality assay was performed to evaluate the toxicity of the plant species. Five aqueous extracts [from *Dichapetalum cymosum* bark (> 65% mortality), *Gardenia volkensii* roots (> 95% mortality), *Trichilia emetica* roots (100% mortality) and *Waltheria indica* whole plant (100% mortality)] and 10 organic plant extracts (Dichloromethane: methanol, 1:1) showed toxicity both after 24 and 48 hours. Most blood purification remedies are decoctions and taken orally prior to breakfast. Combinations such as the roots of *T. sericea* with *Combretum molle* are infused in warm water and taken as enema for internal body cleansing. The wide variety of plant species used for

blood purification stress the importance to scientifically evaluate these plant species for its efficacy and toxicity.

APPENDIX C: Ethical clearance

UNIVERSITY OF ZULULAND
RESEARCH ETHICS COMMITTEE
(Reg No: UZREC 171110-030)

RESEARCH & INNOVATION
Website: http://www.unizulu.ac.za
Private Bag X1001
KwaDlangezwa 3886
Tel: 035 902 6731
Fax: 035 902 6222
Email: luedallK@unizulu.ac.za

ETHICAL CLEARANCE CERTIFICATE

Certificate Number	**UZREC 171110-030 PGM 2018/609**							
Project Title	**AN ETHNOBOTANICAL AND ETHNOPHARMACOLOGICAL STUDY OF PLANTS USED FOR BLOOD PURIFICATION BY RURAL COMMUNITIES IN NORTHERN MAPUTALAND, SOUTH AFRICA**							
Principal Researcher/ Investigator	NS Zwane							
Supervisor and Co-supervisor	Prof H De Wet		Prof SF Van Vuuren					
Department	Botany							
Faculty	Science and Agriculture							
Type of Risk	Low Risk – Data collection – desktop, fieldwork or laboratory							
Nature of Project	Honours/4th Year		Master's	x	Doctoral		Departmental	

The University of Zululand's Research Ethics Committee (UZREC) hereby gives ethical approval in respect of the undertakings contained in the above-mentioned project. The Researcher may therefore commence with data collection as from the date of this Certificate, using the certificate number indicated above.

Special conditions:

(1) This certificate is valid for 1 year from the date of issue.
(2) Principal researcher must provide an annual report to the UZREC in the prescribed format [due date-18 June 2021]
(3) Principal researcher must submit a report at the end of project in respect of ethical compliance.
(4) The UZREC must be informed immediately of any material change in the conditions or undertakings mentioned in the documents that were presented to the meeting.

The UZREC wishes the researcher well in conducting research.

Professor Mashupye R. Kgaphola
University Research Ethics Committee
Deputy Vice-Chancellor: Research & Innovation

22 July 2020

CHAIRPERSON
UNIVERSITY OF ZULULAND RESEARCH
ETHICS COMMITTEE (UZREC)
REG NO: UZREC 171110-30
22-07-2020
RESEARCH & INNOVATION OFFICE

APPENDIX D: Questionnaire used for survey interviews.

RESEARCH QUESTIONNAIRE

Date: _____ Questionnaire No.: _____

Socio-demographic Information

Name of participant: _____

Age: _____ Gender: _____

Educational background: _____ Religion: _____

Particulars of Area

Name of Area: _____ Name of village:

_____

GPS readings: _____

Plant Information

1. Do you know what blood purification is? Are any symptoms related to blood purification? Do you take anything to clean blood (Epson salt, laxatives, blood cleanser products)?

_____

_____

_____

_____

_____

2. Which plant(s) are used for blood cleansing, if any known?

Vernacular name (Zulu)	Scientific name

3. Can these plant species be used to children?

_____

4. Do you take anything for blood thinning?

_____

5. Plant source:

Growing in the wild	Cultivated home	Buy

Other: _____

6. Plant part used:

_____

7. Preparation method:

_____

_____

_____

_____

_____

_____

_____

_____

_____

8. Administration and dosage

8.1 Method of administration (oral, snuff, enema):

_____

_____

8.2 Amount taken per dosage (half a mug, tip of a finger/spoon):

_____

9. How often do you take the medication (1x per day) and for how long (for a week)?

_____

_____

10. Does the plant(s) have any side effects? If yes, what are they?

_____

_____

_____

_____

_____

11. How do you know the plant species are effective?

_____

_____

12. Is the plant used as a preventive/ treatment?

_____

13. Is it safe to use the plant when taking prescribed western medication? If no, why?

_____

14. Are there other ailments the plant is recommended for? If yes name them.

_____

_____

_____

15. Is the plant used in combination with any other plant(s)? If yes, name them and the method of preparation

_____

_____

_____

_____

16. Where did you acquire the knowledge you have about the plant?

_____

17. Do you transfer this information to other people, children perhaps?

_____

_____

18. Any other information about plant or blood purification?

_____

_____

_____

19. Do you prefer clinic or medicinal plant species for blood cleansing? Why?

_____

_____

APPENDIX E: Consent form signed by participants of the study.

INFORMED CONSENT DECLARATION

PARTICIPANT'S DECLARATION

PROJECT TITLE: An ethnobotanical and ethnopharmacological study of plant species used for blood purification by a rural communities in northern Maputaland (South Africa).

Nontobeko Zwane from the Department of Botany, University of Zululand have requested permission to participate in the above-mentioned research project.

The nature and the purpose of the research project and of this informed consent declaration have been explained to me in a language that I understand.

I am aware that:

1. The purpose of the research project is to document information on plant species use to purify/cleanse/detoxify blood.
2. The University of Zululand has given ethical clearance to this project and I have seen or may request to see the clearance certificate.
3. By participating on this research project I will be contributing towards the documentation of these traditional and plant species used by the people in northern KwaZulu-Natal.
4. I will participate in this research project by allowing the researcher to ask me questions and I will give them accurate information concerning the uses of traditions and plant species asked about.
5. My participation is entirely voluntary and should I at any stage wish to withdraw from participating; I may do so without any negative consequences.
6. I will not be paid any salary for participating in this research project, but will accept a token of appreciation from the interviewer, which can be a bag of 2 kg rice and/or a packet of rooibos tea.

7. No risks are associated with my participation in this research project.
8. The researchers intend to publish the research results in the form of a research paper. However, privacy and anonymity of records will be maintained and my name and identity will not be exposed to anyone who has not been involved in the conduct of the research.
9. Any further questions that I might have concerning the research or my participation will be answered by Prof. H de Wet at the University of Zululand, Department of Botany (035 902 6108 / e-mail: deweth@unizulu.ac.za or nontobekoshokahle@gmail.com).
10. By signing this informed consent declaration I am not waiving any legal claims, rights or remedies.
11. A copy of this informed consent declaration will be given to me and the original will be kept on record.

I ---------- have read the above information and everything was clearly explained to me in a language that I understand and I am aware of this document's contents. I fully understand what is expected of me during the research.

I have not been forced in any way and I have freely agreed to participate in the above-mentioned project.

---------- ----------

Participant's signature Date

RESEARCHERS' DECLARATION

I Nontobeko Zwane declare that:

- I explained the information on this document to ----------
- Requested him/her to ask questions if anything was unclear and we have answered them to the best of our ability.

- I am pleased that he/she sufficiently understands all aspects of the research so as to make an informed decision on whether to participate or not.
- The discussion took place in isiZulu or English.
- I interpret the questionnaire.

---------- ----------

Researchers' signature Date