A toxicological evaluation and anti-*Candidal* activity of plants used by women in northern Maputaland (South Africa) for the treatment of gynaecological and obstetrics ailments

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200903145

FACULTY OF SCIENCE, AGRICULTURE AND ENGINEERING

In fulfilment of the requirements for the degree

MSc. Botany

In the Department of Botany

University of Zululand

Supervisor: Prof. H. de Wet

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2021

DECLARATION

I, Samukelisiwe Clerance Ngubane declares that this dissertation entitled **"A toxicological evaluation and anti-*Candidal* activity of plants used by women in northern maputaland (South Africa) for the treatment of gynaecological and obstetrics complaints"** is my own work. The research documented in this dissertation was conducted in the departments of Botany (University of Zululand) and Pharmacy and Pharmacology (University of Witwatersrand) under the supervision of Prof. H. de Wet and Prof. S.F. van Vuuren. The study has not been previously submitted in any form for any degree or examination at this or any other University. I have to best of my knowledge have complied with the University's Plagiarism Policy and acknowledged all the sources of information used in this study.

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Samukelisiwe Clerance Ngubane

ABSTRACT

Medicinal plants still play an important role in the primary healthcare of lay people in northern Maputaland in spite of the availability of hospitals and clinics. According to an ethnobotanical survey conducted in 2014, the lay people in northern Maputaland use plant species independently and in combination to treat gynaecology and obstetrics medical conditions. These plant species were generally regarded as safe by the lay people except for one plant species, *Trichilia dregeana.* Consequently, this study's aim was to investigate the safety of medicinal plant species used by the lay people in northern Maputaland. Furthermore, as these plant species were used to treat medical conditions specifically related to woman, the inclusion of the efficacy of these plant species against *Candida* stains was included due to the prevalence of vaginal thrush.

The aqueous and organic (1:1 methanol-dichloromethane) extracts were prepared from 51 plant samples (including leaf samples collected for potential substitution for the roots). Toxicology of these plants was assessed using the brine shrimp lethality assay (BSLA) and the Ames assay (using *Salmonella typhimurium* TA98 and TA100 strains) for mutagenicity. The anti-Candidal activity was assessed using the antimicrobial micro-dilution assay to determine the minimum inhibitory concentration of of the plant samples aginst *Candida albicans* ATCC 10231*, C. tropicalis* ATCC 750 and *C. glabrata* ATCC 900300.

There were three plant spescies (*Acalypha villicaulis* root*, Grewia occidentalis* root and *Gymnosporia senegalensis* leaves) that indicated neither toxicity nor mutagenicity in this study. All the toxic plants samples (in BSLA) were further subjected to two-fold dilution and demonstrated acceptable toxic concentrations, which were found to range from 0.98 to 0.10 mg/ml. However, *Hermannia boraginiflora, Sapium integerrimum, Scadoxus puniceus and Tabernaemontana elegans* remained toxic even after diluted to the lowest concentration of 0.031 mg/ml.

Plant species combinations that were found to be non-toxic in BSLA in both aqueous and organic extract were *Euphobia tirucalli* (root) + *Ozoroa engleri* (bark) + *Scadoxus puniceus* (bulb) + *Senecio serratuloides* (whole plant), *Bridelia cathartica* (root) +

Opuntia stricta (stem) + *Searsia nebulosa* (bark) and *B. cathartica* (root) + *Erythrina humeana* (root).

In the Ames test, plant samples that appeared to be non-mutagenic against both *S. typhimurium* TA98 and TA100 strains were *A. villicaulis* root*, Cyperus natalensis* root, *Euclea natalensis* leaves, *G. occidentalis* root*, Ochna natalitia* leaves*, S. integerrimum* leaves *and S. puniceus* bulb. However, *Hypoxis hemerocallidea* and *O. stricta* appeared to be the most mutagenic against both the *S. typhimurium* TA98 and TA100 strains with both aqueous and organic extracts showing mutagenicity.

The antimicrobial microdilution assay indicated a small number of plant species that were active against *Candida* strains and were in most cases these were the methanol-dichloromethane extracts. A moderate activity against *C. albicans* was observed with the aqueous extract of *Euclea natalensis* root and *Rhoicissus digitata* leaves. The methanol-dichloromethane extracs of O*. stricta* stem, *P. africanum* root and *S. birrea* stem were also active (moderately) against *C. albicans*. Against the *C. tropicalis,* a moderate activity was observed against *A. villicaulis* leaves, *Acanthospermum glabratum* whole plants, *B. cathartica* leaves, *Cassytha filiformis* whole plant, *Euphorbia tirucalli* stem and *Garcinia livingstonei* root*.* A noteworthy anti-*Candidal* activity was observed with *Commiphora neglecta* root and leaves both with the minimum inhibitory concentration (MIC) of 0.13 mg/ml against *Candida tropicalis*. There was no activity observed against *C. glabrata.*

This study has indicated that medicinal plant species may have toxic and/ or mutagenic effects, even without any noteworthy signs after consumption. However, it was determined that toxicity can be reduced by carefully managing the dose. The reduction of concentration is not known whether it may affect the efficacy, therefore further studies on the efficacy are recommended.

CONFERENCE PRESENTATIONS

1. **De Wet H**., Ngubane S.C. Traditional herbal remedies used by women in a rural community in South Africa for the treatment of gynaecological and obstetrics complaints. 14th International Congress of Ethnopharmacology (ISE) Hotel Patagonico, Puerto Varas, Chile, 23–26 September 2014 (Appendix A)

2. **Ngubane S.C**., De Wet H., Van Vuuren S.F. Ethnobotany and mutagenicity of some medicinal plants used by women in northern Maputaland, to treat gynaecology and obstetrics complaints. 46th Annual conference of the South African Association of Botanists, Department of Plant Sciences, University of the Free State, Qwaqwa, 07–10 January 2020 (Appendix B).

PUBLICATION

De Wet H., Ngubane S.C., 2014. Traditional herbal remedies used by women in a rural community in northern Maputaland (South Africa) for the treatment of gynaecology and obstetrics complaints. South African Journal of Botany 94, 129–139.
(Appendix C)

ACKNOWLEDGEMENTS

- I would like to express my deepest gratitude to my supervisor Prof H. de Wet for her invaluable advice, comments, follow-up and patience from the beginning to the completion of this work. No words can be able to express my appreciation towards you.
- Secondly, I would like to extend my sincere thanks to my co-supervisor Prof S.F. van Vuuren for her guidance, input and kindness throughout this study. Without you this project could not have been carried out.
- My gratitude also goes to the University of Zululand Research Committee for their financial support to carry out this project. Without your support this study would not have been possible.
- I also like to thank University of Zululand Human Resources for sponsoring my postgraduate study.
- To the staff of the department of Botany, thank you so much for your time, help and kindness during the frustration times.
- To the staff and students at University of Witwatersrand (Department of Pharmacy and Pharmacology), particularly Mrs Phumzile Madondo, thank you for the laboratory assistance.
- I would also like to thank all the participants in northern Maputaland who offered their time and share their valuable information with me and for their hospitality.
- I would also like to acknowledge my family, particularly my mother and daughter Ziyanda who give me the reasons to want to do better every day.
- Above all, I thank God for giving me the opportunity, wisdom and the strength to carry out this research

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ABBREVIATIONS

AC	Aqueous combination
ATC	Acceptable toxic concentration
BSLA	Brine shrimp lethality assay
CCL4	Carbon tetrachloride
CFUs	Colony forming units
DCM	Dichloromethane
ºC	Degrees celsius
DMSO	Dimethylsulfoxide
DNA	Deoxyribonucleic acid
FIC	Fractional inhibitory concentration
g	Grams
g/kg	Grams per kilograms
hrs	Hours
HPV	Human papillomavirus
IDP	Integrated development plan
INT	Iodonitrotetrazolium chloride
L	Litre
LC_{50}	Median lethal concentration
MeOH	Methanol
mg/kg	Milligram per kilogram
mg/L	Milligram per litre
MIC	Minimum inhibitory concentration
min	Minutes
MTT	3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide
4NQO	4-Nitroquinoline 1-oxide
N/A	Not available
ND	Not determined
OC	Organic combination
%	Percentage
PH	Potential of hydrogen
µg/L	Microgram per litre

µg/ml	Microgram per millilitre
µl	Microliter
µM	Micromole
RNA	Ribonucleic acid
Spp.	Species
TSA	Tryptone Soya agar
TSB	Tryptone Soya broth
50X VB salts	Vogel-Bonner medium salts
V/V	Volume per volume
VVC	Volvo-vaginal candidiasis
W/W	Weight per weight
ΣFIC	Sum of the fractional inhibitory concentrations

CHAPTER 1
Introduction to the study

1.1 Background of the study

This study is a follow up of an ethnobotanical survey that was conducted by De Wet and Ngubane (2014) where medicinal plant species used for gynaecological and obstetric conditions were investigated (50 interviews) and documented. According to De Wet and Ngubane (2014), the plant species were used mostly by women in their reproductive age i.e. from menarche (first menstrual cycle/period) to menopause (end of menstrual cycle/period). Women can experience gynaecological problems at any point in their lifetime, especially in their reproductive ages. Obstetric problems are prevalent at child-bearing age, during a pregnancy term. According to Abam (2015), gynaecological conditions can result in infertility, chronic diseases and may also be fatal. The prevalence of gynaecological and obstetric problems can be further increased by sexual activity, when women experience vaginal tract infections such as human papillomavirus (HPV) and candidiasis (Abam, 2015; Gao et al., 2021).

The women in northern Maputaland understand and associate gynaecological conditions to vaginal warts, fibroids and menstrual problems, which are mostly detected by pain and bleeding abnormalities. They associate obstetrics problem to conditions such as infertility, miscarriage, antepartum haemorrhage, preterm birth, delayed labour, insufficient milk production and postpartum haemorrhage.

1.1.1 Medicinal plant species use for gynaecology and obstetric conditions in Maputaland

De Wet and Ngubane (2014) documented (Table 1.1) 36 traditionally used medicinal plant species for the treatment of gynaecological and obstetric conditions by the lay women in northern Maputaland. The most frequently reported plant species for various women conditions was *Bridelia cathartica* G.Bertol. (mentioned by 36% of the participants), followed by *Ranunculus multifidus* Forssk. (by 26% of the participants). *Bridelia cathartica* was reported for conditions that includes dysmenorrhoea, infertility, amenorrhoea, menorrhagia, oligomenorrhoea, to prevent premature birth and blood

cleansing. *Ranunculus multifidus* was reported to work against genital warts and to suppress the swelling of limbs during pregnancy. Although the current study focuses on medicinal plant species that are used by mostly women in their reproductive age (between menarche to menopause), these plant species are also used for other health conditions in northern Maputaland such as diarrhoea (De Wet et al., 2010), respiratory infections (York et al., 2012), sexually transmitted infections (De Wet et al., 2012), skin disorders (De Wet et al., 2013) and hypertension (De Wet et al., 2016). These studies signify the importance of these medicinal plant species for the healthcare of the lay people of northern Maputaland.

Table 1.1 Medicinal plant species used for gynaecological and obstetric conditions (De Wet and Ngubane, 2014)

Plant names	**Part used**	**Number of times mentioned (Total = 50)**	**Ailments treated**
Acalypha villicaulis Hochst. ex A.Rich.	Roots	3	Dysmenorrhoea, blood cleansing and delivery
Acanthospermum glabratum (DC.) Wild	Whole plant	1	Cervical pain
Bridelia cathartica G.Bertol.	Roots	18	Dysmenorrhoea, infertility, amenorrhoea, menorrhagia, oligomenorrhoea, prevent premature birth and blood cleansing
Cassytha filiformis L.	Whole plant	1	Induce lactation
Commiphora neglecta I.Verd.	Roots	1	Dysmenorrhoea, infertility, menorrhagia, oligomenorrhoea,

Plant names	Part used	Number of times mentioned (Total = 50)	Ailments treated
			prevent premature birth and blood cleansing
Crotalaria monteiroi Taub. ex Baker f. var. *galpinii* Burtt Davy	Roots	1	Dysmenorrhoea, infertility, menorrhagia, oligomenorrhoea, prevent premature birth and blood cleansing
Cyperus natalensis Hochst. ex. Krauss	Roots	1	Menorrhagia
Diospyros villosa (L.) De Winter	Roots	1	Dysmenorrhoea
Erythrina humeana Spreng	Roots	4	Dysmenorrhoea, infertility, fibroids, menorrhagia and miscarriage
Euclea natalensis A.DC.	Roots	1	Blood cleansing
Euphorbia tirucalli L.	Stem	1	Genital warts
Fleuggea virosa (Wild.) Voigt	Modified roof	1	After-birth pain and preterm birth
Garcinia livingstonei T.Anderson	Roots	2	Dysmenorrhoea and post-partum haemorrhage
Grewia occidentalis L.	Roots	1	Dysmenorrhoea, infertility, menorrhagia, oligomenorrhoea, blood cleansing and prevent premature birth

Plant names	Part used	Number of times mentioned (Total = 50)	Ailments treated
Gymnosporia senegalensis (Lam.) Loes.	Roots	3	Prevent infantile colic when the baby is born and infertility
Hermannia boraginiflora Hook.	Roots	1	Dysmenorrhoea and labour pains
Hyphaene coriacea Gaertn.	Stem	7	Dysmenorrhoea, infertility, after-birth pain, post-partum haemorrhage and labour pains
Hypoxis* cf. *longifolia Baker	Corm	3	Warts, menorrhagia and blood cleansing
Hypoxis hemerocallidea Fisch., C.A.Mey. & Avé-Lall.	Corm	6	Cervical pain, infertility, genital warts and menorrhagia
Kigelia africana (Lam.) Benth.	Bark	3	Cervical pain, blood cleansing, induce lactation and dysmenorrhoea
Ochna natalitia Walp.	Roots	8	Dysmenorrhoea, infertility, menorrhagia, oligomenorrhoea, prevent premature birth, labour pains and blood cleansing
Opuntia stricta Haw.	Stem	3	Cervical pain and blood cleansing
Ozoroa engleri R.Fern & A.Fern.	Bark	3	Dysmenorrhoea, after-birth pain, infertility,

Plant names	Part used	Number of times mentioned (Total = 50)	Ailments treated
			menorrhagia, oligomenorhoea, prevent premature birth, genital warts and blood cleansing
Peltophorum africanum Sond.	Roots	3	Dysmenorrhoea, amenorrhoea and blood cleansing
Ranunculus multifidus Forssk.	Whole plant	13	Genital warts and swelling during pregnancy
Rhoicissus digitata (L.f.) Gilg & M.Brandt	Roots	3	Dysmenorrhoea, infertility, menorrhagia, oligomenorrhia, prevent premature birth and blood cleansing
Sapium integerrimum (Hochst. ex. Krauss) J.Léonard	Roots	1	Dysmenorrhoea
Scadoxus puniceus (L.) Friis Norda	Bulb	1	Blood cleansing
Sclerocarya birrea (A. Rich.) Hochst.	Bark	1	Abortion
Searsia nebulosa (Schoenland) Moffett forma *nebulosa*	Bark	3	Dysmenorrhoea, infertility and genital warts
Senecio deltoideus Less	Whole plant	1	Warts, infertility and pelvic pain

Plant names	Part used	Number of times mentioned (Total = 50)	Ailments treated
Senecio serratuloides DC.	Whole plant	9	Cervical pain, infertility, blood cleansing and genital warts
Senegalia burkei (Benth.) Kyal. & Boatwr. (= *Acacia burkei* Benth.)	Bark	1	Blood cleansing
Tabernaemontana elegans Stapf	Roots	1	Dysmenorrhoea and infertility
Trichilia dregeana Harv. & Sond.	Roots	8	Abortion
	Leaves	1	Labour pains
	Bark	1	Infertility
Vangueria infausta Burch. subsp. *Infausta*	Leaves	1	Menorrhagia and ante-partum haemorrhage

Bold – No plant material was collected; hence it was excluded in the current study.

1.1.2 The use of medicinal plants for pregnancy-related conditions

The use of medicinal plant species during pregnancy is common, especially in Africa (Nergard et al., 2015). Women rely on medicinal plant species to induce or facilitate labour, prevent preterm birth or miscarriage, or as a preventative health tonic during pregnancy (Steenkamp, 2003; Malan and Nauba, 2011; Nergard et al., 2015). In spite of the availability of medical healthcare facilities in northern Maputaland, the women in this area still believe in the use of medicinal plants during pregnancy especially as a health tonic for blood cleansing. The women in northern Maputaland believe that the uncleansed blood during pregnancy could result in pregnancy complications including complicated labour and delivery (De Wet and Ngubane, 2014).

1.1.3 The use of medicinal plants for gynaecological conditions

According to De Wet and Ngubane (2014), the women also rely on medicinal plants for conditions associated with menstruation i.e. dysmenorrhoea, amenorrhoea, menorrhagia and oligomenorrhoea. Some of the women believed that traditional medicine is the only medicine that is effective to cure dysmenorrhoea, while allopathic medicine only temporarily supresses the pain.

1.1.4 Plant species combinations used for gynaecology and obstetric conditions in Maputaland

Some of the plant species in Maputaland were used in combination by the women to treat gynaecological and obstetric conditions. In the study conducted by De Wet and Ngubane (2014), 17 plant species combinations were documented (Table 1.2). Plant species combinations are believed to be more effective and also to reduce toxicity (Van Vuuren and Viljoen, 2011; Mundy et al., 2016). It is evident in South Africa that lay people, and other medicinal plant users know the importance of combining medicinal plants for increased therapeutic efficacy (Varga and Veale, 1997; Van Vuuren and Viljoen, 2008; York et al., 2012; De Wet and Ngubane, 2014). For example, *Isihlambezo* is an herbal mixture prepared from a combination of medicinal plant species and is widely used in KwaZulu-Natal province as a health tonic during pregnancy to promote a good term of pregnancy and facilitate easy labour and delivery (Varga and Veale, 1997). According to Naidoo et al. (2013), in some cases, plant species combinations may exhibit a greater potential for toxicity. *Syzygium cordatum* Krauss and *S. birrea* are used in northern Maputaland to treat sexually transmitted infections (De Wet et al., 2012) and it is documented that both contain potentially toxic metabolites (gallic acid and ellagic acid) (Ojewole, 2003). Cytotoxicity of the two metabolites is enhanced when these plant species are used in combination (Naidoo et al., 2013). Therefore, these results emphasise the importance of assessing safety of plant species combinations.

In the ethnobotanical study done by De Wet and Mngubane (2014), none of the 17 plant species combinations have been previously assessed for safety or anti-*Candidal* activity. Although there has been a toxicological study on plant species combinations

used for hypertension in northern Maputaland (Ramulondi et al., 2019), the plant species combinations were different from those examined in the current study.

1.1.5 Relevance of the study

There is a vast number of medicinal plant species that have been documented in this Northern Maputaland, but the toxicological aspect of these plant species is poorly explored. Most recently, Ramulondi et al., (2018) documented toxicity and mutagenicity of some medicinal plant species used to treat hypertension in this area. In that study, some of the plant species were toxic, indicating that medicinal plants can be toxic even though the lay people often refer to them as safe based on the long-term use without any noticeable poisonous effect.

Table 1.2 Plant species combinations used in Maputaland to treat gynaecological and obstetric conditions (De Wet and Ngubane, 2014)

Plant species combinations	**Number of times mentioned (Total = 50)**	**Ailments treated**
B. cathartica (roots) + *C. neglecta* (roots) + *C. monteiroi* (root) + *G. livingstonei* (root) + *G. occidentalis* (root) + *O. natalitia* (root) + *R. digitata* (root)	1	Dysmenorrhoea, menorrhagia, amenorrhoea, oligomenorrhoea, infertility, prevent premature birth and blood cleansing.
E. tirucalli (root) + *O. engleri* (bark) + *S. puniceus* (bulb) + *S. serratuloides* (whole plant)	1	Blood cleansing
B. cathartica (root) + *E. humeana* (root) + *O. natalitia* (root) + *T. elegans* (root) + *S. nebulosa* (bark)	1	Dysmenorrhoea and infertility

Plant species combinations	Number of times mentioned (Total = 50)	Ailments treated
A. villicaulis (root) + *B. cathartica* (root) + *S. nebulosa* (bark)	1	Dysmenorrhoea and infertility
B. cathartica (root) + *P. africanum* (root) + *R. digitata* (root)	2	Dysmenorrhoea and amenorrhoea
B. cathartica (root) + *H. coriacea* (stem) + *O. engleri* (bark)	6	Dysmenorrhoea and infertility, after-birth pain, postpartum haemorrhage and labour
R. multifidus (whole plant) + *S. serratuloides* (whole plant)	5	Warts
B. cathartica (root) + *O. stricta* (stem) + *S. nebulosa* (bark)	3	Blood cleansing
R. multifidus (whole plant) + *H. hemerocallidea* (corm)	2	Blood cleansing and warts
K. africana (bark) + *C. filiformis* (whole plant)	1	After-birth pain
E. humeana (root) + *O. natalitia* (root)	1	Menorrhagia,
B. cathartica (root) + *E. humeana* (root)	1	Menorrhagia
Senecio deltoideus (whole plant) + *Senecio serratuloides* (whole plant)	1	Ease labour
G. senegalensis (root) + *H. hemerocallidea* (corm)	3	Infantile colic
K. africana (bark) + *S. nebulosa* (bark)	1	Dysmenorrhoea
B. cathartica (root) + *O. natalitia* (root)	1	Dysmenorrhoea and infertility
S. birrea (bark) + *T. dregeana* (bark)	1	Induce abortion

Bold – plant material not collected, hence the combination was excluded in the current study.

Apart from the menstruation and pregnancy conditions that were reported by De Wet and Ngubane (2014), there is a need to also assess these plants for potential anti-*Candidal* activity. *Candida* spp. are among the most common micro-organisms that cause vaginal infections in women. There were only 11 medicinal plant species in the current study that have been previously assessed for anti-*Candidal* activity.

1.2 Toxicity of medicinal plant species

Scientists throughout the world have developed an interest in evaluating safety of medicinal plants despite their long-term traditional uses, like studies from Europe (Masullo et al., 2015; Kristanc and Kreft, 2016) and Africa (Edziri et al., 2011; Adewale et al., 2016). Toxicological studies investigate the deleterious effect of any agent or substance capable of harming the biological system (Kuete, 2014). Such agents are known as toxicants or xenobiotics. A toxicant can induce adverse and irreversible effects that could change the organism's physiology either permanently or temporarily (Kuete, 2014). Various methods can be used to assess the toxicity of medicinal plant species such as the brine shrimp lethality assay (BSLA) and the Ames test to assess toxicity and mutagenicity respectively. It is plausible to assume that the long-term traditional use of medicinal plants and herbal remedies do not always guarantee safety (Madingou et al., 2016). The most notable factors that influences human poisoning from using medicinal plants include the misidentification and unintentional use of toxic plant species and/or over dosage of home preparations (Ndhlala et al., 2013).

In South Africa, the human intoxication by plants is common. According to hospital data, traditional herbal medicine has caused about 2.4% cases of acute toxicity in South Africa (Malangu and Ogunbanjo, 2009; Malangu, 2011). Children have been reported to constitute most of these cases in South Africa (Fennell et al., 2004). It should be acknowledged that there are many similar unreported incidents that are mostly treated at home and therefore cannot be found on health records. According to the literature, quite a few medicinal plant species used in South Africa to treat various conditions have been reported to also have toxic effects. These plants include: *Dioscorea dregeana* (Kunth) T. Durand & Schinz, *Flueggea virosa* (Roxb. ex Willd.) Voigt (Ndhlala et al., 2013), *Catharanthus roseus* (L.) G.Don (Verschaeve and Van Staden, 2008), *Trichilia emetica* Vahl, *Erythrina caffra* Thunb. and *Tetradenia riparia*

(Hochst.) Codd (Tamokou and Kuete, 2014) just to name a few. The seeds of *Ricinus communis* are known to be toxic when ingested because of the compound toxalbumin which the plant produces and inhibit mitosis and protein synthesis, and eventually results in gastrointestinal toxicity and body organ failure. Ndhlala et al. (2013) reported that South Africans continue to use some toxic plant species for their medicinal properties without knowing the short or long-term consequences. Sometimes toxic effects may only show after years of repeated use of the plant/s, and in such cases it is difficult to relate the symptoms to an herbal medicine.

1.2.1 Acute and sub-acute toxicity

Like any other toxic compounds, medicinal plant toxicants may vary in intensity as well as in the severity of damage to the human body. The various degrees of toxicity include acute toxicity, sub-acute toxicity and/or chronic toxicity after administration. Acute toxicity is the kind of toxicity that occurs shortly after a single or multiple drug administration within 24 hrs. This type of toxicity is easy to test in the laboratory because results can be observed in a short period of time. Sub-acute toxicity is usually observed after repeated administration of a drug over a period of days to weeks (Teke and Kuete, 2014). Chronic toxicity differs from acute and sub-acute toxicity because of the adverse effects that occur only after a repeated exposure to a drug. Symptoms may be experienced only after a number of months to years (Adeneye, 2014). The toxicity of substances may vary from minor adversities to fatalities. The extent of toxicity by herbal preparations may sometimes vary depending on the route of administration, amount of the medicine consumed, growth stage of a plant or plant part used as well as the plant species used (Ndhlala et al., 2013), other factors include the health status of the consumer as some plant can induce diarrhoea, nausea or an upset stomach.

1.2.2 Mutagenicity and carcinogenicity

Mutagenicity is the type of toxicity that results in the change of DNA sequence, which can either be chromosomal or the gene mutation as a result of exposure to chemical substances (Richardson et al., 2007). Carcinogenicity is described by Tomakou and Kuete (2014) as the ability of a chemical to induce cancer/tumours. According to Ames et al. (1975), the majority (85%) of carcinogens that have been tested reacted as

mutagens, which implies that their sources may be similar. Agents that bring about the changes in the genetic material are called mutagens. The mechanism of mutagenicity varies. Sometimes the mutagens work directly by chemically promoting the changes in the DNA sequence, or by damaging the building blocks of the DNA, which will eventually damage the DNA (Reha-Krantz, 2013). Other mutagens work indirectly by inhibiting the ability of the cell to repair damage to the DNA. Exogenous or environmental mutagens that are usually introduced into the body are found in food, nicotine, sunlight, industrial by-products as well as in medicinal plant species (Reid et al., 2006; Verschaeve and Van Staden, 2008; Edziri et al., 2011; Ndhlala et al., 2013; Reha-Krantz, 2013; Tamokou and Kuete, 2014). Some medicinal plants in South Africa that have been reported to have mutagenic effects are: *Ekebergia capensis* Sparrm, *Helichrysum herbacea* (Andrews) Sweet*, Helichrysum regulosum* Less.*,* and *Helichrysum simillimum* DC. (Reid et al., 2006; Verschaeve and Van Staden, 2008; Mulaudzi et al., 2013). It is thus important to make practitioners of medicinal plants use aware of plant species that cause side effects, especially as these mutagenic effects are most likely to progress into cancer (Razak et al., 2007).

1.2.3 Genotoxicity and teratogenicity

Genotoxicity is a chemical-induced damage of genetic information within the cell. The resulting mutations can cause malignancies. Genotoxic agents may result in chromosomal abnormality or changes in the DNA structure, which will eventually affect the reliability of the portrayed message (Sponchiado et al., 2016). Some plant species that are commonly used in traditional medicine can induce genotoxicity (Ananthi et al., 2010; Seukep et al., 2014; Sponchiado et al., 2016). Medicinal plant species used in South Africa that have demonstrated genotoxic potential are: *C. roseus, H. hemerocallidea, S. birrea,* and *T. emetica* (Seukep et al., 2014).

Teratogenicity is referred to as congenital toxicity that results in malformation or abnormalities to physiological development of the foetus and children (Zeliger, 2011; Cassina et al., 2012; Seukep et al., 2014). Teratogenic effects can also extend to cause abnormalities at puberty (Seukep et al., 2014). Susceptibility to teratogenic substances is higher at early stages of foetal development (first trimester). Teratogens are present in the environment, xenobiotics as well as untested herbal medications (Zeliger, 2011; Cassina et al., 2012; Seukep et al., 2014; Mohammed et al., 2016). A

few sources of teratogens include air pollution, organic solvents, wood preservative chemicals, pesticides, chemical radiation mixture, alcohol, paint and cigarette smoke (Zeliger, 2011; Cassina et al., 2012).

Mohammed et al. (2016) reported that consumption of herbal remedies at an early stage of pregnancy (first trimester) might not be safe for foetal development as it may result in embryo toxicity. Some plant species used in Africa (including South Africa) that have teratogenic effects are: *Asparagus rasemosus* Willd*, Cannabis sativa* L.*, Nicotiana glauca* Graham*, Solanum tuberosum* L. and *Sorghum bicolor* (l.) Moench (Seukep et al., 2014). Garcia-Algar et al. (2016) reported that dosages that are usually harmless to adults may cause adverse effect in children.

1.2.4 Hepatotoxicity

The liver plays a central role in transforming and clearing chemicals that enter the body (Chaundhary et al., 2016). The toxicity associated with the liver is called hepatotoxicity. More than 900 drugs have been associated with liver damage and this subsequently resulted in their withdrawal from the market (Chaundhary et al., 2016). Hepatotoxicity may result in liver damage that is mild, asymptomatic or the cause of severe liver failure. Liver susceptibility to drug toxicity is higher when it is exposed to higher concentrations of oral administration (Attia, 2010). According to Chaundhary et al. (2016), medicinal plants are also capable of causing hepatotoxicity and may even cause lethal hepatic dysfunctions (Olaleye and Rocha, 2008). In South Africa *Senecio* and *Crotalaria* species are among medicinal plant species that have been reported to have hepatotoxic effects (Botha and Penrith, 2008).

1.2.5 Nephrotoxicity

Nephrotoxicity is the poisonous effect of substances that causes damage to kidneys (Yao et al., 2007; Souza et al., 2016). When the kidney is exposed to nephrotoxins, their function becomes impaired. Renal filtration declines and eventually results in an increased concentrations of blood urea, nitrogen and serum creatine (Chandrakumar et al., 2016). As the kidney pursues its function of metabolizing xenobiotics (a chemical compound foreign to a given biological system), this increases susceptibility to nephrotoxicity (Ekor, 2014). Medicinal plant species such as *Solanum grandiflorum*

Ruiz & Pav. have nephrotoxic effects when fruit extracts are consumed (Ekor, 2014). In South Africa, some plant species including *Aloe ferox* Mill., *Callilepsis laureola* DC. have been associated with nephrotoxicity (Liwa and Jaka, 2016).

1.2.6 Cytotoxicity

While many studies focus on testing plant extracts and isolating new potential bioactive phytochemicals for therapeutic exploration, it is always important to also test for their potential cytotoxicity. Any chemical substance that has a quality of being deleterious to the cell is referred to as cytotoxic. Medicinal plant extracts have also been known to be cytotoxic (Booth et al., 2012; Nemati et al., 2013). Plant toxins may result in a variety of cell fates such as necrosis, apoptosis, autophagy or the cells may cease to proliferate (McGaw et al., 2014). *Dicoma capensis* Less. is a South African medicinal plant species where the leaves are used to treat cancer, fever and hypertension (Steenkamp and Gouws, 2006). The aqueous infusion of this plant species was found to have cytotoxic effects when tested against DU-145 prostate cancer cells, MCF-7 breast cancer cells and MCF-12A non-malignant breast cells (Steenkamp and Gouws, 2006).

1.3 Anti-*Candidal* activity of medicinal plant species

Candida species are the most common fungal pathogens that are capable of causing the opportunistic vulvovaginal candidiasis/ infection (VVC) (Donders et al., 2018), also known as vaginal thrush. *Candida* is a fungal yeast normally found in the mouth, digestive system, and on the skin without causing any problems to the host (Vilander et al., 2016). However, when conditions are favourable (i.e. in immunocompromised conditions), this yeast can cause an infection. The *Candida* strains such as *C. albicans, C. glabrata,* and *C. tropicalis* are among the species that have been reported to cause the majority of VVC in women (Vilander et al., 2016). It has been reported that a woman is likely to contract a yeast infection at least once in her lifetime. An estimated one in every ten women may experience a recurrence more than three episodes per year (Emeribe et al., 2015; Donders et al., 2018). *Candida* infection may cause an irritation, dysuria, discharge and intense itchiness in the vulva and vagina. The risk factors that are associated with causing VVC include hormonal changes (e.g. elevated oestrogen), diabetes mellitus, immunosuppression and broad-spectrum

antibiotic use (which can kill the normal vaginal flora including *Lactobacillus* species that produce hydrogen peroxide which inhibit the excessive yeast growth) (Jeanmonod and Jeanmonod, 2019). Some medicinal plant species have demonstrated good anti-*Candidal* activities. Plant species such as *Allium sativum* L., *Glycyrriza glabra* Linn., *Polygala myrtifolia* L., *Solanum nigrescens* M. Martens & Galeotti (Giron et al., 1988), *Tulbaghia violacea* Harv., *Vertiveria zazinioides* (L.) Nash *and Warburgia salutaris* (Bertol.f.) Chiov. (Motsei, 2003) have been reported to have anti-*Candidal* activity at low concentrations. An *in-vitro* minimum inhibitory concentration test is often used to determine susceptibility and the lowest concentration of a sample that could inhibit the growth of this yeast (Samie et al., 2010; Soliman et al., 2017; Jayachandran et al., 2018). However, most studies have focused on a single strain of *Candida*, especially *C. albicans* (Buwa and Van Staden, 2006; More et al., 2008; Shai et al., 2008; Samie et al., 2010; Mamba et al. 2016; Owotade et al., 2016; Ohikhena et al., 2017). Other strains such as *C. glabrata* (Akhalwaya, 2017) and *C. tropicalis* are poorly studied in South Africa and therefore require exploration.

1.4 The study area

Medicinal plant species that are used in this study are reported to treat gynaecology and obstetrics conditions in northern Maputaland. Northern Maputaland is a biodiverse rich area located in the north eastern part of KwaZulu-Natal between 32°22’S and 32°52’S latitudes and 27°15’E and 27°30’E longitudes (De Wet et al., 2010), as shown in Figure 1.1. It falls under the Umhlabuyalingana municipality with an estimated population of 172 077 people and an average of five people per homestead (Umhlabuyalingana municipality IDP, 2017/2018). About 99% of this area is classified as rural, with a high rate of poverty, where 44.9% of the households do not have a stable monthly income. Umhlabuyalingana municipality has 17 clinics and two hospitals, but people must walk long distances (approximately 10 km) to access these healthcare services (Umhlabuyalingana municipality IDP, 2017/2018). Therefore, people rely heavily on medicinal plants growing in and around the homesteads as their primary source of healthcare that is also influenced by their traditional beliefs. The villages reflected in this study are Mabibi, Mseleni, Kwajozana and Tshongwe.

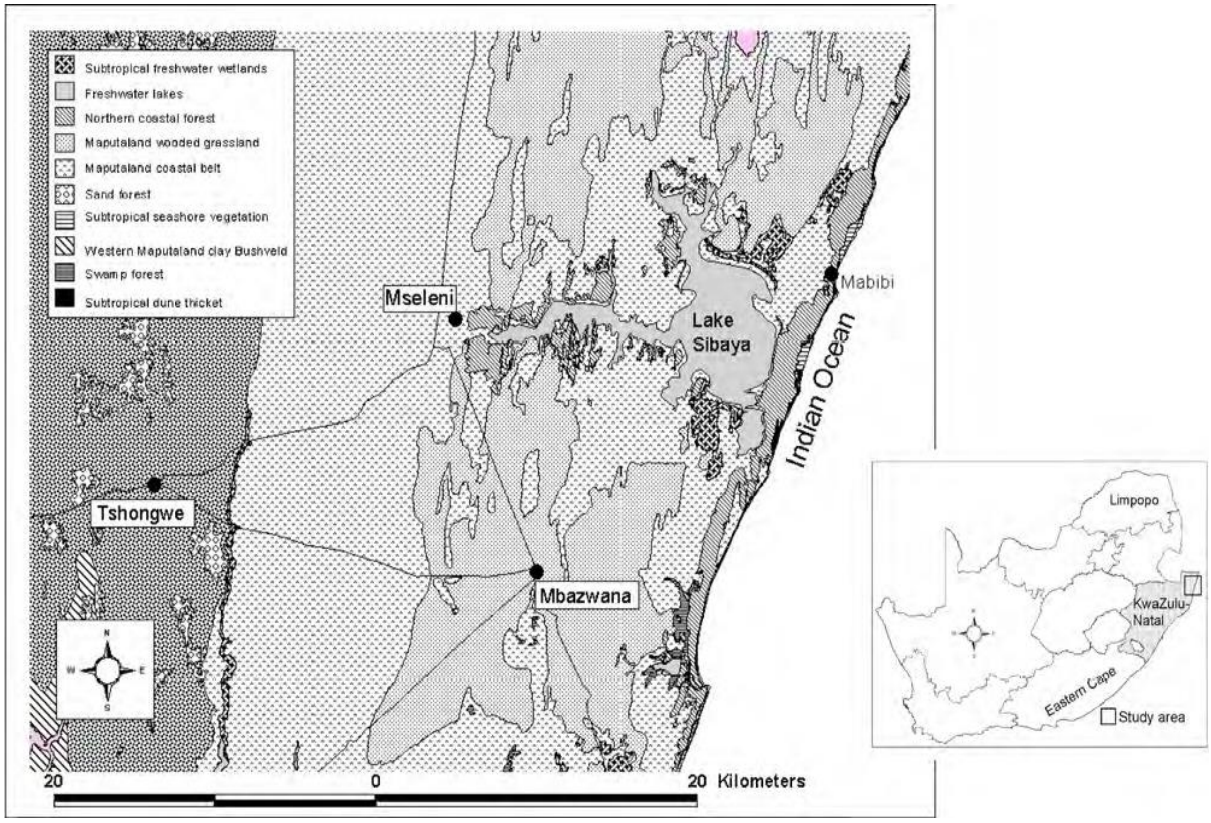

Figure 1.1: Study area: northern Maputaland located in KwaZulu-Natal, South Africa (De Wet et al., 2010).

1.5 Hypothesis

Based on the scientific studies that have been conducted on the toxicity of medicinal plant species, it can be hypothesised that some of the medicinal plant species used by women in northern Maputaland to treat gynaecology and obstetrics complaints possess various degrees of toxicity, genotoxicity and anti-*Candidal* effects.

1.6 Aim and objectives of the study

This study intended to evaluate the safety and potential anti-*Candidal* activity of 36 medicinal plant species and 17 plant species combinations used by women in northern Maputaland to treat gynaecology and obstetrics complaints (De Wet and Ngubane, 2014).

However, due to the seasonal variations some of the plants were not available for collection during the time of study. In order to conduct this study, the following objectives were followed;

- To collect plant material of the plant species mentioned in the study of De Wet and Ngubane (2014).
- To prepare aqueous (using sterile distilled water) and organic extracts (using dichloromethane: methanol at 1:1 ratio).
- To perform the BSLA on plant extracts (individual plant species and combinations documented).
- To perform an Ames test on plant extracts (independently and different combinations documented).
- To perform a minimum inhibitory concentration assay on the plant extracts (independently and in combination) against several *Candida* strains.
- To assess the interactions between the plant species used in combination using the sum of the fractional inhibitory concentration (ΣFIC) to measure the interaction efficacy.

1.7 Significance of this study

There are twelwe plant species that have not been tested previously for any toxicity, namely, *Acalypha villicaulis, Acanthospermum glabratum, Crotalaria monteiroi, Cyperus natalensis, Diospyros villicaulis, Erythrina humeana, Hermannia boraginiflora, Opuntia stricta, Rhoicissus digitata, Sapium integerrimum, Searsia nebulosa* and *Senegalia burkei*. These results will contribute towards the toxicological and ethno-pharmacological data base of South African medicinal plants. The leaves were collected for all the plant species where the roots were mentioned for medicinal use, this will help determine the safety of the leaves for potential root substituents. This study will also help raise awareness to lay people to use certain medicinal plant species with caution.

CHAPTER 2

Toxicity evaluation of plant extracts using the brine shrimp lethality assay

2.1 Introduction

For the current study, the brine shrimp lethality assay (BSLA) was used as a preliminary screen of medicinal plant extracts for toxicity. This technique is very common because it is quick, simple and inexpensive. It can also give results at a very low concentration of toxicant in a micro-well scale (Krishnaraju et al., 2005). The BSLA is easy to accommodate and maintain under laboratory conditions and it has high adaptability to various testing conditions (Libralato et al., 2016). *Artemia* spp. are a group of crustaceans that is used in the BSLA. These salt water animals are extensively used in pharmacology and toxicology to screen various biologically active substances and to assess the effects of various toxicants (Anufriieva and Shadrin, 2014). These invertebrates have been recommended as a suitable alternative model to mammals for pre-screening pharmaceutical substances for toxicity (Nunes et al., 2006). Considering that the life span of *Artemia* spp. varies between 2 – 4 months depending on the living conditions such as temperature, salinity and species-specific characteristics, these animal species can be used to screen for acute (short-term) and chronic (long-term) effects of toxicants (Libralato et al., 2016). In a laboratory experiment, toxicity is commonly articulated as a lethal concentration that results in the death of 50% of the group of test organisms (Libralato et al., 2016). Acute toxicity can be declared in the BSLA when 50% death is observed within 48 hrs of exposure. According to Coe et al. (2010), when a concentration above 1.00 mg/mL is required to exhibit a toxic effect against *Artemia* spp., the test sample is considered non-toxic in nature. The BLSA does not have a capacity to provide sufficient information with regards to the mechanism of action of the toxicant. Nevertheless, it is very useful in evaluating and indicating the toxicity potential of various medicinal plant extracts (Hamidi et al., 2014). This assay can also be used in the evaluation of heavy metals, pesticides and medicine (Wu, 2014). The results from this chapter will preliminarily

indicate whether the plant species used by the woment in northern Maputaland for gynaecological and obstetric conditions are safe or toxic.

2.2 Material and methods

2.2.1 Plant material

Plant materials were collected from four areas (Mseleni, Kwajozana, Tshongwe and Mabibi) in northern Maputaland (KwaZulu-Natal) following all ethical considerations as per the University of Zululand Research Ethics Committee (Appendix D), with the permission from the home owners. Voucher specimens were prepared and deposited in the Botany Herbarium (ZULU), University of Zululand. Botanical identification of plant species was done by Dr T.H.C. Mostert (Department of Botany, University of Zululand) and Mr M. Ngwenya (SANBI, KwaZulu-Natal Herbarium). Table 2.1 represents the 33 plant species collected with their voucher numbers. Among the 35 collected plant samples (33 different plant species), roots of 16 species were collected (De Wet and Ngubane, 2014). However, the harvesting of roots is considered not sustainable in terms of conservation, therefore, leaves of these plants were also collected to compare results. According to Manohar (2012), different parts of a plant can share the same properties, therefore it is important to find out whether the leaves can be used instead of the roots.

Table 2.1 Plant species that were collected and their voucher numbers

Plant name	Family	Plant part used	Voucher number
Acalypha villicaulis Hochst. Ex A.Rich.	Euphorbiaceae	Root bark and **leaves**	SC Ngubane 14
Acanthospermum glabratum (DC.) Wild	Asteraceae	Whole plant	SC Ngubane 29
Bridelia cathartica G.Bertol.	Euphorbiaceae	Roots and **leaves**	SC Ngubane 9
Cassytha filiformis L.	Lauraceae	Whole plant	SC Ngubane 18

Plant name	Family	Plant part used	Voucher number
Commiphora neglecta I.Verd.	Burseraceae	Roots and **leaves**	SC Ngubane 28
Crotalaria monteiroi Taub. Ex Baker f. var. *galpinii* Burtt Davy	Papilionaceae	Roots and **leaves**	SC Ngubane 31
Cyperus natalensis Hochst. Ex. Krauss	Cyperaceae	Roots and **leaves**	SC Ngubane 32
Diospyros villosa (L.) De Winter	Ebenaceae	Roots and **leaves**	SC Ngubane 17
Erythrina humeana Spreng	Leguminosae	Roots	SC Ngubane 10
Euclea natalensis A.DC.	Ebenaceae	Roots and **leaves**	SC Ngubane 5
Euphorbia tirucalli L.	Euphorbiaceae	Stem	SC Ngubane 7
Garcinia livingstonei T.Anderson	Clusiaceae	Roots and **leaves**	SC Ngubane 8
Grewia occidentalis L.	Tiliaceae	Roots and **leaves**	SC Ngubane 16
Gymnosporia senegalensis (Lam.) Loes.	Celastraceae	Roots and **leaves**	SC Ngubane 3
Hermannia boraginiflora Hook.	Sterculiaceae	Roots and **leaves**	SC Ngubane 27
Hyphaene coriacea Gaertn.	Arecaceae	Stem	SC Ngubane 35
Hypoxis hemerocallidea Fisch., C.A.Mey. & Avé-Lall.	Hypoxidaceae	Corm	SC Ngubane 15
Kigelia africana (Lam.) Benth.	Bignoniaceae	Bark	SC Ngubane 19
Ochna natalitia Walp.	Ochnaceae	Roots and **leaves**	SC Ngubane 4
Opuntia stricta Haw.	Cactaceae	Stem	NZ-41
Ozoroa engleri R.Fern & A.Fern.	Anacardiaceae	Bark	SC Ngubane 1
Peltophorum africanum Sond.	Leguminosae	Roots and **leaves**	SC Ngubane 20
Ranunculus multifidus Forssk.	Ranunculaceae	Whole plant	NZ-36
Rhoicissus digitata (L.f.) Gilg & M.Brandt	Vitaceae	Roots and **leaves**	SC Ngubane 2

Plant name	Family	Plant part used	Voucher number
Sapium integerrimum (Hochst. Ex. Krauss) J.Léonard	Euphorbiaceae	Roots and **leaves**	SC Ngubane 6
Scadoxus puniceus (L.) Friis Norda	Amaryllidaceae	Bulb	TYORK 5
Sclerocarya birrea (A. Rich.) Hochst.	Anacardiaceae	Bark	S. Nciki 17
Searsia nebulosa (Schoenland) Moffett forma *nebulosa*	Anacardiaceae	Bark	SC Ngubane 25
Senecio serratuloides DC.	Asteraceae	Whole plant	S. Nciki 1
Senegalia burkei (Benth.) Kyal. & Boatwr. (= *Acacia burkei* Benth.)	Fabaceae	Bark	SC Ngubane 26
Tabernaemontana elegans Stapf	Apocynaceae	Roots and **leaves**	SC Ngubane 13
Trichilia dregeana Harv. & Sond.	Meliaceae	Bark, roots and leaves	SC Ngubane 11
Vangueria infausta Burch. subsp*. Infausta*	Rubiaceae	Leaves	SC Ngubane 21

Bold = plant parts not specified by lay people for the treatment gynaecology and obstetrics complaints

2.2.2 Preparation of plant extracts

Collected plant materials were chopped into small pieces and left to dry at room temperature. The dried plant material was then ground into fine powder using a Scientec RSA hammer mill. The powdered plant material was used to prepare aqueous and organic extracts.

2.2.2.1 Aqueous extracts

Aqueous extracts were prepared in a similar method as described by interviewees in northern Maputaland (De Wet and Ngubane, 2014). Two basic preparation methods were considered for this study, and were a decoction and an infusion, to mimic the instructions given by the lay people during the ethnobotanical survey (De Wet and Ngubane, 2014). To prepare a decoction, 10.00 g of dried plant material was boiled in 200 ml of water for 30 min, and for an infusion the same amount of plant material was

soaked in 200 ml of warm water and the preparation was left for 24 hrs on a platform shaker. The extract was then filtered and frozen using the ultra-low temperature -80 °C freezer (VF120-86) for 24 hrs and lyophilized using CHRIST Alpha 1-2 LD plus. The dried aqueous extract was stored at room temperature away from sunlight until needed.

2.2.2.2 Organic extracts

Two hundred millilitres of methanol-dichloromethane (1:1) solvent was used to immerse 10.00 g of ground plant material. This preparation method was used to ensure that both polar and non-polar compounds are extracted. The extraction solution was then left for 24 hrs on a platform shaker at 37 °C after which, it was filtered through a 90 mm grade 3 hw filter paper (Whatman). The liquid extract was kept in a fume hood to evaporate the solvent, and the resulted solid extract was stored in a refrigerator (4 °C) until it was needed.

2.2.3 Percentage yield

After the solid extract was obtained, the percentage yield was calculated for organic and aqueous extracts. This was obtained by dividing the total mass of the extract by the total mass of plant material that was used for the preparation of the extract (w/w) (Table 2.2). *Ozoroa engleri* bark gave the highest percentage yield in both aqueous (32.16%) and organic (32.73%) extracts. The lowest yield was found with the aqueous root extract of *C. natalensis* (1.01%) and the organic root extract of *G. senegalensis* (1.18%).

Table 2.2 Percentage yield of organic and aqueous plant extracts

Plant name	Plant part used	Extract yield in percentage (%)	
		Organic	Aqueous
Acalypha villicaulis	Root	9.38	7.78
	Leaves	14.68	16.76
Acanthospermum glabratum	Whole plant	14.77	16.64
Bridelia cathartica	Roots	2.19	2.52

Plant name	Plant part used	Extract yield in percentage (%)	
		Organic	Aqueous
	Leaves	20.86	13.22
Cassytha filiformis	Whole plant	1.28	19.53
Commiphora neglecta	Roots	9.76	3.62
	Leaves	4.23	14.29
Crotalaria monteiroi var. *galpinii*	Roots	3.85	8.11
	Leaves	5.82	7.04
Cyperus natalensis	Roots	1.01	13.83
	Shoot	19.59	7.68
Diospyros villosa	Roots	5.12	16.03
	Leaves	10.12	17.08
Erythrina humeana	Roots	3.13	13.39
Euclea natalensis	Roots	8.46	6.87
	Leaves	20.33	5.09
Euphorbia tirucalli L.	Stem	7.88	17.87
Garcinia livingstonei	Roots	10.71	18.54
	Leaves	7.71	9.35
Grewia occidentalis	Roots	4.97	10.01
	Leaves	8.01	14.45
Gymnosporia senegalensis	Roots	1.18	5.85
	Leaves	9.22	17.46
Hermannia boraginiflora	Roots	7.76	15.91
	Leaves	9.34	10.13
Hyphaene coriacea	Stem	6.28	17.76
Hypoxis hemerocallidea	Corm	19.81	26.21
Kigelia africana	Bark	20.13	18.89
Ochna natalitia	Roots	6.58	13.78

Plant name	Plant part used	Extract yield in percentage (%)	
		Organic	Aqueous
	Leaves	11.72	5.74
Opuntia stricta	Stem	15.69	19.97
Ozoroa engleri	Bark	32.16	32.73
Peltophorum africanum	Roots	19.61	6.77
	Leaves	14.15	12.74
Ranunculus multifidus	Whole plant	9.62	21.62
Rhoicissus digitata	Roots	18.07	11.03
	Leaves	4.20	11.09
Sapium integerrimum	Roots	7.58	9.19
	Leaves	11.08	21.35
Scadoxus puniceus	Bulb	4.16	10.18
Sclerocarya birrea	Bark	16.02	10.45
Searsia nebulosa	Bark	6.56	8.16
Senecio serratuloides	Whole plant	15.87	21.09
Senegalia burkei	Bark	13.37	6.18
Tabernaemontana elegans	Roots	2.45	2.87
	Leaves	9.99	17.34
Trichilia dregeana	Roots	3.69	6.34
	Leaves	5.32	14.21
	Bark	17.51	16.02
Vangueria infausta subsp. *Infausta*	Leaves	18.92	16.98

2.2.4 Sample preparation for the BSLA

Dimethyl sulfoxide (DMSO) (2%) was used to dissolve the organic extracts. Aqueous extracts were dissolved in distilled deionised water (Figure 2.1). Considering that a concentration of 1.00 mg/ml or lower is required for the BSLA, a concentration of 2.00 mg/ml was prepared for all plant extracts to be tested.

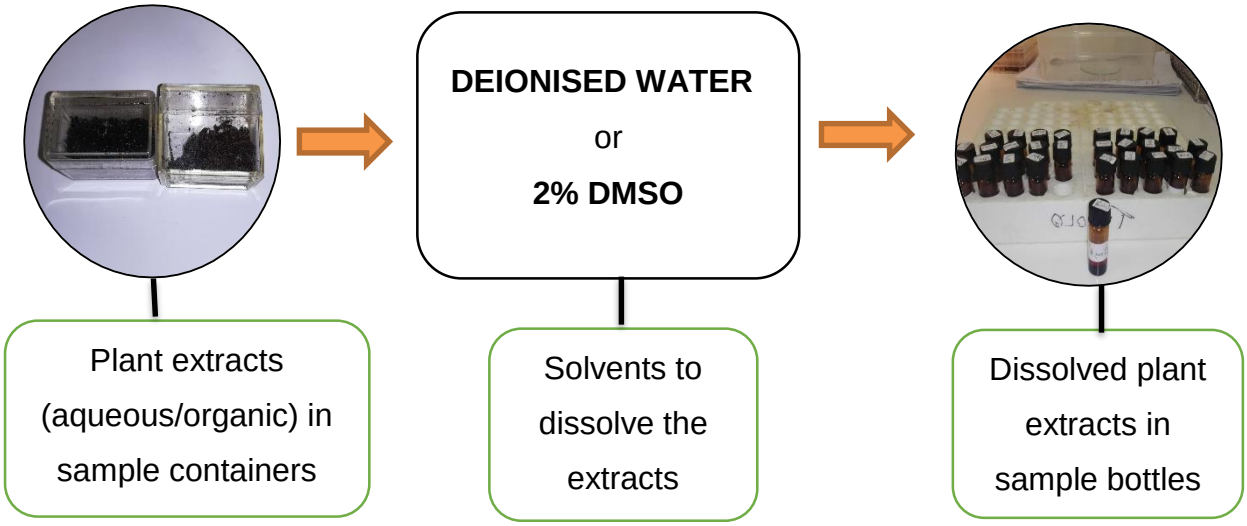

Figure 2.1 A diagrammatic illustration of sample preparation for BSLA. Photo credit: S.C. Ngubane

2.2.5 Hatchery of the brine shrimp eggs

Growth medium was prepared by dissolving 16.00 g of Tropic Marine® Sea Salt in 500.00 ml of distilled water to make artificial sea water (Figure 2.2). The prepared medium was then transferred into an inverted, bottomless plastic container, into which the dried eggs of brine shrimp (*Artemia franciscana*) (0.50 g) were added. The culture was aerated with a rotary pump and kept at constant temperature, and a light source of 220 – 240V to ensure a high hatch rate. The eggs were incubated for 18 – 24 hrs at 25 °C. After the incubation, saltwater containing hatched brine shrimp was transferred into a shallow plastic container, which was tilted so that the solution lies on one side of the container. Light was placed over the side for approximately 30 min to attract the shrimp on one side.

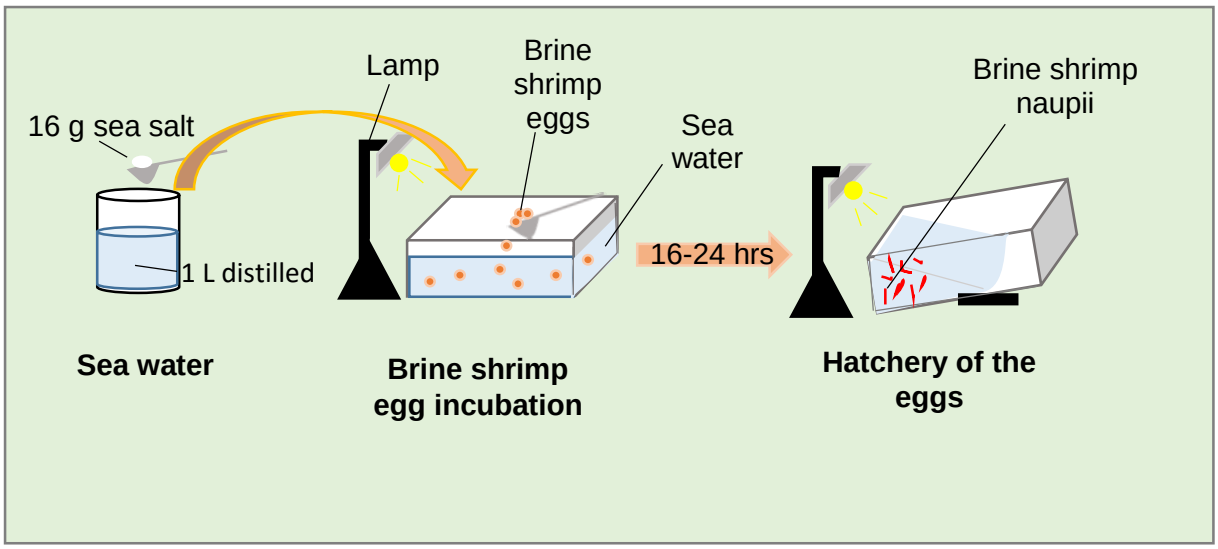

Figure 2.2 A diagrammatic representation of the hatchery of brine shrimp eggs.

2.2.6 The brine shrimp lethality assay

To assess the toxicity of the individual extracts, 400.00 µl of salt water (containing 40–60 live brine shrimp) were added along with 400.00 µl of the plant extract into 48-well microtiter plates (Figure 2.3). The number of dead brine shrimp was counted immediately after adding the plant extract using a light microscope (Olympus). The experiment for each extract was done in triplicate and repeated on consecutive days allowing for at least six repeats per sample. Four hundred microliters of artificial sea water (32.00 g/L) was added to 400.00 µl of sea water with live brine shrimp as a negative control (toxin-free). Potasium dichromate (AR grade, Chem-Supply) was used as the standard reference positive control for the brine shrimp assay. The positive control was prepared by adding 400.00 µl of potassium dichromate (1.60 mg/ml) into 400.00 µl of sea water with live brine shrimp. Counting of dead brine shrimp was done using a light microscope (Olympus) after 24 and 48 hrs. After the dead brine-shrimp count at 48 hrs, a lethal dose of 50.00 µl of glacial acetic acid (100% v/v) was added to each well. Then the total dead shrimp were counted. Plant extracts that were toxic in BSLA were further analysed at various concentrations of 0.031, 0.063, 0.125, 0.25 and 0.5 mg/ml to determine the dose response effects.

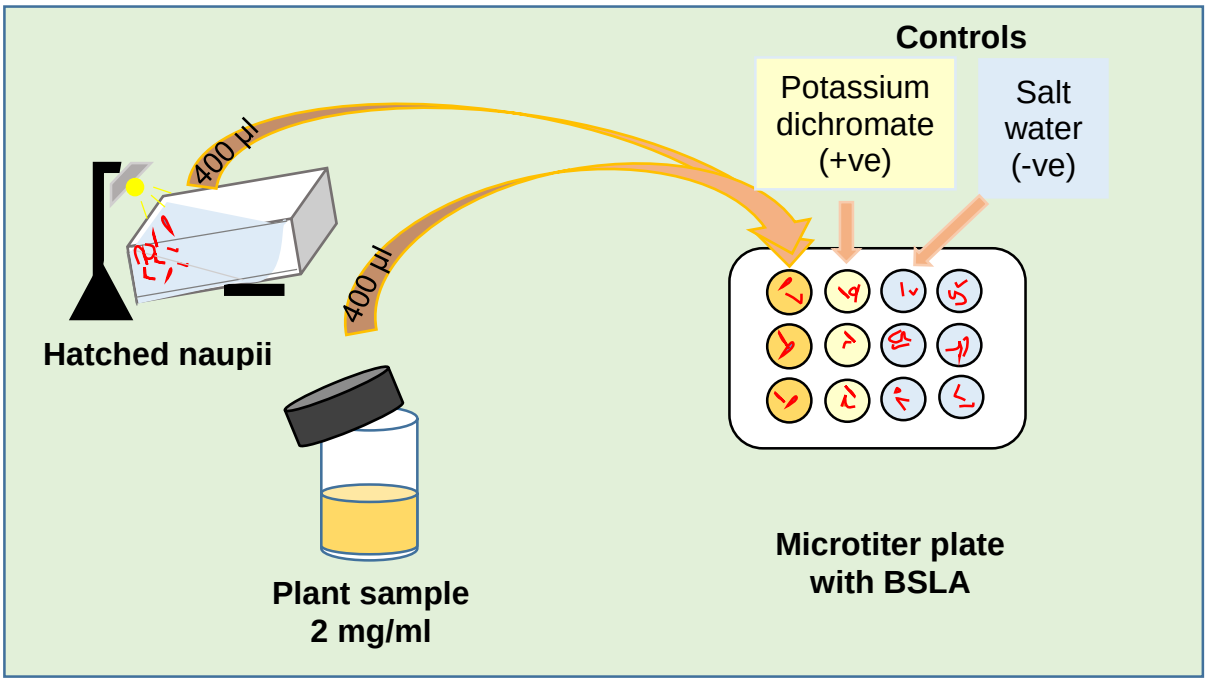

Figure 2.3 A diagrammatic illustration of the BSLA.

The percentage mortality was calculated for each plant extract using Equation 2.1,

$$\% \text{ Mortality} = \frac{\text{dead shrimp at 48 hrs (before acetic acid)} - \text{dead shrimp (time 0)} \times 100}{\text{dead shrimp (after acetic acid)}}$$

Time zero being the time immediately after the plant samples were added with the brine shrimp. Plant samples that induced a percentage mortality of 50% and above were considered toxic in nature (Cock and Ruebhart, 2009; Libralato et al., 2016).

2.3 Results and discussion

2.3.1 Toxicity analysis of plant extracts towards brine shrimp larvae

The results in Table 2.3 show the average percentage mortality of brine shrimp when exposed to 1.00 mg/ml of organic and aqueous plant extracts. Among the 51 plant extracts tested, 35 were prepared from plant parts (root, bark, leaves, bulb, corm, etc.) that were specified by lay people to treat gynaecology and obstetrics complaints. Where traditional use referred specifically to root material (16 plant species) additional leaf samples were prepared to determine whether substitution of plant parts might play

a role in conservation (over harvesting of roots can be detrimental to plants). As expected, there was an increase in the toxicity from 24 to 48 hrs of exposure. An overall 24% of aqueous extracts showed toxicity (≥ 50% mortality) effects on the brine shrimp within 24 hrs of exposure and this increased to 55% after 48 hrs.

The toxicity of the aqueous extracts varied from 0% to 100% mortality after 24 hrs and 1% to 100% after 48 hrs. The organic extracts varied from 0% to 78% mortality after 24 hrs and 0% to 100% after 48 hrs. For the purpose of this study, the extracts that demonstrated >80% mortality were observed as having extremely high toxic effects and mortality below 10% was considered as having minimal toxicity and recognised as "safe". The aqueous extracts that demonstrated the least toxic effects (<10% mortality after 48 hrs) in this study were *A. villicaulis* (roots, 1%), *B. cathartica* (roots, 9%), *G. occidentalis* (roots, 2%), *S. integerrimum* (roots, 8%) and *S. nebulosa* (bark, 1%). The aqueous extract of *E. tirucalli* was the most toxic among all extracts by killing all (100%) brine shrimp within 24 hrs. This plant species is reported in numerous studies as having toxic activities (Silva et al., 2007; Schlamovitz et al., 2009; Kumar et al., 2010). The majority (86%) of the plant extracts had a major increase in percentage mortality from 24 to 48 hrs, whereas 14% had very little to no change throughout the test period. The increase in toxicity in the BSLA with the increase in the period of exposure to the test sample has also been observed in a toxicological study by Ramulondi et al. (2018).

Table 2.3 Average percentage mortality of brine shrimp when exposed to aqueous and organic plant extracts

Plant names	**Plant part used**	**Aqueous extract**		**Organic Extract**	
		24 hrs	**48 hrs**	**24 hrs**	**48 hrs**
Acalypha villicaulis	Roots	0	1	17	27
	Leaves	**90**	**93**	4	24
Acanthospermum glabratum	Whole plant	10	17	29	34
Bridelia cathartica	Roots	3	9	2	**89**
	Leaves	3	**87**	28	**93**
Cassytha filiformis	Whole plant	6	29	20	**69**

Plant names	Plant part used	Aqueous extract		Organic Extract	
		24 hrs	48 hrs	24 hrs	48 hrs
Commiphora neglecta	Roots	18	32	0	40
	Leaves	11	**92**	0	**97**
Crotalaria monteiroi	Roots	**55**	**74**	0	**68**
	Leaves	16	30	13	14
Cyperus natalensis	Roots	21	38	45	**72**
	Shoot	47	**74**	45	**96**
Diospyros villosa	Roots	6	14	35	**61**
	Leaves	**59**	**80**	14	28
Erythrina humeana	Roots	22	**92**	5	49
Euclea natalensis	Roots	2	24	1	**96**
	Leaves	15	**81**	0	**87**
Euphorbia tirucalli	Stems	**100**	**100**	**78**	**90**
Garcinia livingstonei	Roots	1	30	8	45
	Leaves	27	**67**	0	22
Grewia occidentalis	Roots	0	2	4	44
	Leaves	45	**94**	26	44
Gymnosporia senegalensis	Roots	4	13	0	1
	Leaves	10	14	1	1
Hermannia boraginiflora	Roots	28	**86**	13	**91**
	Leaves	**76**	**98**	**59**	**89**
Hyphaene coriacea	Stems	4	14	25	38
Hypoxis hemerocallidea	Corms	4	40	28	**66**
Kigelia africana	Bark	13	40	30	35
Ochna natalitia	Roots	1	15	**52**	**55**
	Leaves	6	**67**	**59**	**68**
Opuntia stricta	Stems	**79**	**88**	19	38
Ozoroa engleri	Bark	**50**	**74**	23	**54**
Peltophorum africanum	Roots	47	49	23	28
	Leaves	45	47	37	**53**
Ranunculus multifidus	Whole plant	11	15	17	34
Rhoicissus digitata	Roots	22	43	38	**80**

Plant names	Plant part used	Aqueous extract		Organic Extract	
		24 hrs	48 hrs	24 hrs	48 hrs
	Leaves	28	**51**	27	**56**
Sapium integerrimum	Roots	3	8	**65**	**100**
	Leaves	**87**	**93**	**61**	**81**
Scadoxus puniceus	Bulb	**50**	**93**	22	**94**
Sclerocarya birrea	Bark	3	17	23	**90**
Searsia nebulosa	Bark	1	1	1	2
Senecio serratuloides	Whole plant	4	14	0	0
Senegalia burkei	Bark	17	48	3	4
Tabernaemontana elegans	Roots	19	**85**	27	**96**
	Leaves	18	**89**	0	0
Trichilia dregeana	Roots	**75**	**92**	0	0
	Leaves	1	27	1	1
	Bark	20	**100**	25	**79**
Vangueria infausta	Leaves	**67**	**73**	**66**	**95**
Potassium dichromate (positive control)		**100**	**100**	**100**	**100**
Distilled water /2% DMSO (negative control)		1	7	1	8

Bold = toxic

Acalypha villicaulis root is administered twice a day for various menstruation and pregnancy related problems in Maputaland (De Wet and Ngubane, 2014). In this study, both the aqueous and organic extracts of the root of *A. villicaulis* did not show toxic activity in the BSLA (Table 2.3). However, the aqueous extracts of the leaves were toxic with over 90% death of brine shrimp within 24 hrs. The organic extract of the leaves was not toxic. As established from the current toxicity results, the leaves (aqueous extract) of this plant cannot be used to substitute the roots and the roots still remain a safer choice for medicinal purposes. No information could be found on toxicity of *A. villicaulis* in the literature. However, other species of the genus such as *Acalypha wilkesiana* have been studied and was reported to be non-toxic to Wistar albino rats at 3000.00 mg/kg (Olukunle et al., 2015), but have been reported to cause lesions in the kidney and liver tissue (Olukunle et al., 2015). A clinical study on

Acalypha indica revealed effects such as acute hemolysis and methemoglobinemia (Alli et al., 2015).

Acanthospermum glabratum was recorded only once and used for cervical pains during pregnancy (De Wet and Ngubane, 2014). In the current toxicity study *A. glabratum* (both the organic and aqueous extracts) was non-toxic when tested in the BSLA for (Table 2.3). There were no previous studies on the toxicity of the crude extract of this plant. However, the *in vivo* and *in vitro* cytotoxic constituents including melapholides and cis, cis-germacranolides from this plant species has been reported (Lotter et al., 1979; Ashidi et al., 2010). Other metabolites in the crude extract may also impact on the overall toxicity of the plant. When the other species of the same genus *(A. hispidum)* was tested for toxicity, it was also regarded as non-toxic with the LC_{50} >5000.00 mg/kg when tested in the rat model (Chika et al., 2018).

Bridelia cathartica root was the most mentioned (18 times, 36%) plant species for treating various menstrual and pregnancy related problems (De Wet and Ngubane, 2014). When the current toxicity study was conducted, the aqueous root extract of *B. cathartica* demonstrated non-toxicity in the BSLA for the full 48 hrs of exposure. However, the organic extract was non-toxic (2%) in the first 24 hrs and escalated to 89% toxicity after 48 hrs. The leaves of this plant were toxic in both aqueous and organic extracts after 48 hrs. In a previous toxicity study of this plant, 20% aqueous-ethanol extract of the stem-bark of *B. cathartica* has been reported as non-toxic with the LC_{50} of 58.45 µg/ml in the BSLA (Moshi et al., 2004). The American National Cancer Institute considered crude extracts that have LC_{50} <30.00 µg/ml as toxic (Fadeyi et al., 2013). When these results are compared with the current study on the roots and the leaves, it is understandable that the roots are the preferred choice of plant part for medical purposes.

The aqueous extract of *C. filiformis* was not toxic when tested in the BLSA with the percentage mortality of 29% (48 hrs exposure). However, Hoet at el. (2004), reported that the aqueous extract of this plant species contains cytotoxic aporphine alkaloids when tested against the Hela cell line using the MTT assay. These findings focus on isolated compounds, as opposed to the current study, which looks at the crude extract and thus is not directly comparable. It is also possible that while selected compounds are toxic, when combined in the whole extract, toxicity can be reduced due to the

synergistic interaction of compounds. The study conducted by Mythili et al. (2011) tested the aqueous extract of *C. filiformis* in male Wister albino rats, and concluded that an aqueous herbal extract of this plant is less likely to result in severe toxic effects when administered at normal therapeutic doses (Mythili et al., 2011). The organic extracts of *C. filiformis* correlate with the Mythili et al. (2011) and Prayong et al. (2008). Prayong et al. (2008) reported that the ethanol extract of this plant exhibited cytotoxic effects against the HepG2 cell line. This plant was reported in Maputaland as used to induce lactation (De Wet and Ngubane, 2014).

Commiphora neglecta root is used in Maputaland for menstruation and pregnancy problems including infertility (De Wet and Ngubane, 2014). In this study, both aqueous and organic extracts of *C. neglecta* root demonstrated no toxicity. There was no information in the literature about the toxicity of the root extract on this plant species. In the current study, both the aqueous and organic leaf extract of this plant species exhibited toxicity after 48 hrs of exposure. Paraskeva et al. (2008), also reported cytotoxic activity of the leaves against the Graham 293 cell line with an LC_{50} of 11.50 g/ml using the MTT assay. Haemolytic activity was also reported on the leaves of *C. neglecta* (Van Zyl and Viljoen, 2009). Therefore, based on these results, the use of roots is likely to be the safer choice.

The aqueous and organic root extracts of *C. monteiroi* were mostly toxic in the current study (Table 2.3). *Crotalaria monteiroi* was reported by Botha et al. (2012), to have somewhat toxic metabolites such as the pyrlizidine alkaloids. The pyrrolizidine alkaloids were reported to have a pneumotoxic effect when tested in a horse's respiratory system (Botha et al., 2012) and were also associated with hepatotoxicity (Neuman et al., 2015). A long-term consumption of herbal medicine containing pyrrolizidine alkaloids is reported to potentially result in cancer (Neuman et al., 2015). This plant species was only mentioned once in Maputaland for medicinal use to treat menstrual problems such as dysmenorrhoea, menorrhagia, oligomenorrhoea, infertility and to prevent premature birth; and to cleanse the blood when pregnant (De Wet and Ngubane, 2014). The unpopularity of this plant species could be associated with its toxicity. In the current study, both the aqueous and organic extracts of the leaves were non-toxic. Although the current study indicates the leaves as "safe", the previous study on the whole plant has indicated a potential toxicity of this plant

(Botha et al., 2012). Therefore, caution needs to be taken when using this plant species for medicinal purposes.

The aqueous root extract of *C. natalensis* (Table 2.3) was non-toxic in this study, but, the organic extract exhibited toxicity after 48 hrs. The result indicates that the traditional aqueous extraction method may be extracting fewer toxic compounds, and therefore may be safer to use. The shoot (leaves) of this plant species was, however, toxic in both aqueous (74%) and organic extract (96%) after 48 hrs of exposure. The women in Maputaland use the roots to treat menorrhagia. Therefore, roots remain the safer parts of the plant species in this study. No additional toxicity information was found in the literature on *C. natalensis.*

The aqueous root extracts of *D. villosa* demonstrated no toxicity in the BSLA, but the aqueous leaf extract was highly toxic (80% at 48 hrs). However, the organic root extract was toxic (61% at 48 hrs) but the organic leaves extract was non-toxic (28% at 48 hrs). These results shows that the traditional (aqueous preparation) use of the roots is safer than the leaves. The root is traditionally used during menstruation to treat period pains (De Wet and Ngubane, 2014). Therefore, in this case, leaves may not be used as a substitute for the roots. No information could be found in the literature on the toxicity of this plant species.

In this study, the aqueous extract of *E. humeana* roots exhibited toxicity of 92% mortality in BSLA after 48 hrs (Table 2.3). However, the organic extract of the plant species was non-toxic, showing 5% and 49% mortality after 24 and 48 hrs respectively. No information was found in the literature on the toxicity of *E. humeana.* However, the bark of another species of this genus (*Erythrina senegalensis*) was reported to be moderately safe at low doses in short-term use, but produced lesions on the liver and heart tissue after the prolonged use (Udem et al., 2010). The roots of this plant species are medicinally used for illnesses such as bronchitis, tuberculosis (Pillay et al., 2001) dysmenorrhea and infertility (De Wet and Ngubane, 2014). Therefore, this plant species should be used with caution.

The aqueous root extract of *E. natalensis* (Table 2.3) demonstrated minimal toxicity in the BSLA with 2% and 24% percentage mortality of the brine shrimp after 24 and 48 hrs respectively. However, the organic roots extract had a high percentage mortality

(96%). A MTT assay has previously indicated the aqueous root extract of *E. natalensis* as having LC_{50} of 0.20 mg/ml towards the primary vervet monkey kidney cells (Lall et al., 2005[a]). Numerous other studies were found that tested the toxicity of the organic extracts. The current results correlated with the ethanol root extract of *E. natalensis* that was reported by Moshi et al. (2006) to have some degree of toxicity in the BSLA with an LC_{50} of 19.33 µg/ml. Lall et al. (2005[b]), reported that a chloroform root extract had an LC_{50} of 64.87 µg/ml on Vero cells. The root has been reported to contain cytotoxic phytochemicals against selected cancer cells (Kishore et al., 2014). The leaves of *E. natalensis* exhibited toxicity (81% and 87% respectively) to brine shrimp in both aqueous and organic extractions. These results suggest that the leaves may not be an alternative of the roots for medicinal purposes in terms of safety. Cytotoxicity of the ethanol leaf extract against the Vero cell line, had a LC_{50} of 285.10 µg/ml (More et al., 2008) which was less toxic and different from the current results. The solvent used in these tests may have an influence on the range of phytochemicals extracted. In spite of the toxicity reports on this plant species, *E. natalensis* is widely used to treat various diseases such as sexually transmitted infections (Tshikalange et al., 2005) schistosomiasis (Sparg et al., 2000), diabetes (Deutschlander et al., 2009) and as a blood purifier for pregnant women (De Wet and Ngubane, 2014).

The stem extract of *E. tirucalli* was the most toxic extract among all plant species tested (Table 2.3). This plant species demonstrated 100% mortality in BSLA after 24 and 48 hrs of exposure to the aqueous extract and the organic extract exhibited 78% and 90% mortality after 24 and 48 hrs of exposure respectively. This plant species leaks a white latex when cut or wounded. This latex has been tested in numerous studies for toxicity. It was reported to be non-toxic in the embryo development of rat (Silva et al., 2007). However, the majority of the studies reported this plant to have toxic effects in various *in vivo* tests. Kumar et al. (2010) reported the aqueous latex extract as being toxic to cat fish (*Heteropneustes fossilis*) with the LC_{50} of 3.45 µl/L after 24 hrs and 1.31 µl/L after 96 hrs. The latex powder was toxic when it was tested on *Colisa fasciatus* with the LC_{50} of 8.14 mg/L after 24 hrs and LC_{50} of 9.01 on *Channa panctatus* (Kumar et al., 2010). When the latex was tested on *Tillapia zilli*, it also demonstrated toxic effects with LC_{50} of 1.20 mg/L after 96 hrs of exposure (Kumar et al., 2010). The diterpene ester constituent of the latex is reported to promote tumor growth and to also have ocular toxicity (Schlamovitz et al., 2009). A single woman in

Maputaland reported this plant species as used in a concoction to cleanse blood during pregnancy (De Wet and Ngubane, 2014). This plant species was not popular to treat gynaecological conditions in Maputaland, which may relate to it being toxic and previously reported as an abortifacient (Samuelsson et al., 1992).

The *G. livingstonei* root extract was non-toxic in the BSLA (Table 2.3) with 30% and 45% mortality in both aqueous and organic extracts after 48 hrs respectively. The aqueous leaf extract of this plant demonstrated toxicity with 67% mortality (48 hrs) and non-toxicity in the organic extracts (22% mortality) after 48 hrs. No studies were found in the literature on the toxicity of either the roots or leaves of *G. livingstonei.* However, other plant parts have been assessed including the the ethyl acetate extract of the fruit peel, which was reported to be highly toxic with 100% MeWo cell death within 48 hrs (Mulholland et al., 2013). Some of the compounds isolated from the stem bark such as morrelloflavone, morrelloflavone-7''-sulphate and sargaol demonstrated up to 20% cell death, with the highest observed with guttiferone A, which demonstrated approximately 80% cell death at 25.00 µM (Mulholland et al., 2013). The other *G. livingstonei* active xenthone compounds exhibited gastric toxicity which damages the hydrophobic lipid layer of the gastric mucosa (Markiewicz et al., 2017). The roots of this plant species were reported to treat various gynaecology and pregnancy problems in Maputaland (De Wet and Ngubane, 2014).

The root extracts of *G. occidentalis* showed no toxicity (<50%) in this study in both the aqueous and organic extracts. In Maputaland the root is traditionally used by women to treat dysmenorrhoea, menorrhagia, infertility, oligomenorrhoea, premature birth, and for blood purification during pregnancy (De Wet and Ngubane, 2014). However, the leaves demonstrated toxicity (94%) in the aqueous extract after 48 hrs, whereas the organic extract was non-toxic (44%). No previous studies on toxicity were found on the roots, which are traditionally used in this study. However, the current results of the leaves contrast the previous study where the methanol extract of the leaves was reported to have weak cytotoxic effects, which resulted in 17% death of lung carcinoma cells (A-549), 13.5% colon carcinoma cells (HCT-116), 5.7% hepatocellular carcinoma (HepG2) and non-cytotoxic effects (0%) on breast carcinoma (MCF-7) using the MTT assay (Moustafa et al., 2014). The difference in the results could be influenced by the different tests and solvents used in these studies. Regarding the

traditional use (aqueous root extract) of this plant species, the results in this study supports the use of roots instead of leaves as medicine.

The aqueous extracts of the roots and leaves of *G. senegalensis* (Table 2.3) were non-toxic (13% mortality against root and 14% against the leaves) in the BSLA. The methanol dichloromethane extract of both plant parts were also non-toxic (1% mortality in both plant parts). These results correlated to Ahmed et al. (2013), which demonstrated that the acetone leaf extract was non-cytotoxic (LC_{50} of 87.62 µg/ml) against the Vero African green monkey kidney cell line. The results from the current study show that the organic extract of *G. senegalensis* was less toxic than the aqueous extract. Therefore, the leaves of *G. senegalensis* have a potential to substitute for the roots in terms of toxicity, however, efficacy must be evaluated. The roots are used to treat infertility and during pregnancy prevent infantile colic when the baby is born (De Wet and Ngubane, 2014).

The aqueous and organic extract of *H. boraginiflora* roots and leaves (Table 2.3) demonstrated toxicity in the BSLA after 48 hrs. The root demonstrated toxic activity only after 48 hrs in both aqueous and organic extracts whereas the leaves were toxic at both 24 and 48 hrs of exposure. This plant species was only mentioned once in the ethnobotanical study to treat dysmenorrhea and to ease labor and delivery (De Wet and Ngubane, 2014). No other information was found in the literature to verify its medicinal use. To date, no information was found on the toxicity of this plant species in the literature.

Both the aqueous and methanol-dichloromethane extract of *H. coriacea* stem was non-toxic in the BSLA (Table 2.3). The extracts demonstrated percentage mortality below 50% (14% and 38%) after 48 hrs for the aqueous and organic extracts respectively. No information was found in the literature on toxicity of *H. coriacea* stem extracts. However, the other plant parts of this species have been tested for toxicity. The methanol fruit extract was reported to be non-cytotoxic against human colon carcinoma (DLD-1) cells in the MTT assay (El Seoud et al., 2003). The aqueous extract of the root demonstrated toxic activity in the BSLA and the methanol-dichloromethane extract was toxic with percentage mortality of 67% on brine shrimp after 48 hrs of exposure (Ramulondi et al., 2018). The different results to this study maybe due to different plant parts used in these assays, and therefore they are not entirely

comparative. *Hyphaene coriacea* was reported to treat dysmenorrhea, reduce labour pains and to control postpartum bleeding (De Wet and Ngubane, 2014).

The corm extract of *H. hemerocallidea* was non-toxic (40% mortality) in the aqueous extract, but the methanol-dichloromethane extract demonstrated toxicity (66% mortality) after 48 hrs of exposure (Table 2.3). This study corresponds with previous similar studies. The aqueous extract of *H. hemerocallidea* was reported to be non-cytotoxic in the MTT assay against prostate cancer cells (Du-145), breast cancer cells (MDA–MB-23 and MCF-7) and non-malignant breast cancer cells (MCF-12A) (Steenkamp and Gouws, 2006). Non-cytotoxicity of the aqueous extract of *H. hemerocallidea* was reported against the human epithelial cell line (Naidoo et al., 2013). The methanol-dichloromethane (organic) extract of this plant was reported to be toxic (54% mortality) against the brine shrimp (Ramulondi et al., 2018), which corresponds with the current findings. The organic extract was reported to be non-cytotoxic against the human epithelial cell line (Naidoo et al., 2013) in the MTT assay. This variance of the toxicity results could be influenced by the difference in the methods used. The use of *H. hemerocallidea* was reported in association to conditions such as pelvic pains, genital warts, infertility and menorrhagia (De Wet and Ngubane, 2014).

The *K. africana* bark extracts demonstrated non-toxicity against the brine shrimp, with both aqueous (40%) and organic (35%) extracts showing less than 50% mortality after 48 hrs (Table 2.3). These results are in agreement with a previous study by Adoum, (2009), where the bark was non-toxic with the LC_{50} of 1000.00 µg/ml against brine shrimp. Zofou et al. (2011), assessed the cytotoxicity of *K. africana* using the MTT assay. When the bark was tested against the monkey epithelial cells (LLC-MK2), the hexane extract had a cytotoxic concentration (LC_{50}) of 125.00 µg/ml and the ethyl acetate extract of LC_{50} of 125.00 µg/ml and this was considered as non-cytotoxic. The women in Maputaland use *K. africana* during pregnancy to cleanse the blood and after delivery to induce lactation. It was also reported to treat dysmenorrhea and genital warts (De Wet and Ngubane, 2014).

The aqueous extract of *O. natalitia* roots (Table 2.3) was non-toxic (15% mortality) in the BSLA over a period of 48 hrs. However, the methanol-dichloromethane extract of

the root demonstrated toxicity activity against brine shrimp with 55% mortality after 48 hrs. No information was found in the literature on the toxicity of the root extract of this plant species. The root is traditionally used in Maputaland to treat infertility and menstrual disorders (period pains, oligomenorrhoea and menorrhagia). During pregnancy it is used for blood cleansing, to prevent pre-term birth, to ease labour and minimize complications during delivery (De Wet and Ngubane, 2014). When the leaves were tested, mortality was observed in both aqueous (67%) and organic (68%) extracts. The leaf acetone extract was reported to have cytotoxic activity against Vero kidney cell line LC_{50} of 0.05 mg/ml, Bovine dermis cell line (LC_{50} of 0.07 mg/ml) and C3A liver cell line (LC_{50} of 0.10 mg/ml) (Makhafola et al., 2014). Suleiman et al. (2010), also reported that the leaf acetone extract had high cytotoxicity, resulting in the death of 80% of Vero monkey kidney cells when treated with 1.00 mg/ml of the extract. The leaves seem to have more toxicity than the roots, therefore, the potential to substitute the roots with leaves is not suitable.

The stem extract (aqueous) of *O. stricta* was toxic towards the brine shrimp, with a percentage mortality of 88% after 48 hrs. The toxicity potential of the aqueous extract is concerning as the plant species is used during pregnancy to cleanse blood and to aid in cervical dilation during child birth (De Wet and Ngubane, 2014). However, the methanol-dichloromethane extract was non-toxic, with only 38% mortality after 48 hrs. No studies were found in the literature that have investigated the toxicity of the *O. stricta* stem. As the results indicated high toxicity, this is of concern for the traditional use, and therefore other toxicity assays and different range of concentrations are recommended to determine a safe dose.

The *O. engleri* bark extract was found to be toxic towards brine shrimp in this assay. The aqueous extract resulted in 74% mortality and the organic extract 54% after 48 hrs. A study reported that the bark extract (dichloromethane) was toxic with a LC_{50} of 35.00 µg/ml (Prozesky et al., 2001). In another study, the aqueous extract of the roots was found to be non-toxic against brine shrimp with a mortality of 7% over 48 hrs and the methanol-dichloromethane extract resulted in a mortality of 58% (Ramulondi et al., 2018). The differences in these results occur because of different plant parts being tested. The previous study focused on the roots, while the current study focused on the bark. It is known that different plant parts can yield different

pharmacological activities (Raya et al., 2015; Altemimi et al., 2017). The bark of *O. engleri* is used in Maputaland to treat period pains, irregular menses, excessive bleeding, infertility, after-birth pains, to prevent preterm birth, and also used for blood purification (De Wet and Ngubane, 2014).

The root extract of *P. africanum* was non-toxic in this assay. The mortality percentage values were 49% (aqueous) and 28% (organic) after a maximum period of 48 hrs. The value of the aqueous root extract was very close to the borderline value to be toxic (50%). A previous study on the toxicity of this plant reported that the roots were non-toxic in the BSLA as well as when tested against the Vero monkey cell line (Naidoo et al., 2013; Madikizela et al., 2017). The root of *P. africanum* is used in Maputaland for period pains, heavy bleeding during menstruation and blood purification during pregnancy (De Wet and Ngubane, 2014). The aqueous leaf extract of this plant species was also non-toxic, but the organic extract had a mortality percentage of 53% which is classified as toxic. No studies were found in the literature to verify the current results found for the toxicity levels in the leaves.

After a maximum duration of 48 hrs, both organic and aqueous extracts of *R. multifidus* were non-toxic against brine shrimp, with percentage mortalities of 15% and 34%, respectively. A study by Naidoo et al. (2013), reported that *R. multifidus* was non-toxic against the human kidney epithelial cells in the MTT assay, which corresponds with the current results. There were no other studies found in the literature with the toxicological information of this plant species. It is a popular medicinal plant species used (reported 13 times, 26%) among the women in Maputaland for the treatment of genital warts (De Wet and Ngubane, 2014).

The aqueous root extract of *R. digitata* was non-toxic in the BSLA with a mortality of 43% after 48 hrs. However, the methanol dichloromethane extract was toxic by killing 80% of brine shrimp in the assay after 48 hrs exposure. The roots of this plant species are used in Maputaland for various medical conditions specifically related to the female conditions such as infertility, menstrual disorders (period pains, amenorrhoea, oligomenorrhoea and menorrhagia) and during pregnancy as a blood purifier and to prevent pre-term birth (De Wet and Ngubane, 2014). The leaves of *R. digitata* demonstrated toxic effects in both the aqueous (51%) and organic (56%) extracts after 48 hrs. No previous documentation was found on the toxicity of this plant species. The

traditional use of the plant species which is the aqueous root appears to be safe for lay people, as found in the current study.

Sapium integerrimum is traditionally used in Maputaland to treat menstrual pains (De Wet and Ngubane, 2014). The aqueous root extract of *S. integerrimum* presented no toxicity in the BSLA, with a percentage mortality of 8% after 48 hrs. However, the organic extract of the roots was highly toxic by killing all (100%) of the brine shrimp in the assay after 48 hrs. The leaves of this plant species also demonstrated a high toxic activity. The aqueous leaf extract resulted to 93% mortality of the brine shrimp and the organic extract killed 93% of brine shrimp after a duration of 48 hrs. No studies were found in the literature on the toxicity of this plant species. However, according to the current results, the traditional use (aqueous extract) of this plant species is non-toxic and the roots cannot be substituted by the leaves as the leaves demonstrated higher toxicity than the roots.

The aqueous extract of *S. puniceus* showed 50% toxicity towards the brine shrimp after 24 hrs and this increased to 93% after 48 hrs. The organic extracts also resulted in the death of 94% of the brine shrimp after 48 hrs. This plant species has demonstrated toxicity in numerous other studies. It was reported by Nair and Van Staden, (2013) that *S. puniceus* extract contains alkaloids such as haemanthamine, haemanthidine and 6-hydroxycrinamine, that are cytotoxic. These compounds are capable of causing adverse effects on the central nervous system, on visual impairment, and may even be fatal (Nair and Van Staden, 2013). A BSLA study by Coe et al. (2010) also reported toxicity for this plant species supporting the current findings. In spite of the toxic nature of this plant species, it is one of the widely used medicinal plants for various ailments such as cleansing blood during pregnancy and ensuring safe delivery of the baby during birth (Ndhlala et al., 2013; De Wet and Ngubane, 2014). The concern is that the concoction of this plant species is supposed to be taken daily during pregnancy, which increases the exposure to the toxins (De Wet and Ngubane, 2014).

The aqueous extract of *S. birrea* bark demonstrated non-toxicity in the BSLA, with a mortality percentage of 17% after 48 hrs. There was no previous study found in the literature that was done using BSLA. However, when the extract was tested using the MTT assay on human embryonic kidney epithelial cell line (Graham HEK-293), it was

found to be non-cytotoxic (Naidoo et al., 2013). In the current study, a percentage mortality of 90% (highly toxic) was observed in the organic extract after 48 hrs. Numerous toxicity studies of the organic extract of *S. birrea* have reported a variation in toxicological results. Naidoo et al. (2013) reported that the methanol-dichloromethane extract demonstrated no cytotoxic effect against the human embryonic kidney cell line (Graham HEK-293) in the MTT assay. Sharma and Lall, (2014) reported that the ethanol extract had low cytotoxicity against the mouse melanocytes (B16-F10) with a LC_{50} of 92.07 µg/ml. The methanol extract, however, had an LC_{50} of >200.00 µg/ml against the HepG2 and it was >400 µg/ml against the human dermal fibroblast (Russo et al., 2018). The difference in toxicity results of this plant could be influenced by the different extraction solvents and the assay used. Furthermore, geographical variation could yield variant chemotypes resulting in differences of toxicity levels. Future studies should focus on identifying geographical variability within this species. Nonetheless, *S. birrea* was reported by women in Maputaland as an abortifacient (De Wet and Ngubane, 2014), and concern is warranted regarding possible toxic effects.

Both the organic and aqueous extracts of *S. nebulosa* bark were non-toxic when tested in the BSLA. A brine shrimp mortality of 1% (aqueous extract) and 2% (organic extract) was observed after 48 hrs of exposure. To the best of my knowledge, this is the first report on the toxicity profiles of *S. nebulosa.* The women in Maputaland use *S. nebulosa* daily for infertility and during menstruation for period pains (De Wet and Ngubane, 2014).

Both aqueous and organic extracts of *S. serratuloides* were non-toxic (14% and 0% mortality, respectively) in the BSLA after a maximum duration of 48 hrs. When the same study was conducted by Ramulondi et al. (2018), both aqueous and organic extracts were also reported as non-toxic with the highest mortality of 36% (48 hrs) from the organic extracts. This plant species is used by women in Maputaland to treat genital warts, infertility and pelvic pains (De Wet and Ngubane, 2014).

The aqueous and organic extracts of *S. burkei* bark demonstrated no toxicity in the BSLA at 1.00 mg/ml after 24 and 48 hrs of continuous exposure. No information could be found in literature pertaining the toxicity of this plant species. *Senegalia burkei* was

mentioned once to maintain healthy pregnancy (De Wet and Ngubane, 2014). According to the current study, this plant species is non-toxic. Another plant species of the same genus, *S. nilotica* root, was reported to be safe for a single dose administration >250.00 mg/kg body weight, but potential hepatotoxicity was reported with repeated administration (Alli et al., 2015). Therefore, *S. burkei* may also need to be evaluated for repeated long-term use.

Both the aqueous (85% mortality) and organic root (96% mortality) extracts of *T. elegans* demonstrated high toxicity after 48 hrs. The aqueous leaf extracts also demonstrated toxicity (89% after 48 hrs), but no toxicity was observed for the organic leaf extract after 48 hrs. Lou et al. (2011), reported that the ethanol root extracts were cytotoxic towards the human monocytic THP -1 cells with the LC_{50} <4.00 µg/ml. The acetone leaf extract demonstrated an LC_{50} of 32.35 µg/ml towards the Vero cells (Fouche et al., 2016). *Tabernaemontana elegans* is traditionally used by women to treat dysmenorrhoea, amenorrhoea and infertility (Steenkamp, 2003; De Wet and Ngubane, 2014). The medicinal use of this plant species has therefore been proven to be unsafe, especially when used under unmonitored circumstances such as home-made concoctions.

The aqueous root extract of *T. dregeana* demonstrated high toxic effects when tested in the BSLA. This extract induced death of 75% of the brine shrimp within 24 hrs and up to a total of 92% after 48 hrs. This therefore implies that the roots were toxic in the BSLA. In the ethnobotanical study by De Wet and Ngubane, (2014), the roots were reported to be used as an enema to treat infertility. When the stem-bark aqueous extract was tested, the results after 24 hrs demonstrated less toxicity (20%). However, this escalated to the extreme toxicity in killing all (100%) the brine shrimp after 48 hrs. The women in Maputaland had frequently mentioned the use of the stem-bark of *T. dregeana* as an abortifacient (De Wet and Ngubane, 2014). When correlating this with the toxicity results, one can see why this plant species was used as an abortifacient. The aqueous leaf extract of *T. dregeana* was also tested for toxicity and the results were non-toxic. This extract had a percentage mortality of 27% and 1% after 48 hrs for the aqueous and organic extracts respectively. Naidoo et al. (2013), also reported that the aqueous leaf extracts of *T*. *dregeana* to be non-cytotoxic when tested against the human embryonic kidney epithelial cells (Graham, Hek-293). The

non-toxicity of the leaves compared to the roots that are traditionally used for medicine, leads to the possibility for substituting the toxic roots with the non-toxic leaves. The leaves are used topically for women to massage their belly during labour to ease the pain (De Wet and Ngubane, 2014).

The aqueous and organic leaf extracts of *V. infausta* were toxic towards the brine shrimp. The aqueous extract demonstrated 73% (aqueous) and 95% (organic) mortality of brine shrimp after 48 hrs of exposure. *Vangueria infausta* was one of the four plant species in this study which demonstrated toxicity at both exposure periods (24 and 48 hrs) and both extracts (aqueous and organic). *Vangueria infausta* is used for various female related medicinal treatments such as dysmenorrhoea, infertility, menorrhagia, labour induction and antepartum haemorrhage (Steenkamp, 2003; Bruschi et al., 2011; De Wet and Ngubane, 2014). Therefore, the toxic impact is alarming and it suggests that the medicinal use of this plant species must be taken with caution. However, a previous study by Ramulondi et al. (2018), reported that the leaf extract of *V. infausta* to be non-toxic with mortality percentage of 5% and 26% for aqueous and organic extracts respectively using BSLA. Discrepancy, of the results could be associated to the variation of the individual plant chemotype. This variation usually causes the individuals of the species or sub-species to have differences in the quality and quantity of chemical components as a result of differences in genetic expression (Polatoglu, 2013). These differences may also be due to environmental factors. Different plants of the same species can have different phytochemical profiles when grown in different areas.

2.3.2 Overview on toxicity of plant extracts specified by lay people towards the brine shrimp larvae

Overall, among the 35 aqueous extracts studied, 14 aqueous extracts were found to have toxicities in the BSLA. Seven of these extracts (*C. monteiroi*, *E. tirucalli, O. stricta, O. engleri, S. puniceus, T. dregeana* and *V. infausta*) exhibited signs of toxicity within 24 hrs. The highest mortality observed after 48 hrs was 100% with *E. tirucalli* stem (100%) and *T. dregeana* bark (100%) extracts. There were no toxicity reports on *T. dregeana* bark to support the current results. The other aqueous extracts that demonstrated high mortality >80% after 48 hrs were *E. humeana* root, *H. boraginiflora* roots, *O. stricta* stem, *S. puniceus* bulb, *T. elegans* root and *T. dregeana* root. In this

study, the toxicity of the aqueous extracts is more important because this extraction method is traditionally used by the lay people. The organic extracts of the same plant species demonstrated 16% toxicity in the first 24 hrs and this increased to 55% after 48 hrs. In the first 24 hrs the lowest mortality value was 0%, and that was observed in nine different extracts, however, the highest value was 78% and it was obtained from *E. tirucalli* extract. After 48 hrs the highest mortality was 100%, which was obtained from *S. integerrimum* root extract. It is often expected that organic extracts will have a higher rate of toxicity because of its ability to extract both polar and non-polar compounds when compared to the aqueous extracts. This is also true for the plant species tested in this study. Overall, the organic extracts were more toxic than aqueous extracts. The organic plant extract demonstrating the highest toxicity (>80%) at both 24 and 48 hrs was only *E. tirucalli,* while the extracts that demonstrating the least toxicity (≤ 10%) were *G. senegalensis* (both roots and leaves), *S. nebulosa* (bark) *S. serratuloides* (whole plant), *S. burkei* (bark)*, T. elegans* (leaves) and *T. elegans* (all plant parts tested).

2.3.3 Comparative toxicity between different plant species parts

In Table 2.4, the outcome of the toxicity study of 16 leaf extracts (potential root substituents) are presented alongside their respective roots for comparison. Fourteen of these leaf extracts demonstrated toxicity in the BSLA. Eight of these leaf extracts (*B. cathartica, C. neglecta, C. natalensis, E. natalensis, H. boraginiflora, O. natalitia, R. digitata and S. integerrimum*) were toxic in both aqueous and organic extracts. The other six (*A. villicaulis, D. villosa, G. livingstonei, G. occidentalis, P. africanum and T. elegans*) were toxic in either aqueous or organic extract (with five of them showing toxicity only in aqueous extracts except for *P. africanum,* which demonstrated toxicity only in organic extract). It was established that these leaves have limited potential to substitute the roots as the respective roots were mostly non-toxic. However, *H. boraginiflora* demonstrated toxicity with both leaves and roots when testing both in the aqueous and organic extracts. Only two leaf extracts were non-toxic in the current study. The leaf extracts which demonstrated a potential substitute were *C. monteiroi* and *G. senegalensis.* These leaves were non-toxic in both aqueous and organic extracts. Interestingly, the roots of *C. monteiroi* had demonstrated toxicity in both aqueous and organic extracts in this study. Therefore, the leaves may be a better

substitute, should the leaf be verified as having the same medicinal efficacies. In spite of *G. senegalensis* root also being non-toxic, leaves can be a better alternative for safeguarding the trees. The high toxicity rate of the leaf extracts overrides the hyphothesis that the leaves from these plants can possibly be used to substitute for the roots.

Table 2.4 Summary of the toxicity of the roots versus the leaves

Plant names	Plant part used	Toxicity of aqueous extracts	Toxicity of organic extracts
Acalypha villicaulis	Roots	Non-toxic	Non-toxic
	Leaves	**Toxic**	Non-toxic
Bridelia cathartica	Roots	Non-toxic	Toxic
	Leaves	**Toxic**	**Toxic**
Commiphora neglecta	Roots	Non-toxic	Non-toxic
	Leaves	**Toxic**	**Toxic**
Crotalaria monteiroi	Roots	Toxic	Toxic
	Leaves	Non-toxic	Non-toxic
Cyperus natalensis	Roots	Non-toxic	Toxic
	Leaves	**Toxic**	**Toxic**
Diospyros villosa	Roots	Non-toxic	Toxic
	Leaves	**Toxic**	Non-toxic
Euclea natalensis	Roots	Non-toxic	Toxic
	Leaves	**Toxic**	**Toxic**
Garcinia livingstonei	Roots	Non-toxic	Non-toxic
	Leaves	**Toxic**	Non-toxic
Grewia occidentalis	Roots	Non-toxic	Non-toxic
	Leaves	**Toxic**	Non-toxic
Gymnosporia senegalensis	Roots	Non-toxic	Non-toxic
	Leaves	Non-toxic	Non-toxic
Hermannia boraginiflora	Roots	Toxic	Toxic
	Leaves	**Toxic**	**Toxic**
Ochna natalitia	Roots	Non-toxic	Toxic
	Leaves	**Toxic**	**Toxic**
Peltophorum africanum	Roots	Non-toxic	Non-toxic
	Leaves	Non-toxic	**Toxic**
Rhoicissus digitata	Roots	Non-toxic	Toxic
	Leaves	**Toxic**	**Toxic**
Sapium integerrimum	Roots	Non-toxic	Toxic
	Leaves	**Toxic**	**Toxic**
Tabernaemontana elegans	Roots	Toxic	Toxic
	Leaves	**Toxic**	Non-toxic

Bold = Toxic parts not specified by lay people

2.3.4 Dose response on plant extracts

When the aqueous extracts were tested for toxicity at a concentration of 1.00 mg/ml, a total of 24 extracts were toxic. Among the toxic extracts, 11 plant extracts formed a major component of traditional medicinal usage for women in Maputaland. The other 13 extracts were the leaves, which were initially tested for comparison to their respective roots for potential substitution. Therefore, these toxic extracts were further analysed at different concentrations to determine whether their toxicity is concentration dependant and at what concentration the plant species could be used without any toxic effects. The results of the aqueous extracts were toxic are given in Figures 2.4 to 2.7. Organic extracts are presented in Figures 2.8 to 2.11. The negative control used was distilled water for aqueous extracts and a 2% DMSO for organic extracts. The positive control was potassium dichromate. The results from the controls are not displayed in the figures for the sake of brevity. However, they responded as expected and correlate with control values in Table 2.3.

The results of the aqueous extracts (dose response) are divided into two groups (the plant parts specified by lay people and the leaves for potential substitution) and these were further sub-divided into four figures. Figure 2.4 present the aqueous extracts specified by lay people which had demonstrated toxicity at both 24 and 48 hrs and Figure 2.5 presenting only the ones toxic only after 48 hrs. Figure 2.6 and 2.7 were used to represent the aqueous leaf extracts (potential substituents) in similar divisions as described for the extracts specified by lay people.

All the aqueous extracts presented in Figure 2.4 demonstrated an expected dose response. Toxicity of the aqueous extracts was proportional to the concentration of the extract at both 24 and 48 hrs. It was also noted that acceptable toxic concentrations (ATCs) were higher after 24 hrs and lower after 48 hrs. This indicated that the period of exposure affected the safety of the plant species samples against the brine shrimp. The ATC can be defined as the toxic concentration that intercepts the dotted red line separating toxic (>50%) from non-toxic (<50%) values. The best response observed after 24 hrs was among the five plant extracts that has the ATC between 0.50 and 0.94 mg/ml, and those were *C. monteiroi, O. engleri, S. puniceus, T. dregeana* and *V. infausta. Euphorbia tirucalli* and *O. stricta* demonstrated lower ATC below 0.35 and

0.25 mg/ml, respectively. After 48 hrs, the best results decrease and ranged between 0.48 and 0.44 mg/ml. The extract that demonstrated the most toxicity was *E. tirucalli* (ATC of 0.15 mg/ml). This is linked to this extract being the most toxic (100%) at 1.00 mg/ml at both 24 and 48 hrs.

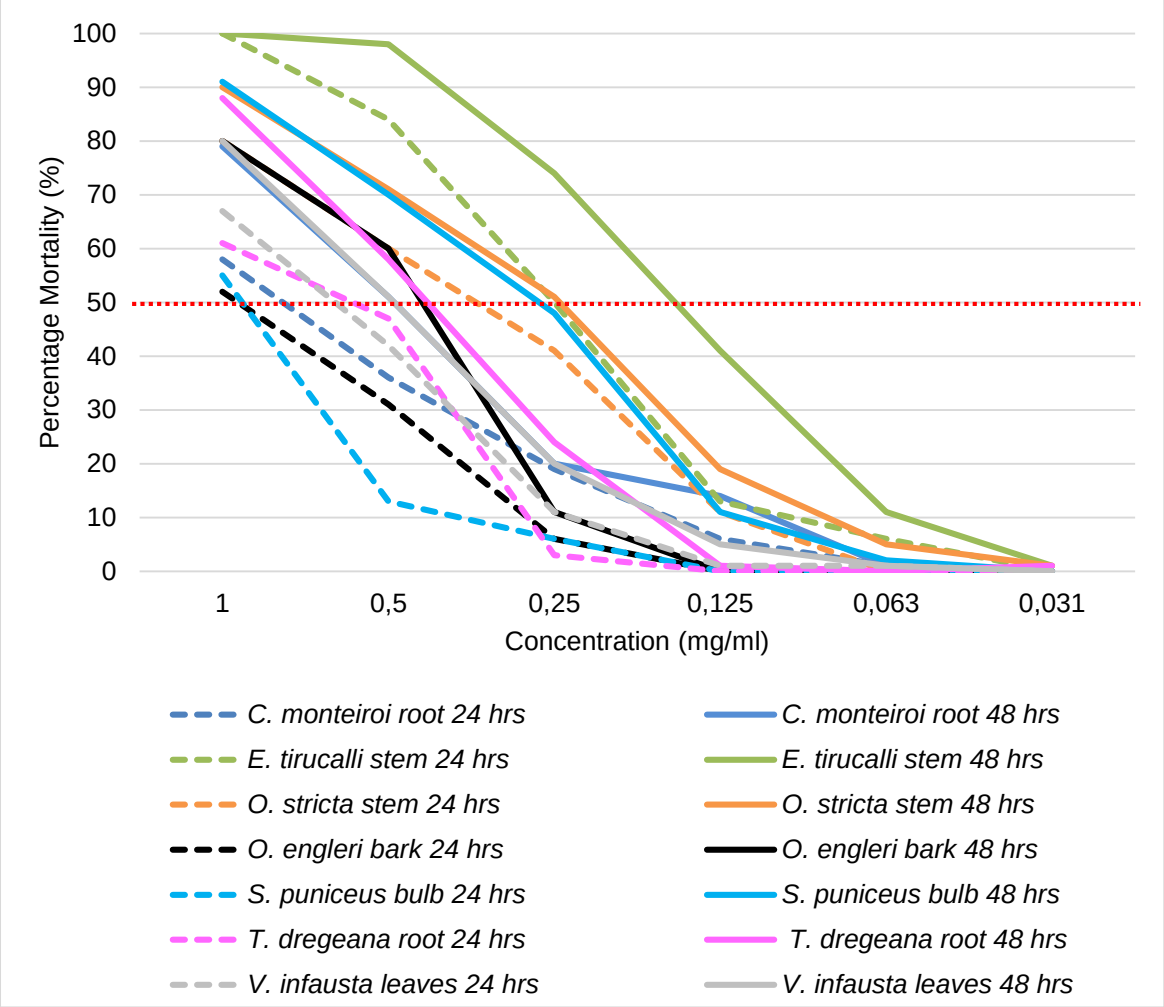

Figure 2.4 Dose response of aqueous extracts that were toxic at 1.00 mg/ml (at both 24 and 48 hrs) against brine shrimp. Dotted red line delineates toxicity (>50%) from non-toxicity (<50%).

Extracts that had demonstrated toxicity only after 48 hrs are presented in Figure 2.5. An overall response to the dilutions demonstrated that the percentage mortality of brine shrimp decreased with the decrease in extract concentration. Three of the four extracts demonstrated an ATC between 0.50 and 0.98 mg/ml. The extract that appeared to be more toxic than others was *H. boraginiflora* root, with the ATC of

0.34 mg/ml. This plant extract had also demonstrated high mortality of 98% at 1.00 mg/ml and demonstrated the least favourable toxic profile in this group. The best results were observed with *T. dregeana* bark having an ATC of 0.98 mg/ml.

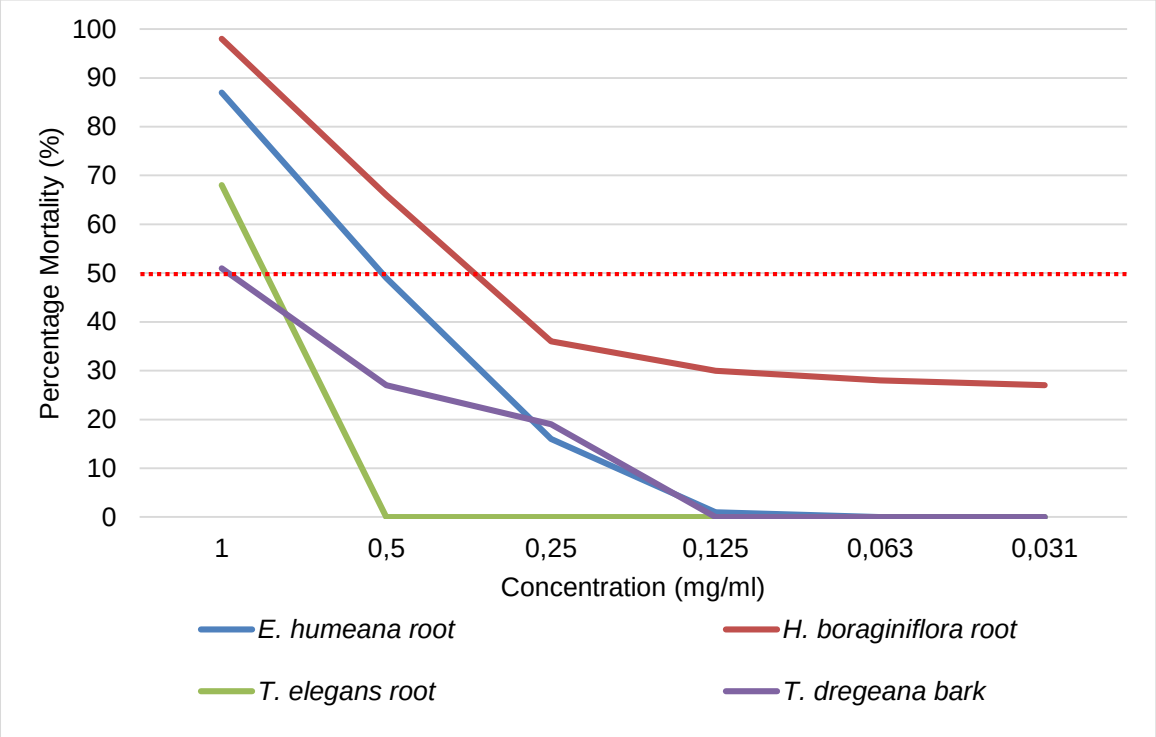

Figure 2.5 Dose response of aqueous extracts that were toxic at 1.00 mg/ml (after 48 hrs) against brine shrimp. Dotted red line delineates toxicity (>50%) from non-toxicity (<50%).

All the aqueous leaf extracts (potential substituents) that demonstrated toxicity at both 24 and 48 hrs are presented in Figure 2.6. After a 24 hrs period, the ATC ranged between 0.41 mg/ml (*H. boraginiflora)* and 0.92 mg/ml (*D. villosa*) across all the leaf extracts. However, after 48 hrs the ATC ranged between 0.25 mg/ml (*H. boraginiflora*) and 0.50 mg/ml (*D. villosa).* Some of the leaf extracts that were only toxic after 48 hrs at 1.00 mg/ml were studied in different dose concentrations and the results are presented in Figure 2.7. The lowest ATC observed with these extracts was 0.33 mg/ml from *C. neglecta*. This plant had demonstrated the highest mortality (100%) when it was tested at 1.00 mg/ml. The organic extracts were also diluted to provide a dose response to brine shrimp and were likewise divided into plant parts used traditionally and the leaves which were considered as substitutes for root material. Each was then

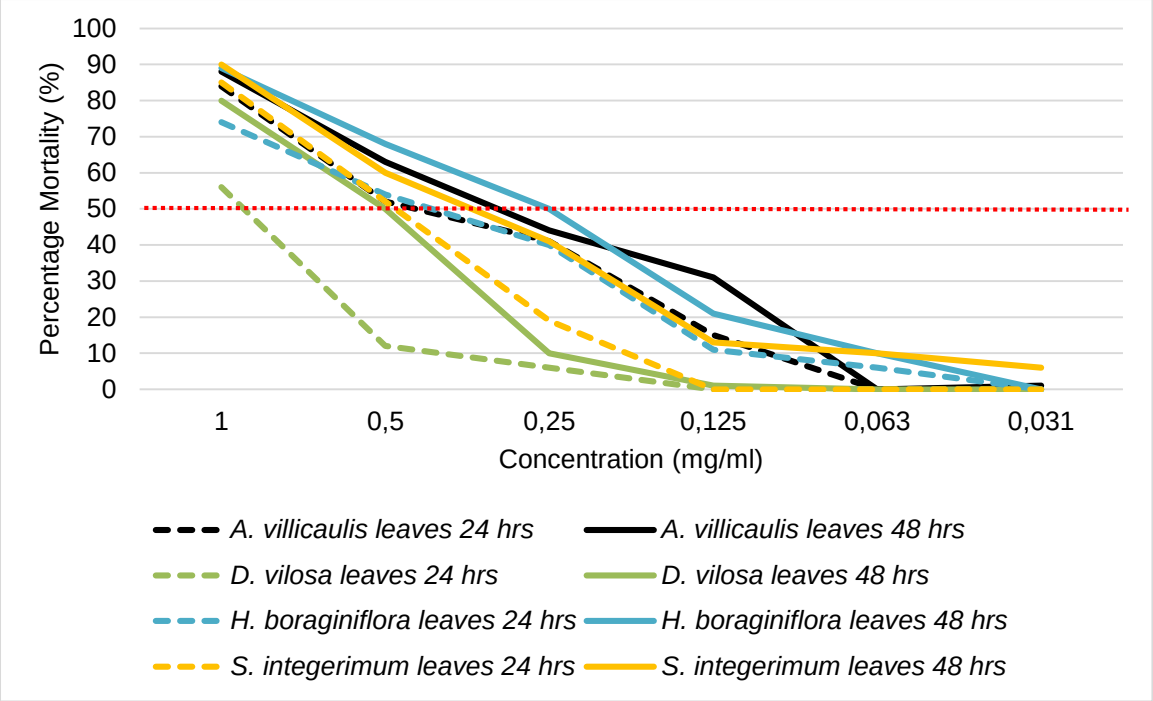

Figure 2.6 Dose response of aqueous leaf extract (potential substituents) that were toxic at 1.00 mg/ml (at both 24 and 48 hrs) against brine shrimp. Dotted red line delineates toxicity (>50%) from non-toxicity (<50%).

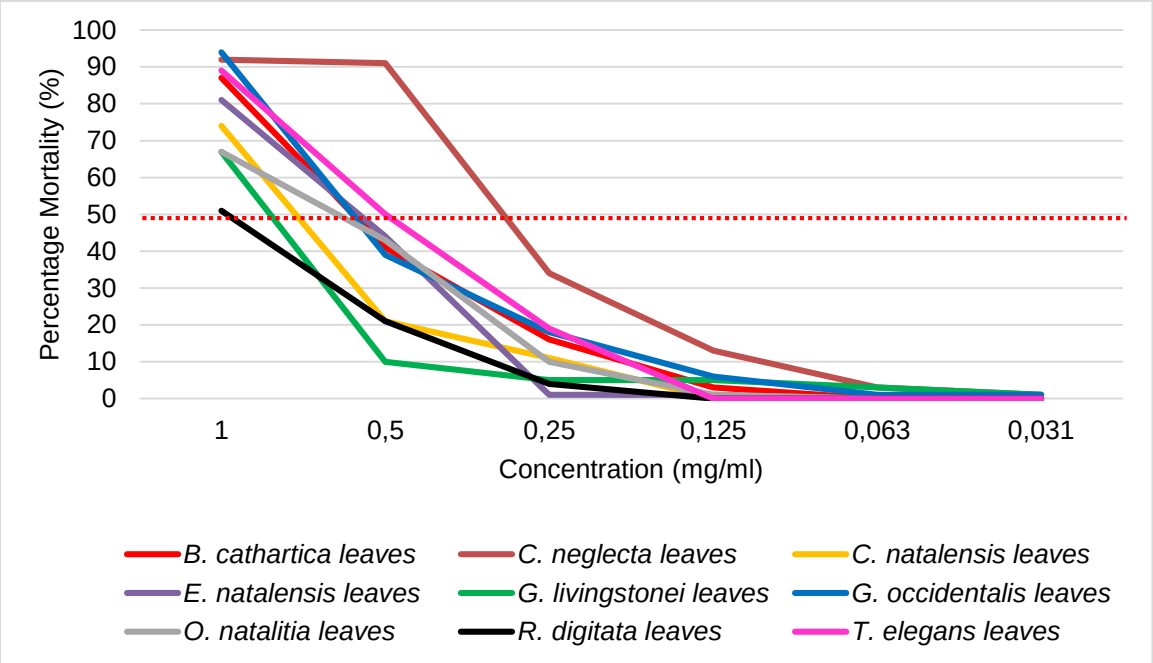

Figure 2.7 Dose response of aqueous leaf extract (substituents) that were toxic at 1.00 mg/ml (after 48 hrs) against brine shrimp. Dotted red line delineates toxicity (>50%) from non-toxicity (<50%).

subdivided into extracts that were toxic at 24 and 48 hrs (Figure 2.8 and 2.10), and those toxic only after 48 hrs (Figure 2.9 and 2.11).

The organic extracts that were toxic at both 24 and 48 hrs (Figure 2.8), indicated a decrease in toxicity with a decrease in concentration against brine shrimp as expected. However, *S. integerrimum* was toxic at all concentrations after 48 hrs, with a mortality percentage of 61% at the lowest concentration. *Sapium integerrimum* (roots) did not show toxicity with the aqueous extract. However, the methanol dichloromethane extract in this case demonstrated a notable impact a solvent may have during extraction. Clearly more or different toxic compounds were extracted during the process of extraction.

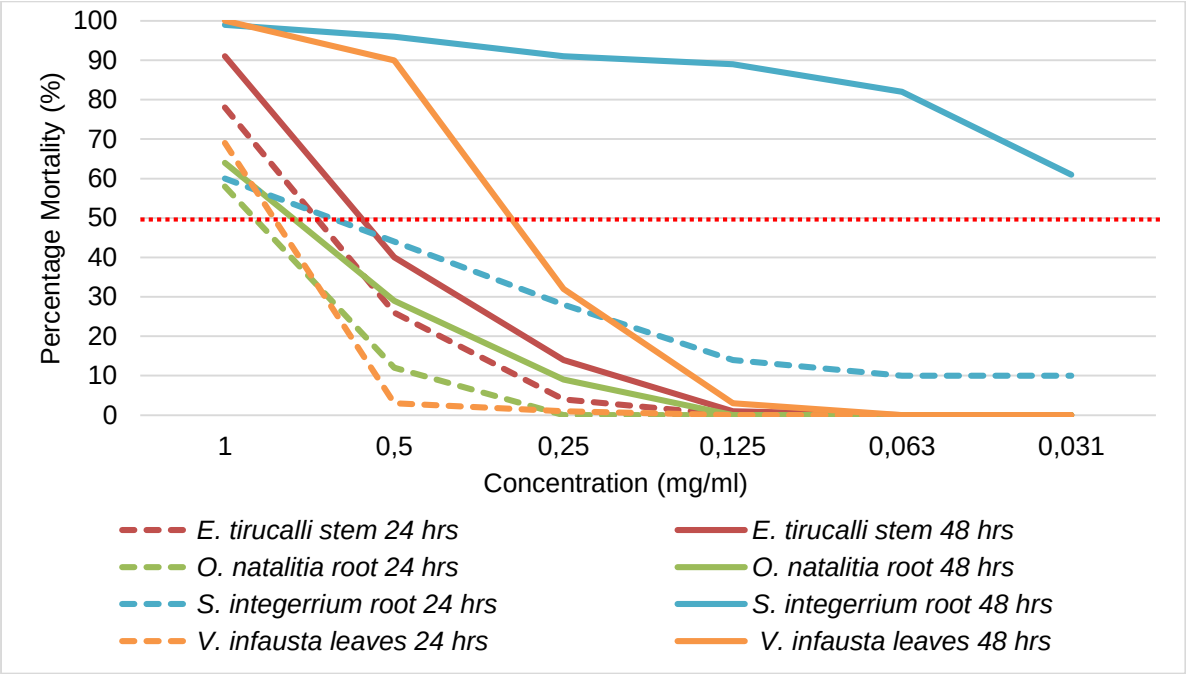

Figure 2.8 Dose response of organic extracts that were toxic at 1.00 mg/ml (at both 24 and 48 hrs) against brine shrimp. Dotted red line delineates toxicity (>50%) from non-toxicity (<50%).

In Figure 2.9, 14 organic extracts that demonstrated toxicity only after 48 hrs at 1.00 mg/ml were studied. The lowest ATC was observed with the *E. natalensis* roots extract at 0.12 mg/ml and the least toxic (highest ATC) was *H. hemerocallidea* with the highest ATC of 0.80 mg/ml. However, three extracts remained toxic throughout the dilution

process and therefore there was no ATC. Those extracts were *S. puniceus* bulb (by 66% mortality), *H. boraginiflora* root (by 65%) and *T. elegans* root (by 58%), demonstrating toxicity even at the lowest concentration tested.

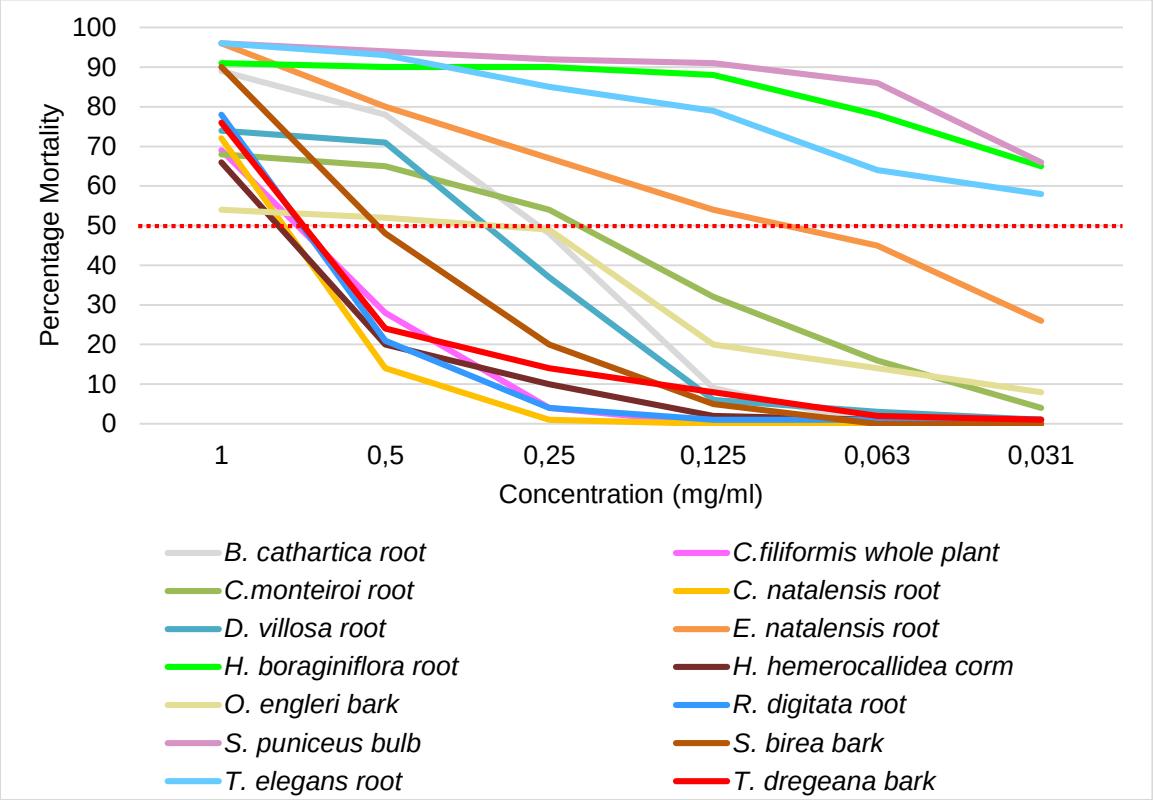

Figure 2.9 Dose response of organic extracts that were toxic at 1.00 mg/ml (after 48 hrs) against brine shrimp. Dotted red line delineates toxicity (>50%) from non-toxicity (<50%).

Figure 2.10 shows the dose response of the organic leaf extracts that were toxic at 1.00 mg/ml (at both 24 and 48 hrs). The dose response was as expected, although it was observed with *H. boraginiflora* after 48 hrs (ATC at 0.10 mg/ml) that the ATC was much lower than the rest of the extracts. This was the lowest ATC in this study. *Hermannia boraginiflora* was initially (at 1.00 mg/ml) indicated to be toxic in both aqueous and organic extracts, which may be the reason it was only mentioned once for medicinal use (De Wet and Ngubane, 2014). The overall dose response of the extracts against the brine shrimp indicated that aqueous extracts had higher ATCs (less toxic at higher concentrations) than the organic extracts.

In Figure 2.11 the dose response of the organic leaf extracts that were toxic at 1.00 mg/ml after 48 hrs were further studied at different concentrations. The ATCs of all six extracts was determined. The best result (highest ATC) was found with *P. africanum* at 0.94 mg/ml and the lowest ATC was 0.22 with *B. cathartica.*

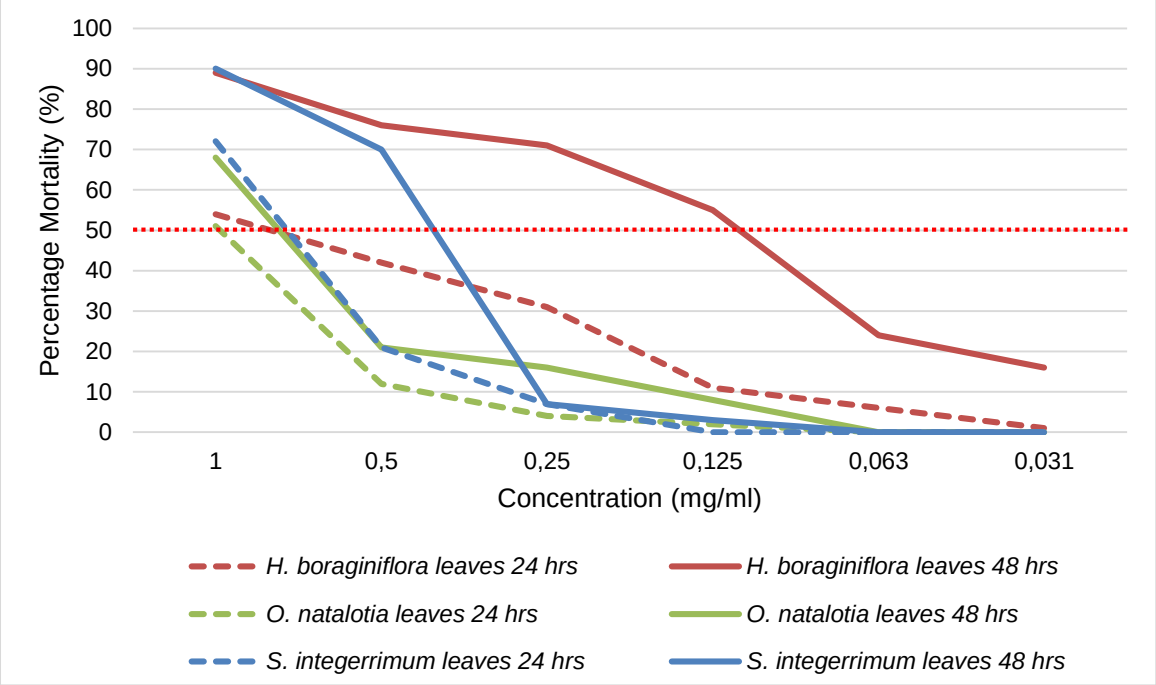

Figure 2.10 Dose response of organic leaf extract (substituents) that were toxic at 1.00 mg/ml (at both 24 and 48 hrs) against brine shrimp. Dotted red line delineates toxicity (>50%) from non-toxicity (<50%).

Table 2.5 is a summary of the ATC for all samples that demonstrated toxicity when initially tested at 1.00 mg/ml and further diluted as per Figures 2.4 to 2.11. The ATCs presented reflect the results recorded after the maximum exposure period (48 hrs) for this study. The duration of 48 hrs was selected as it would indicate the most appropriate results, considering that it is unknown for how long these extracts may remain active in the system after consumption.

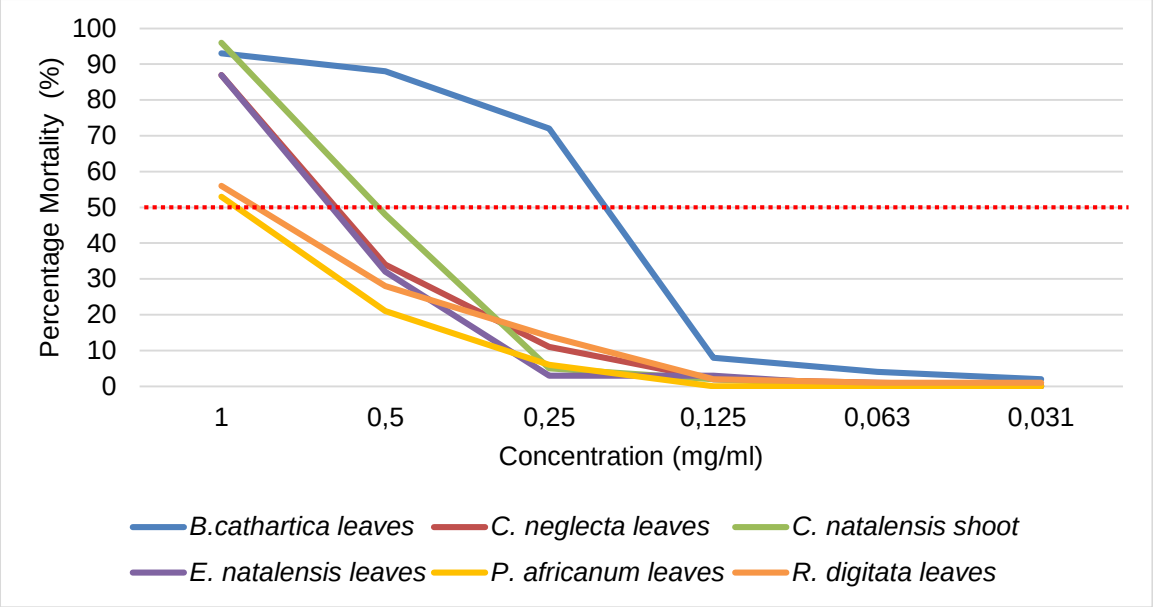

Figure 2.11 Dose response of organic leaf extract (substituents) that were toxic at 1.00 mg/ml (after 48 hrs) against brine shrimp. Dotted red line delineates toxicity (>50%) from non-toxicity (<50%).

Table 2.5 The acceptable toxic concentration of aqueous and organic extracts when tested in varied concentrations after 48 hrs

Plant scientific name	Toxic plant part	ATC (mg/ml)	
		Aqueous	Organic
A. villicaulis	Leaves	0.34	—
B. cathartica	Roots	—	0.26
	Leaves	0.57	0.22
C. filiformis	Whole plant	—	0.72
C. neglecta	Leaves	0.74	0.65
C. monteiroi	Roots	0.48	0.23
C. natalensis	Roots	—	0.80
	Shoot	0.78	0.60
D. villosa	Roots	—	0.35
	Leaves	0.50	—
E. humeana	Roots	0.50	—

Plant scientific name	Toxic plant part	ATC (mg/ml)	
		Aqueous	Organic
E. natalensis	Roots	—	0.12
	Leaves	0.75	0.65
E. tirucalli L.	Stem	0.5	0.58
G. livingstonei	Leaves	0.89	—
G. occidentalis	Leaves	0.58	—
H. boraginiflora	Roots	0.34	Toxic
	Leaves	0.25	0.10
H. hemerocallidea	Corm	—	0.80
O. natalitia	Roots	—	0.75
	Leaves	0.64	0.83
O. stricta	Stem	0.25	—
O. engleri	Bark	0.45	0.45
P. africanum	Leaves	—	0.94
R. digitata	Roots	—	0.71
	Leaves	0.98	0.85
S. integerrimum	Roots	—	Toxic
	Leaves	0.38	0.43
S. puniceus	Bulb	0.30	Toxic
S. birrea	Bark	—	0.53
T. elegans	roots	0.83	Toxic
	Leaves	0.50	—
T. dregeana	Roots	0.44	—
	Bark	0.89	0.70
V. infausta	Leaves	0.48	0.34

— = Non-toxic at 1.00 mg/ml, therefore no further dose response was determined

Four of the organic extracts (*H. boraginiflora* root*, S. integerrimum* root, *S. puniceus* bulb and *T. elegans* root) remained toxic in all concentrations*.* These extracts had initially indicated mortality of between 91% and 100% towards brine shrimp at 1.00 mg/ml concentration. However, at 0.031 mg/ml the mortality decreased and

ranged between 58% and 66%, with *T. elegans* showing the lowest mortality (58%). Reducing the dose of toxic extracts is of importance to determine a safe concentration. Although there are no previous studies on the dose response of the plant species extracts used in this study, the response correlated with numerous previous studies, which indicated similar responses to concentrations in toxicity studies (Ali et al., 2011; Apu et al., 2013; Abesede et al., 2015; Kolbeck and Tintjer, 2016; Ahmed et al., 2018*).* The same response was also observed with some medicinal plant species (*C. roceus* and *Citrus limon* (L.) Burm.) from Maputaland (Ramulondi et al., 2018).

2.4 Summary

- In this study, a total of 51 aqueous and methanol dichloromethane plant extracts were tested for toxicity using the BSLA assay at 1.00 mg/ml. Extracts that demonstrated toxicity were diluted further to determine a dose response concentration.
- The toxicity of the extracts increased when the exposure time to brine shrimp increased from 24 hrs to 48 hrs.
- The methanol-dichloromethane extracts were generally more toxic than the aqueous extracts.
- Thirteen of the 35 medicinally mentioned aqueous extracts were toxic with the highest mortality (100%) observed with *E. tirucalli* at both 24 and 48 hrs. This plant species was reported only once and used regularly during pregnancy to cleanse the blood.
- Only three aqueous leaf extracts were non-toxic (*C. monteiroi, G. senegalensis* and *P. africanum*) when tested for potential in substituting for the roots, therefore substitution with leaves are generally not suitable if toxicity is the only biological parameter considered.
- Nineteen of the 35 organic extracts demonstrated toxic activity in the BSLA, with the highest mortality of 100% observed with *S. integerrimum.* This plant species was reported once to treat dysmenorrhea.
- All toxic aqueous and organic extracts demonstrated a decrease in toxicity when concentrations were decreased and ATCs were attained.
- The organic extracts of *H. boraginiflora* root, *S. puniceus* bulb*, S. integerrimum* and *T. elegans* root remained toxic even at the lowest concentration (0.031 mg/ml)

tested. These plant species were unpopular for medicinal use by women in Maputaland as they were only reported once during the survey.

CHAPTER 3

Toxicity evaluation of plant species combinations using the brine shrimp lethality assay

3.1 Introduction

The plant species combinations to be evaluated in this study were reported by women in Maputaland for treating gynaecology and obstetrics problems (De Wet and Ngubane, 2014). Plant combinations in this study referred to two or more medicinal plant species that are mixed together to form a medicinal concoction based on the knowledge given by the women in Maputaland (De Wet and Ngubane, 2014).

The BSLA can preliminarily screen for toxicity of the selected plant species combinations as explained in Chapter 2, Section 2.1. The results from this study can give a good indication on the toxicity when plant species are combined. The women in Maputaland prepare these concoctions using water. Therefore, in this study, the aqueous extracts of the combinations were examined for toxicity. The organic (methanol-dichloromethane) combinations were also tested for comparison purposes. The plant species combination is then compared to the toxicity of the individual plant species (Chapter 2) using the sum of fractional inhibitory concentration (ΣFIC) calculation.

3.2 Materials and methods

The plant materials used in this assay were collected in the four areas of northern Maputaland as mentioned in Chapter 2 (Section 2.2.1). This study was performed using 16 different plant combinations encompassing a total of 25 different plant species (Table 3.1). The aqueous and organic extracts were initially prepared from the plant materials independently (Chapter 2, Section 2.2.2), to be later incorporated into the various combinations. Plant combinations incorporated two to a maximum of seven plant species within a combination, which were mixed in equal ratios depending on the number of plant species to be incorporated (see Figure 3.1). For the toxicity assessment (BSLA), the method explained in Chapter 2 (Section 2.2.4 to 2.2.6) was followed.

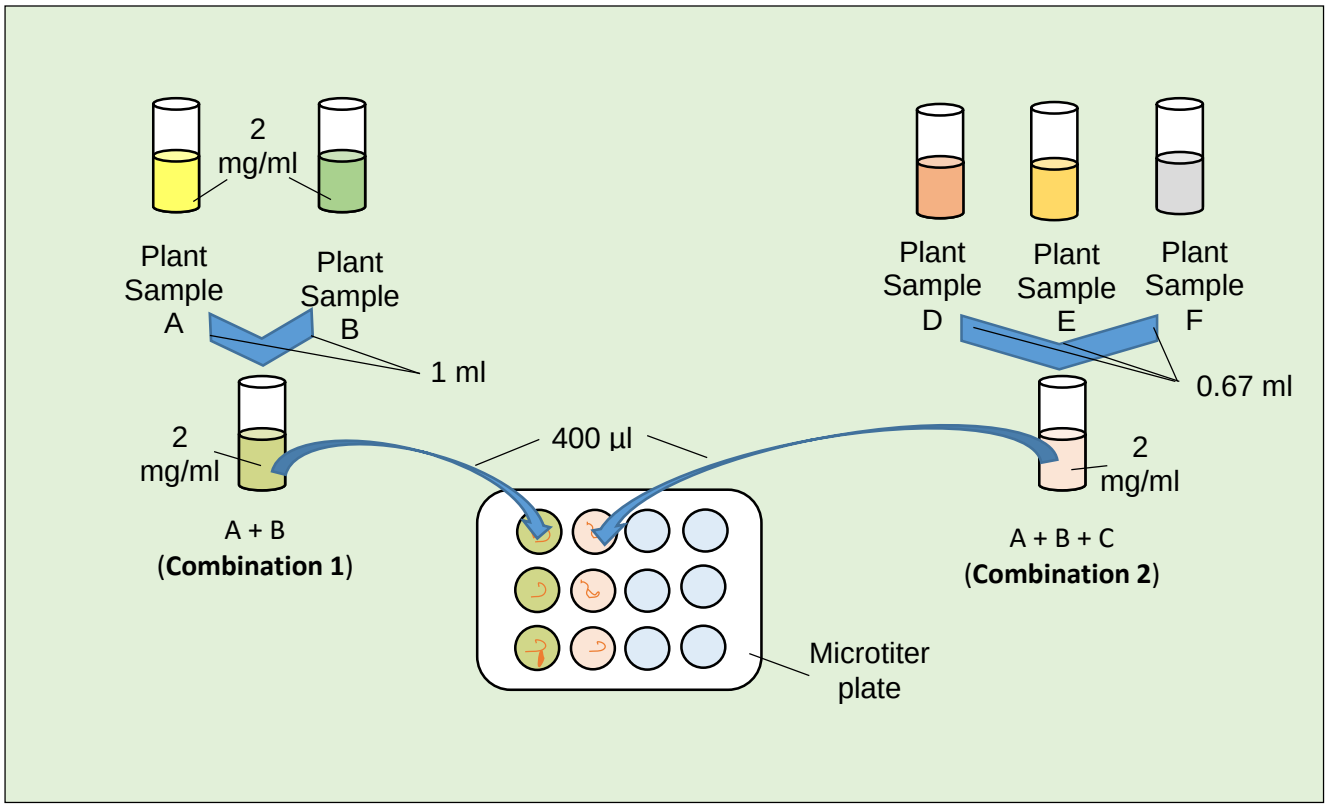

Figure 3.1 A typical example for plant combination preparation.

Table 3.1 Plant combinations that were evaluated and the plant species parts used

Combination of plant species	Combination code
B. cathartica (roots) + *C. neglecta* (roots) + *C. monteiroi* (root) + *G. livingstonei* (root) + *G. occidentalis* (root) + *O. natalitia* (root) + *R. digitata* (root)	AC1 / OC1
E. tirucalli (root) + *O. engleri* (bark) + *S. puniceus* (bulb)+ *S. serratuloides* (whole plant)	AC2 / OC2
B. cathartica (root) + *E. humeana* (root) + *O. natalitia* (root) + *T. elegans* (root) + *S. nebulosa* (bark)	AC3 / OC3
A. villicaulis (root) + *B. cathartica* (root) + *S. nebulosa* (bark)	AC4 / OC4
B. cathartica (root) + *P. africanum* (root) + *R. digitata* (root)	AC5 / OC5
B. cathartica (root)+ *H. coriacea* (stem) + *O. engleri* (bark)	AC6 / OC6
R. multifidus (whole plant) + *S. serratuloides* (whole plant)	AC7 / OC7
B. cathartica (root) + *O. stricta* (stem) + *S. nebulosa* (bark)	AC8 / OC8
R. multifidus (whole plant) + *H. hemerocallidea* (corm)	AC9 / OC9
K. africana (bark) + *C. filiformis* (whole plant)	AC10 / OC10

Combination of plant species	Combination code
E. humeana (root) + *O. natalitia* (root)	AC11 / OC11
B. cathartica (root) + *E. humeana* (root)	AC12 / OC12
G. senegalensis (root) + *H. hemerocallidea* (corm)	AC13 / OC13
K. africana (bark) + *S. nebulosa* (bark)	AC14 / OC14
B. cathartica (root) + *O. natalitia* (root)	AC15 / OC15
S. birrea (bark) + *T. dregeana* (bark)	AC16 / OC16

AC = aqueous combination, OC = organic combination

Plant species combinations were further assessed to determine whether the combination therapy is more or less toxic (antagonism or synergism respectively) when compared to the toxicity of the extracts tested independently (Chapter 2). This was determined using Equation 3.1 to calculate the sum of fractional concentration (ΣFIC).

$$FIC^{(i)} = \frac{\text{Toxicity (a) in combination with (b)}}{\text{Toxicity (a) independently}}$$

$$FIC^{(ii)} = \frac{\text{Toxicity (a) in combination with (b)}}{\text{Toxicity (b) independently}}$$

$$\Sigma FIC = FIC^{(i)} + FIC^{(ii)}$$

Equation 3.1

The letters (a) and (b) represent different plant extracts. In some instances, more than two plant species are combined and then the equation is expanded to include each of these components, and (iii), (iv) etc. were then calculated.

When you look at other studies, potentiated toxicity particularly in substances used to kill a particular thing (such as fungi and insects) and in anticancer therapy is often regarded as synergy. However, in this instance, a lower toxicity is considered a positive effect. Therefore, when toxicity is reduced, it becomes a synergistic effect, and thatb was determined as follows; the ΣFIC value ≤ 0.50 was regarded as synergistic; >0.50 – 1.00 as additive; >1.00 – 4.00 as indifferent and >4.00 as

antagonistic (Hall et al., 1983). A synergistic interaction was interpreted as a combination resulting in a remarkably reduced toxicity when it is compared to the respective extracts independently. The interaction was additive when the combination produced a slightly better result when compared to the independent extracts. When the combinations produced results that were similar than those exhibited by plant species independently, it was regarded as indifferent. Lastly, the antagonistic interactions were interpreted as combinations that are highly toxic when compared to the individual extracts working independently (Cedergreen, 2014; Hubsch et al., 2014).

3.3 Results and discussion

An overview of the 16 plant species combinations (Table 3.2) indicated that combinations were generally less toxic when tested in the aqueous form. No aqueous combination demonstrated toxicity in the BSLA in the first 24 hrs. However, three (19%) aqueous combinations (AC7, AC9, and AC13) were toxic after 48 hrs of exposure. Among the three aqueous combinations that were toxic, it was noted that plant species such as *R. multifidus* and/or *H. hemerocallidea* were included. Other plant species involved were *G. senegalensis* and *S. serratuloides.* When these plant species were tested individually (aqueous extract), they were all non-toxic. No previous studies have been found which studied the toxicity of these combinations. However, according to Naidoo et al. (2013), the interactions within the combination can potentially bring about toxic activity, regardless of the non-toxic nature of the individual extracts. The highest percentage mortality acquired with the aqueous combination was 69.33%, which was obtained from combination AC7 (Table 3.2) after 48 hrs. The lowest mortality observed after 48 hrs of brine shrimp exposure to the aqueous combinations was 0.23% with the plant species combination AC2.

When the organic combinations were tested, seven (44%) combinations indicated toxicity against the brine shrimp after 24 hrs and this increased to 81% after 48 hrs. After 48 hrs, the highest percentage mortality for organic combinations was 92% from the combination OC7; and the lowest mortality was 40% with the combination OC8. All combinations in this study are tested for the first time for toxicity, hence no comparison with previous studies could be done. However, two of the plant species (*H. hemerocallidea* and *S. serratuloides*) have been previously mentioned in other

medically related combinations and their toxicity using the BSLA assay has been documented (Ramulondi et al., 2018).

In the study by Ramulondi et al., (2018), *H. hemerocallidea* was incorporated in four different combinations, and two of these combinations (*H. hemerocallidea* + *C. roseus* and *Aloe marlothii* + *H. hemerocallidea* + *Mamordica balsamina*) were toxic to brine shrimp. *Senecio serratuloides* was incorporated in four combinations of which two were toxic (*Albertisia delagoensis* + *S. serratuloides,* both aqueous and organic extracts were toxic at 2.00 mg/ml; and organic extracts of *M. balsamina* + *S. serratuloides* was toxic at 2.00 mg/ml).

In the current study, *S. serratuloides* was used in two combinations (AC2/OC2 and AC7/OC7), where AC2/OC2 was non-toxic and AC7/OC7 was toxic in aqueous and organic combinations. *Hypoxis hemerocallidea* was used in AC9/OC9 and AC13/OC13 combinations, which were both toxic. Although some similarities can be observed with these combinations (where the same plant species as the current study were used) comparing them is not possible as they incorporate totally different plant species apart from the two discussed.

All plant combinations were further analysed using the ΣFIC calculation (Table 3.3). When studying the toxicity of combinations each plant species involved is important as it can increase or reduce the toxicity of the combination. However, in some combinations, the ΣFIC could not be calculated as the mortality percentages of some extracts were 0%. In that case the ΣFIC value was not determined, but the interaction was estimated. Some combinations indicated a change in the interaction between 24 and 48 hrs, e.g., a synergistic demonstration after 24 hrs changing to antagonistic/ indifferent interaction after 48 hrs. A longer period may have allowed these combinations to undergo some processes such as excretion, metabolization, transportation, uptake and binding at the target sites (Cock and Ruebhart, 2009; Cedergreen, 2014) and thus the difference in interaction was observed. For this reason, the discussion of the results in this section focuses more on the results obtained after the maximum period of 48 hrs. However, the 24 hrs results were presented in Table 3.3 to indicate the reaction progress.

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Table 3.2 Average percentage mortality of brine shrimp when exposed to aqueous and organic combinations of plant extracts

Plant combination codes	Plants in combination	Average percentage mortality			
		Aqueous combination (AC)		Organic combination (OC)	
		24 hrs	48 hrs	24 hrs	48 hrs
AC1/ OC1	*B. cathartica* (roots) + *C. neglecta* (roots)+ *C. monteiroi* (root) + *G. livingstonei* (root) + *G. occidentalis* (root) + *O. natalitia* (root) + *R. digitata* (root)	0.67	10.33	45.67	**75.33**
AC2/ OC2	*E. tirucalli* (root) + *O. engleri* (bark)+ *S. puniceus* (bulb) + *S. serratuloides* (whole plant)	0.09	0.23	32.00	49.00
AC3/ OC3	*B. cathartica* (root) + *E. humeana* (root) + *O. natalitia* (root) + *T. elegans* (root) + *S. nebulosa* (bark)	0.67	23.33	30.00	**61.67**
AC4/ OC4	*A. villicaulis* (root) + *B. cathartica* (root) + *S. nebulosa* (bark)	1.67	3.33	**68.33**	**75.33**
AC5/ OC5	*B. cathartica* (root) + *P. africanum* (root) + *R. digitata* (root)	5.33	22.00	**55.67**	**66.33**
AC6/ OC6	*B. cathartica* (root)+ *H. coriacea* (stem) + *O. engleri* (bark)	20.00	38.33	45.67	**69.67**
AC7/ OC7	*R. multifidus* (whole plant) + *S. serratuloides* (whole plant)	11.67	**69.33**	**86.33**	**92.00**
AC8/ OC8	*B. cathartica* (root) + *O. stricta* (stem) + *S. nebulosa* (bark)	0.02	34.00	21.00	40.00
AC9/ OC9	*R. multifidus* (whole plant) + *H. hemerocallidea* (corm)	4.33	**66.67**	**74.67**	**88.67**
AC10/ OC10	*K. africana* (bark) + *C. filiformis* (whole plant)	0.00	0.67	**78.00**	**78.67**
AC11/ OC11	*E. humeana* (root) + *O. natalitia* (root)	9.67	40.67	26.33	**60.67**
AC12/ OC12	*B. cathartica* (root) + *E. humeana* (root)	0.01	36.00	24.00	48.00
AC13/ OC13	*G. senegalensis* (root) + *H. hemerocallidea* (corm)	0.00	**52.33**	**53.67**	**82.33**
AC14/ OC14	*K. africana* (bark)+ *S. nebulosa* (bark)	0.00	0.67	44.00	**57.00**
AC15/ OC15	*B. cathartica* (root) + *O. natalitia* (root)	0.00	5.67	**56.33**	**57.67**

Plant combination codes	Plants in combination	Average percentage mortality			
		Aqueous combination (AC)		Organic combination (OC)	
		24 hrs	48 hrs	24 hrs	48 hrs
AC16/ OC16	*S. birrea* (bark) + *T. dregeana* (bark)	16.67	49.33	36.00	**57.67**
Controls					
Potassium dichromate (positive control)		**100**	**100**	**100**	**100**
Salt water (negative control for aqueous extracts)		0.00	2.00	-	-
2% DMSO (negative control for organic extracts)		-	-	0.00	0.00

Values in bold – Toxic

Having a look at the type of interaction in the aqueous combinations after 48 hrs, the most frequently encountered was an antagonistic interaction, observed in nine (56%) combinations. This indicated that toxicity of the extracts in a combination was enhanced compared to the independent extracts. There were five (31%) combinations that demonstrated indifferent interaction with ΣFIC values between 1.00 and 4.00. This implies that the joint effect of these combinations compared to the individual plant species studied had little to no notable impact from a toxicity point of view. Observing the results after 48 hrs, synergistic interactions were detected in two combinations, namely the AC2 and AC10 (Table 3.3) with the ΣFIC value of 0.02 and 0.10, respectively. These two combinations were considered as having the most reduced toxicity among the aqueous combinations over the exposure period of 48 hrs. The aqueous combination that demonstrated the highest ΣFIC value was OC8 with the ΣFIC value of 38.16 after 48 hrs. Therefore, this combination is an indication of the most enhanced toxicity among the aqueous combinations when compared to their relative independently studied extracts.

Although there has been reports that plant species combinations give better benefits than single plant species. The results of this study shows that these combinations are also likely to result in adverse effects. The popular combinations in Maputaland such as AC7 and AC9 were referred to 13 times (26%) as a traditional treatment for genital warts (De Wet and Ngubane, 2014). In this study, these combinations were toxic and also demonstrated an antagonistic interaction. The other popular combination (AC16) was a two-plant combination traditionally reported as an abortifacient when the *T. dregeana* bark concentration was higher than *S. birrea* bark concentration in the mixture (De Wet and Ngubane, 2014). *Trichilia dregeana* killed 100% of brine shrimp whereas *S. birrea* killed 17% and in combination 49% of brine shrimp were killed, which was considered non-toxic. The ΣFIC of this combination indicated that the interaction between these two extracts is indifferent. This shows the effect that *S. birrea* extract have in this combination by reducing the toxicity level of *T. dregeana.* However, *T dregeana* also increased the toxicity of *S, birrea* hence the interaction is indifferent.

The ΣFIC of the organic extract combinations (Table 3.4) indicated 50% of the extracts to be antagonistic and the other 50% being indifferent. This was an observation after

Table 3.3 Interactions (ΣFIC) and interpretation for the aqueous extracts in combination

Plant species combinations [% mortality after 24 and 48 hrs of brine shrimp when investigated individually]	**Codes**	**Time in hrs**	**% mortality of combination**	**ΣFIC index value**	**Toxicological interactions**
B. cathartica (root) [3.00; 9.00] + *C. neglecta* (root) [18.00; 32.00] + *C. monteiroi* (root) [55.00; 74.00] + *G. occidentalis* (root) [0.00; 2.00] + *O. natalitia* (root) [1.00; 15.00] + *R. digitata* (root) [22.00; 43.00] + *G. livingstonei* (root) [1.00; 30.00]	AC1	24	1.00	ND	Synergistic [(1)]
		48	10.00	7.80	Antagonistic
E. tirucalli (root) [100.00; 100.00] + *O. engleri* (bark) [50.00; 74.00] + *S. puniceus* (bulb) [50.00; 93.00] + *S. serratuloides* (whole plant) [4.00; 14.00]	AC2	24	0.09	0.03	Synergistic
		48	0.23	**0.02**	**Synergistic**
B. cathartica (root) [3.00; 9.00] + *E. humeana* (root) [22.00; 92.00] + *O. natalitia* (root) [1.00; 15.00] + *T. elegans* (root) [19.00; 85.00] + *S. nebulosa* (bark) [1.00; 1.00]	AC3	24	1.00	2.40	Indifferent
		48	23.00	27.60	Antagonistic

Plant species combinations [% mortality after 24 and 48 hrs of brine shrimp when investigated individually]	**Codes**	**Time in hrs**	**% mortality of combination**	**ΣFIC index value**	**Toxicological interactions**
A. villicaulis (root) [0.00; 1.00] + *B. cathartica* (root) [3.00; 9.00] + *S. nebulosa (bark)* [1.00; 1.00]	AC4	24	2.00	ND	Indifferent
		48	3.00	6.30	Antagonistic
B. cathartica (root) [3.00; 9.00] + *P. africanum* (root) [47.00; 49.00] + *R. digitata* (root) [22.00; 43.00]	AC5	24	5.00	2.00	Indifferent
		48	22.00	3.40	Indifferent
B. cathartica (root) [3.0; 9.00] + *H. coriacea* (stem) [4.00; 14.00] + *O. engleri* (bark) [50.00; 74.00]	AC6	24	20.00	12.10	Antagonistic
		48	38.00	7.50	Antagonistic
R. multifidus (whole plant) [11.00; 15.00] + *S. serratuloides* (whole plant) [4.00; 14.00]	AC7	24	12.00	4.09	Antagonistic
		48	69.00	9.53	Antagonistic
B. cathartica (root) [3.00; 9.00] + *O. stricta* (stem) [79.00; 88.00] + *S. nebulosa* (bark) [1.00; 1.00]	AC8	24	0.02	0.03	Synergistic
		48	34.00	38.16	Antagonistic
R. multifidus (whole plant) [11.00; 15.00] + *H. hemerocallidea* (corm) [4.00; 40.00]	AC9	24	4.00	1.36	Indifferent
		48	67.00	6.14	Antagonistic

Plant species combinations [% mortality after 24 and 48 hrs of brine shrimp when investigated individually]	**Codes**	**Time in hrs**	**% mortality of combination**	**ΣFIC index value**	**Toxicological interactions**
K. africana (bark) [13.00; 40.00] + *C. filiformis* (whole plant) [6.00; 29.00]	AC10	24	0.00	0.00	Synergistic
		48	**1.00**	**0.10**	**Synergistic**
B. cathartica (root) [3.00; 9.00] + *E. humeana* (root) [22.00; 92.00]	AC11	24	0.01	0.00	Synergistic
		48	36.00	4.39	Antagonistic
B. cathartica (root) [3.00; 9.00] + *E. humeana* (root) [22.00; 92.00]	AC12	24	0.01	0.003	Synergistic
		48	36.00	4.39	Antagonistic
G. senegalensis (root) [4.00; 13.00] + *H. hemerocallidea* (corm) [4.00; 40.00]	AC13	24	0.00	0.00	Synergistic
		48	52.00	5.30	Antagonistic
K. africana (bark) [13.00; 40.00] + *S. nebulosa* (bark) [1.00; 1.00]	AC14	24	0.00	0.00	Synergistic
		48	1.00	1.10	Indifferent
B. cathartica (root) [3.00; 9.00] + *O. natalitia* (root) [1.00; 15.00]	AC15	24	0.00	0.00	Synergistic
		48	6.00	1.10	Indifferent
S. birrea (bark) [3.00; 17.00] + *T. dregeana* (bark) [20.00; 100.00]	AC16	24	17.00	6.50	Antagonistic
		48	49.00	3.40	Indifferent

ND – not determined, (1) – estimated interaction, Shaded area indicates 48 hrs for analysis, **bold** – synergy after 48

Table 3.4 Interaction among the organic extracts involved in combinations using the ΣFIC calculation

Plant species combinations [% mortality after 24 and 48 hrs of brine shrimp when investigated individually]	**Codes**	**Time in hours**	**% mortality of combination**	**ΣFIC index value**	**Toxicological interactions**
B. cathartica (root) [2.00; 89.00] + *C. neglecta* (root) [0.00; 40.00] + *C. monteiroi* (root) [0.00; 68.00] + *G. occidentalis* (root) [4.00; 44.00] + *O. natalitia* (root) [52.00; 55.00] + *R. digitata* (root) [38.00; 80.00] + *G. livingstonei* (root) [8.00 ;45.00]	OC1	24	46.00	ND	Antagonistic (1)
		48	75.00	9.50	Antagonistic
E. tirucalli (root) [78.00; 90.00] + *O. engleri* (bark) [23.00 ;54.00] + *S. puniceus* (bulb) [22.00; 94.00] + *S. serratuloides* (whole plant) [0.00; 0.00]	OC2	24	32.00	ND	Antagonistic (1)
		48	49.00	ND	Antagonistic (1)
B. cathartica (root) [2 .00; 89.00] + *E. humeana* (root) [5.00; 49.00] + *O. natalitia* (root) [52.00; 55.00] + *T. elegans* (root) [27.00; 96.00] + *S. nebulosa* (bark) [1.00; 2.00]	OC3	24	30.00	52.70	Antagonistic
		48	62.00	34.70	Antagonistic

Plant combinations [% mortality after 24 and 48 hrs of brine shrimp when investigated individually]	**Codes**	**Time in hours**	**% mortality of combination**	**ΣFIC index value**	**Toxicological interactions**
B. cathartica (root) [2.00; 89.00] + *P. africanum* (root) [23.00; 28.00] + *R. digitata* (root) [38.00; 80.00]	OC5	24	56.00	31.90	Antagonistic
		48	66.00	3.90	Indifferent
B. cathartica (root) [2.00; 89.00] + *H. coriacea* (stem) [25.00; 38.00] + *O. engleri* (bark) [23.00; 54.00]	OC6	24	46.00	25.70	Antagonistic
		48	70.00	3.90	Indifferent
R. multifidus (whole plant) [17.00; 34.00] + *S. serratuloides* (whole plant) [0.00; 0.00]	OC7	24	86.00	ND	Antagonistic (1)
		48	92.00	ND	Antagonistic (1)
B. cathartica (root) [2.00; 9.00] + *O. stricta* (stem) [19.00; 38.00] + *S. nebulosa* (bark) [1.00; 2.00]	OC8	24	21.00	32.61	Antagonistic
		48	40.00	21.50	Antagonistic
R. multifidus (whole plant) [17.00; 34.00] + *H. hemerocallidea* (corm) [28.00; 66.00]	OC9	24	75.00	3.70	Indifferent
		48	89.00	2.30	Indifferent
K. africana (bark) [30.00; 35.00] + *C. filiformis* (whole plant) [20.00; 69.00]	OC10	24	78.00	6.50	Antagonistic
		48	79.00	3.40	Indifferent

Plant combinations [% mortality after 24 and 48 hrs of brine shrimp when investigated individually]	**Codes**	**Time in hours**	**% mortality of combination**	**ΣFIC index value**	**Toxicological interactions**
B. cathartica (root) [2.00; 98.00] + *E. humeana* (root) [5.00; 49.00]	OC12	24	24.00	16.80	Antagonistic
		48	48.00	1.46	Indifferent
G. senegalensis (root) [0.00; 1.00] + *H. hemerocallidea* (corm) [28.00; 66.00]	OC13	24	53.00	ND	Antagonistic (1)
		48	82.00	83.20	Antagonistic
K. africana (bark) [30.00; 35.00] + *S. nebulosa* (bark) [1.00; 2.00]	OC14	24	44.00	45.50	Antagonistic
		48	57.00	30.10	Antagonistic
B. cathartica (root) [2.00; 89.00] + *O. natalitia* (root) [52.00*, 55.00]	OC15	24	56.00	29.08	Antagonistic
		48	58.00	1.70	Indifferent
S. birrea (bark) [23.00*, 90.00] + *T. dregeana* (bark) [25.00; 79.00]	OC16	24	36.00	3.00	Indifferent
		48	58.00	1.40	Indifferent

ND – not determined, (1) – estimated interaction, Shaded area indicates 48 hrs for analysis

a maximum period of 48 hrs. There were no synergistic or additive interactions observed with organic combinations. The lowest acquired ΣFIC value was 1.40 (indifferent), which was obtained with the combination OC16. The highest ΣFIC value obtained was 83.20 (antagonistic) with combination OC13. It was noted that with the organic extracts, the ΣFIC values were lower after 48 hrs when compared to 24 hrs. For example, some combinations such as OC5, OC6, OC10, OC12 and OC15 were initially (after 24 hrs) antagonistic and later (after 48 hrs) an indifferent interaction. This indicated that the exposure time have an influence on the interactions among the extracts. Other factors such as bioavailability of the toxic phytochemicals and /or the target site for binding the phytochemicals may have declined with the extended time (Cedergreen, 2014).

3.3.1 Dose response of combinations

As with individual plant species samples, those aqueous combinations (AC7, AC9 and AC13) that demonstrated toxicity after 48 hrs (Table 3.2) were tested further at varying lower concentrations to determine the dose response and ATC to cause <50% mortality in the BSLA (Figure 3.1). It was noted that combinations AC7 and AC9 were non-toxic at concentrations below 0.62 mg/ml and 0.59 mg/ml, respectively, and AC13 was non-toxic at a concentration below 0.88 mg/ml. Overall, these aqueous combinations had demonstrated a potential for being used at safer (lower) concentrations (0.62 mg/ml for AC7, 0.59 mg/ml for AC9 and 0.88 mg/ml for AC13).

Traditionally, a decoction of AC7 or AC9 is taken orally by women to treat vaginal warts. The AC13 combination is taken during the third trimester by a pregnant woman to prevent infantile colic when the baby is born (De Wet and Ngubane, 2014). A total of 13 organic extract combinations (based on results obtained from Table 3.2) were subjected to serial dilution from a concentration of 1.00 mg/ml to 0.031 mg/ml. To simplify the results, a set of two figures (Figures 3.2 and 3.3) was used to present the response of toxic organic combinations to brine shrimp after dilution. Figure 3.2, represents a set of seven organic combinations that were toxic at both 24 and 48 hrs of exposure time. The overall results of this set indicated that these combinations demonstrate non-toxicity after dilution. After 24 hrs these extract-combinations were

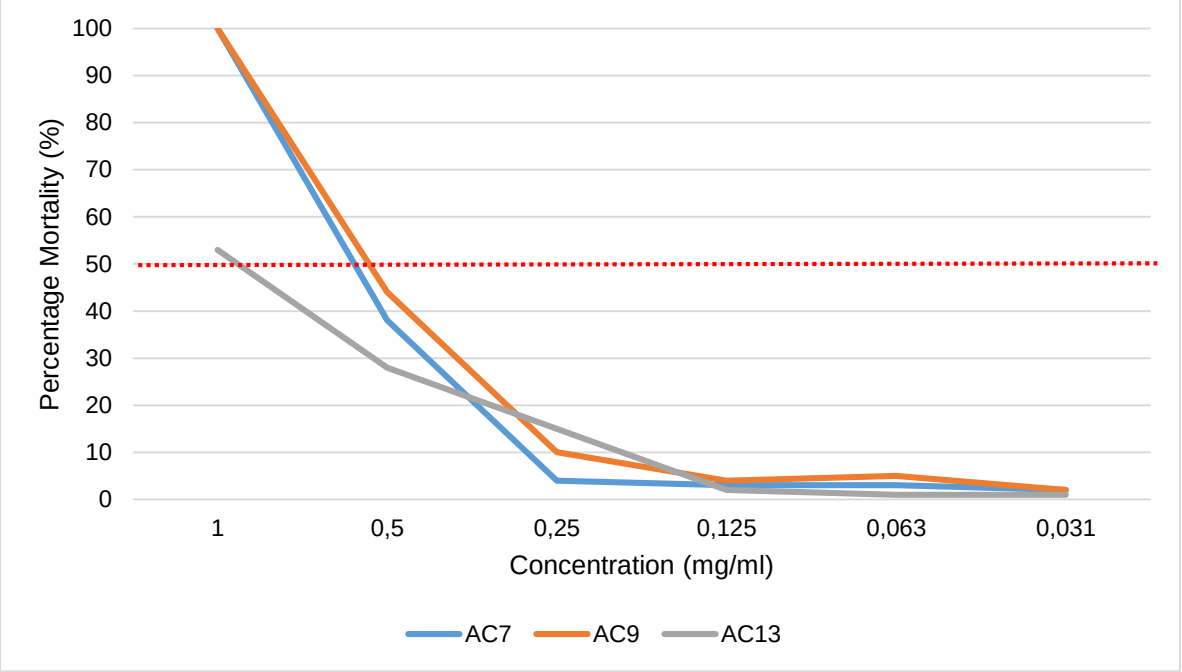

Figure 3.2 Dose response of aqueous combinations that were toxic at 1.00 mg/ml against brine shrimp. Dotted red line delineate toxicity (>50%) from non-toxicity (<50%).

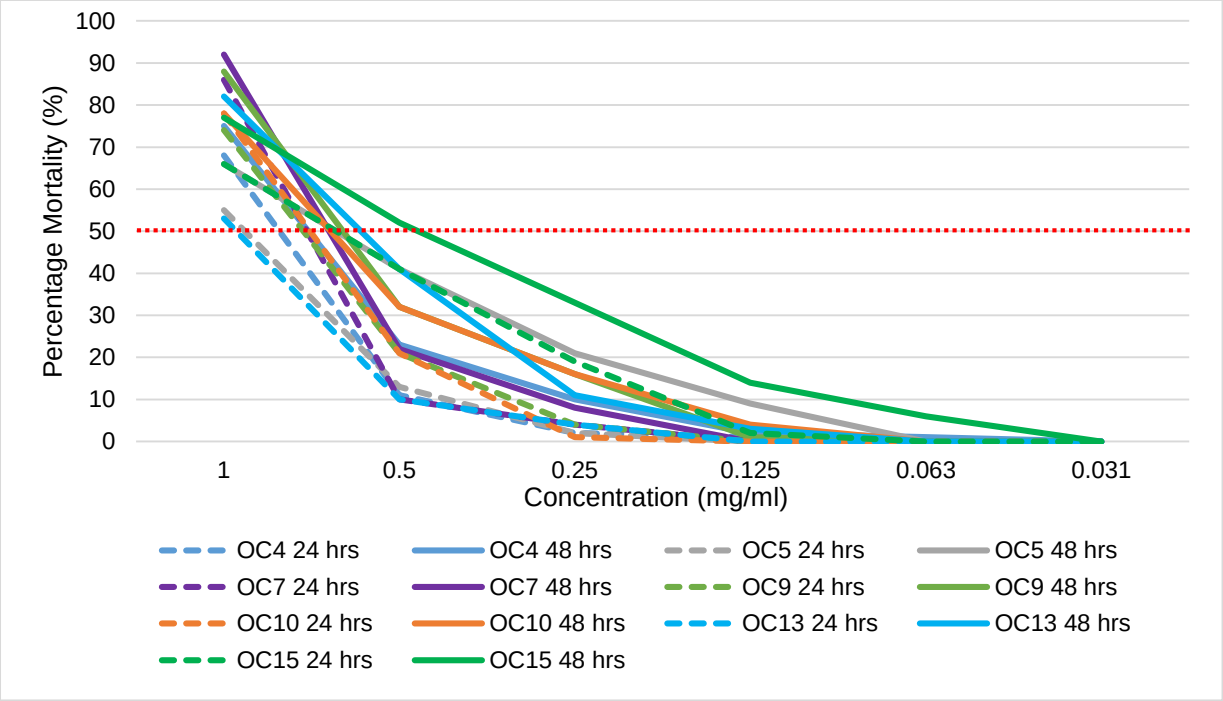

Figure 3.3 Dose response of organic combinations that were toxic at 1.00 mg/ml (at both 24 and 48 hrs) against brine shrimp. Dotted red line delineate toxicity (>50%) from non-toxicity (<50%).

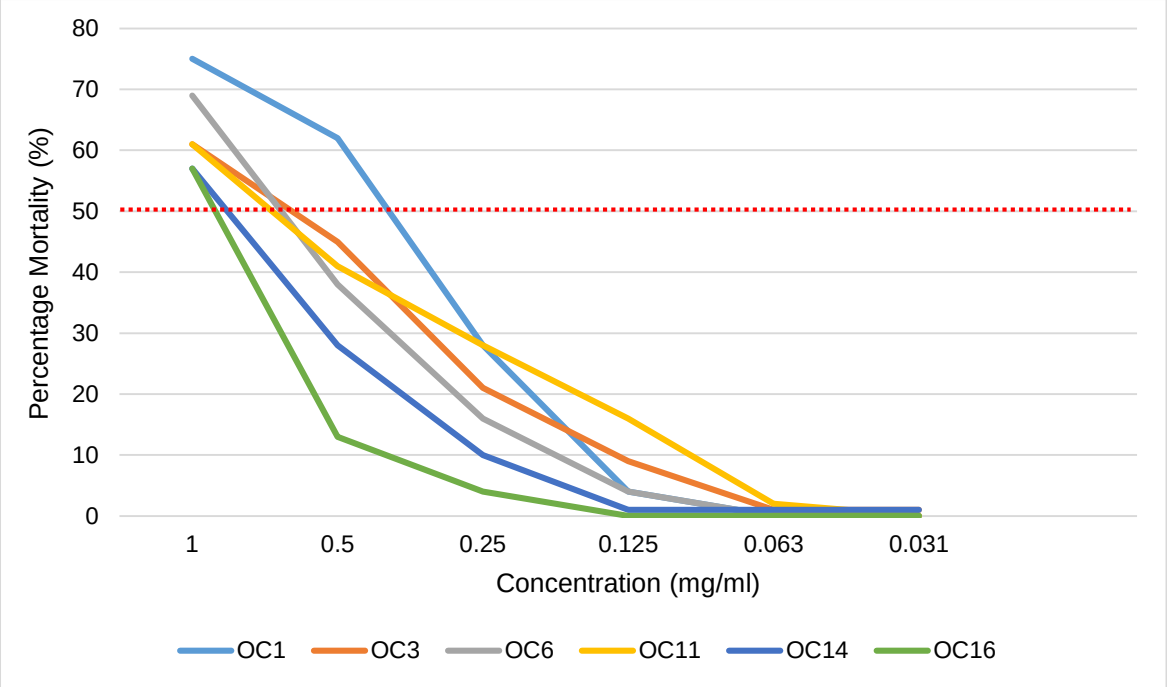

Figure 3.4 Dose response of organic combinations that were toxic only after 48 hrs at 1.00 mg/ml against brine shrimp. Dotted red line delineate toxicity (>50%) from non-toxicity (<50%).

non-toxic at concentrations between 0.71 mg/ml and 0.93 mg/ml, and after 48 hrs revealed non-toxic concentrations between 0.45 mg/ml and 0.75 mg/ml.

All combinations were successfully diluted and ATC were attained. In this set of combinations, the ATCs obtained were ranging between 0.42 mg/ml and 0.94 mg/ml. The ATCs of all aqueous and organic combinations are collectively presented in Table 3.5. This table indicated that all aqueous extracts were non-toxic at concentrations below 0.59 mg/ml and organic extracts were non-toxic at concentrations below 0.42 mg/ml. Overall, both the aqueous and the organic extract combinations could be successfully diluted to non-toxic concentrations when tested in the BSLA. Therefore, the current results (Figures 3.1 – 3.3) indicate that the concentration of toxic combinations can be diluted to an ATC and lower concentrations that would be safer. However, these combinations are used for self-medication by women, and it is common that preparations may differ from person to person, which also includes the difference in doses. It was established that the women in Maputaland

Table 3.5 The ATC of aqueous and organic combinations after 48 hrs in BSLA

Combination of plant species	Combination code (a/b)	ATC of aqueous combination (AC) (mg/ml)	ATC of organic combination (OC) (mg/ml)
B. cathartica (roots) + *C. neglecta* (roots)+ *C. monteiroi* (root) + *G. livingstonei* (root) + *G. occidentalis* (root) + *O. natalitia* (root) + *R. digitata* (root)	AC1 / OC1	—	0.42
B. cathartica (root) + *E. humeana* (root) + *O. natalitia* (root) + *T. elegans* (root) + *S. nebulosa* (bark)	AC3 / OC3	—	0.67
A. villicaulis (root) + *B. cathartica* (root) + *S. nebulosa* (bark)	AC4 / OC4	—	0.75
B. cathartica (root) + *P. africanum* (root) + *R. digitata* (root)	AC5 / OC5	—	0.68
B. cathartica (root) + *H. coriacea* (stem) + *O. engleri* (bark)	AC6 / OC6	—	0.70
R. multifidus (whole plant) + *S. serratuloides* (whole plant)	AC7 / OC7	0.62	0.70
R. multifidus (whole plant) + *H. hemerocallidea* (corm)	AC9 / OC9	0.59	0.66
K. africana (bark) + *C. filiformis* (whole plant)	AC10 / OC10	—	0.71
E. humeana (root) + *O. natalitia* (root)	AC11 / OC11	—	0.74
G. senegalensis (root) + *H. hemerocallidea* (corm)	AC13 / OC13	0.95	0.60
K. africana (bark) + *S. nebulosa* (bark)	AC14 / OC14	—	0.88
S. birrea (bark) + *T. dregeana* (bark)	AC16 / OC16	—	0.94

— = Non-toxic at 1.00 mg/ml therefore no further dose response was determined, a/b **–** aqueous combination/organic combination

mostly do not have a standard procedure and they are also not aware of the concentrations they used for their herbal medicine.

What is alarming is that this study demonstrated that many of the plant species combinations had some level of toxicity. Therefore, the primary users of these concoctions should be made aware and educated on the importance of dosage when preparing herbal medicine. In this study, the plant species combination determined to be the safest was *E. tirucalli* (root) + *O. engleri* (bark) + *S. puniceus* (bulb) + *S. serratuloides* (whole plant) (AC2/OC2). In this combination, these plant species were non-toxic in both aqueous and organic extracts and demonstrated the lowest percentage mortality (0.23%) after 48 hrs. An enema of this combination was reported only once for blood cleansing during pregnancy (De Wet and Ngubane, 2014).

In the study conducted in the same study area by Naidoo, et al. (2013), 92% of the plant species combinations used for sexually transmitted diseases were non-toxic and the ΣFIC indicated that they were also synergistic. The traditional use of these medicinal plant species by the people of Maputaland was then validated as non-toxic. However, in the current study, 81% of the combinations that were investigated were toxic and the ΣFIC indicated that the majority (56%) being antagonistic and 31% being indifferent. The differences in these results shows how the general perception of plant species combinations as safe could be wrong. As it was observed with the aqueous combinations in this study, more extracts that were synergistic and non-toxic in the first 24 hrs, changed later to either antagonistic, indifferent or toxic. This shows not only the importance of giving the experimentation more time before taking the final results when assessing acute toxicity, but it also indicates that the adverse effect of these combinations may show up rather later than expected. Overall, it was established that aqueous extracts in combination are safer than the organic extracts.

3.4 Summary

- A total of 16 aqueous and organic plant species combinations were tested for toxicity using the BSLA.
- These combinations were further analyzed using the ΣFIC method, to determine whether a combination increases or decreases toxicity when compared to individual extracts tested independently.

- All toxic aqueous and organic extracts were diluted to assess dose response and determine the ATC concentration.
- When the 16 plant species combinations were tested, three aqueous-extract combinations were toxic (AC7, AC9 and AC13) after 48 hrs.
- Thirteen organic-extract combinations were toxic after 48 hrs.
- It was determined that toxic extract combinations can be diluted to ATCs that are non-toxic.
- The interactions of aqueous extracts in combination demonstrated nine combinations as having antagonistic, five indifferent and two demonstrated synergisms. Those were AC2 and AC10 after the maximum period of 48 hrs.
- Eight (50%) of the organic combinations demonstrated antagonistic interaction.
- The best combination in this study is AC2/OC2, since it is non-toxic in both aqueous and organic extract combinations. The ΣFIC also indicated that the aqueous extract of this combination is synergistic. Even though the organic extract combinations were antagonistic, it was still non-toxic.

CHAPTER 4

Mutagenicity of some medicinal plant species traditionally used for the treatment of gynaecology and obstetrics

4.1 Introduction

The most widely used assay to test for the mutagenicity of medical plant species is the Ames test (also known as the *Salmonella typhimurium* microsome assay) (Elgorashi et al., 2003; Mashele and Fuku, 2011; Della et al., 2011; Moosavi et al*.,* 2013; Eren et al., 2015; Lee et al*.,* 2018). This test is widely accepted for the detection of substances that can cause damage to genes or lead to gene mutation (Mortelmans and Zeiger, 2000). The Ames test uses previously mutated strains of *S. typhimurium.* These adaptive mutations hinder the bacteria to synthesize essential amino acid histidine, which is important for protein synthesis and as result, the mutant bacteria cannot grow to form colonies (Mortelmans and Zeiger, 2000). During the assay, the mutant strains are exposed to the test sample and bacterial growth is observed. The test substance or extract is considered mutagenic (toxic) if it can cause the growth of colonies (revertants) greater than two-fold when compared to the negative control (Resende et al., 2015). Colony growth is an indication that the applied substance has resulted in a reverse mutation that restored the gene function to synthesize histidine and thus the bacteria can grow.

The most commonly used *S. typhimurium* strains in the Ames test are, TA98 and TA100. These strains have high sensitivity and the ability to identify a large range of mutagenic substances (Sui et al*.,* 2009). The TA98 strain carries a frameshift mutation while TA100 have a base-pair substitution (Alanyali et al., 2011). The Ames test can be conducted in the present or absence of a metabolic enzyme called the S9 microsomal fraction, which can enhance the bio-activation of the test samples (Hakura et al., 1999). In the absence of the metabolic activation enzymes, a phosphate buffer is used to maintain a constant pH of the test. This buffer is reported to be isotonic and non-toxic to most cells (Wieczorowska-Tobis et al., 2001). In the absence of the activation enzymes, the Ames test is used to identify the direct-acting substances or chemicals as mutagens. For positive controls, direct-acting mutagens such as sodium azide (TA100 strain) and 4-nitroquinoline 1-oxide (4NQO) (TA98 strain) are used.

Medicinal plant extracts are among the popular substances that have been tested using the Ames test for safety. Therefore, this chapter aims to evaluate the potential mutagenic effects of 35 medicinal plant samples (from 33 plant species) traditionally reported to treat gynecology and obstetrics complaints in the study area Maputaland, KwaZulu-Natal, South Africa.

4.2 Materials and methods

4.2.1 Sample and culture preparation

The mutagenicity of plant extracts was evaluated using the standard Ames test method that was established by Ames et al., (1973). Organic plant extracts used in this study were dissolved in 10% DMSO to a concentration of 5 mg/ml. The aqueous extracts were dissolved in distilled water to the same concentration as the organic extracts. *Staphylococcus typhimurium* TA98 and TA100 bacterial strains were used in this assay to test mutagenicity of the extracts. The strains were obtained from Prof. Lyndy McGaw, Phytomedicine group, University of Pretoria, South Africa. A 0.1 ml of the stock culture was sub-cultured into 10 ml of Nutrient broth (Oxoid) and incubated at 37 °C for 18 to 24 hrs before it was used in the assay.

4.2.2 Media preparation

To perform the Ames test, two types of media were prepared i.e. the minimum glucose plates (Figure 4.1) and the top agar with traces of L-histidine (Sigma-Aldrich) and biotin (Sigma-Aldrich). Minimum glucose plates were prepared by mixing 15 g of agar (Oxoid agar bacteriological) in 930 ml of distilled water and autoclaving. When the agar was slightly cooled down to approximately 50 °C, 20 ml of the Vogel Bonner medium/salts (50X VB) and 40% glucose was added, gently mixed and poured into the petri dishes. The 50X VB medium was prepared by adding a series of salts [10 g of magnesium sulphate (Sigma-Aldrich), 100 g of citric acid monohydrate (ChemLab), 500 g of potassium phosphate (dibasic) (Sigma-Aldrich) and 175 g sodium ammonium phosphate (ChemLab)] in sequence into 1 L of distilled water and dissolving each one completely before the next one could be added. A 40% glucose solution was prepared by dissolving 400 g of D-glucose (Sigma-Aldrich) into 1 L of distilled water. The top agar was prepared by first preparing a histidine/biotin solution, which was prepared by

dissolving 12 mg of D-biotin (Sigma-Aldrich) and 10.5 mg L-histidine (Sigma-Aldrich) in 100 ml of autoclaved distilled water. The agar was prepared by mixing 6 g of bacteriological agar, and 5 g of sodium chloride (Merck) in 1 L of distilled water and autoclaved. Then 10 ml of biotin /histidine solution was added to 100 ml of the top agar. The bottles were then stored in 50 °C in a water bath until used.

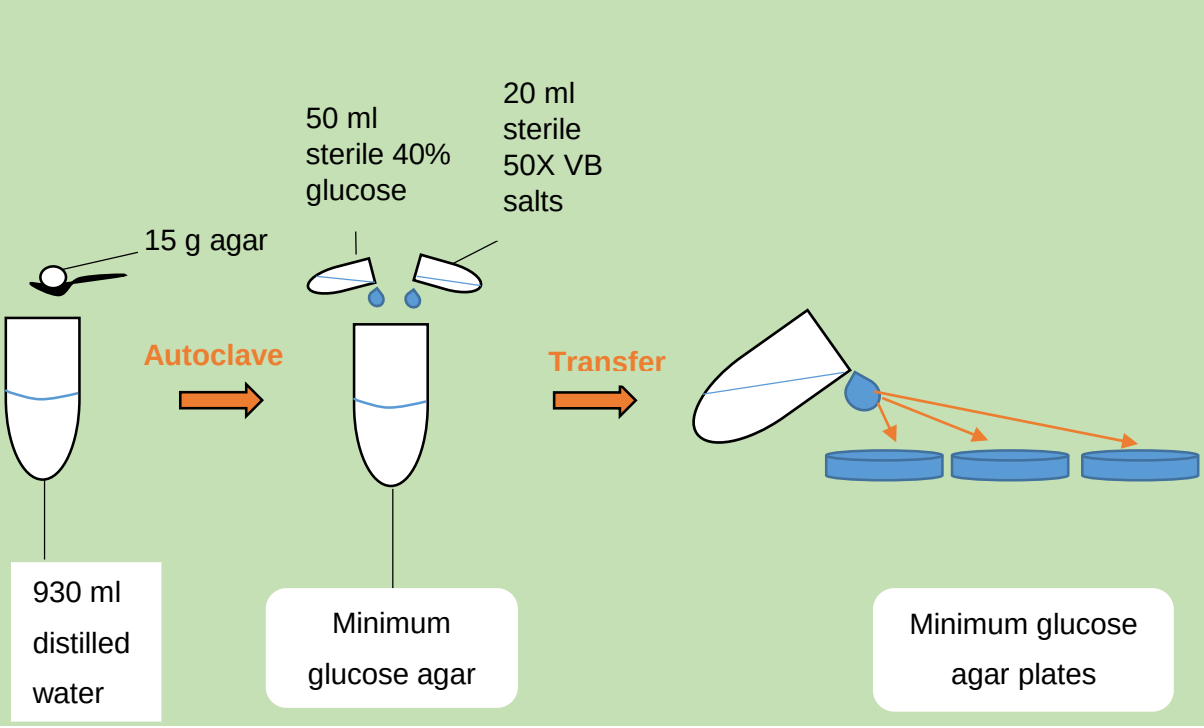

Figure 4.1 A diagrammatic summary of the preparation of the minimum glucose agar plates.

4.2.3 The Ames assay

Positive controls for the assay were 4NQO for TA98 strain at 2.5 mg/ml and sodium azide (Sigma-Aldrich) for TA100 at 2 mg/ml. The negative controls were distilled water for aqueous samples and 10% DMSO for the organic samples. A phosphate buffer (Sigma-Aldrich) was prepared to yield a pH of 7.4. The assay was performed by adding 100 µl of the test sample (positive/negative control or the plant extract), 500 µl of phosphate buffer and 100 µl of the overnight culture to a sterile test tube, in triplicates. Then 2 ml of the top agar was added into the sample-culture mixture. The test tube content was then vortexed and poured over the surface of the minimum glucose plates (labelled). Plates were incubated at 37 °C for 48 hrs. After the incubation period,

bacterial colonies were counted manually and the reversion rate was compared with the control plates (Figure 4.2). The test sample was considered mutagenic if the number of colony-forming units (CFU) in a plate was double the number of colonies in the negative control plates (distilled water and 10% DMSO).

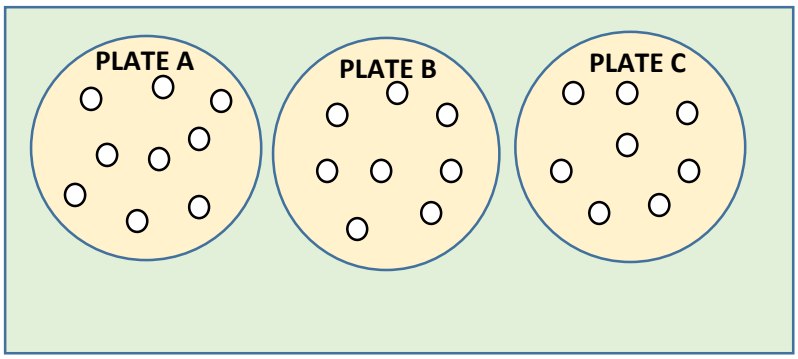

Figure 4.2 A typical Ames assay plate with colonies.

4.3 Results and discussion

4.3.1 Effects of aqueous extracts towards *S. typhimurium* TA98 strain

The colony count with the negative control against *S. typhimurium* TA98 was 131 colony forming units (CFU). Mutagenicity of the plant extracts was determined by the average number of revertant colonies more than 262 CFU (Table 4.1). However, the majority (92%) of the aqueous extracts showed no mutagenic effects towards TA98 strain. Only four aqueous extracts demonstrated a mutagenicity potential against TA98 strain, i.e. *E. tirucalli* stem (by 1205 CFU), *H. hemerocallidea* corm (by 1266 CFU), *O. stricta* stem (by 2413 CFU) and *O. engleri* bark (by 494 CFU). Mutagenicity is one of the causes of cancer by causing genetic alterations that leads to uncontrolled cell growth and tumour development (National Cancer Institute, 2017). According to Paiva et al., (2011), the latex that exude from the stem of *E. tirucalli* has the potential to cause cancer. Waczuk et al., 2015 also reported that the aqueous extract of *E. tirucallii* induced genetic damage in the Comet assay. It was noted that the potential mutagenicity or genetic damage of this plant extract may vary as was observed in the current study. Such variation was observed where the aqueous extract was mutagenic towards the TA98 strain and non-mutagenic towards the TA100 strain. In this study, the extract was active in the frame shift mutation (TA98 strain) and non-active in the

base-pair mutation (TA100 strain) (Franzen et al*.,* 1998). In a similar study by Ramulondi et al., (2018), mutagenicity results of *H. hemerocallidea* were different from the current test. In this study, the aqueous extract of *H. hemerocallidea* was found to be non-mutagenic. In spite of these plant species collected from the same region (Maputaland), it shows that each individual plant species may have a different chemotype, which could make it produce different results from the previously tested plants. The other two plants species *O. engleri* and *O. stricta* had no previous reports of mutagenicity. The extracts which resulted to the highest number of revertant colonies were *O. stricta* stem (2413 CFU) and *H. hemerocallidea* corm (1266 CFU).

4.3.2 The aqueous extracts against *S. typhimurium* TA100 strain

The colony count of the negative control against the *S. typhimurium* TA100 strain was 174 colony forming units (CFU). Any colony count that was more than 348 CFU was considered mutagenic. There were approximately 28% aqueous extracts (Table 4.1) which demonstrated mutagenicity towards the TA100 strain. Only two aqueous extracts (*C. filiformis* and *S. serratuloides*) have been previously assessed against the TA100 strain. A previous study (Wu et al., 2017) was found to have similar results for *C. filiformis,* where it indicated that this plant species is mutagenic. However, *S. serratuloides* had different results (non-mutagenic) in the Ames test according to Ramulondi et al., (2018). The environmental conditions and differences of individual chemotypes could be the reason for the differences of these results. While comparisons could be made with some previous studies, many plant species (17) have not been previously studied (Table 4.1). The aqueous extracts that resulted to the highest number of revertant colonies were *C. natalensis* shoot (1814 CFU) and *E. humeana* roots (1350 CFU).

4.3.3 Mutagenicity of organic extracts against *S. typhimurium* TA98 strain

In the current study, the negative control (10% DMSO) against the *S. typhimurium* TA98 resulted to the colony count of 142 CFU. Therefore, mutagenicity of the organic extracts against the TA98 strain was determined by the growth of revertant colonies more than 284 CFU. In this study, a total of 14 (27%) organic extracts demonstrated mutagenicity potential towards the TA98 strain (Table 4.1). These extracts were

C. filiformis plant (1617 CFU), *C. monteiroi* roots (1402 CFU), C. natalensis shoot (1866 CFU), E. humeana roots (1331 CFU), E. *tirucalli* stem (2069 CFU), *H. boraginiflora* leaves (2721 CFU) and roots (356 CFU), *H. coriacea* stem (2092 CFU), *H. hemerocallidea* corm (1241 CFU), *O. stricta* stem (2848 CFU), *P. africanum* leaves (2462 CFU), *R. digitata* leaves (1006 CFU), *S. nebulosa* bark (1836 CFU) and *T. elegans* roots (1583 CFU). It was observed that the number of mutagenic extracts were higher for the organic extracts when compared to the aqueous extracts. The number of revertant colonies also increased. These results can be correlated to the fact that organic solvents are able to extract more compounds when compared to water, which can extract only the polar compounds. With that said, the aqueous extract of *O. engleri* was mutagenic (494 CFU) against TA98 strain whereas the organic extract was non-mutagenic (145 CFU). In this case, the organic extract could have had extra compounds (nonpolar) that were able to inhibit the mutagenic compounds or to correct the damaged genes, hence less mutagenic. The extracts which resulted to the highest number of revertant colonies were *O. stricta* stem (2848 CFU) and *H. boraginiflora* leaves (2721 CFU). *Hyphaene coriacea, H. hemerocallidea* and *E. tirucalli* were reported in previous studies (Table 4.1) as non-mutagenic. However, these plant extracts were mutagenic in the current study. The solvents used for the previous study were different from the current study, which could be the reason why the results are not the same. *Hypoxis hemerocallidea* and *S. serratuloides* also demonstrated different results to the previous study (Ramulondi et al., 2018), even though the same solvent (methanol-dichloromethane) was used. The differences of these results could be caused by the differences of the chemotypes of individual plants.

4.3.4 The organic extracts against *S. typhimurium* TA100 strain

Considering that the negative control (10% DMSO) in the current study had 274 CFU, all extracts that had more than 548 CFU was considered mutagenic. Therefore, the majority (69%) of organic extracts were mutagenic towards the TA100 strain. The extracts such as *K. africana* bark, *G. senegalensis*, *T. dregeana* bark, leaves and roots were among the extracts that showed different results when compare to previous studies (as shown in Table 4.1). Such results have been observed also on previous studies where different results can be obtained from the same pant species, e.g.

G. senegalensis was reported to be non-mutagenic towards the TA100 by Mulaudzi et al. (2012) and demonstrated mutagenicity in Verschaeve and Van Staden, (2008) study. *Sapium integerrimum* roots (2455 CFU) and *H. boraginiflora* (2456 CFU) had the highest number of revertant colonies.

Plant extracts that showed the highest mutagenicity in both organic and aqueous extracts against both TA98 and TA100 strains were *C. filiformis, H. hemerocallidea* and *O. stricta.* These extracts were able to result in revertant growth of over five times that of the negative control. There were plant extracts in this study that demonstrated no mutagenicity in both *Salmonella* test strains. These extracts include *A. villicaulis* roots, *C. natalensis* roots, *D. villosa* leaves, *E. natalensis* leaves, *G. occidentalis* roots, *O. natalitia* leaves, *S. integerrimum* leaves and *S. puniceus* bulb.

Other plant extracts showed varying mutagenic activity. The aqueous and organic extract of *S. burkei* bark was non-mutagenic towards *S. typhimurium* TA98 strain in the Ames test. However, when it was tested against the *S. typhimurium* TA100 strain, only the organic extract demonstrated mutagenic potential. This plant species was reported in Maputaland to help facilitate healthy and safe pregnancy when the infusion is taken orally or by rectal administration during pregnancy (De Wet and Ngubane, 2014). The mutagenic potential of *S. burkei* extract raises some concerns for the health of an unborn baby as well as the pregnant mother. Howerver, with mutagenicity observed with the organic extracts of *S. burkei*, and the lay people use the aqueous extracts when preparing the medicine, which was non-mutagenic in this study, it means that there is a potential that this plant could cause mutagenicity. In most cases, when traditional herbal medicine is prepared, there is no strict consistency with the measurements, which affects the doses of the end product. Therefore, in this case there is a possibility that when the extract is prepared too strong, it may have enough properties to result into mutagenicity. No previous studies have been found in the literature about the mutagenicity of *S. burkei*.

It can then be speculated that these plants were not popular in the area for treating gynaecological and obstetric complaints when compared to the other plants, which were reported several times. Such plants include *C. filiformis* used to help stimulate

Table 4.1 The results of medicinal plant species used in Maputaland to treat gynaecology and obstetric ailments with reference to mutagenicity including previous mutagenicity studies

Plant names	Part used	TA98 (CFU)		TA100 (CFU)		Previous reports on mutagenicity
		AQ	ORG	AQ	ORG	
Acalypha villicaulis	Roots	78	116	98	54	N/A
	Leaves	29	115	91	**721**	N/A
Acanthospermum glabratum	Whole plant	29	185	73	**737**	N/A
Bridelia cathartica	Roots	10	21	**944**	**876**	N/A
	Leaves	20	7	126	**634**	N/A
Cassytha filiformis	Whole plant	255	**1617**	**652**	**1829**	Mutagenic (Wu et al., 2017)
Commiphora neglecta	Roots	111	111	74	**693**	N/A
	Leaves	26	68	344	**733**	N/A
Crotalaria monteiroi	Roots	91	**1402**	81	**1703**	N/A
	Leaves	26	64	**900**	**1182**	N/A
Cyperus natalensis	Roots	110	69	83	150	N/A
	Shoot	33	**1866**	**1814**	**584**	N/A
Diospyros villosa	Roots	79	156	**431**	403	N/A
	Leaves	32	110	76	515	N/A
Erythrina humeana	Roots	34	**1331**	**1350**	118	N/A
Euclea natalensis	Roots	81	178	**509**	242	Non-mutagenic (bark) (Elgorashi et al., 2003; Taylor et al., 2003)
	Leaves	19	120	56	123	N/A
Euphorbia tirucalli	Stems	**1205**	**2069**	77	**663**	Latex (non-mutagenic) (Paiva et al., 2011), genotoxic (Waczuk et al., 2015)

Plant names	Part used	TA98 (CFU)		TA100 (CFU)		Previous reports on mutagenicity
		AQ	ORG	AQ	ORG	
Garcinia livingstonei	Roots	62	117	177	**1151**	N/A
	Leaves	19	162	**715**	159	N/A
Grewia occidentalis	Roots	101	140	259	344	Non-mutagenic (Mulaudzi et al., 2013)
	Leaves	21	178	**979**	195	N/A
Gymnosporia senegalensis	Roots	185	108	**1087**	**774**	Non-mutagenic (Mulaudzi et al., 2012), mutagenic with S9 (Verschaeve and Van Staden, 2008)
	Leaves	28	76	63	**1019**	N/A
Hermannia boraginiflora	Roots	94	**356**	74	328	N/A
	Leaves	29	**2721**	75	**2456**	N/A
Hyphaene coriacea	Stem	198	**2021**	122	76	Non mutagenic (Ramulondi et al., 2018)
Hypoxis hemerocallidea	Corms	**1266**	**1241**	**1307**	**1887**	Non-mutagenic (Elgorashi et al., 2003; Reid et al., 2006; Ramulondi et al., 2018)
Kigelia africana	Bark	59	140	72	**948**	Non-mutagenic (fruit) (Elgorashi et al., 2003; Verschaeve and Van Staden, 2008)
Ochna natalitia	Roots	28	94	58	**1808**	N/A
	Leaves	28	196	319	283	Non-mutagenic (Makhafola et al., 2014)
Opuntia stricta	Stems	**2413**	**2848**	**930**	**770**	N/A
Ozoroa engleri	Bark	**494**	145	**1000**	**1032**1	Mutagenic (Ramulondi et al., 2018)
	Roots	58	47	26	**1142**	N/A

Plant names	Part used	TA98 (CFU)		TA100 (CFU)		Previous reports on mutagenicity
		AQ	ORG	AQ	ORG	
Peltophorum africanum	Leaves	21	**2462**	73	84	Non-mutagenic (bark) (Mulaudzi et al., 2013)
Ranunculus multifidus	Whole plant	103	235	166	**1875**	N/A
Rhoicissus digitata	Roots	78	132	86	**1535**	N/A
	Leaves	23	**1006**	68	414	N /A
Sapium integerrimum	Roots	61	121	29	**2455**	N/A
	Leaves	34	91	61	74	N/A
Scadoxus puniceus	Bulb	133	90	99	71	Non-mutagenic (Nair and Van Staden, 2013)
Sclerocarya birrea	Bark	25	245	**659**	**1993**	Non-mutagenic (Verschaeve and Van Staden, 2008)
Searsia nebulosa	Bark	78	**1836**	69	**1362**	N/A
Senecio serratuloides	Whole plant	77	170	**514**	**868**	Non-mutagenic (Elgorashi et al., 2003; Ramulondi et al., 2018), Genotoxic (Tamokou and Kuete, 2014)
Senegalia burkei	Bark	21	62	75	**790**	N/A
Tabernaemontana elegans	Roots	177	**1583**	**1074**	**1088**	N/A
	Leaves	131	116	**984**	**1546**	N/A
Trichilia dregeana	Roots	142	141	215	**814**	Non-mutagenic (Eldeen et al., 2005)
	Leaves	164	154	**895**	**2103**	Non-mutagenic (Eldeen et al., 2005)
	Bark	25	79	127	**717**	Non-mutagenic (Eldeen et al., 2005)
Vangueria infausta	Leaves	33	81	234	**718**	Non-mutagenic (Ramulondi et al., 2018)

Plant names	Part used	TA98 (CFU)		TA100 (CFU)		Previous reports on mutagenicity
		AQ	ORG	AQ	ORG	
Water (negative control)		131	-	174	-	N/A
DMSO (negative control)		-	142	-	274	N/A
4NQO (positive control)		**341**	-	-	-	N/A
Sodium azide (positive control)		-	-	**624**	-	N/A

Bold – Mutagenic; N/A – Not available; CFU – colony forming units; (*) – Negative control

lactation after giving birth (De Wet and Ngubane, 2014). The aqueous extract was noted as mutagenic (652 CFU) against the *S. typhimurium* TA100 strain. Other aqueous extracts that demonstrated a mutagenic response towards TA100 and were only reported once in the ethnobotany survey, were *D. villosa* root and *E. natalensis* roots. *Diospyros villosa* was reported in the survey to treat dysmenorrhoea (period pains), and *E. natalensis* is used to "cleanse the blood" during pregnancy (De Wet and Ngubane, 2014). With these plant species being mutagenic further studies can be done with lower dosages to find a non-mutagenic concentration. These plants are sometimes used in combination e.g *B. cathartica, H. hemerocallidea, S. serratulosed* and *R. multifidus* to name a few, hence the incorporation of other species may reduce the mutagenic potential.

The roots of *A. villicaulis* are used in northern Maputaland to alleviate period pains, and during pregnancy to sustain a healthy pregnancy and uncomplicated delivery (De Wet and Ngubane, 2014). This plant species has also been reported as traditionally used as an abortifacient (Steenkamp, 2003). In this study, both aqueous and organic extract results from the Ames test indicated no mutagenic effects against both the TA98 strain and TA100 strains. To the best of my knowledge, this is the only mutagenicity study to date that has been conducted on the roots of *A. villicaulis.* This plant is important as it has a wide range of other medicinal uses such as to treat coughs, diarrhoea, asthma and constipation in babies (Schmelzer, 2007). When the leaf extracts of this plant were tested for comparison purposes, the aqueous extract demonstrated no mutagenic activity (29 CFU) towards *S. typhimurium* TA98 strain, although the methanol-dichloromethane extract was mutagenic (721 CFU) towards the TA100 strain.

The herbaceous weed *A. glabratum* is used by women in Maputaland to alleviate cervical pains during pregnancy (De Wet and Ngubane 2014). In this study, the crude aqueous extract demonstrated no mutagenic potential with both the *S. typhimurium* TA98 strain and TA100 strains. However, the methanol-dichloromethane extract of this plant species indicated mutagenic activity towards the TA100 strain. There were no other mutagenic reports of this plant species found in the literature. The mutagenicity result from this study are important as the plant species is used during pregnancy. It is now known that it must be used with caution.

Bridelia cathartica is one of the most frequently used plants (18 times, 36%) in northern Maputaland for a variety of women-related ailments such as, infertility, dysmenorrhoea, amenorrhoea, menorrhagia, oligomenorrhoea, and to prevent preterm-birth (De Wet and Ngubane 2014). This plant species is also used worldwide to treat various diseases. However, no information on mutagenicity of this plant species was found in the literature. The current study indicated no mutagenicity of the aqueous leaf extracts. However, the aqueous root extract was mutagenic towards the *S. typhimurium* TA100 strain. The methanol-dichloromethane extract from both the leaves and root of *B. cathartica* were mutagenic towards the *S. typhimurium* TA100 strain. These results are concerning, considering that many people are relying on this plant species and use it with no knowledge of the adversity it may possess. During pregnancy, this plant is advised to be taken daily throughout the course of pregnancy for blood cleansing, and to prevent preterm or immature birth (De Wet and Ngubane, 2014). The mutagenic properties of the plant species may pose a threat to the unborn baby by causing cancer, Crohn's disease, cystic fibrosis, sickle cell anaemia or other associated genetic disorders. Usually, people in Maputaland believe that medicinal plants cannot cause harm besides the few plant species that the community is aware of such as *T. dregeana,* which was reported to induce abortion and diarrhoea when consumed in access (De Wet and Ngubane, 2014).

The extracts from *A. villicaulis* roots and leaves, *A. glabratum* whole plant*, B. cathartica* leaves, *C. neglecta* roots and leaves, *G. livingstonei* roots*, G. senegalensis* leaves, *K. africana* bark, *O. natalitia* roots, *P. africanum* roots, *R. multifidus* whole plant*, S. integerrimum* roots*, S. burkei* bark, *T. dregeana* roots and bark and *V. infausta* leaves demonstrated mutagenicity only towards the TA100 strain when the methanol-dichloromethane extracts were tested. No records were found in the literature on these plant species for comparison, therefore these findings were observed as the only existing data at the time of the study except *T. dregeana* bark extract, which was reported to have no mutagenic effects when tested against the *S. typhimurium* TA98 strain (Eldeen et al., 2005). This result is in line with the current findings. However, no other results were found for this plant extract against the TA100 strain. Other correlating results were observed in *C. filiformis* (mutagenic) (Wu et al*.,* 2017) *G. occidentalis* roots (Mulaudzi et al., 2013), *O. natalitia* leaves (Makhafola et

al., 2014) and *S. puniceus* (Nair and Van Staden, 2013) bulb which were non-mutagenic.

4.4 Summary

- Among the 51 plant extracts that was tested for mutagenicity, four (8%) aqueous- and 14 (27%) organic extracts demonstrated mutagenic effect towards the TA98 strain.
- With the TA100 strain, a higher rate of mutagenicity was observed with both the aqueous (28%) and organic (67%) samples.
- *Hypoxis hemerocallidea* and *O. stricta* were the most mutagenic, demonstrating mutagenicity with both aqueous and organic extracts across both strains.
- The variation of the results in aqueous versus organic extracts and *S. typhimurium* TA98 versus TA100 indicate the importance of incorporating different strains and extraction methods to get a broader indication of activity.
- The plant species that were most frequently reported for various gynaecological and obstetric conditions (*B. cathartica, R. multifidus* and *S. serratuloides*) were mutagenic against the TA100 strain.
- *Diospyros villosa, E. natalensis, O. natalitia, and S. integerrimum* leaves were non-mutagenic compared to their respective roots.

CHAPTER 5

Anti-*Candidal* assessment of some medicinal plant species used in northern Maputaland

5.1 Introduction

This Chapter intends to investigate the anti-C*andidal* activity of some medicinal plant species that were reported by women in northern Maputaland to treat various gynaecology and obstetrics related ailments (De Wet and Ngubane, 2014). The plant species were tested independently and in combination as documented in De Wet and Ngubane (2014) using the minimum inhibitory concentration (MIC) assay. The MIC assay has been successfully used to screen medicinal plants species against *Candida* species (Motsei et al., 2003; Runyoro et al., 2006; Steenkamp et al., 2007; Shai et al., 2008; Soliman et al., 2017). This method has the ability to screen for the active extract against the pathogen and also indicates the lowest concentration of the extract that is effective (Eloff, 1998). The MIC has been the preferred method for most antifungal studies for its reliability and efficiency and it was validated as precise and accurate (Veiga et al., 2019).

In the ethnobotanical study conducted by De Wet and Ngubane, (2014) none of the reported plant species were mentioned to be specifically used for genital yeast infections, which is mostly associated with *Candida* spp. (Dan et al., 2010). However, yeast infections are mostly prevalent in women, it would be beneficial to test the plant species that the women already use for other “women ailments” to determine if they could also be used for *Candidal* infections. The symptoms of a *Candida* infection can include itching and irritation in the vagina and vulva, burning during intercourse or while urinating, redness, pain or swelling of the vulva or the presence of a discharge. These all relate to gynaecological complaints in woman and thus validates the necessity to investigate further. The positive results from this study would have a great benefit to the women who depend on these plant species for medicinal use. Most importantly, with further testing, these plant species could yield alternative sources of treatment for *Candidal* infections.

5.2 Materials and methods

For the purpose of this study, the overnight *Candidal* cultures were prepared by adding 1 ml of the American Type Culture Collection (ATCC) reference stock cultures (*C. albicans* ATCC 10231, *C. glabrata* ATCC 900300 or *C. tropicalis* ATCC 750) into 10 ml of Tryptone Soya broth (TSB) and incubated for 48 hrs at 37 °C. The test samples were prepared by dissolving the plant extract to a concentration of 32.00 mg/ml using distilled water for aqueous extracts and acetone 100% v/v for methanol-dichloromethane (organic) extracts.

5.2.1 The MIC assay for independent plant extracts

Ninety-six well microtiter plates were aseptically prepared by placing 100.00 µl of the TSB in all wells. Then, 100 µl of the extract samples, solvent and broth were added in rows A1 to A12. The controls for this study were nystatin for positive control, the broth for culture control and a solvent for the negative control. These test samples were added in duplicate. A serial dilution was then performed by transferring 100 µl each time from the well A1 to B1, B1 to C1 wells and so forth from 8 mg/ml to 0.06 mg/ml (Figure 5.1).

After a serial dilution, the culture (*Candida* strains) were inoculated. During the culture inoculation, a 0.5 McFarland standard was performed by adding 1 ml of the properly vortexed overnight culture into approximately 10.00 ml of a sterile broth in the test tube. The McFarland standard was then vortexed and 1.00 ml added into 100.00 ml of the TSB to make a 1:100 dilution. Using a multichannel pipette, 100.00 µl of the 1:100 dilutions was aseptically transferred into each well and the plates were then sealed. A streak plate was also prepared for the cultures using Tryptone Soya agar (TSA). The microtiter plates and the streak plates were incubated at 37 °C for 48 hrs and sterile TSB was kept at room temperature. After the incubation period, the sterile TSB was observed for any turbidity, which could be an indication of contamination. The streak plates were also observed for pure culture growth and lastly the 1:100 dilution was observed for a successful yeast growth. When all the controls indicated no contamination and a successful growth of *Candida* strains was satisfactory, 40.00 µl of *p*-iodonitrotetrazolium chloride (INT) reagent was aseptically added into each well. A colorimetric INT reagent was used in this study to indicate the presence of a

redox reaction which is associated with an active microbial growth by turning to pinkish colour and remains clear where there is no microbial viability detected (Lall et al., 2013). The INT binds to living yeast cells and colourises red when viable. Plates were then allowed to stand for 24 hrs at room temperature. Thus, concentrations that are clear with the reagent are considered as having anti-C*andidal* activity. The MIC is determined as the lowest concentration to have a clear broth after the colorimetric reagent is added. The results were then read and recorded. According to Pauw and Eloff, (2014) medicinal plant extract having the MIC value of ≤ 0.16 mg/ml should be regarded as having a good activity and values between >0.16 and below 0.62 mg/ml as having moderate activity.

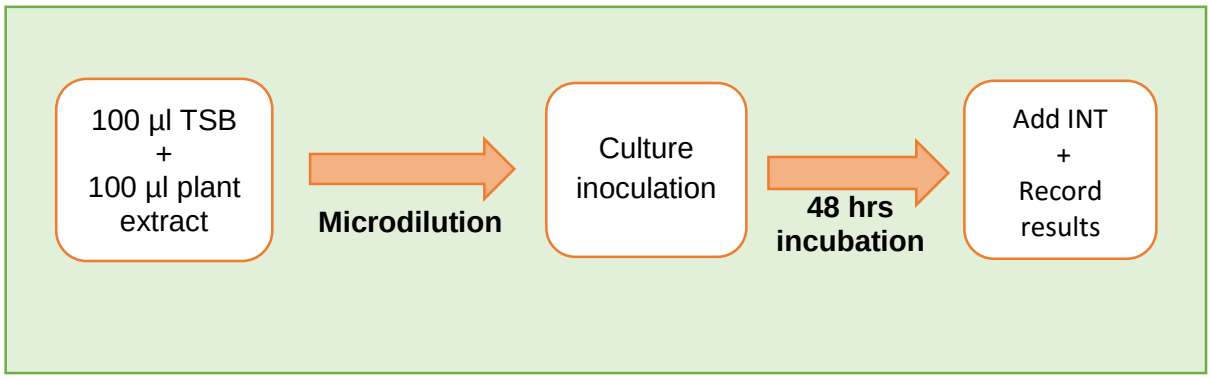

Figure 5.1 A summarized microdilution assay.

5.2.2 The MIC assay for combined plant extracts

Plant combination samples were prepared by dissolving the extracts to a concentration of 32.00 ml using distilled water and acetone 100% v/v for aqueous and organic extracts respectively. The equal volumes of the dissolved extracts (depending on the number of extracts making a combination) were then transfered into a single container and vortexed to obtain a 32 mg/ml plant combination. The MIC assay was then performed as previously explained in Section 5.2.1. The recorded results were further used to calculate the sum of fractional inhibitory concentration (ΣFIC) also known as the FIC index (Berenbaum et al., 1978). The FIC index is used to predict whether the interaction of the combined extracts is synergistic, additive, antagonistic or indifferent towards executing an antimicrobial effect. These classifications are based on the antimicrobial effect of a plant species combination in comparison to the effect of

individual plant species when tested independently. Where a concentration of each plant extract in a combination is treated as a fraction of concentration capable of producing the same antimicrobial effect when used independently.

A synergistic interaction of the extracts involved in a combination is important as it affects its activity against the pathogens. When extracts involved in a combination are having a synergistic interaction it means that their combined effect is greater than the sum of their individual effects at the same doses (Doldan-Martelli and Miguez, 2015, Basri and Sandra, 2016). The synergistic interactions in combination are perceived as having the benefits such as enhanced efficacy, reduce toxicity, lower the therapeutic dose and also reduce the increasing antimicrobial resistance (Van Vuuren and Viljoen, 2011). Combinations may also have indifferent interaction where there is neither positive nor negative effect when compared to the individual extracts acting separately. The other form of interaction is additive interactions where a slight improvement may be seen in a combination. However, some combinations may show an antagonistic interaction, when this interaction occurs, the effect of the combination is reduced when compared to the individually tested samples (Basri and Sandra, 2016). Equation 5.1 was used to determine the ΣFIC.

$$\Sigma FIC = FIC^{(i)} + FIC^{(ii)}$$

$$FIC^{(i)} = \frac{\text{MIC (a) in combination with (b)}}{\text{MIC (a) independently}}$$

$$FIC^{(ii)} = \frac{\text{MIC (a) in combination with (b)}}{\text{MIC (b) independently}}$$

Equation 5.1

(a) and (b) represent different plant samples, factor (c), (d) etc. can be added depending on the number of plant samples in a combination.

The results from Equation 5.1 were interpreted as follows; where the ΣFIC was ≤0.50, it was regarded as synergistic, >0.50 – 1.00 as additive, >1.00 – 4.00 as indifferent and >4.00 as antagonistic. In some cases, where the MIC was not determined at the highest concentration tested the ΣFIC could not be calculated. In that case, the ΣFIC value was indicated as “not determined” (ND).

5.3 Results and discussion

5.3.1 Plant species studied independently against *Candida* stains

5.3.1.1 *Candida albicans*

The majority of the aqueous extracts demonstrated low to no activity when tested against *C. albicans* (Table 5.1). Only 23 plant extracts (45%) indicated anti-*Candidal* activity at concentrations tested in this study (0.06 – 8.00 mg/ml). All the aqueous extracts demonstrated an MIC value above 0.16 mg/ml, hence no noteworthy activity. However, some moderate activities were observed on two aqueous extracts and those were *E. natalensis* roots (0.38 mg/ml) and *R. digitata* leaves (0.50 mg/ml).

Candida albicans is one of the most studied strains of all *Candida* spp. This can relate to it being the most isolated strain in clinical cases of yeast infections. A number of aqueous extracts assessed in this study have been previously investigated for anti-C*andidal* activity. These plant speices were *S. burkei* (>8.00 mg/ml), *E. tirucalli* (>8.00 mg/ml), *H. hemerocallidea* (>25.00 mg/ml , >8.00 mg/ml) (Motsei et al., 2003; Nciki et al., 2016), *K. africana* (16.00 mg/ml) (Naidoo et al., 2013), *O. engleri* (0.75 mg/ml, 8.00 mg/ml) (Naidoo et al., 2013; Nciki et al., 2016), *P. africanum* (4.00 mg/ml) Naidoo et al., 2013), *R. multifidus* (12.00 mg/ml, >8.00 mg/ml), *S. birrea* (>16.00 mg/ml, 1.00 mg/ml), *S. serratuloides* (2.00 mg/ml, >8.00 mg/ml) (Naidoo et al., 2013; Nciki et al., 2016), *S. puniceus* (3.00 mg/ml) (Mabona et al., 2013), *T. elegans* (3.30 mg/ml, 0.38 mg/ml, 8.00 mg/ml) (Steenkamp et al., 2007; Naidoo e al., 2013; Nciki et al., 2016) and *T. dregeana* (>16.00 mg/ml) (Naidoo et al., 2013). Similar results were obtained in the current assessment, where these extracts did not demonstrate any noteworthy activity.

There was also no noteworthy activity amongst the organic extracts when tested against *C. albicans* (Table 5.1). Most (42) of these extracts demonstrated the MIC value between 1.00 and 4.00 mg/ml. Some (five) of these extracts demonstrated a very high (>4.00 mg/ml) MIC to no activity (>8.00 mg/ml) based on the highest concentration tested in this study. There were a few (four) other plant extracts that demonstrated moderate anti-C*andidal* activity. These were *E. natalensis* roots (0.45 mg/ml), *O. stricta* stem (0.50 mg/ml), *P. africanum* roots (0.38 mg/ml) and *S. birrea*

bark (0.38 mg/ml). *Euclea natalensis* root extract was the only extract that demonstrated a noticeable (moderate) activity with both aqueous (0.38 mg/ml) and organic (0.43 mg/ml) extracts. In a similar study, Van Vuuren and Naidoo, (2010) reported that the leaf extract of *E. natalensis* had a MIC value of 0.5 mg/ml (aqueous) and 3.00 mg.ml (organic) against *C. albicans*. However, in this study, the leaves demonstrated a MIC value of 1.50 mg/ml and 2.00 mg/ml for the aqueous and organic extracts, respectively.

A few of the plant species have been previously tested against the *C. albicans* strain using different solvents for extraction. These plant species were *C. neglecta* bark, *P. africanum* root and *T. elegans* roots. In the current study, the organic extract of *C. neglecta* had a MIC value of 1.00 mg/ml, and this result was similar to Paraskeva et al., (2008) where a MIC value of 2.00 mg/ml was reported in a chloroform-methanol extract. The anti-C*andidal* activity of *E. tirucalli* was previously tested using the disc diffusion method (Rathi et al., 2012) and it was reported to have the best results at 100.00 mg/ml with 19 mm using n-hexane as a solvent. These results, however, differ greatly to the current findings using the MIC method with methanol-dichloromethane as a solvent where the anti-C*andidal* activity was observed at 1.00 mg/ml. Also, Rathi et al, 2012, tested the extract at a very high concentration compared to the current study, hence this study is not comparable.

The methanol-dichloromethane extract of *K. africana* bark in the current study demonstrated an MIC value of 1.00 mg/ml. However, a similar study by Shai et al., (2008) on this plant species using n-hexane, dichloromethane and acetone extracts reported the *K. africana* bark to have MIC values <0.45 mg/ml. The differences could be because of the different solvents used. The root extract of *P. africanum* was reported to have activity against *C. albicans* (Mazimba, 2014). However, Steenkamp et al., (2007) reported a MIC value of 16.00 mg/ml using a methanol extract, and these results correlate to the current study where the organic root extract demonstrated no noteworthy activity against *C. albicans* (MIC value of 1.00 mg/ml).

Table 5.1 The anti-C*andidal* activity of the aqueous and organic extracts against *C. albicans* (MIC value with standard deviation where n=±>3)

Plant Names	Part used	Aqueous extract (mg/ml)	Methanol-dichloromethane extract (mg/ml)
Acalypha villicaulis	Root	>8.00±0.00	1.00±0.00
	Leaves	2.00±0.00	1.00±0.00
Acanthospermum glabratum	Whole plant	4.00±0.00	3.00±0.00
Bridelia cathartica	Root	>8.00±0.00	2.00±0.00
	Leaves	>8.00±0.00	1.25±0.00
Cassytha filiformis	Whole plant	>8.00±0.00	2.00±0.00
Commiphora neglecta	Root	>8.00±0.00	2.00±0.00
	Leaves	>8.00±0.00	1.00±0.00
Crotalaria monteiroi	Root	>8.00±0.00	3.00±1.00
	Leaves	>8.00±0.00	4.00±0.00
Cyperus natalensis	Root	>8.00±0.00	1.00±0.00
	Leaves	>8.00±0.00	8.00±0.00
Diospyros villosa	Root	>8.00±0.00	4.00±0.00
	Leaves	2.00±0.00	4.00±0.00
Erythrina humeana	Root	>8.00±0.00	>8.00±0.00
Euclea natalensis	Root	**0.38±0.00**	**0.43±0.10**
	Leaves	1.50±0.00	2.00±0.00
Euphorbia tirucalli	Stem	>8.00±0.00	1.00±0.00
Garcinia livingstonei	Root	1.00±0.00	1.00±0.00
	Leaves	6.00±0.00	2.00±0.00
Grewia occidentalis	Root	8.00±0.00	4.00±0.00
	Leaves	>8.00±0.00	2.00±0.00
Gymnosporia senegalensis	Root	8.00±0.00	2.00±1.00
	Leaves	4.00±0.00	2.00±0.00
Hermannia boraginiflora	Root	8.00±0.00	1.00±0.00

Plant Names	Part used	Aqueous extract (mg/ml)	Methanol-dichloromethane extract (mg/ml)
	Leaves	8.00±0.00	3.00±1.00
Hyphaene coriacea	Stem	>8.00±0.00	>8.00±0.00
Hypoxis hemerocallidea	Corm	1.50±0.00	5.33±1.89
Kigelia africana	Bark	2.00±0.00	1.00±0.00
Ochna natalitia	Root	>8.00±0.00	1.00±0.00
	Leaves	3.00±0.00	2.00±0.00
Opuntia stricta	Stem	>8.00±0.00	**0.50±0.00**
Ozoroa engleri	Bark	>8.00±0.00	6.00±2.00
Peltophorum africanum	Root	>8.00±0.00	**0.38±0.13**
	Leaves	6.00±0.00	2.00±0.00
Ranunculus multifidus	Whole plant	>8.00±0.00	4.00±0.00
Rhoicissus digitata	Root	>8.00±0.00	1.00±0.00
	Leaves	**0.50±0.00**	2.00±0.00
Sapium integerrimum	Root	>8.00±0.00	2.00±0.00
	Leaves	3.00±0.00	2.00±0.00
Scadoxus puniceus	Bulb	>8.00±0.00	1.00±0.00
Sclerocarya birrea	Bark	>8.00±0.00	**0.38±0.13**
Searsia nebulosa	Bark	>8.00±0.00	2.00±0.00
Senecio serratuloides	Whole plant	2.00±0.00	3.00±1.00
Senegalia burkei	Bark	>8.00±0.00	2.00±0.00
Tabernaemontana elegans	Root	0.75±0.00	2.00±0.00
	Leaves	4.00±0.00	2.00±0.00
Trichilia dregeana	Root	6.00±0.00	6.00±0.00
	Bark	>8.00±0.00	1.00±0.00
	Leaves	>8.00±0.00	2.00±0.00
Vangueria infausta	Leaves	4.00±0.00	2.00±0.00
Nystatin (positive control)		0.001±0.00	
Culture control		>8.00±0.00	
Negative control		>8.00±0.00	

Bold- Moderate anti-C*andidal* activity

5.3.1.2 *Candida tropicalis*

The results found in this study (Table 5.2) shows that none of the aqueous extracts demonstrated a strong activity against *Candida tropicalis*. The three extracts which demonstrated the lowest MIC values of 0.75 mg/ml were *E. natalensis* roots, *G. livingstonei* root and *S. nebulosa* bark. No previous studies could be found in the literature to compare with the current results against this *Candidal* strain. However, in the current study, the *E. tirucalli* extract indicated to have no anti-*Candidal* activity with the MIC of 6.00 mg/ml.

When *C. tropicalis* was tested against the organic extracts, most of the extracts did not show any noteworthy activity. However, there were six organic extracts that demonstrated moderate activity. These extracts were *A. villicaulis* leaves, *A. glabratum* whole plant, *B. cathartica* leaves, *C. filiformis* whole plant, *E. tirucalli* stem and *G. livingstonei* root. Among all the organic extracts tested in this study against *C. tropicalis,* only *K. africana* bark had been previously tested. *Kigelia africana* currently demonstrated an MIC value of 1.00 mg/ml against *C. tropicalis.* However, in the study conducted by Jain and Belsare, (2009), this plant demonstrated activity with MIC values of 2.50 µg/ml (petroleum ether extract), 1.25 µg/ml (chloroform extract) and 2.50 µg/ml (methanol extract) against *C. tropicalis* using different solvents. The difference when comparing the results could be associated to the different geographical locations of the plants used, different extraction methods as well as the solvents used. There was only one plant species that demonstrated noteworthy anti-C*andidal* activity against *C. tropicalis,* namely *C. neglecta,* which had the lowest MIC value of 0.13 mg/ml in both the organic roots and leaf extracts. The roots of this plant are traditionally used to treat dysmenorrhoea, menorrhagia, oligomenorrhea, infertility and also to prevent pre-term birth during pregnancy (De Wet and Ngubane, 2014). There was no previous data found on this plant against *C. tropicalis.* However, it was reported to have potential anti-C*andidal* activity when it was tested against *C. albicans* (Paraskeva et al., 2008).

Table 5.2 The anti-C*andidal* activity of the single aqueous and organic extracts against *C. tropicalis*

Plant names	Part used	Aqueous extract (mg/ml)	Methanol-dichloromethane extract (mg/ml)
Acalypha villicaulis	Root	>8.00±0.00	>8.00±0.00
	Leaves	>8.00±0.00	**0.50±0.00**
Acanthospermum glabratum	Whole plant	4.00±0.00	**0.25±0.00**
Bridelia cathartica	Root	1.50±0.50	4.00±0.00
	Leaves	>8.00±0.00	**0.25±0.00**
Cassytha filiformis	Whole plant	>8.00±0.00	**0.25±0.00**
Commiphora neglecta	Root	>8.00±0.00	***0.13±0.00***
	Leaves	>8.00±0.00	***0.13±0.00***
Crotalaria monteiroi	Root	>8.00±0.00	2.00±0.00
	Leaves	>8.00±0.00	1.00±0.00
Cyperus natalensis	Root	>8.00±0.00	8.00±0.00
	Leaves	>8.00±0.00	>8.00±0.00
Diospyros villosa	Root	>8.00±0.00	>8.00±0.00
	Leaves	8.00±0.00	1.00±0.00
Erythrina humeana	Root	>8.00±0.00	1.50±0.00
Euclea natalensis	Root	0.75±0.25	2.00±0.00
	Leaves	2.00±0.00	2.00±0.00
Euphorbia tirucalli	Stem	6.00±2.00	**0.50±0.00**
Garcinia livingstonei	Root	0.75±0.25	**0.50±0.00**
	Leaves	>8.00±0.00	1.00±0.00
Grewia occidentalis	Root	>8.00±0.00	8.00±0.00
	Leaves	6.00±2.00	2.00±0.00
Gymnosporia senegalensis	Root	3.00±0.10	>8.00±0.00
	Leaves	1.00±0.00	1.00±0.00
Hermannia boraginiflora	Root	8.00±0.00	6.00±0.00
	Leaves	6.00±2.00	2.00±0.00
Hyphaene coriacea	Stem	8.00±0.00	2.00±0.00

Plant names	Part used	Aqueous extract (mg/ml)	Methanol-dichloromethane extract (mg/ml)
Hypoxis hemerocallidea	Corm	1.50±0.50	4.00±0.00
Kigelia africana	Bark	3.00±1.00	2.00±0.00
Ochna natalitia	Root	>8.00±0.00	2.00±0.00
	Leaves	>8.00±0.00	1.00±0.00
Opuntia stricta	Stem	>8.00±0.00	>8.00±0.00
Ozoroa engleri	Bark	>8.00±0.00	>8.00±0.00
Peltophorum africanum	Root	2.00±0.00	1.00±0.00
	Leaves	1.50±0.50	4.00±0.00
Ranunculus multifidus	Whole plant	>8.00±0.00	4.00±0.00
Rhoicissus digitata	Root	>8.00±0.00	2.00±0.00
	Leaves	1.00±0.00	1.00±0.00
Sapium integerrimum	Root	2.00±0.00	2.00±0.00
	Leaves	3.00±1.00	1.00±0.00
Scadoxus puniceus	Bulb	8.00±0.00	>8.00±0.00
Sclerocarya birrea	Bark	4.00±0.00	>8.00±0.00
Searsia nebulosa	Bark	0.75±0.25	2.00±0.00
Senecio serratuloides	Whole plant	7.00±1.73	4.00±0.00
Senegalia burkei	Bark	>8.00±0.00	2.00±0.00
Tabernaemontana elegans	Root	4.00±0.00	4.00±0.00
	Leaves	2.00±0.00	1.00±0.00
Trichilia dregeana	Root	>8.00±0.00	8.00±0.00
	Bark	>8.00±0.00	>8.00±0.00
	Leaves	4.00±0.00	2.00±0.00
Vangueria infausta	Leaves	2.00±0.00	1.00±0.00
Nystatin (positive control)		0.002±0.00	
Negative control		>8.00±0.00	
Culture control		>8.00±0.00	

Bold – moderate anti-*Candidal* activity, bold and italic – noteworthy anti-C*andidal* activity

5.3.1.3 *Candida glabrata*

The antimicrobial assay conducted against *C. glabrata* (Table 5.3) indicated poor anti-*Candidal* activity with both the aqueous and organic extracts. This demonstrates that this strain is more resilient (resistant) when compared to the other two strains (*C. albicans* and *C. tropicalis*) that were also screened in this study. Considering that the highest concentration used in this study was 8 mg/ml, only five of the 51 aqueous extracts exhibited a slight inhibition. The two aqueous extracts that exhibited the lowest MIC in this study were *G. livingstonei* roots and *S. integerrimum* leaves with an MIC of 4.00 mg/ml. It was noted that despite *C. glabrata* being among *Candida* strains that cause most human fungal infections, this strain is not frequently included in medical plant screenings (Kolaczkowski et al., 2009). There was no record of any of these aqueous extracts being previously tested against *C. glabrata* to compare with the current results.

There was a slight activity with the organic extracts when compared to the aqueous extracts, even though none of the extracts exhibited any noteworthy activity against *C. glabrata.* Approximately half (49%) of these extracts had a MIC value of 2.00 mg/ml followed by 45% having the MIC value of 1.00 mg/ml (Table 5.3). *Acalypha villicaulis* roots and *T. dregeana* roots had a MIC value of 4.00 mg/ml and 3.00 mg/ml respectively. *Euphorbia tirucalli and H. hemerocallidea* were the only plants that had been previously screened against *C. glabrata*, but different organic solvents were used. In the current study, the organic extract of *E. tirucalli* demonstrated an MIC value of 2.00 mg/ml. Previously, a n-hexane extract of *E. tirucalli* was reported to have an inhibition zone of 19 mm at 100 mg/ml using the disc diffusion method (Rathi et al., 2012), which was also a poor activity as observed with MIC method in the current study. A previous test that was conducted by Mwinga et al. (2019), reported *H. hemerocallidea* (petroleum ether extract and 50% methanol extract) as having a MIC value of 6.25 mg/ml and 3.13 mg/ml respectively. However, the organic extract of *H. hemerocallidea* in this study indicated a MIC value of 2 mg/ml. None of these extracts indicate a potential anti- anti-C*andidal* activity of *H. hemerocallidea* against the *C. glabrata* strain. The resistance of this strain corresponds to the reports that *C. glabrata* is the most resistant strain towards antifungal drugs when compared to the

other *Candida* strains (Musa et al., 2018). *Candida glabrata* is among the fungi strains that lack a membrane lipid called glucosylceramides (GlcCer) which is responsible for permeability and intracellular transportation, thus leading to resistance (Van Meer et al., 2003; Soliman et al., 2017).

Table 5.3. The anti- anti-C*andidal* activity of the aqueous and organic extracts against *C. glabrata*

Plant names	Part used	Aqueous extract (mg/ml)	Methanol-dichloromethane extract (mg/ml)
Acalypha villicaulis	Root	>8.00±0.00	4.00±0.00
	Leaves	>8.00±0.00	2.00±0.00
Acanthospermum glabratum	Whole plant	>8.00±0.00	1.00±0.00
Bridelia cathartica	Root	8.00±0.00	1.00±0.00
	Leaves	>8.00±0.00	1.00±0.00
Cassytha filiformis	Whole plant	>8.00±0.00	1.00±0.00
Commiphora neglecta	Root	>8.00±0.00	2.00±0.00
	Leaves	>8.00±0.00	2.00±0.00
Crotalaria monteiroi	Root	>8.00±0.00	2.00±0.00
	Leaves	>8.00±0.00	2.00±0.00
Cyperus natalensis	Root	>8.00±0.00	2.00±0.00
	Leaves	>8.00±0.00	2.00±0.00
Diospyros villosa	Root	>8.00±0.00	1.00±0.00
	Leaves	>8.00±0.00	2.00±0.00
Erythrina humeana	Root	>8.00±0.00	2.00±0.00
Euclea natalensis	Root	8.00±0.00	1.00±0.00
	Leaves	>8.00±0.00	1.00±0.00
Euphorbia tirucalli	Stem	>8.00±0.00	2.00±0.00
Garcinia livingstonei	Root	4.00±0.00	1.00±0.00
	Leaves	>8.00±0.00	2.00±0.00

Plant names	Part used	Aqueous extract (mg/ml)	Methanol-dichloromethane extract (mg/ml)
Grewia occidentalis	Root	>8.00±0.00	2.00±0.00
	Leaves	>8.00±0.00	2.00±0.00
Gymnosporia senegalensis	Root	>8.00±0.00	2.00±0.00
	Leaves	>8.00±0.00	2.00±0.00
Hermania boraginiflora	Root	>8.00±0.00	2.00±0.00
	Leaves	>8.00±0.00	2.00±0.00
Hyphaene coriacea	Stem	>8.00±0.00	2.00±0.00
Hypoxis hemerocallidea	Corm	8.00±0.00	2.00±0.00
Kigelia africana	Bark	>8.00±0.00	2.00±0.00
Ochna natalitia	Root	>8.00±0.00	1.00±0.00
	Leaves	>8.00±0.00	2.00±0.00
Opuntia stricta	Stem	>8.00±0.00	>8.00±0.00
Ozoroa engleri	Bark	>8.00±0.00	1.00±0.00
Peltophorum africanum	Root	>8.00±0.00	1.00±0.00
	Leaves	>8.00±0.00	1.00±0.00
Ranunculus multifidus	Whole plant	>8.00±0.00	1.00±0.00
Rhoicissus digitata	Root	>8.00±0.00	2.00±0.00
	Leaves	>8.00±0.00	1.00±0.00
Sapium integerrimum	Root	>8.00±0.00	2.00±0.00
	Leaves	4.00±0.00	1.50±0.50
Scadoxus puniceus	Bulb	>8.00±0.00	2.00±0.00
Sclerocarya birrea	Bark	>8.00±0.00	1.00±0.00
Searsia nebulosa	Bark	>8.00±0.00	1.00±0.00
Senecio serratuloides	Whole plant	>8.00±0.00	1.00±0.00
Senegalia burkei	Bark	>8.00±0.00	1.00±0.00
Tabernaemontana elegans	Root	>8.00±0.00	1.00±0.00
	Leaves	>8.00±0.00	1.00±0.00

Plant names	Part used	Aqueous extract (mg/ml)	Methanol-dichloromethane extract (mg/ml)
Trichilia dregeana	Root	>8.00±0.00	3.00±1.00
	Bark	>8.00±0.00	2.00±0.00
	Leaves	>8.00±0.00	1.00±0.00
Vangueria infausta	Leaves	>8.00±0.00	1.00±0.00
Nystatin (positive control)		0.002±0.00	
Culture control		>8.00±0.00	
Negative control		>8.00±0.00	

5.3.2 The anti-*Candidal* activity of the leaves for potential substitution of the roots

Because there was no previous reference of the roots in this study against *Candida* spp., the activity of the leaves could not be compared to that of the respective roots for the purpose of a potential substitution. Nonetheless, there was one plant species in this study that demonstrated activity for both their roots and leaves. This plant species was *C. neglecta,* which was tested against the *C. tropicalis* strain. Based on these results, the leaves of this plant species can be substituted for the roots.

5.3.3 Plant species studied in combination

The overall results of plant species combinations (Tables 5.4 – 5.6) did not differ much to the single plant species in this study across all the *Candidal* strains tested. Throughout the screening, the strains showed more resistance to the aqueous extract compared to organic extracts. All the plant species combinations tested in this study were screened for the first time against these *Candidal* strains, hence, no previous data was found for comparison.

5.3.3.1 *Candida albicans*

For the majority of the aqueous combinations tested in this study, MIC values were not determined, and the extracts/ combinations were only tested at a highest

concentration of 8.00 mg/ml. There were only two combinations that demonstrated any inhibition at the concentrations tested against the *C. albicans* strain, and these combinations were AC16 with MIC of 6.00 mg/ml and AC15 at 8.00 mg/ml (Table 5.4). In both of these combinations, the plant extracts showed no activity when tested independently. These two combinations could have slightly increased the effectiveness of these plant extracts, but not enough to result in an effective concentration. Those combinations also resulted in a poor activity against *C. albicans.*

With the majority of the aqueous extracts not showing anti-C*andidal* activity at the highest concentration tested independently and in combinations, MIC values were reported as >8 mg/ml. As a result, the ΣFIC values for these combinations could not be calculated. For the purpose of this study, these were recorded as “not determined” (ND) in Table 5.4. Without the ΣFIC to assess the class of interaction of the extracts involved in combinations, the interactions were estimated based on the MICs obtained with the extracts tested independently, compared to the MIC of the combination. It was observed that 13 of the combinations indicated an indifferent interaction. Therefore, neither the individual aqueous extracts nor the combinations had promising activity against *C. albicans.* The AC13 combination was the only combination that showed an antagonistic interaction for anti-C*andidal* activity against *C. albicans*. There were two aqueous combinations that demonstrated an additive interaction and those were AC15 and AC16.

As opposed to the aqueous combinations, all organic plant combinations had measurable MICs against *C. albicans.* Nine of these combinations had an MIC value of 1.00 mg/ml, whereas the other seven demonstrated an inhibition at lower concentrations (Table 5.5). In comparison with the aqueous extracts, the organic extracts showed better activity against *C. albicans.* The best results in this study was found at 0.30 mg/ml, which indicated moderate activity for the OC1 combination. Other moderate activities were observed at the MIC value of 0.50 mg/ml against the OC8 and OC5 combinations. All these extracts individually had MIC values between 1.00 mg/ml and 4.00 mg/ml, yet in combination these plant extracts demonstrated noteworthy activity. None of the organic extract combinations have been previously tested against *C. albicans.* However, some plant species (*T. dregeana, S. serratuloides, H. hemerocallidea, K. africana, S. birrea, T. elegans, R. multifidus*

Table 5.4 anti-C*andidal* activity of aqueous-combination extracts against *C. albicans* and the interactions among the extracts

Combinations **[MIC values in mg/ml for plant species investigated independently]**	**Codes**	**MIC of aqueous combination (mg/ml)**	**ΣFIC**	**Interactions among the extracts***
B. cathartica (root) [>8.00] + *C. neglecta* (root) [>8.00] + *C. monteiroi* (root) [>8.00] + *G. occidentalis* (root) [8.00] + *O. natalitia* (root) [>8.00] + *R. digitata* (root) [>8.00] + *G. livingstonei (root)* [1.00]	AC1	>8.00±0.00	ND	Indifferent
E. tirucalli (stem) [>8.00] + O*. engleri* (bark) [>8.00] + *S. puniceus* (bulb) [>8.00] + *S. serratuloides* (whole plant) [2.00]	AC2	>8.00±0.00	ND	Indifferent
B. cathartica (root) [>8.00] + *E. humeana* (root) [>8.00] + *O. natalitia* (root) [>8.00] + *T. elegans* (root) [0.75] + *S. nebulosa* (bark) [>8.00]	AC3	>8.00±0.00	ND	Indifferent
A. villicaulis (root) [>8.00] + *B. cathartica* (root) [>8.00] + *S. nebulosa* (bark) [>8.00]	AC4	>8.00±0.00	ND	Indifferent
B. cathartica (root) [>8.00] + *P. africanum* (root) [>8.00] + *R. digitata* (root) [>8.00]	AC5	>8.00±0.00	ND	Indifferent

Combinations **[MIC values in mg/ml for plant species investigated independently]**	**Codes**	**MIC of aqueous combination (mg/ml)**	**ΣFIC**	**Interactions among the extracts***
B. cathartica (root) [>8.00] + *H. coriacea* (stem) [>8.00] + *O. engleri* (bark) [>8.00]	AC6	>8.00±0.00	ND	Indifferent
R. multifidus (whole plant) [>8.00] + *S. serratuloides* (whole plant) [2.00]	AC7	>8.00±0.00	ND	Indifferent
B. cathartica (root) [>8.00] + *O. stricta* (stem) [>8.00] + *S. nebulosa* (bark) [>8.00]	AC8	>8.00±0.00	ND	Indifferent
R. multifidus (whole plant) [>8.00] + *H. hemerocallidea* (corm) [1.50]	AC9	>8.00±0.00	ND	Indifferent
K. africana (bark) [2.00] + *C. filiformis* (whole plant) [>8.00]	AC10	>8.00±0.00	ND	Indifferent
E. humeana (root) [>8.00] + *O. natalitia* (root) [>8.00]	AC11	>8.00±0.00	ND	Indifferent
B. cathartica (root) [>8.00] + *E. humeana* (root) [>8.00]	AC12	>8.00±0.00	ND	Indifferent
G. senegalensis (root) [8.00] + *H. hemerocallidea* (corm) [1.50]	AC13	>8.00±0.00	ND	Antagonistic
K. africana (bark) [2.00] + *S. nebulosa* (bark) [>8.00]	AC14	>8.00±0.00	ND	Indifferent
B. cathartica (root) [>8.00] + *O. natalitia* (root) [>8.00]	AC15	8.00±0.00	ND	Additive
S. birrea (bark) [>8.00] + *T. dregeana* (bark) [>8.00]	AC16	6.00±2.00	ND	Additive

Combinations [MIC values in mg/ml for plant species investigated independently]	Codes	MIC of aqueous combination (mg/ml)	ΣFIC	Interactions among the extracts*
Nystatin (positive control)		0.001±0.00		
Culture control		>8.00±0.00		
Negative control		>8.00±0.00		

* – Estimated interaction, ND - Not determined

Table 5.5 The anti-C*andidal* activity of organic-combination extracts against *C. albicans* and the interactions among the extracts

Combinations **[MIC values in mg/ml for plant species investigated independently]**	**Codes**	**MIC of Methanol-dichloromethane combination (mg/ml)**	**ΣFIC**	**Interaction among the extracts**
B. cathartica (root) [2.00] + *C. neglecta* (root) [2.00] + *C. monteiroi* (root) [3.00] + *G. occidentalis* (root) 4.00] + O. natalitia (root) [1.00] + *R. digitata* (root) [1.00] + *G. livingstonei* (root*)* [1.00]	OC1	**0.31±0.19**	1.47	Indifferent
E. tirucalli (stem) [1.00] + O*. engleri* (bark) [6.00] + *S. puniceus* (bulb) [1.00] + *S. serratuloides* (whole plant) [3.00]	OC2	0.75±0.25	1.88	Indifferent
B. cathartica (root) [2.00] + *E. humeana* (root) [>8.00] + *O. natalitia* (root) [1.00] + *T. elegans* (root) [2] + *S. nebulosa* (bark) [2.00]	OC3	1.00±0.00	ND	Indifferent*
A. villicaulis (root) [1.00] + *B. cathartica* (root) [2.00] + *S. nebulosa(bark)* [2.00]	OC4	1.00±0.00	2.00	Indifferent
B. cathartica (root) [2.00] + *P. africanum* (root) [0.38] + *R. digitata* (root) [1.00]	OC5	**0.50±0.00**	2.07	Indifferent
B. cathartica (root) [2.00] + *H. coriacea* (stem) [>8.00] + *O. engleri* (bark) [6.00]	OC6	1.00±0.00	ND	Additive*

Combinations **[MIC values in mg/ml for plant species investigated independently]**	**Codes**	**MIC of Methanol-dichloromethane combination (mg/ml)**	**ΣFIC**	**Interaction among the extracts**
R. multifidus (whole plant) [4.00] + *S. serratuloides* (whole plant) [3.00]	OC7	0.75±0.25	0.44	Synergistic
B. cathartica (root) [2.00] + *O. stricta* (stem) [0.50] + *S. nebulosa* (bark) [2.00]	OC8	**0.50±0.00**	1.50	Indifferent
R. multifidus (whole plant) [4.00] + *H. hemerocallidea* (corm) [5.33]	OC9	1.00±0.00	0.44	Synergistic
K. africana (bark) [1.00] + *C. filiformis* (whole plant) [2.00]	OC10	1.00±0.00	1.50	Indifferent
E. humeana (root) [>8.00]+ *O. natalitia* (root) [0.50]	OC11	0.75±0.25	ND	Indifferent*
B. cathartica (root) [2.00] + *E. humeana* (root) [>8.00]	OC12	1.00±0.00	ND	Additive*
G. senegalensis (root) [2.00] + *H. hemerocallidea* (corm) [5.33]	OC13	1.00±0.00	0.69	Additive
K. africana (bark) [1.00] + *S. nebulosa* (bark) [2.00]	OC14	1.00±0.00	1.50	Indifferent
B. cathartica (root) [2.00] + *O. natalitia* (root) [1.00]	OC15	1.00±0.00	1.50	Indifferent
S. birrea (bark) [0.38] + *T. dregeana* (bark) [1.00]	OC16	0.75±0.25	2.72	Indifferent
Nystatin		0.001±0.00		

Combinations [MIC values in mg/ml for plant species investigated independently]	Codes	MIC of Methanol-dichloromethane combination (mg/ml)	ΣFIC	Interaction among the extracts
Culture control		>8.00±0.00		
Negative control		>8.00±0.00		

Bold- Moderate activity, * – Estimated interaction, ND - Not determined

and *O. engleri*) in these combinations have been previously tested in combinations with different plant species where no noteworthy activity was reported (Naidoo et al., 2013).

When the ΣFIC was calculated for the 16 combinations assessed in this study, various interactions were observed, with two combinations having synergistic interactions These combinations were OC7 and OC9, both with the ΣFIC of 0.44. There were three combinations that demonstrated an additive interaction. Those combinations were OC12, OC13 and OC6. None of the organic extract combinations demonstrated an antagonistic interaction against *C. albicans*. These results indicate the importance of combination therapy. Singularly, the plant extracts may not be effective, but combining with the other plant extracts could activate and enhance the activity of the combination.

5.3.3.2 *Candida tropicalis*

The majority (12) of the aqueous combinations did not demonstrate any noteworthy activity against *C. tropicalis.* Only four combinations demonstrated anti-*Candidal* activity (Table 5.6). Twelve others showed no activity against *C. tropicalis* at the highest concentration of 8.00 mg/ml in this study. The lowest MIC obtained against *C. tropicalis* was 4.00 mg/ml, which was obtained in two combinations, namely, the AC15 and AC13. However, the aqueous extracts are usually expected to have a poor anti-C*andidal* activity when compared to the organic extracts as water can only extract the polar compounds, compared to the organic solvents which can extract both polar and non-polar compounds. The attempt to calculate the ΣFIC was not successful for most of the aqueous combinations against *C. tropicalis.* Most of the independent extracts and combinations did not present with an absolute MIC value at the highest level of concentration tested in this study (8.00 mg/ml). As the ΣFIC can only be calculated on absolute values, no quantitative value could be assigned to the interaction. Hence the interaction was estimated.

There was one combination in which the MIC values were determined while testing both the independent extracts as well as a combination, and that combination was AC13*.* This combination indicated an indifferent interaction when compared to the activity of the independent extracts with an MIC value of 4.00 mg/ml. All other combinations were compared in relation to the independent extracts and they were

mostly found to be indifferent (that was 15 combinations). There was one combination (AC14) that had an antagonistic interaction. No data was found in the literature for any plant. Synergism was observed in four organic combinations (Table 5.7), which were OC7, OC9, OC13 and OC16 in this study against *C. tropicalis.* The two organic combinations (OC7 and OC16) demonstrated both synergism as well as moderate activity. These combinations also demonstrated a synergistic interaction when compared to their respective extracts that were tested independently. However, organic extracts demonstrated an overall better result when compared to the aqueous extracts.

5.3.3.3 *Candida glabrata*

Candida glabrata strain also demonstrated resistance to most aqueous plant combinations. Only six out of the 16 plant combinations tested had some activity (Table 5.8). Five of these combinations had an MIC value of 8.00 mg/ml with only one combination (AC9) which showed a lower MIC value of 4.00 mg/ml. The ΣFIC of all aqueous combinations against *C. glabrata* could not be calculated as either the combination or an independent extract did not have a quantitative value assigned. Hence, all ΣFICs were recorded as “not determined” (ND) and for the interest of this study, all interactions were estimated. The estimated interactions indicated that the majority (12) of the combinations were indifferent with three showing non-interactive results. The only aqueous combination that demonstrated positive interaction was AC9 with an additive interaction. It is important to note that no adverse reaction (no antagonism) was observed.

The overall results of the organic combinations (Table 5.9) demonstrated better activity when compared to the aqueous extracts as expected. All combinations had an anti-C*andidal* activity against *C. glabrata*. However, it was noted that these combinations were active at slightly higher concentrations when compared to the active concentrations found against the other two strains (*C. albicans* and *C. tropicalis*) tested in this study. The highest active concentration obtained against *C. glabrata* was 3.00 mg/ml from the OC6 combination. The lowest MIC obtained was 0.75 mg/ml from the OC12 combination. The overall high MIC values of these combinations could be because this strain is resistant to many anti-*Candidal* drugs (Musa et al., 2018). The ΣFIC values also did not show any remarkable improvements in plant combinations

Table 5.6 The anti-C*andidal* activity of the aqueous-combination extracts against *C. tropicalis* and interactions among the extracts

Combinations **[MIC values in mg/ml for plant species investigated independently]**	**Codes**	**MIC of aqueous combination (mg/ml)**	**ΣFIC**	**Interactions among the extracts***
B. cathartica (root) [1.50] + *C. neglecta* (root) [>8±0] + *C. monteiroi* (root) [>8±0] + *G. occidentalis* (root) [>8±0] + *O. natalitia* (root) [>8±0] + *R. digitata* (root) [>8±0] + *G. livingstonei (root)* [0.75]	AC1	>8.00±0.00	ND	Indifferent
E. tirucalli (stem) [6.00] + O*. engleri* (bark) [>8±0] + *S. puniceus* (bulb) [>8±0] + *S. serratuloides* (whole plant) [7.00]	AC2	>8.00±0.00	ND	Indifferent
B. cathartica (root) [1.50] + *E. humeana* (root) [>8±0] + *O. natalitia* (root) [>8±0] + *T. elegans* (root) [4.00] + *S. nebulosa* (bark) [0.75]	AC3	>8.00±0.00	ND	Indiffereni
A. villicaulis (root) [>8±0] + *B. cathartica* (root) [1.50] + *S. nebulosa(bark)* [0.75]	AC4	>8.00±0.00	ND	Indifferent
B. cathartica (root) [1.50] + *P. africanum* (root) [2.00] + *R. digitata* (root) [>8±0]	AC5	>8.00±0.00	ND	Indifferent
B. cathartica (root) [1.50] + *H. coriacea* (stem) [8±0] + *O. engleri* (bark) [>8±0]	AC6	>8.00±0.00	ND	Indifferent

Combinations **[MIC values in mg/ml for plant species investigated independently]**	**Codes**	**MIC of aqueous combination (mg/ml)**	**ΣFIC**	**Interactions among the extracts***
R. multifidus (whole plant) [>8±0] + *S. serratuloides* (whole plant) [7.00]	AC7	>8.00±0.00	ND	Indifferent
B. cathartica (root) [1.50] + *O. stricta* (stem) [>8±0] + *S. nebulosa* (bark) [0.75]	AC8	8.00±0.00	ND	Indifferent
R. multifidus (whole plant) [>8±0] + *H. hemerocallidea* (corm) [1.50]	AC9	>8.00±0.00	ND	Indifferent
K. africana (bark) [3.00] + *C. filiformis* (whole plant) [>8±0]	AC10	>8.00±0.00	ND	Indifferent
E. humeana (root) [>8±0] + *O. natalitia* (root) [>8±0]	AC11	>8.00±0.00	ND	Indifferent
B. cathartica (root) [1.50] + *E. humeana* (root) [>8±0]	AC12	>8.00±0.00	ND	Indifferent
G. senegalensis (root) [3.00] + *H. hemerocallidea* (corm) [1.50]	AC13	4.00±0.00	4.00	Indifferent
K. africana (bark) [3.00] + *S. nebulosa* (bark) [0.75]	AC14	>8.00±0.00	ND	Antagonistic
B. cathartica (root) [1.50] + *O. natalitia* (root) [>8±0]	AC15	4.00±0.00	ND	Indifferent
S. birrea (bark) [4.00] + *T. dregeana* (bark) [>8±0]	AC16	6.00±2.00	ND	Indifferent
Nystatin		0.003±0.00		
Culture control		>8.00±0.00		

Combinations **[MIC values in mg/ml for plant species investigated independently]**	**Codes**	**MIC of aqueous combination (mg/ml)**	**ΣFIC**	**Interactions among the extracts***
Negative control	Codes	>8.00±0.00		

* – Estimated interaction, ND - Not determined

Table 5.7 The anti-C*andidal* activity of the organic-combination extracts against *C. tropicalis* and interactions among the extracts

Combinations **[MIC values in mg/ml for plant species investigated independently]**	**Codes**	**MIC of methanol-dichloromethane combination (mg/ml)**	**ΣFIC**	**Interactions among the extracts**
B. cathartica (root) [4.00] + *C. neglecta* (root) [0.13] + *C. monteiroi* (root) [2.00] + *G. occidentalis* (root) [8.00] + *O. natalitia* (root) [2.00] + *R. digitata* (root) [2.00] + *G. livingstonei (root)* [0.50]	OC1	1.00±0.00	11.75	Antagonistic
E. tirucalli (stem) [0.50] + O*. engleri* (bark) [>8.00] + *S. puniceus* (bulb) [>8.00] +*S. serratuloides* (whole plant) [4.00]	OC2	0.75±0.00	ND	Indifferent*
B. cathartica (root) [4.00] + *E. humeana* (root) [1.50] + *O. natalitia* (root) [2.00] + *T. elegans* (root) [4.00] + *S. nebulosa* (bark) [2.00]	OC3	2.00±0.00	4.33	Antagonistic
A. villicaulis (root) [>8.00] + *B. cathartica* (root) [4.00] + *S. nebulosa(bark)* [2.00]	OC4	1.00±0.00	ND	Additive*
B. cathartica (root) [4.00] + *P. africanum* (root) [1.00] + *R. digitata* (root) [2.00]	OC5	1.00±0.00	1.75	Indifferent
B. cathartica (root) [4.00] + *H. coriacea* (stem) [2.00] + *O. engleri* (bark) [>8.00]	OC6	2.00±0.00	ND	Indifferent*

Combinations **[MIC values in mg/ml for plant species investigated independently]**	**Codes**	**MIC of methanol-dichloromethane combination (mg/ml)**	**ΣFIC**	**Interactions among the extracts**
R. multifidus (whole plant) [4.00] + *S. serratuloides* (whole plant) [4.00]	OC7	0.50±0.50	0.25	Synergistic
B. cathartica (root) [4.00] + *O. stricta* (stem) [>8.00] + *S. nebulosa* (bark) [2.00]	OC8	1.00±0.00	ND	Additive*
R. multifidus (whole plant) [4.00] + *H. hemerocallidea* (corm) [4.00]	OC9	1.00±0.00	0.50	Synergistic
K. africana (bark) [2.00] + *C. filiformis* (whole plant) [0.25]	OC10	1.00±0.00	4.50	Antagonistic
E. humeana (root) [1.50] + *O. natalitia* (root) [2.00]	OC11	1.00±0.00	1.17	Indifferent
B. cathartica (root) [4.00] + *E. humeana* (root) [1.50]	OC12	0.75±0.25	0.69	Additive
G. senegalensis (root) [>8.00] + *H. hemerocallidea* (corm) [4.00]	OC13	1.00±0.00	ND	Synergistic*
K. africana (bark) [2.00] + *S. nebulosa* (bark) [2.00]	OC14	0.75±0.00	0.75	Additive
B. cathartica (root) [4.00] + *O. natalitia* (root) [2.00]	OC15	1.00±0.00	0.75	Additive
S. birrea (bark) [>8.00] + *T. dregeana* (bark) [>8.00]	OC16	0.50±0.00	ND	Synergistic*
Nystatin		0.003±0.00		

Combinations [MIC values in mg/ml for plant species investigated independently]	Codes	MIC of methanol-dichloromethane combination (mg/ml)	ΣFIC	Interactions among the extracts
Culture control		>8.00±0.00		
Negative control		>8.00±0.00		

Bold- Moderate activity, * – Estimated interaction, ND - Not determine

Table 5.8 The anti-C*andidal* activity of the aqueous-combination extracts against *C. glabrata* and interactions among the extracts

Combinations **[MIC values in mg/ml for plant species investigated independently]**	**Codes**	**MIC of aqueous combination (mg/ml)**	**ΣFIC**	**Interactions among the extracts**
B. cathartica (root) [8.00] +*C. neglecta* (root) [>8±0] + *C. monteiroi* (root) [>8±0] + *G. occidentalis* (root) [>8±0] + *O. natalitia* (root) [>8±0] + *R. digitata* (root) [>8±0] + *G. livingstonei* (root) [4.00]	AC1	>8.00±0.00	ND	Non-interactive*
E. tirucalli (stem) [>8±0] + O*. engleri* (bark) [>8±0] + *S. puniceus* (bulb) [>8±0] + *S. serratuloides* (whole plant) [>8±0]	AC2	>8.00±0.00	ND	Non-interactive *
B. cathartica (root) [8.00] + *E. humeana* (root) [>8±0] + *O. natalitia* (root) [>8±0] + *T. elegans* (root) [>8±0] + *S. nebulosa* (bark) [>8±0]	AC3	>8.00±0.00	ND	Non-interactive *
A. villicaulis (root) [>8±0] + *B. cathartica* (root) [8.00] + *S. nebulosa (bark)* [>8±0]	AC4	8.00±0.00	ND	Indifferent*
B. cathartica (root) [8.00] + *P. africanum* (root) [>8±0] + *R. digitata* (root) [>8±0]	AC5	8.00±0.00	ND	Indifferent*
B. cathartica (root) [8.00] + *H. coriacea* (stem) [>8±0] + *O. engleri* (bark) [>8±0]	AC6	8.00±0.00	ND	Indifferent*

Combinations **[MIC values in mg/ml for plant species investigated independently]**	**Codes**	**MIC of aqueous combination (mg/ml)**	**ΣFIC**	**Interactions among the extracts**
R. multifidus (whole plant) [>8±0] + *S. serratuloides* (whole plant) [>8±0]	AC7	>8.00±0.00	ND	Indifferent*
B. cathartica (root) [8.00] + *O. stricta* (stem) [>8±0] + *S. nebulosa* (bark) [>8±0]	AC8	8.00±0.00	ND	Indifferent*
R. multifidus (whole plant) [>8±0] + *H. hemerocallidea* (corm) [8.00]	AC9	4.00±0.00	ND	Additive*
K. africana (bark) [>8±0] + *C. filiformis* (whole plant) [>8±0]	AC10	>8.00±0.00	ND	Indifferent*
E. humeana (root) [>8±0] + *O. natalitia* (root) [>8±0]	AC11	>8.00±0.00	ND	Indifferent*
B. cathartica (root) [8.00] + *E. humeana* (root) [>8±0]	AC12	>8.00±0.00	ND	Indifferent*
G. senegalensis (root) [>8±0] + *H. hemerocallidea* (corm) [8.00]	AC13	>8.00±0.00	ND	Indifferent*
K. africana (bark) [>8±0] + *S. nebulosa* (bark) [>8±0]	AC14	>8.00±0.00	ND	Indifferent*
B. cathartica (root) [8.00] + *O. natalitia* (root) [>8±0]	AC15	>8.00±0.00	ND	Indifferent*
S. birrea (bark) [>8±0] + *T. dregeana* (bark) [>8±0]	AC16	8.00±0.00	ND	Indifferent*
Nystatin		0.01±0.00		
Culture control		>8.00±0.00		

Combinations [MIC values in mg/ml for plant species investigated independently]	Codes	MIC of aqueous combination (mg/ml)	ΣFIC	Interactions among the extracts
Negative control		>8.00±0.00		

Bold- Moderate activity, * – Estimated interaction, ND - Not determine

Table 5.9. The anti-C*andidal* activity of the organic-combination extracts against *C. glabrata*

Combinations **[MIC values in mg/ml for plant species investigated independently]**	**Codes**	**MIC of methanol-dichloromethane combination (mg/ml)**	**ΣFIC**	**Interactions among the extracts**
B. cathartica (root) [1.00] + *C. neglecta* (root) [2.00] + *C. monteiroi* (root) [2.00] +*G. occidentalis* (root) [2.00] + *O. natalitia* (root) [1.00] + *R. digitata* (root) [2.00] + *G. livingstonei* (root) [2.00]	OC1	1.00±0.00	4.50	Antagonistic
E. tirucalli (stem) [2.00] + O*. engleri* (bark) [1.00] + *S. puniceus* (bulb) [2.00] + *S. serratuloides* (whole plant) [1.00]	OC2	2.00±0.00	6.00	Antagonistic
B. cathartica (root) [1.00] + *E. humeana* (root) [2.00] + *O. natalitia* (root) [1.00] + *T. elegans* (root) [1.00] + *S. nebulosa* (bark) [1.00]	OC3	1.00±0.00	4.50	Antagonistic
A. villicaulis (root) [4.00] + *B. cathartica* (root) [1.00] + *S. nebulosa (bark)* [1.00]	OC4	2.00±0.00	4.50	Antagonistic
B. cathartica (root) [1.00] + *P. africanum* (root) [1.00] + *R. digitata* (root) [2.00]	OC5	1.00±0.00	2.50	Indifferent
B. cathartica (root) [1.00] + *H. coriacea* (stem) [2.00] + *O. engleri* (bark) [1.00]	OC	3.00±1.00	7.50	Antagonistic

Combinations **[MIC values in mg/ml for plant species investigated independently]**	**Codes**	**MIC of methanol-dichloromethane combination (mg/ml)**	**ΣFIC**	**Interactions among the extracts**
R. multifidus (whole plant) [1.00] + *S. serratuloides* (whole plant) [1.00]	OC7	1.00±0.00	2.00	Indifferent
B. cathartica (root) [1.00] + *O. stricta* (stem) [>8.00] + *S. nebulosa* (bark) [1.00]	OC8	2.00±0.00	ND	Non-interactive*
R. multifidus (whole plant) [1.00] + *H. hemerocallidea* (corm) [2.00]	OC9	1.50±0.50	2.25	Indifferent
K. africana (bark) [2.00] + *C. filiformis* (whole plant) [1.00]	OC10	1.50±0.50	2.25	Indifferent
E. humeana (root) [2.00] + *O. natalitia* (root) [1.00]	OC11	2.00±0.00	4.00	Indifferent
B. cathartica (root) [1.00] + *E. humeana* (root) [2.00]	OC12	0.75±0.25	1.13	Indifferent
G. senegalensis (root) [2.00] + *H. hemerocallidea* (corm) [2.00]	OC13	1.00±0.00	1.00	Additive
K. africana (bark) [2.00] + *S. nebulosa* (bark) [1.00]	OC14	1.50±0.50	2.25	Indifferent
B. cathartica (root) [1.00] + *O. natalitia* (root) [1.00]	OC15	1.00±0.00	2.00	Indifferent
S. birrea (bark) [1.00] + *T. dregeana* (bark) [2.00]	OC16	1.00±0.00	1.50	Indifferent
Nystatin		0.01±0.00		

Combinations [MIC values in mg/ml for plant species investigated independently]	Codes	MIC of methanol-dichloromethane combination (mg/ml)	ΣFIC	Interactions among the extracts
Culture control		>8.00±0.00		
Negative control		>8.00±0.00		

* – Estimated interaction, ND - Not determined

when compared to individual extracts. The interactions were mostly indifferent and antagonistic. Therefore, *C. glabrata* was the most resilient of all *Candidal* strains.

5.4 Summary

- In this study, 51 independent and 16 different combinations (aqueous and organic extracts) were tested for anti-C*andidal* activity against *C. albicans, C. tropicalis* and *C. glabrata* using the broth microdilution (MIC) assay.
- These *Candida* strains demonstrated a very strong resistance to the extracts. However, the methanol-dichloromethane extracts demonstrated a stronger activity when compared to the aqueous extracts.
- Only the organic solvent leaf and root extracts of *C. neglecta* demonstrated a noteworthy anti-C*andidal* activity against *C. tropicalis* with an MIC value of 0.13 mg/ml.
- The OC7 and OC9 were the two organic extract combinations to show synergistic interactions against both *C. albicans* and *C. tropicalis.* The OC13 combination demonstrated a positive (additive) interaction across all three *Candida* strains in this study.
- The most synergistic combination was OC7 against *C. tropicalis.*
- The most antagonististic interaction was observed with OC1 against *C. tropicalis.*

CHAPTER 6
Conclusion and future recommendations

This current study is a follow up from the study on the ethnobotany survey conducted in Maputaland (2014) on the medicinal plant species used for gynaecology and obstetrics ailments. In the survey, the women reported that they use medicinal plants regularly for problems mostly associated with menstruation, pregnancy, infertility and many more medical conditions specifically related to women. The women were confident that these plant species are safe except for one plant species (*T. dregeana*), which was known to most women as an abortifacient and in high concentrations it can induce diarrhoea (De Wet and Ngubane, 2014). In the current study, a set of objectives (Chapter 1, Section 1.6) were established to evaluate the safety of these plants. To achieve the first to third objectives of this study, the relevant plant species were selected from the previous ethnobotanical survey and collected. In addition, the respective aqueous and methanol-dichloromethane (organic) extracts were prepared.

6.1 Toxicity evaluation of the selected plant species

The forth objective of the study was to assess toxicity of the plant extracts at 1.00 mg/ml by means of the BSLA. The most frequently used plant species according to De Wet and Ngubane (2014) were toxic when the organic extracts (*B. cathartica, H. hemerocallidea, O. natalitia* and *T. dregeana*) were tested in the BSLA. Two exceptions were *S. serratuloides* and *R. multifidus*. However, most of these frequently used plant species were non-toxic in the aqueous extracts (preferred traditional used method) except for *T. dregeana* bark (100% mortality) and roots (92% mortality), which was also reported by the lay women as harmful. All plant extracts where toxicity was found were subjected to further tests at lower concentrations (0.50, 0.25, 0.125, 0.063, and 0.031 mg/ml). All plant extracts were non-toxic at concentrations below 0.120 mg/ml, except for the organic extracts of *H. boraginiflora* root, *S. integerrimum* root, *S. puniceus* bulb and *T. elegans* root, which remained toxic. *Crotalaria monteiroi* and *G. senegalensis* leaf extracts were non-toxic and therefore can be explored for potential root substitution.

6.2 Mutagenicity potential of the selected plant species

The fifth objective of the study was to investigate mutagenicity of the plant extracts using the Ames assay against *Salmonella typhimurium* TA98 and TA100 strains at 5.00 mg/ml. Among the 51 plant extracts that were tested, six (*A. villicaulis* root, *C. natalensis* root, *E. natalensis* leaves, *G. occidentalis* root, *O. natalitia* leaves, *S. integerrimum* leaves and *S. puniceus* bulb) were non mutagenic against both strains. The most frequently reported plant species used for medical conditions related to women (such as *B. cathartica, H. hemerocallidea, O. natalitia, R. multifidus, S. serratuloides* and *T. dregeana*) were mutagenic in the Ames assay.

6.3 Anti-*Candidal* assessment of the selected plant species

To accomplish the sixth objective of the study, plant species were assessed for anti-*Candida* activity against *C. albicans, C. tropicalis* and *C. glabrata* using the microdilution MIC assay. No anti-*Candidal* activity was observed with the aqueous extracts. Six extracts [*E. natalensis* root (aqueous and organic)*, O. stricta* stem (organic), *P. africanum* root (organic), *R. digitata* leaves (aqueous) and *S. birrea* stem (organic)] were moderately active (MIC range between 0.16 and 0.62 mg/ml) against *C. albicans.* No plant extract indicated noteworthy activity against *C. glabrata.* The organic root and leaf extract of *C. neglecta* demonstrated a noteworthy activity against *C. tropicalis* with the MIC of 0.13 mg/ml.

6.4 Interaction of plant species in combination

The last objective of the study was to assess the interaction among the plant extracts used in combination and compare it to the efficacy of plant extracts independently. Sixteen plant combinations were assessed for toxicity and anti-*Candidal* activity. Three plant combinations were non-toxic in the BSLA and those were *E. tirucalli* (root) + *O. engleri* (bark) + *S. puniceus* (bulb) + *S. serratuloides* (whole plant), *B. cathartica* (root) + *O. stricta* (stem) + *S. nebulosa* (bark) and *B. cathartica* (root) + *E. humeana* (root). Where toxicity was found, lower concentrations were tested to find the acceptable toxic concentration (ATC). The results indicated that all plant combinations were non-toxic at a concentration below 0.42 mg/ml. The ΣFIC was calculated and the aqueous combinations such as *E. tirucalli* (root) + *O. engleri* (bark) + *S. puniceus* (bulb)

+ *S. serratuloides* (whole plant) and *K. africana* (bark) + *C. filiformis* (whole plant) were found to be synergistic and non-toxic.

In the anti-*Candidal* study, none of the aqueous combinations demonstrated noteworthy activity or synergy against the *Candida* strains. The organic combinations that demonstrated anti-*Candidal* activity (moderate) against *C. albicans* were OC1, (0.31 mg/ml MIC), OC5 (0.50 mg/ml MIC) and OC8 (0.50 mg/ml). Against *C. tropicalis*, OC7 (0.50 mg/ml MIC) and OC16 (0.50 mg.ml MIC) were moderately active. With the ΣFIC calculations, synergistic interactions were observed in combinations OC7 and OC9 against *C. albicans* and *C. tropicalis.* The organic combination of *S. birrea* (bark) + *T. dregeana* (bark) was synergistic against *C. tropicalis*.

6.5 The correlation between toxicity, mutagenicity and anti-*Candida* activity of individual plant species and combinations

Table 6.1 provides an overview of all the parameters studied and a summary of the results. In examining this overview, there are 17 aqueous extracts and five organic extracts that were neither toxic nor mutagenic. However, only *A. villicaulis* and *G. occidentalis* roots were non-toxic and non-mutagenic in both aqueous and organic extracts. It was also established that concentrations when diluted, were found to be non-toxic at lower concentrations (between 0.98 and 0.12 mg/ml). Plant species such as *A. villicaulis* leaves, *A. glabratum* whole plant, *C. filiformis* whole plant, *C. neglecta* root, *E. tirucalli* stem, *G. livingstonei* root, *O. stricta* stem, *P. africanum* root, *R. digitata* leaves and *S. birrea* bark held some promise as they demonstrated anti-*Candidal* activity at concentrations that were non-toxic in the BSLA. The leaves that were evaluated for potential substitution for roots cannot be used as they were toxic and/ or mutagenic.

In Table 6.2, the organic combinations such as *B. cathartica* (roots) + *C. neglecta* (roots) + *C. monteiroi* (root) + *G. livingstonei* (root) + *G. occidentalis* (root) + *O. natalitia* (root) + *R. digitata* (root) (0.31 mg/ml MIC), *B. cathartica* (root) + *P. africanum* (root) + *R. digitata* (root) (0.50 mg.ml MIC) and *B. cathartica* (root) + *O. stricta* (stem) + *S. nebulosa* (bark) (0.50 mg/ml MIC) against *C. albicans,* and *R. multifidus* (whole plant) + *S. serratuloides* (whole plant) (0.5. mg/ml MIC) and *S. birrea* (bark) +

Table 6.1 Summary of BSLA, Ames test and anti-C*andidal* activity of individual aqueous and methanol-dichloromethane (organic) plant extracts

Plant extracts	Plant part used	Traditional use	No. of reports	Extract	BSLA	Ames test	Anti-*Candida* activity
Acalypha villicaulis	**Root**	Dys, Lab and Blc	1	Aqueous	Non-toxic	Non-mutagenic	Poor activity
				MeOH: DCM	Non-toxic	Non-mutagenic	Poor activity
	Leaves	—	—	Aqueous	Toxic	Non-mutagenic	Poor activity
				MeOH: DCM	Non-toxic	Mutagenic	Moderate activity against *C. tropicalis* ATCC 750
Acanthospermum glabratum	Whole plant	Cervical pain		Aqueous	Non-toxic	Non-mutagenic	Poor activity
				MeOH: DCM	Non-toxic	Mutagenic	Moderate activity against *C. tropicalis* ATCC 750
Bridelia cathartica	Roots	Dys, Inf, Ame, Men, Oli, Pmb and Blc	8	Aqueous	Non-toxic	Mutagenic	Poor activity
				MeOH: DCM	Toxic	Mutagenic	Poor activity
	Leaves	—	—	Aqueous	Toxic	Non-mutagenic	Poor activity
				MeOH: DCM	Toxic	Mutagenic	Moderate activity against *C. tropicalis* ATCC 750
Cassytha filiformis	Whole plant	Induce lactation	1	Aqueous	Non-toxic	Mutagenic	Poor activity
				MeOH: DCM	Toxic	Mutagenic	Poor activity
Commiphora neglecta	Roots		1	Aqueous	Non-toxic	Non-mutagenic	Poor activity

Plant extracts	Plant part used	Traditional use	No. of reports	Extract	BSLA	Ames test	Anti-*Candida* activity
		Dys, Inf, Men, Oli, Pmb and Blc		MeOH: DCM	Non-toxic	Mutagenic	Noteworthy activity against *C. tropicalis* ATCC 750
	Leaves	—	—	Aqueous	Toxic	Non-mutagenic	Poor activity
				MeOH: DCM	Toxic	Mutagenic	Noteworthy activity against *C. tropicalis* ATCC 750
Crotalaria monteiroi	Roots	Dys, Inf, Men, Oli, Pmb and Blc	1	Aqueous	Toxic	Non-mutagenic	Poor activity
				MeOH: DCM	Toxic	Mutagenic	Poor activity
	Leaves	—	—	Aqueous	Non-toxic	Mutagenic	Poor activity
				MeOH: DCM	Non-toxic	Mutagenic	Poor activity
Cyperus natalensis	Roots	Men	1	Aqueous	Non-toxic	Non-mutagenic	Poor activity
				MeOH: DCM	Toxic	Non-mutagenic	Poor activity
	Shoot	—	—	Aqueous	Toxic	Mutagenic	Poor activity
				MeOH: DCM	Toxic	Mutagenic	Poor activity
Diospyros villosa	Roots	Dys	1	Aqueous	Non-toxic	Mutagenic	Poor activity
				MeOH: DCM	Toxic	Non-mutagenic	Poor activity
	Leaves	—	—	Aqueous	Toxic	Mutagenic	Poor activity
				MeOH: DCM	Non-toxic	Non-mutagenic	Poor activity
Erythrina humeana	Roots		4	Aqueous	Toxic	Mutagenic	Poor activity

Plant extracts	Plant part used	Traditional use	No. of reports	Extract	BSLA	Ames test	Anti-*Candida* activity
		Dys, Inf, Fib, Men, Mis		MeOH: DCM	Non-toxic	Mutagenic	Poor activity
Euclea natalensis	Roots	Blc	1	Aqueous	Non-toxic	Mutagenic	Moderate activity against *C. albicans* ATCC 10231
				MeOH: DCM	Toxic	Non-mutagenic	Moderate activity against *C. albicans* ATCC 10231
	Leaves	—	—	Aqueous	Toxic	Non-mutagenic	Poor activity
				MeOH: DCM	Toxic	Non-mutagenic	Poor activity
Euphorbia tirucalli	Stem	Wrt	1	Aqueous	Toxic	Mutagenic	Poor activity
				MeOH: DCM	Toxic	Mutagenic	Moderate activity against *C. tropicalis* ATCC 750
Garcinia livingstonei	Roots	Dys, Pph	2	Aqueous	Non-toxic	Non-mutagenic	Poor activity
				MeOH: DCM	Non-toxic	Mutagenic	Moderate activity against *C. tropicalis* ATCC 750
	Leaves	—	—	Aqueous	Toxic	Mutagenic	Poor activity
				MeOH: DCM	Non-toxic	Non-mutagenic	Poor activity
Grewia occidentalis	**Roots**	Dys,Inf, Men, Oli, Pmb and Blc	1	Aqueous	Non-toxic	Non-mutagenic	Poor activity
				MeOH: DCM	Non-toxic	Non-mutagenic	Poor activity
	Leaves	—	—	Aqueous	Toxic	Mutagenic	Poor activity
				MeOH: DCM	Non-toxic	Non-mutagenic	Poor activity
Gymnosporia senegalensis	Roots	Col, Inf	3	Aqueous	Non-toxic	Mutagenic	Poor activity
				MeOH: DCM	Non-toxic	Mutagenic	Poor activity

Plant extracts	Plant part used	Traditional use	No. of reports	Extract	BSLA	Ames test	Anti-*Candida* activity
	Leaves	—	—	Aqueous	Non-toxic	Non-mutagenic	Poor activity
				MeOH: DCM	Non-toxic	Mutagenic	Poor activity
Hermannia boraginiflora	Roots	Dys, Lab	1	Aqueous	Toxic	Non-mutagenic	Poor activity
				MeOH: DCM	Toxic	Mutagenic	Poor activity
	Leaves	—	—	Aqueous	Toxic	Non-mutagenic	Poor activity
				MeOH: DCM	Toxic	Mutagenic	Poor activity
Hyphaene coriacea	Stem	Dys, Inf, Abp, Pph and Lab	7	Aqueous	Non-toxic	Non-mutagenic	Poor activity
				MeOH: DCM	Non-toxic	Mutagenic	Poor activity
Hypoxis hemerocallidea	Corm	Cervical pain, Inf, Wrt and Men	6	Aqueous	Non-toxic	Mutagenic	Poor activity
				MeOH: DCM	Toxic	Mutagenic	Poor activity
Kigelia Africana	Bark	Cervical pain, Blc, Lac and Dys	3	Aqueous	Non-toxic	Non-mutagenic	Poor activity
				MeOH: DCM	Non-toxic	Mutagenic	Poor activity
Ochna natalitia	Roots	Dys,Inf, Men, Oli, Pmb, Lab and Blc	8	Aqueous	Non-toxic	Non-mutagenic	Poor activity
				MeOH: DCM	Toxic	Mutagenic	Poor activity
	Leaves	—	—	Aqueous	Toxic	Non-mutagenic	Poor activity

Plant extracts	Plant part used	Traditional use	No. of reports	Extract	BSLA	Ames test	Anti-*Candida* activity
				MeOH: DCM	Toxic	Non-mutagenic	Poor activity
Opuntia stricta	Stem	Cervical pain and Blc	3	Aqueous	Toxic	Mutagenic	Poor activity
				MeOH: DCM	Non-toxic	Mutagenic	Moderate activity against *C. albicans* ATCC 10231
Ozoroa engleri	Bark	Dys, Abp, Inf, Men, Oli, Pmb, Wrt and Blc	3	Aqueous	Toxic	Mutagenic	Poor activity
				MeOH: DCM	Toxic	Mutagenic	Poor activity
Peltophorum africanum	Roots	Dys, Ame and Blc	3	Aqueous	Non-toxic	Non-mutagenic	Poor activity
				MeOH: DCM	Non-toxic	Mutagenic	Moderate activity against *C. albicans* ATCC 10231
	Leaves	—	—	Aqueous	Non-toxic	Non-mutagenic	Poor activity
				MeOH: DCM	Toxic	Mutagenic	Poor activity
Ranunculus multifidus	Whole plant	Wrt and Swl	13	Aqueous	Non-toxic	Non-mutagenic	Poor activity
				MeOH: DCM	Non-toxic	Mutagenic	Poor activity
Rhoicissus digitata	Roots	Dys,Inf, Men, Oli, Pmb, and Blc	3	Aqueous	Non-toxic	Non-mutagenic	Poor activity
				MeOH: DCM	Toxic	Mutagenic	Poor activity
	Leaves	—	—	Aqueous	Toxic	Non-mutagenic	Moderate activity against *C. albicans* ATCC 10231
				MeOH: DCM	Toxic	Mutagenic	Poor activity
Sapium integerrimum	Roots	Dys	1	Aqueous	Non-toxic	Non-mutagenic	Poor activity

Plant extracts	Plant part used	Traditional use	No. of reports	Extract	BSLA	Ames test	Anti-*Candida* activity
				MeOH: DCM	Toxic	Mutagenic	Poor activity
	Leaves	—	—	Aqueous	Toxic	Non-mutagenic	Poor activity
				MeOH: DCM	Toxic	Non-mutagenic	Poor activity
Scadoxus puniceus	Bulb	Blc	1	Aqueous	Toxic	Non-mutagenic	Poor activity
				MeOH: DCM	Toxic	Non-mutagenic	Poor activity
Sclerocarya birrea	Bark	Abt	1	Aqueous	Non-toxic	Mutagenic	Poor activity
				MeOH: DCM	Toxic	Mutagenic	Moderate activity against *C. albicans* ATCC 10231
Searsia nebulosa	Bark	Dys, Inf and Wrt	3	Aqueous	Non-toxic	Non-mutagenic	Poor activity
				MeOH: DCM	Non-toxic	Mutagenic	Poor activity
Senecio serratuloides	Whole plant	Cervical pain, Inf, Blc and Wrt	9	Aqueous	Non-toxic	Mutagenic	Poor activity
				MeOH: DCM	Non-toxic	Mutagenic	Poor activity
Senegalia burkei	Bark	Blc	1	Aqueous	Non-toxic	Non-mutagenic	Poor activity
				MeOH: DCM	Non-toxic	Mutagenic	Poor activity
Tabernaemontana elegans	Roots	Dys and Inf	1	Aqueous	Toxic	Mutagenic	Poor activity
				MeOH: DCM	Toxic	Mutagenic	Poor activity
	Leaves	—	—	Aqueous	Toxic	Mutagenic	Poor activity

Plant extracts	Plant part used	Traditional use	No. of reports	Extract	BSLA	Ames test	Anti-*Candida* activity
				MeOH: DCM	Non-toxic	Mutagenic	Poor activity
Trichilia dregeana	Roots	Abt	8	Aqueous	Toxic	Non-mutagenic	Poor activity
				MeOH: DCM	Non-toxic	Mutagenic	Poor activity
	Leaves	Lab	1	Aqueous	Non-toxic	Mutagenic	Poor activity
				MeOH: DCM	Non-toxic	Mutagenic	Poor activity
	Bark	Inf	1	Aqueous	Toxic	Non-mutagenic	Poor activity
				MeOH: DCM	Toxic	Mutagenic	Poor activity
Vangueria infausta	Leaves	Men and Aph	1	Aqueous	Toxic	Non-mutagenic	Poor activity
				MeOH: DCM	Toxic	Mutagenic	Poor activity

Bold – non-toxic and non-mutagenic, MeOH: DCM – Methanol-dichloromethane, Abt – Abortion, Abp – After-birth pains, Ame – Amenorrhoea, , Aph – Antepartum haemorrhage, Blc – Blood cleansing, Col – Infantile Colic, Dys – Dysmenorrhoea, Fib – Fibriods, Inf – Infertility, Lab – Labour, Lac – Lactation, Men – Menorrhagia, Mis – Miscarriage, Oli – Oligomenorrhoea, Pmb – Premature birth, , Pph – Postpartum haemorrhage, Swl – Swelling, Wrt – Genital warts

Table 6.2 Summary of BSLA, Ames test and anti-*Candidal* activity of aqueous and methanol-dichloromethane (organic) plant combinations

Plant combination	Combination code	Traditional use	No. of reports	Extract	BSLA	Anti-*Candidal* activity
B. cathartica (roots) + *C. neglecta* (roots) + *C. monteiroi* (root) + *G. livingstonei* (root) + *G. occidentalis* (root) + *O. natalitia* (root) + *R. digitata* (root)	AC1/OC1	Dys, Inf, Men, Oli, Pmb, and Blc	1	Aqueous	Non-toxic	Poor activity
				MeOH: DCM	Toxic	Moderate activity against *C. albicans* ATCC 10231
***E. tirucalli* (root) + *O. engleri* (bark) + *S. puniceus* (bulb)+ *S. serratuloides* (whole plant)**	AC2/OC2	Blc	1	Aqueous	Non-toxic	Poor activity
				MeOH: DCM	Non-toxic	Poor activity
B. cathartica (root) + *E. humeana* (root) + *O. natalitia* (root) + *T. elegans* (root) + *S. nebulosa* (bark)	AC3/OC3	Dys and Inf	1	Aqueous	Non-toxic	Poor activity
				MeOH: DCM	Toxic	Poor activity
A. villicaulis (root) + *B. cathartica* (root) + *S. nebulosa* (bark)	AC4/OC4	Dys and Inf	1	Aqueous	Non-toxic	Poor activity
				MeOH: DCM	Toxic	Poor activity
B. cathartica (root) + *P. africanum* (root) + *R. digitata* (root)	AC5/OC5	Dys and Ame	2	Aqueous	Non-toxic	Poor activity
				MeOH: DCM	Toxic	Moderate activity against *C. albicans* ATCC 10231
B. cathartica (root)+ *H. coriacea* (stem) + *O. engleri* (bark)	AC6/OC6	Dys, Inf, Abp, Pph and Lab	6	Aqueous	Non-toxic	Poor activity
				MeOH: DCM	Toxic	Poor activity
R. multifidus (whole plant) + *S. serratuloides* (whole plant)	AC7/OC7	Wrt	5	Aqueous	Toxic	Poor activity
				MeOH: DCM	Toxic	Moderate activity against *C. tropicalis* ATCC 750

Plant combination	Combination code	Traditional use	No. of reports	Extract	BSLA	Anti-*Candidal* activity
***B. cathartica* (root) + *O. stricta* (stem) + *S. nebulosa* (bark)**	AC8/OC8	Blc	3	Aqueous	Non-toxic	Poor activity
				MeOH: DCM	Non-toxic	Moderate activity against *C. albicans* ATCC 10231
R. multifidus (whole plant) + *H. hemerocallidea* (corm)	AC9/OC9	Wrt	2	Aqueous	Toxic	Poor activity
				MeOH: DCM	Toxic	Poor activity
K. africana (bark) + *C. filiformis* (whole plant)	AC10/OC10	Abp	1	Aqueous	Non-toxic	Poor activity
				MeOH: DCM	Toxic	Poor activity
E. humeana (root) + *O. natalitia* (root)	AC11/OC11	Men	1	Aqueous	Non-toxic	Poor activity
				MeOH: DCM	Toxic	Poor activity
***B. cathartica* (root) + *E. humeana* (root)**	AC12/OC12	Men	1	Aqueous	Non-toxic	Poor activity
				MeOH: DCM	Non-toxic	Poor activity
G. senegalensis (root) + *H. hemerocallidea* (corm)	AC13/OC13	Col	3	Aqueous	Toxic	Poor activity
				MeOH: DCM	Toxic	Poor activity
K. africana (bark) + *S. nebulosa* (bark)	AC14/OC14	Dys	1	Aqueous	Non-toxic	Poor activity
				MeOH: DCM	Toxic	Poor activity
B. cathartica (root) + *O. natalitia* (root)	AC15/OC15	Dys and Inf	4	Aqueous	Non-toxic	Poor activity
				MeOH: DCM	Toxic	Poor activity
S. birrea (bark) + *T. dregeana* (bark)	AC16/OC16	Abt	1	Aqueous	Non-toxic	Poor activity
				MeOH: DCM	Toxic	Moderate activity against *C. tropicalis* ATCC 750

Bold – non-toxic, MeOH: DCM – Methanol-dichloromethane, Abt – Abortion, Abp – After-birth pains, Ame – Amenorrhoea, Blc – Blood cleansing, Col – Infantile Colic, Dys – Dysmenorrhoea, Inf – Infertility, Lab – Labour, Men – Menorrhagia, Oli – Oligomenorrhoea, Pmb – Premature birth, Pph – Postpartum haemorrhage, Wrt – Genital warts

T. dregeana (bark) against *C. tropicalis* indicated activity at concentrations that were non-toxic in the BSLA. All plant extracts and combinations that indicated anti-C*andidal* activity were mutagenic at 5.00 mg/ml.

6.6 Do the current findings correlate with the traditional reports about the plant species used for gynaecological and obstetric conditions?

The plant species evaluated in this study were reported as safe (as used in aqueous form) by the lay people in Maputaland, except for *T. dregeana.* In this study, 49% of these traditionally used plant species (aqueous extracts) were non-toxic and non-mutagenic and include the popular plant species *H. coriacea, O. natalitia and R. multifidus*. However, the other popular plant species such as *B. cathartica, H. hemerocallidea* and *S. serratuloides* were mutagenic, and *B. cathartica* was also toxic. *Trichilia dregeana* was reported as a “poisonous plant” by the lay people and in this study the roots and bark of this plant species was also identified as toxic and the leaves were mutagenic.

While the focus is still on the traditionally used form (aqueous) of the extracts, Table 6.3 presents the plant species that demonstrate no toxicity and no mutagenicity alongside the ailments they are used for. These plant species can be used as alternatives for the toxic plant species in treating the same ailment e.g. replacing *O. stricta* stem with *O. natalitia* root for blood cleansing. One also needs to consider that the lower concentrations (below 0.25 mg/ml) demonstrated non-toxicity and lower doses of plant material should also be recommended for use.

The plant species combinations that demonstrated no toxicity are presented in Table 6.4 alongside the ailments they are used for. Two (AC7 and AC9) of the three toxic combinations were only used topically for warts. The third combination (AC13) was used during pregnancy to prevent infantile colic when the baby is born. However, at concentrations below 0.59 mg/ml all combinations were non-toxic. The most popular combination [*B. cathartica* (root) + *P. africanum* (root) + *R. digitata* (root)] for various women gynaecological conditions was non-toxic in the BSLA. However, another

popular combination (*R. multifidus* (whole plant) + *S. serratuloides* (whole plant) used for genital warts was toxic.

It is important to note that none of the plant species in this study was reported by the lay people to treat vaginal thrush. However, these plant species were explored for possible anti-C*andidal* activity as *Candida* being the most common cause of yeast infection in women. Furthermore, vaginal thrush often presents with symptoms indistinguishable from other gynaecological complaints. The study thus sought to include this component to determine if the plant species indeed did have some anti-C*andidal* activity. It was determined that the traditionally used form (aqueous form) of all these plant species including the plant species combinations had poor activity against *Candida* strains tested (*C. albicans, C. tropicalis* and *C. glabrata*).

Table 6.3 Traditionally used plant species (aqueous extract) that indicated no toxicity and no mutagenicity when tested in both the BSLA and Ames assays. The ailments they are used for are also listed.

Ailment	**Non-toxic and non-mutagenic plant species (aqueous extracts)**
After-birth pain	*H. coriacea*
Amenorrhoea	*P. africanum*
Blood-cleansing	*A. villicaulis, C. neglecta, G. occidentalis, O. natalitia, P. africanum, R. digitata* and *S, burkei*
Cervical pain	*A. glabratum*
Dysmenorrhoea	*A. villicaulis, C. neglecta, C. monteiroi, G. livingstonei, G. occidentalis, H. coriacea, K. africana, O. natalitia, P. africanum, S. integerrimum* and *S. nebulosa*
Infertility	*C. neglecta, H. coriacea, R. digitata* and *S. nebulosa*
Labour	*A. villicaulis, H. coriacea* and *O. natalitia*
Lactation	*K. Africana*
Menorrhagia	*C. neglecta, C. natalensis, G. occidentalis, O. natalitia* and *R. digitata*
Oligomenorrhoea	*C. neglecta, G. occidentalis, O. natalitia* and *R. digitata*
Postpartum haemorrhage	*H. coriacea*
Prevent premature birth	*C. neglecta, G. occidentalis, O. natalitia* and *R. digitata*

Ailment	Non-toxic and non-mutagenic plant species (aqueous extracts)
Swelling of limbs	*R. multifidus*
Warts	*R. multifidus, S. nebulosa* and *S. serratuloides*

Table 6.4 Traditionally used plant species combinations (aqueous extracts) that indicated no toxicity in the BSLA and the ailments they are traditionally used to treat

Ailment	Non-toxic plant species combinations (aqueous extracts)
Abortion	AC16
After-birth pain	AC6 or AC10
Amenorrhoea	AC5
Blood-cleansing	AC1, AC2 and AC8
Dysmenorrhoea	AC1, AC3, AC4, AC5, AC6, AC14 and AC15
Infertility	AC1, AC3, AC4, AC6 and AC13
Menorrhagia	AC1, AC11 and AC12
Oligomenorrhoea	AC1
Prevent premature birth	AC1

6.7 Final conclusion

This study agrees with the hypotheses that some of the plant species used by women in northern Maputaland possesses some degree of toxicity, mutageniciry and anti-*Candidal* activity. Thereafter, it brought out valuable information on the safety of the plant species used in this study using two approaches i.e. toxicity and mutagenicity. It has also managed to screen the plant species that have a potential for further assessments for *Candidal* treatment using *C. albicans, C. tropicalis* and *C. glabrata* strains. Although 51% of the traditionally used plant species (aqueous) were toxic and mutagenic it was demonstrated that at lower concentrations most plant species were non-toxic. There were plant species in this study that were assessed for the first time for any toxicity. These included *C. natalitia, D. villosa, H. boraginiflora, O. stricta, S. nebulosa and S. integerrimum.* The interaction studies (ΣFIC) can play an important

role in the future where the concentration of one extract in a combination can be increased /decreased to determine whether it can result in a more enhanced efficacy and reduced toxicity.

As people continue to use medicinal plants in their primary healthcare, it is important that they practice appropriate preparation methods and to be mindful that high doses can change a therapeutic concoction into a poisonous infusion. This study highlights that not all natural products are necessary safe. Even if a plant is determined to be toxic, it may still be useful (although care would be needed) if its therapeutic concentration is much lower than the toxic concentration. Conversely, extracts that appear low toxicity may be of little use if their activity is also correspondingly low. Alternative and allopathic medicine systems need to work together to infuse the holistic approach of herbal medicine and the scientific approach of western medicine to make it safe for consumers.

6.8 Recommendation for future studies

The results in this study are linked to the extraction from water and methanol-dichloromethane (1:1 ratio) as solvents to extract active plant compounds. It is recommended that in the future other solvents (such as acetone, chloroform, ethanol etc.) and different ratios of methanol-dichloromethane be considered to increase the extraction yield of phytochemicals (Osmic et al., 2019, Truong et al., 2019).

Although the aqueous extracts showed less toxicity, many of the organic extracts demonstrated toxic and mutagenic effects when tested in the BSLA and Ames assay. This broached the question of how safe are these medicinal plant species, especially if it is used over a long period of time, as it was indicated in the study done by De Wet and Ngubane (2014) that some of these plant species are used regularly. Plant species such as *B. cathartica* are used regularly for infertility problems. Some plants (e.g. *O. natalitia*) are used monthly for the menstrual cycle or during the entire course of pregnancy (e.g. *S. serratuloides*). Therefore, it is recommended that toxicity is always considered when studying the efficacy of medicinal plants species. Other toxicological studies such as cytotoxicity, hepatotoxicity, carcinogenicity etc. to cover a broader and more organ/system specific areas of potential plant toxicity should be further explored. With mutagenicity, the use of other strains such as *S. typhimurium*

TA97 and TA102 is recommended, especially for extracts that were non-mutagenic in this study. Future studies are recommended on the mutagnicity of plant species combinstions in this study. Unfortunately, due to the Covid-19 pandemic restrictions, this couldn't be done in the current study. According to Maron and Ames, (1983), these strains may allow for the detection of mutagens that are poorly or not detected by other strains. *Escherichia coli* WP2 can also be used as it has a high sensitivity to oxidative mutagens (Blanco et al., 1998).

The plant species used in this study were reported mostly for menstrual problems such as dysmenorrhea, irregular menses and other gynaecological conditions including infertility (De Wet and Ngubane, 2014). In the future, studies such as antispasmodic, anti-inflammatory (especially for plant species recommended to be taken during the menstrual period) and hormonal influence of these plant species should be considered to verify the holistic efficacy. Plant species are also used during pregnancy. Some phytochemicals can cross the placenta to the foetus, and may also be safe in some trimester than the other (Bernstein et al., 2020). Therefore, future studies should look at teratogenicity as well as their likelihood to cause miscarriage and abortion. Also, the overall therapeutic indexes of these plants and combinations should be determined, in order to relate the therapy wirh the safety of these plants.

More importantly, the information from scientific studies should be taken back to the lay people as they are using these plants daily. Unfortunately, at the time of the submission of this thesis, the valuable feedback given to the local communities was put on hold as a result of the restrictions that were put in place due to the Covid-19 pandemic. When feasible these results should be shared with the local communities at schools or community centres.

REFERENCES

Abam, D.S., 2015. Overview of gynaecological emergencies. Contemporary Gynaecologic Practice. Atef Darwish, IntechOpen, DOI: 10.5772/59107.

Abesede, O.W., Sunday, A., Jide, A.A., 2015. Toxicological investigation of *Aloe ferox* Mill. Extracts using brine shrimp (*Artemia salina* L) assay. Pakistan Journal of Pharmaceutical Sciences 28 (2), 635–640.

Adeneye, A.A., 2014. Sub-chronic and chronic toxicities of African medicinal plants. In: Kuete, V. (Ed.), Toxicological Survey of African Medicinal Plants. Elsevier Insights, USA, 99–133.

Adewale, O.B., Onasanya, A., Anadozie, S.O., Abu, M.F., Akintan, I.A., Ogbole, C.J., Olayide, I.I., Afolabi, O.B., Jaiyesimi, K.F., Ajiboye, B.O., Fadaka, A.O., 2016. Evaluation of acute and subacute toxicity of aqueous extract of *Crassocephalum rubens* leaves in rats. Journal of Ethnopharmacology 188, 153–158.

Adoum, O.A., 2009. Determination of toxicity levels of some Savannah plants using brine shrimp test (BST). Bayero Journal of Pure Applied Sciences 2(1), 135–138.

Ahmed, A.S., McGaw, L.J., Eloff, J.N., 2013. Evaluation of pharmacological activities, cytotoxicity and phenolic composition of four *Maytenus* species used in southern African traditional medicine to treat intestinal infections and diarrhoeal diseases. BMC Complementary and Alternative Medicine 13(100), 1–15.

Ahmed, F., Akter, D., Muhit, M.A., Raihan, S.Z., Faroque, A.B.M., 2018. DPPH free-radical scavenging and cytotoxicity activities of *Leea macrophylla.* Bangladesh Medical Research Council Bulletin 44, 77–81.

Akhalwaya, S., 2017. The antimicrobial investigation of indigenous South African medicinal plants against oral pathogens. MSc Thesis, University of Witwatersrand, Johannesburg, South Africa.

Alanyali, F.S., Artagan, O., Yuksel, S., 2011. Mutagenicity studies of some substituted benzylideneaniline derivatives. International Journal of Pharmacology 7 (2), 278–282.

Ali, N., Ahmed, G., Shah, S.W.A., Shah, I., Ghias, M., Khan, I., 2011. Acute toxicity, brine shrimp cytotoxicity and relaxant activity of fruits of *Callistemon citrinus* Curtis. BioMed Central Complementary and Alternative Medicine 11(99), DIO: 10.1186/1472-6882-11-99.

Alli, L.A., Adesokan, A.A., Salawu, O.A., Akanji, M.A., 2015. Toxicological studies of aqueous extract of *Acacia nilotica* root. Interdisciplinary Toxicity 8 (1), 48–54.

Altemimi, A., Lakhssassi, N., Baharlouei, A., Watson, D.G., 2017. Phytochemicals: extraction, isolation and identification of bioactive compounds from plant extracts, Plants Multidisciplinary Digital Publishing Institute 6(4), DOI: 10.3390/plants6040042.

Ames, B.N., Durston, W.E., Yamasaki, E., Lee, F.D., 1973. Carcinogens are mutagens: A simple test system combining liver homogenates for activation and bacteria for detection. Proceedings of the National Academy of Sciences of the United States of America 70 (8), 2281–2285.

Ames, B.N., McCann, J., Yamasaki, E., 1975. Methods for detecting carcinogens and mutagens with the Salmonella/ mammalian-microsome mutagenicity test. Mutation research 31, 347–346.

Ananthi, R., Chandra, N., Santhiya, S.T., Ramesh, A., 2010. Genotoxic and antigenotoxic effects of *Hemidesmus indicus* R. Br. root extract in cultured lymphocytes. Journal of Ethnopharmacology 127, 558–560.

Anufriieva, E., Shadrin, N.V., 2014. The swimming behaviour of *Artemia* (Anostraca): new experimental and observational data. Zoology 117, 415–421.

Apu, A.S., Bhuyan, S.H., Khatun, F., Liza, M.S., Matin, M., Hossain, M.F., 2013. Assessment of cytotoxic activity of two medicinal plants using brine shrimp

(*Artemia salina*) as an experimental tool. International Journal of Pharmaceutical Sciences and Research 4(3), 1125–1130.

Ashidi, J.S., Houghton, P.J., Hylands, P.J., Efferth, T., 2010. Ethnobotanical survey and cytotoxicity testing of plants of South-western Nigeria used to treat cancer, with isolation of cytotoxic constituents from *Cajanus cajan* Millsp. leaves. Journal of Ethnopharmacology 128, 501–512.

Attia, S.M., 2010. Deleterious effects of reactive metabolites. Oxidative Medicine and Cellular Longevity 3(4), 238–253.

Basri, D.F., Sandra, V., 2016. Synergistic interaction of methanol extract from *Canarium odontophyllum* Miq. leaf in combination with oxacillin against methicillin-resistant *Staphylococcus aureus* (MRSA) ATCC 33591. International Journal of Microbiology 2016, 1-7, DOI: 10.1155/2016/5249534.

Berenbaum, M.C., 1978. A method for testing synergy with any number of agents. Journal of Infectious Diseases 137(2), 122–130.

Bernstein, N., Akram, M., Yaniv-Bachrach, Z., Daniyal, M., 2020. Is it safe to consume traditional medicinal plants duting pregnancy? Research Pathology, DOI: 10.1002/ptr.6935.

Blanco, M., Urios, A., Martinez, A., 1998. New *Eschericha coli* WP2 tester strain highly sensitive to reversion by oxidative mutagens. Mutation Research 413, 95−101.

Booth, G.M., Malmstrom, R.D., Kipp, E., Paul, A., 2012. Cytotoxicity of selected medicinal and nonmedicinal plant extracts to microbial and cervical cancer cells**.** Journal of Biomedicine and Biotechnology 2012(4), 106746, DOI:10.1155/2012/106746.

Botha, C.J., Lewis, A., Du Plessis, E.C., Clift, S.J., Williams, M.C., 2012. *Crotalariosis equorum* ("jaagsiekte") in horses in southern Mozambique, a rare form of pyrrolizidine. Journal of Veterinary Diagnostic Investigation 24(6), DOI: 10.1177/1040638712460673.

Botha, C.J., Penrith, M.-L., 2008. Poisonous plants of veterinary and human importance in southern Africa. Journal of Ethnopharmacology 119, 549–558.

Bruschi, P., Morganti, M., Mancini, M., Signorini, M.A., 2011. Traditional healers and laypeople: A qualitative and quantitative approach to local knowledge on medicinal plants in Muda (Mozambique). Journal of Ethnopharmacology 138, 543–563.

Buwa, L.V., Van Staden, J., 2006. Antibacterial and antifungal activity of traditional medicinal plants used against venereal diseases in South Africa. Journal of Ethnopharmacology 103, 139–142.

Cassina, M., Salviati, L., Gianantonio, E.D., Clementi, M., 2012. Genetic susceptibility to teratogens: State of the art. Reproductive Toxicology 34, 186–191.

Cedergreen, N., 2014. Quantifying synergy: A systematic review of mixture toxicity studies within environmental toxicity. Plos One, DOI: 10.1371/journal.pone.0096580.

Chandrakumar, A., Mundadan, C.J., Chandrasekhar, D., Sojan, S.T., Nair, S., 2016. Crystal-induced nephrotoxicity associated with acyclovir use in paediatric patient. Journal of the Indian Academy of Paediatrics, Infectious Disease 6(4), 1–3.

Chaundhary, P., Ahmad, S., Khan, N.A., 2016. Herbal plants - A boon for hepatotoxicity. Asian Journal of Pharmaceutical and Clinical Research 9(1), 37–40.

Chika, A., Onyebueke, D.C., Bello, S.O., 2018. Phytochemical analysis and evaluation of antidiabetic effects in alloxan-induced diabetic rats treated with aqueous leaf extract of *Acanthospermum hispidum*. African Journal of Biomedical Research 21, 81–85.

Cock, I.E., Reubhart, D.R., 2009. Comparison of the brine shrimp naupii bioassay and the ToxScreen-II test for the detection of toxicity associated with *Aloe vera* (*Aloe babadensis* Miller) leaf extract. Pharmacognosy Research 1(2), 98–101.

Coe, F.G., Parikh, D.M., Johnson, C.A., 2010. Alkaloid presence and brine shrimp (*Artemia salina*) bioassay of medicinal species of eastern Nicaragua. Pharmaceutical Biology 48(4), 439–445.

Dan, M., Leshem, Y., Yeshaya, A., 2010. Performance of a rapid yeast test in detecting *Candida spp*. in the vagina. Diagnostic Microbiology and Infectious Disease 67, 52–55.

De Wet, H., Nciki, S., Van Vuuren, S.F., 2013. Medicinal plants used for the treatment of various skin disorders by a rural community in northern Maputaland, South Africa. Journal of Ethnobiology and Ethnomedicine 9(51), DOI: 10.1186/1746-4269-9-51.

De Wet, H., Ngubane, S.C. 2014. Traditional herbal remedies used by women in a rural community in northern Maputaland (South Africa) for the treatment of gynaecology and obstetrics complaints. South African Journal of Botany 94, 129–139.

De Wet, H., Nkwanyana, M.N., Van Vuuren, S.F., 2010. Medicinal plants used for the treatment of diarrhoea in northern Maputaland, KwaZulu-Natal Province, South Africa. Journal of Ethnopharmacology 130, 284–289.

De Wet, H., Nzama, V.N., Van Vuuren, S.F., 2012. Medicinal plants used for the treatment of sexually transmitted infections by lay people in northern Maputaland, KwaZulu–Natal Province, South Africa. South African Journal of Botany 78, 12–20.

De Wet, H., Ramulondi, M., Ngcobo, Z.N., 2016. The use of indigenous medicine for the treatment of hypertension by a rural community in northern Maputaland, South Africa. South African Journal of Botany 103, 78–88.

Della, T.A., Albuquerque, L.B.L., Farrapo, N.M., Oshima-Franco, Y., Santos, M.G., Tavares, R.V.S., Rodas, A.C.D., Dal, B.C.A., Cardoso, C.R.P., Varanda, E.A., Groppo, F.C., Lopes, P.S., 2011. Mutagenicity induces by the hydroalcoholic extract of the medicinal plant *Plathymenia reticulata* Benth. The Journal of Venomous Animals and Toxins including Tropical Diseases 17 (2), 190–198.

Deutschlander, M.S., Van de Venter, M., Roux, S., Louw, J., Lall, N., 2009. Hypoglycaemic activity of four plant extracts traditionally used in South Africa for diabetes. Journal of Ethnopharmacology 124(3), 619–624.

Doldan-Martelli, V., Miguez, D.G., 2015. Synergistic interaction between selective drugs in cell population models. Plos One 10 (2), e0117558. DOI: 10.1371/journal.pone.0117558.

Donders, G.G.G., Grinceviciene, S., Bellen, G., Ruban, K., 2018. Is multiple-site colonization with *Candida* spp. related to inadequate response to individualized fluconazole maintenance therapy in women with recurrent Candida vulvo-vaginitis? Diagnostic Microbiology and Infectious Disease 92, 226–229.

Edziri, H., Mastouri, M., Mahjoub, A., Anthonissen, R., Mertens, B., Cammaerts, S., Gevaert, L., Verschaeve, L., 2011. Toxic and mutagenic properties of extracts from Tunisian traditional medicinal plants investigated by the neutral red uptake, VITOTOX and alkaline comet assays. South African Journal of Botany 77, 703–710.

Ekor, M., 2014. The growing use of herbal medicines: issues relating to adverse reactions and challenges in monitoring safety. Frontiers in Pharmacology 4, 1–10.

El Seoud, K.E.A.H.A., Bibby, M.C., Shoeib, N., Wright, C.W., 2003. Evaluation of some Egyptian plant species for *in vitro* antimycobacterial and cytotoxic activities. Pharmaceutical Biology 41(6), 463–465.

Eldeen, I.M.S., Elgorashi, E.E., Van Staden, J., 2005. Antibacterial, anti-inflammatory, anti-cholinestrase and mutagenic effects of extracts obtained from some trees used in South African traditional medicine. Journal of Ethnopharmacology 102, 457–464.

Elgorashi, E.E., Taylor, J.L.S., Maes, A., Van Staden, J., De Kimpe, N., Verschaeve, L., 2003. Screening of medicinal plants used in South African traditional medicine for genotoxic effects. Toxicology Letters 143, 195–207.

Eloff, J.N., 1998. A sensitive and quick microplate method to determine the minimum inhibitory concentration of plant extracts for bacteria. Planta Medica 64 (8), 711–713.

Emeribe, A., Abdullahi, N.I., Onyia, J., Afunanya, A.L., 2015. Prevalence of vulvovaginal candidiasis among non-pregnant women attending a tertiary health care facility in Abuja, Nigeria. Research and Reports in Tropical Medicine 6, 37–42.

Eren, Y., Ozata, A., Konuk, M., Akyil, D., Liman, R., 2015. A mutagenicity and cytotoxicity study on *Limonium effusum* aqueous extracts by Allium, Ames and MTT tests. Cytotoxicity and Genetics 49 (2), 125–133.

Fadeyi, S.A., Fadeyi, O.O., Adejomu, A.A., Okoro, C., 2013. *In-vitro* anticancer screening of 24 locally used Nigerian medicinal plants. BMC Complementary and Alternative Medicine 13 (79), DOI: 10.1186/1472-6882-13-79.

Fennell, C.W., Lindsey, K.L., McGaw, L.J., Sparg, S.G., Starfford, G.I., Elgorashi, E.E., Grace, O.M., Van Staden, J., 2004. Assessing African medicinal plants for efficacy and safety: pharmacological screening and toxicology. Journal of Ethnopharmacology 94, 205–217.

Fouche, G., Sakong, B.M., Adenubi, O.T., Pauw, E., Leboho, T. Wellington, K.W., Eloff, J.N., 2016. Anthelmintic activity of acetone extracts from South African plants used on egg hatching of *Haemonchus contortus*. Onderstepoort Journal of Veterinary Research 83(1), 1–8.

Franzen, R., Gobo, S., Tanabe, K., Morita, M., 1998. Genotoxic activity of chlorinated butenoic acids in *Salmonella typhimurium* strains TA98, TA100 and TA104. Mutation Research 417(1), 31–37.

Gao, B., Liong, Y., Zou, L., Huang, H., Zhang, A., Xu, D., Zhao, X., 2021. The characteristics and risk factors of human papillomavirus infection: an outpatient population-based study in Changsha, Hunan. Scientific Reports 11, DOI: 10.1038/s41598-021-94635-1.

Garcia-Algar, O., Gonzalez, A.C., Falcon, M., 2016. Toxicology screening in paediatrics. Anales de Pediatria 85(3), 160, DOI:http://dx.doi.org/10.1016/j.anpedi.2015.07.136.

Giron, L.M., Aguilar, G.A., Caceres, A., Arroyo, G.L., 1988. Anticandidal activity of plants used for the treatment of vaginitis in Guatemala and clinical trials of a *Solanum nigrescens* preparation. Journal of Ethnopharmacology 22, 307–313.

Hakura, A., Suzuki, S., Satoh, T., 1999. Advantage of the use of human liver S9 in the Ames test. Mutation Research 438 (1), 29–36.

Hall, M.J., Middleton, R.F., Westmacott, D., 1983. The fractional inhibitory concentration (FIC) index as a measure of synergy. Journal of Antimicrobial Chemotherapy 11 (5), 427–433.

Hamidi, M.R., Jovanova, B., Panovska, T.K., 2014. Toxicological evaluation of the plant products using brine shrimp (*Artemia salina* L.) model. Macedonian Pharmaceutical Bulletin 60(1), 9–18.

Hoet S., Opperdoes, F., Brun, R., Adjakidje, V., Quetin-Leclercq, J., 2004. *In vitro* antiplasmodial activity of ethnopharmacologically selected Beninese plants. Journal of Ethnopharmacology 91, 37–42.

Hubsch, Z., Van Zyl, R.L., Cock, I.E., Van Vuuren, S.F., 2014. Interactive antimicrobial and toxicity profiles of conventional antimicrobials with Southern African medicinal plants. South African Journal of Botany 93, 183–197.

Jain, P.S., Belsare, D.P., 2009. Antifungal activity of stem bark of *Kigelia pinnata* Linn. Drug Invention Today 1(1), 66–67.

Jayachandran, A.L., Katragadda, R., Ravinder, T., Vajralevu, L., Manorajan, L., Hemalatha, S., Shanmugam, K., 2018. Antifungal susceptibility pattern among *Candida* species: An evaluation of disc diffusion and broth micro-dilution method. Journal of Microbiology and Infectious Diseases 8(3), 97–101.

Jeanmonod, R., Jeanmonod, D. 2019. Vaginal Candidiasis (Vulvovaginal Candidiasis). In StatPearls; StatPearls Publishing: Treasure Island, Florida, USA.

Kishore, N., Binneman, B., Mahapatra, A., Van De Venter, M., Du Plessis-Stoman, D., Boukes, G., Houghton, P., Meyer, J.J.M., Lall, N., 2014. Cytotoxicity of synthesized 1, 4-naphthoquinone analogues on selected human cancer cell lines. Bioorganic and Medicinal Chemistry 22, 5013–5019.

Kolaczkowski, M., Kolaczkowska, A., Stermitz, F.R., 2009. Modulation of the antifungal activity of new medicinal plant extracts active on the *Candida glabrata* by the major transporters and regulators of the pleiotropic drug-resistance network on *Saccharomyces cerevisiae.* Microbial and Drug Resistance 15(1) 11–7.

Kolbeck, M.K., Tintjer, T.E., 2016. The use of a brine shrimp assay to detect bioactivity in the endophyte-infected grass, *Agrotis hyemalis.* Journal of the Pennsylvania Academy of Science 90 (1), 13–20.

Krishnaraju, A.V., Rao, T.V.N., Sundaraju, D., Vanisree, M., Tsay, H., Subbaraju, G.V., 2005. Assessment of bioactivity of Indian medicinal plants using brine shrimp (*Artemia salina*) lethality assay. International Journal of Applied Science and Engineering 3(2), 125–134.

Kristanc, L., Kreft, S., 2016. European medicinal and edible plants associated with subacute and chronic toxicity part I: Plants with carcinogenic, teratogenic and endocrine-disrupting effects. Food and Chemical Toxicology 92, 150–164.

Kuete, V., 2014. Physical, hematological, and histopathological signs of toxicity Induced by African medicinal plants. In: Kuete, V. (ed.), Toxicological Survey of African Medicinal Plants, 635–657. Elsevier, USA.

Kumar, A., Prasad, M., Mishra, D., Srivastav, S.K., Srivastav, A.K., 2010. Toxicity of aqueous extract of *Euphorbia tirucalli* latex on catfish, *Heteropneustes fossilis*. Ecotoxicology and Environmental Safety 73, 1671–1673.

Lall, N., Henley-Smith, C.J., Canha, M.N., Oosthuizen, C.B., Berrington, D., 2013. Viability reagent, prestoblue, in comparison with other available reagents, utilized in cytotoxicity and antimicrobial assay. International Journal of Microbiology, 2013, 1–5.

Lall, N., Meyer, J.J.M., Taylor, M.B., 2005[a]. Anti-HSV-1 activity of *Euclea natalensis*. South African Journal of Botany 71 (3&4), 444–446.

Lall, N., Meyer, J.J.M., Wang, Y., Bapela, N.B., van Rensburahmed C.E.J., Fourie, B., Franzblau S.G., 2005[b]. Characterization of intracellular activity of antitubercular constituents the roots of *Euclea natalensis*. Pharmaceutical Biology 43(4), 353–357.

Lee, M., Seo, C., Ha, H., Park, E., Kim, J., Shin, H., 2018. The genotoxicity of an aqueous extract of Gyejibokryeong-hwan. BMC Complementary and Alternative Medicine 18 (21), 1–9.

Libralato, G., Prato, E., Migliore, L., Cicero, A.M., Manfra, L., 2016. A review of toxicity testing protocols and endpoints with *Artemia* spp. Ecological Indicators 69, 35–49.

Liwa, A., Jaka, H.M., 2016. Renal diseases and use of medicinal herbal extracts: A concise update of reported literature in Africa. Journal of Nephrology and Renal therapy 2, 008. DOI: 10.24966/NRT-7313/100008.

Lotter, H., Wagner, H., Saleh, A.A., Cordell, G.A., Farnsworth, N.R., 1979. Potential anticancer agents XI. X-Ray structure determination of acantholide. Zeitschrift für Naturforschung C Journal of Biological Sciences 34 (9–10), 677–682.

Lou, X., Pires, D., Ainsa, J.A., Gracia, B., Milhovo, S., Duarte, A., Anes, E., Ferreira, M-J.U., 2011. Antimycobacterial evaluation and preliminary phytochemical investigation of selected medicinal plants traditionally used in Mozambique. Journal of Ethnopharmacology 137, 114–120.

Mabona, U., Viljoen, A., Shikanga, E., Marston, A., Van Vuuren, S., 2013. Antimicrobial activity of southern African medicinal plants with dermatological

relevance: From an ethnopharmacological screening approach, to combination studies and the isolation of a bioactive compound. Journal of Ethnopharmacology 148, 45–55.

Madikizela, B., Ndhlala, A.R., Rengasamy, K.R.R., McGaw L.J., Van Staden J., 2017. Pharmacological evaluation of two South African commercial herbal remedies and their plant constituents. South African Journal of Botany 111, 291–298.

Madingou, N.O.K., Traore, A., Souza A., 2016. Preliminary studies of acute and sub chronic toxicity of the aqueous extract of *Guibourtia tessmannii* (Harms) J. Leonard stem barks (Caesalpiniaceae) in mice and rats. Asian Pacific Journal of Tropical Biomedicine 6 (6), 506–510.

Makhafola, T.J., McGaw, L.J., Eloff, J.N., 2014. *In vitro* cytotoxicity and genotoxicity of five *Ochna* species (Ochnaceae) with excellent antibacterial activity. South African Journal of Botany 91, 9–13.

Malan D.F., Neuba D.F., 2011. Traditional practices and medicinal plants use during pregnancy by Anyi-Ndenye women (Eastern Cote d'Ivoire). African Journal of Reproductive Health 15, 85–93.

Malangu, N., 2011. Acute poisoning in three African countries: Botswana, South Africa and Uganda. PhD thesis, University of Limpopo, Polokwane, South Africa.

Malangu, N., Ogunbanjo, G.A., 2009. A profile of acute poisoning at selected hospitals in South Africa. South African Journal of Epidemiology and Infection 24 (2), 14–16.

Mamba, P., Adebayo, S.A., Tshikalange, T.E., 2016. Anti-microbial, anti-inflamatory, and HIV-1 reverse transcriptase activity of selected South African plants used to treat sexually transmitted diseases. International Journal of Pharmacognosy and Phytochemical Research 8(11), 1870–1876.

Manohar, P.R., 2012. Sustainable harvesting of medicinal plants: Some thoughts in search for solutions. Ancient Science of Life 32(1), 1–2.

Markiewicz, M., Librowski, T., Lipkowska, A., Serda, P., Baczynski, K., Pasenkiewicz-Gierula, M., 2017. Assessing gastric toxicity of xanthone derivatives of anti-inflammatory activity using simulation and experimental approaches. Biophysical Chemistry 220, 20–33.

Maron, D.M., Ames, B.N., 1983. Revise methods for the *Salmonella* mutagenicity test. Mutation Research 113, 173–215.

Mashele, S., Fuku, S.L., 2011. Evaluation of the antimutagenic and mutagenic properties of *Asparagus laricinus*. Medical Technology South Africa 25 (2), 33–36.

Masullo, M., Montoro, P., Mari, A., Pizza, C., Piacente, S., 2015. Medicinal plants in the treatment of women's disorders: Analytical strategies to assure quality, safety and efficacy. Journal of Pharmaceutical and Biomedical Analysis 113, 189–211.

Mazimba, O., 2014. Pharmacology and phytochemistry studies in *Peltophorum africanum.* Bulletin of Faculty of Pharmacy, Cairo University 52, 145–153.

McGaw, L.J., Elgorashi, E.E., Elof, J.N., 2014. Cytotoxicity of African medicinal plants against normal animal and human cells. In: Kuete, V. (ed.), Toxicological Survey of African Medicinal Plants, 235-275. Elsevier, USA.

Mohammed, O.J., Latif, M.L., Pratten, M.K., 2016. Evaluation of embryotoxicity for major components of herbal extracts using the chick embryonic heart micromass and mouse D3 embryonic stem cell systems. Reproductive Toxicology 59, 117–127.

Moosavi, M., Jalali, A., Siahpoosh, A., Farajzadeh-Shikh, A., Kianipur, F, 2013. Assessing mutagenicity of methanolic extract of *Abrus precatorius* seeds using Ames bioassay. Journal of Medical Sciences 13(2), 118–123.

More, G., Tshikalange, T.E., Lall, N., Botha, F., Meyer, J.J.M., 2008. Antimicrobial activity of medicinal plants against oral microorganisms. Journal of Ethnopharmacology 119(3), 473–477.

Mortelmans, K., Zeiger, E., 2000. The Ames *Salmonella*/ microsome mutagenicity assay. Mutation Research 455, 29–60.

Moshi, M.J., Cosam, J.C., Mbwambo, Z.H., Kapingu, M., Nunya, M.H.H., 2004. Testing beyond ethnomedical claims: brine shrimp lethality of some Tanzanian plants. Pharmaceutical Biology 42(7), 547–551.

Moshi, M.J., Mbwambo, Z.H., Nondo, R.S.O., Masimba, P.J., Kamuhabwa, A., Kapingu, M.C., Thomas, P., Richard, M., 2006. Evaluation of ethnomedical claims and brine shrimp toxicity of some plants used in Tanzania as traditional medicines. African Journal of Traditional, Complementary and Alternative Medicines 3(3), 48–58.

Motsei, M.L., 2003. Screening of traditionally used South African medicinal plants against *Candida albicans*. MSc Thesis, University of Natal, Durban, South Africa.

Motsei, M.L., Lindsey, K.L., Van Staden, J., Jäger, A.K., 2003. Screening of traditionally used South African plants for fungal activity against *Candida albicans*. Journal of Ethnopharmacology 86, 235–241.

Moustafa, S.M.A., Menshawi, B.M., Wassel, G.M., Mahmoud, K., Mounier, M.M., 2014. Screening of some plants in Egypt for their cytotoxicity against four human cancer cell lines. International Journal of PharmTech Research 6(3), 1074–1084.

Mulaudzi, R.B., Ndhlala, A.R., Kulkarni, M.G., Finnie, J.F., Van Staden. J., 2013. Anti-inflammatory and mutagenic evaluation of medicinal plants used by Venda people against venereal and related diseases. Journal of Ethnopharmacology 146, 173–179.

Mulaudzi, R.B., Ndhlala, A.R., Kulkarni, M.G., Van Staden., J., 2012. Pharmacological properties and protein binding capacity of phenolic extracts of some Venda medicinal plants used against cough and fever. Journal of Ethnopharmacology 143, 185–193.

Mulholland, D.A., Mwangi, E.M., Dlova, N.C., Plant, N., Crouch, N.R., Coombes, P.H., 2013. Non-toxic melanin producing inhibitors from *Garcinia livingstonei* (Clusiaceae). Journal of Ethnopharmacology 149, 570–575.

Mundy, L., Pendry, B., Rahman, M., 2016. Antimicrobial resistance and synergy in herbal medicine. Journal of Herbal Medicine 6, 53–58.

Musa, K., Ahmed, M.A., Shahpudin, S.N.M., Sandai, R., Tabana, Y., Sandai, D., 2018. Resistance of *Candida glabrata* to drugs and the host immune system. Clinical Microbiology and Infectious Diseases 3(3), 2–4.

Mwinga, J.L., Asong, J.A., Amoo, S.O., Nkadimeng, S.M., McGaw, L.J., Aremu, A.O., Otang-Mbeng, W., 2019. *In vitro* antimicrobial effects of *Hypoxis hemerocallidea* against six pathogens with dermatological relevance and its phytochemical characterization and cytotoxicity evaluation. Journal of Ethnopharmacology 242, 112048–112057.

Mythili, S., Gajalakshmi, S., Sathiavelu, A., Sridharan, T.B., 2011. Pharmacological Activities of *Cassytha filiformis*: A Review. Asian Journal of Plant Science and Research 1(1), 77–83.

Naidoo, D., Van Vuuren, S.F., Van Zyl, R.I., De Wet, H., 2013. Plants traditionally used individually and in combination to treat sexually transmitted infections in northern Maputaland, South Africa: Antimicrobial activity and cytotoxicity. Journal of Ethnopharmacology 149, 656–667.

Nair, J.J., Van Staden, J., 2013. Pharmacological and toxicological insights to the South African Amaryllidaceae. Food and Chemical Toxicology 62, 262–275.

National cancer institute, 2017. The genetics of cancer. https://www.cancer.gov/about-cancer/causes-prevention/genetics. Retrieved on 07 November 2019

Nciki, S., Van Vuuren, S., Van Eyk, A., De Wet, H., 2016. Plants used to treat skin diseases in northern Maputaland, South Africa: antimicrobial activity and *in vitro* permeability studies. Pharmaceutical Biology 54(11), 2420–2436.

Ndhlala, A.R., Ncube, B., Okem, A., Mulaudzi, R.B., Van Staden J., 2013. Toxicology of some important medicinal plants in southern Africa. Food and Chemical Toxicology 62, 609–621.

Nemati, F., Dehpouri, A.A., Eslami, B., Mahdavi, V., Mirzanejad, S., 2013. Cytotoxic properties of some medicinal plant extracts from Mazandaran, Iran. Iran Red Crescent Medical Journal 15(11), DOI: 10.5812/ircmj.8871.

Nergard, C.S., Ho, T.P.T., Diallo, D., Ballo, N., Paulsen, B.S., Nordeng, H., 2015. Attitudes and use of medicinal plants during pregnancy among women at health care centers in three regions of Mali, West-Africa. Journal of Ethnobiology and Ethnomedicine 11(73), DOI: 10.1186/s13002-015-0057-8.

Neuman, M.G., Cohen, L.B., Opris, M., Nanau, R., Jeong, H., 2015. Hepatotoxicity of Pyrrolizidine Alkaloids. Journal of Pharmacy and Pharmaceutical Sciences 18(4), 825–843.

Nunes, S.N., Carvalho, F.D., Guilhermino, L.M., Van Stappen, G., 2006. Use of genus *Artemia* in ecotoxicology testing. Environmental Pollution 144, 453–462.

Ohikhena, F.U., Wintola, O.A., Afolayan, A.J., 2017. Evaluation of the antibacterial and antifungal properties of *Phragmanthera capitata* (Sprengel) Balle (Loranthaceae), a mistletoe growing on rubber tree, using the dilution techniques. The Scientific World Journal 2017, 1–8.

Ojewole, J.A.O., 2003. Evaluation of the anti-inflammatory properties of *Sclerocarya birrea* (A.Rich) Hochst. (Family: Anacardiaceae) stem-bark extracts in rats. Journal of Ethnopharmacology 85, 217–220.

Olaleye, M.T., Rocha, B.T.J., 2008. Acetaminophen-induced liver damage in mice: Effects of some medicinal plants on the oxidative defence system. Experimental and Toxicologic Pathology 59, 319–327.

Olukunle, J.O., Jacobs, E.B., Ajayi, O.L., Biobaku, K.T., Abatan, M.O., 2015. Toxicological evaluation of the aqueous extract of *Acalypha wilskesiana* in Wistar

albino rats. Journal of Complementary and Integrative Medicine 12(1), 53−6, DOI: 10.1515/jcim-2013-0066.

Osmic, S., Begic, S., Micic, V., petrovic, Z., Advic, G., 2019. Effect of solvent and extraction conditions on the antioxidative activity of Sage (*Salvia officinalis* L.) extracts obtained by maceration. Technologica Acta 11(2), 1−8.

Owotade, F.J., Gulube, Z., Ramla, S., Patel, M., 2016. Antifungal susceptibility of *Candida albicans* isolated from the oral cavities of patients with HIV infection and cancer. South African Dental Journal 71(1), 8–11.

Paiva, J.P., Lima L.G.S., Siqueira, C.M., Cardoso, J.S., Holandino, C., 2011. Evaluation of the genotoxic and mutagenic potentials of phytotherapic and homeopathic solutions of *Euphorbia tirucalli* Lineu (Aveloz). International Journal of High Dilution Research 10(35), 71–72.

Paraskeva, M.P., Van Vuuren, S.F., Van Zyl, R.L., Davids, H., Viljoen, A.M., 2008. The *in vitro* biological activity of selected South African *Commiphora* species. Journal of Ethnopharmacology 119, 673–679.

Pauw, E., Eloff, JN., 2014. Which tree orders in southern Africa have the highest antimicrobial activity and selectivity against bacterial and fungal pathogend of animals? BMC Complementary and Alternative Medicine 14, 1–12.

Pillay, C.C.N., Jäger, A.K., Mulholland, D.A., Van Staden, J., 2001. Cyclooxygenase inhibition and anti-bacterial activities of South African *Erythrina* species. Journal of Ethnopharmacology 74, 231–237.

Polatoglu, K., 2013. “Chemotypes” – A fact that should not be ignored in natural product studies. The Natural Products Studies 3, 10–14.

Prayong, P., Barusrux, S., Weerapreeyakul, N., 2008. Cytotoxic activity screening of some indigenous Thai plants. Fitoterapia 79, 598–601.

Prozesky, E.A., Meyer, J.J.M., Louw, A.I., 2001. *In vitro* antiplasmodial activity and cytotoxicity of ethnobotanically selected South African plants. Journal of Ethnopharmacology 76(3), 239–245.

Ramulondi, M., De Wet, H., Van Vuuren, S., 2019. Toxicology of medicinal plants and combinations used in northern KwaZulu-Natal (South Africa) for the treatment of hypertension. Journal of Herbal Medicine. Advance online publication 16(2019), 100251, DOI: 10.1016/j.hermed.2018.12.001.

Rathi, S.G., Patel, K.R., Bhaskar, V.H., 2012. Isolation of herbal plants: Antifungal and antibacterial activities. Journal of Pharmaceutical Science and Bioscientific Research 2 (1), 25–29.

Raya, K.B., Ahmad, S.H., Farhana, S.F., Mohammad, M., Tajidin, N.E., Parvez, A., 2015. Changes in phytochemical contents in different parts of *Clinacanthus nutans* (Burm. f.) lindau due to storage duration. Bragantia 74, 445–452.

Razak, M.A., Aidoo, K.E., Candlish, A.G.G., 2007. Mutagenic and cytotoxic properties of three herbal plants from Southern Asia. Tropical Biomedicine 20(2), 49–59.

Reha-Krantz, L.J., 2013. Mutagens. In: Maloy S., Hughes K. (eds.) Brenner's Encyclopedia of Genetics (2nd Edition). Elsevier, New York, 528–532.

Reid, K.A., Maesa, J., Maesa, A., van Staden, J., De Kimpec, N., Mulholland, D.A., Verschaeve, L., 2006. Evaluation of the mutagenic and antimutagenic effects of South African plants. Journal of Ethnopharmacology 106, 44–50.

Resende, F.A., Campos, D.L., Da Silva, V.C., Grandis, R.A., Souza, L.P., Junior, S.L., Rocha, C.Q., Santos, L.C., Vilegas, W., Varanda, E.A., 2015. Mutagenicity and chemo preventive activities of *Astronium* species assessed by Ames test. Regulatory Toxicology and Pharmacology 72, 506–513.

Richardson, S.D., Plewa, M.J., Wagner, E.D., Schoeny, R., De Marini, D.M., 2007. Occurrence, genotoxicity, and carcinogenicity of regulated and emerging disinfection by-products in drinking water: A review and roadmap for research. Mutation Research 636, 178–242.

Roell, K.R., Reif, D.M., Motsinger-Reif, A.A., 2017. An introduction to terminology and methodology of chemical synergy – perspectives from across disciplines, Frontiers in Pharmacology, DOI: 10.3389/fphar.2017.00158.

Runyoro, D.K.B., Matee, M.I.N., Ngassapa, O.D., Joseph, C.C., Mbwambo, Z.H., 2006. Screening of Tanzanian medicinal plants for anti-Candida activity. BMC Complementary and Alternative Medicine 6(11), 1–10.

Russo, D., Miglionico, R., Carmosino, M., Bisaccia, F., Andrade, P.B., Valentao, P., Milella, L., Armentano, M.F., 2018. A comparative study on phytochemical profiles and biological activities of *Sclerocarya birrea* (A.Rich.) Hochst leaf and bark extracts. International Journal of Molecular Sciences 19(186), 1–14.

Samie, A., Tambani, T., Harshfield, E., Green, E., Ramalivhana, J.N., Bessong, P.O., 2010. Antifungal activities of selected Venda medicinal plants against *Candida albicans, Candida krusei* and *Cryptococcus neoformans* isolates from South African AIDS pacients. African Journal of Biotechnology 9(20), 2965–2976.

Samuelsson, G., Farah, M.H., Claeson, P., Hagos, M., Thulin, M., Hedberg, O., Warfa, A.M., Hassan, A.O., Elmi, A.H., Abdurahman, A.D., Elmi, A.S., Abdi, Y.A., Alin, M.H., 1992. Inventory of plants used in traditional medicine in Somalia. II. Plants of the families Combretaceae to Labiatae. Journal of Ethnopharmacology 37, 47–70.

Schlamovitz, Z., Gupta, M., Diaz, J.A., 2009. A case of acute keratoconjunctivitis from exposure to latex of *Euphorbia tirucalli* (Pencil cactus). The Journal of Emergency Medicine 36(3), 239–241.

Schmelzer, G.H., 2007. *Acalypha villicaulis* Hochst. In: Schmelzer, G.H., and Gurib-Fakim, A., (Ed), PROTA (Plant Resources of Tropical Africa), Wageningen, Netherlands.

Seukep, A.J., Noumedem, J.A.K., Djeussi, D.E., Kuete, V., 2014. Genotoxocity and teratogenicity of African medicinal plants. In: Kuete, V. (ed.), Toxicological Survey of African Medicinal Plants, 235-275. Elsevier, USA.

Shai, L.J., McGaw, L.J., Masoko, P., Eloff, J.N., 2008. Antifungal and antibacterial activity of seven traditionally used South African plant species active against *Candida albicans.* South African Journal of Botany 74, 677–684.

Sharma, R., Lall, N., 2014. Antibacterial, antioxidant activities and cytotoxicity of plants against *Propionibacterium acnes*. South African Journal of Science 110(11/12), 1–8.

Silva, A.C.P., Faria, D.E.P., Borges, N.B.E.S., Souza, I.A., Peters, V.M., Guerra, M.O., 2007. Toxicological screening of *Euphorbia tirucalli* L.: Developmental toxicity studies in rats. Journal of Ethnopharmacology 110, 154–159.

Soliman, S.S.M., Semreem, M.H., El-Keblawy, A.A., Abdullah, A., Uppuluri, P., Ibrahim, A.S., 2017. Assessment of herbal drugs for promising anti-Candidal activity. MBC Complementary and Alternative Medicine 17, 1–9.

Souza, L.F., Pagno, C.H., Medeiros, N.S., Barbosa, S., Dos Santos, P.C.P., Rios, A., Achaval, M., De Jong, E.V., 2016. The effect of the carotenoid bixin and annatto seeds on haematological markers and nephrotoxicity in rats subjected to chronic treatment with cisplatin. Brazilian Journal of Pharmacognosy 1(26), 1–5.

Sparg, S.G., Van Staden, J., Jäger, A.K., 2000. Efficiency of traditionally used South African plants against Schistosomiasis. Journal of Ethnopharmacology 73, 209–214.

Sponchiado, G., Adam, M.L., Silva, C.D., Soley, B.S., De Mello-Sampayo, C., Cabrini, D.A., Correr, C.J., Otuki, M.F., 2016. Quantitative genotoxicity assay for analysis of medicinal plants: A systematic review. Journal of Ethnopharmacology 178, 289–296.

Steenkamp, V., 2003. Traditional herbal remedies used by South African women for gynaecological complaints. Journal of Ethnopharmacology 86, 97–108.

Steenkamp, V., Fernandes, A.C., Van Rensburg C.E.J., 2007. Screening of Venda medicinal plants for antifungal activity against *Candida albicans.* South African Journal of Botany 73, 256–258.

Steenkamp, V., Gouws, M.C., 2006. Cytotoxicity of six South African medicinal plant ectracts used in the treatment of cancer. South African Journal of Botany 72, 630–633.

Sui, H., Kawakami, K., Sakurai, N., Hara, T., Nohmi, T., 2009. Improvement and evaluation of high throughput fluctuation Ames test using 384-well plate with *Salmonella typhimurium* TA100 and TA98. Genes and Environment 31(2), 47–55.

Suleiman, M.M., Bagla, V., Naidoo, V., Eloff, J.N., 2010. Evaluation of selected South African plant species for antioxidants, antiplatelet, and cytotoxic activity. Pharmaceutical Biology 48(6), 643–650.

Tamokou, J., Kuete, V., 2014. Mutagenicity and carcinogenicity of African medicinal plants. In: Kuete, V. (ed.), Toxicological Survey of African Medicinal Plants, 277–322. Elsevier, USA.

Taylor, J.L.S., Elgorashi, E.E., Maes, A., Van Gorp, U., Van Staden, J., Verschaeve, L., 2003. Investigating the safety of plants used in South African traditional medicine: Testing for Genotoxicity in the micronucleus and alkaline comet assay. Environmental and Molecular Mutagenicity 42, 144–154.

Teke, G.N., Kuete, V., 2014. Acute and subacute toxicities of African medicinal plants. In: Kuete, V. (ed.), Toxicological Survey of African Medicinal Plants, 63–98. Elsevier, USA.

Truong, D., Nguyen, D.H., Ta, N.T.A., Bui, A.V., Do, T.H., Nguyen, H.C., 2019. Evaluation of the use of different solvents for phytochemical constituents, antioxidants, and *in-vitro* anti-inflammatory activity of *Severinia buxifolia.* Journal of Food Quality 2019, 1–9.

Tshikalange, T.T., Meyer, J.J.M., Hussein, A.A., 2005. Antimicrobial activity, toxicity and the isolation of a bioactive compound from plants used to treat sexually transmitted diseases. Journal of Ethnopharmacology 96, 515–519.

Udem, S.C., Obidoa, O., Asuzu, I.U., 2010. Acute and chronic toxicity studies of *Erythrina senegalensis* DC stem bark extract in mice. Comparative Clinical Pathology 19, 275–282.

Umhlabuyalingana municipality IDP, 2017/2018. Umhlabuyalingana municipality Integrated Development Plan 2017/2018 – 2022. Cited from: https://www.umhlabuyalingana.gov.za/docs/idp/20170911/UMHLABUYALINGANA_FINAL_IDP_2017-2022_MATHEWS_MTHEMBU.pdf. Retrieved on 18 February 2019.

Van Meer, G., Wolthoorn, J., Degroote, S., 2003. The fate and function of Glycosphinglipids glucosylceramide. Philosophical Transactions: Biological Sciences 358 (1433), 869–873.

Van Vuuren, S.F., Naidoo, D., 2010. An antimicrobial investigation of plants used traditionally in Southern Africa to treat sexually transmitted infections. Journal of Ethnopharmacology 130(3) 552–558.

Van Vuuren, S.F., Viljoen, A.M., 2008. *In vitro* evidence of phyto-synergy for plant part combinations of *Croton gratissumus* (Euphorbiaceae) used in African traditional healing. Journal of Ethnopharmacology 119, 700–704.

Van Vuuren, S.F., Viljoen, A.M., 2011. Plant-based antimicrobial studies – methods and approaches to study the interaction between natural products. Planta Medica 77, 1168–1182.

Van Zyl, R.L., Viljoen, A.M., 2009. The antiplasmodial activity and toxicity profile of ten South African *Commiphora* species. African Journal of Traditional, Complementary and Alternative Medicines 6, 327. Retrieved from https://journals.athmsi.org/index.php/ajtcam/article/view/656.

Varga, C.A., Veale, D.J.H., 1997. Isihlambezo: utilization patterns and potential health effects of pregnancy related traditional herbal medicine. Social Science and Medicine 44, 911–924.

Veiga, A., Toledo, M.G.T., Rossa, L.S., Mengarda, M., Stofella, N.C.F., Oliveira, L.J., Goncalves, A.G., Murakami, F.S., 2019. Colorimetric microdilution assay: Validation of s standard method for determination of MIC, IC_{50} and IC_{90} of antimicrobial compounds. Journal of Microbiological Methods 162, 50–61.

Verschaeve, L., Van Staden, J., 2008. Mutagenic and antimutagenic properties of extracts from South African traditional medicinal plants. Journal of Ethnopharmacology 119, 575–587.

Vilander, A.C., Niles, G.A., Frank, C.B., 2016. Cerebral candidal abscess and bovine viral diarrhoea virus infection in an aborted bovine foetus. Journal of Comparative Pathology 154,161–164.

Waczuk, E.P., Kamdem, J.P., Abolaji, A.O., Meinerz, D.F., Bueno, D.C., Nascimento Gozanga, T.K.S., Canto Dorow, T.S., Boligon, A.A., Athayde, M.L., Rocha, J.B.T., Avila, D.S., 2015. *Euphorbia tirucalli* aqueous extract induces cytotoxicity, genotoxicity and changes in antioxidant gene expression in human leukocytes. Toxicology Research 4, 739–748.

Wieczorowska-Tobis, K., Polubinska, A., Breborowicz, A., Oreopoulos, D.G., 2001. A comparison of the biocompatibility of phosphate-buffered saline and dianeal 386% I the rat model o peritoneal dialysis. Advances in Peritoneal Dialysis 17, 42–46.

Wu, C., 2014. An important player in brine shrimp lethality bioassay: the solvent. Journal of Advanced Pharmaceutical Technology and Research 5(1), 57–58.

Wu, C-S., Wang, T-J., Wu, C-W, Wang, Y-N., Chaw, S-M., 2017. Plastome evolution in the sole hemipaeasitic genus Laurel Dodder (*Cassytha)* and insights into the plastid phylogenomics of Lauraceae. Genome Biology and Evolution 9(10), 2604–2614.

Yao, X., Panichpisal, K., Kurtzman, N., Nugent, K., 2007. Cisplatin nephrotoxicity: A review. The American Journal of the Medical Sciences 334(2), 115–124.

York, T., Van Vuuren, S.F., De Wet, H., 2012. An antimicrobial evaluation of plants used for the treatment of respiratory infections in rural Maputaland, KwaZulu-Natal, South Africa. Journal of Ethnopharmacology 144, 118–127.

Zeliger, H.I., 2011. Teratogens. In: Zeliger H.I. (2nd Edition), Human Toxicology of Chemical Mixtures. Elsevier, UK, 341–353.

Zofou, D., Kengne, A.B.O., Tene, M., Ngemenya, M.N. Tane, P., Titanji, V.P.K., 2011. *In vitro* antiplasmodial activity and cytotoxicity of crude extracts and compounds from the stem bark of *Kigelia africana* (Lam) Benth (Bignoniaceae). Parasitol Research 108, 1383–1390.

APPENDICES

Appendix A: International conference presentation abstract

Traditional herbal remedies used by women in a rural community in South Africa for the treatment of gynaecological and obstetrics complaints

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According to the World Health Organization, there are annually 350 000 maternal deaths in sub-Saharan Africa, with obstetric haemorrhage the most common cause of death. Although maternal mortality can be reduced by health-care interventions such as the provision of family planning, maternity care and access to safe abortion practice, it is not happening in rural areas. Previous studies in a rural community in northern Maputaland (South Africa) had indicated the importance of medicinal plants in their primary health care-system. However, no survey has been done in this region to document the medicinal plants used to treat various gynaecological and obstetric problems. The aim of the study was to conduct an ethnobotanical survey, focussing on lay people's knowledge on plants used to treat gynaecological and obstetrics complaints. A total of 70 lay people were purposively interviewed using structured questionnaires. Thirtytwo plant species from 21 families were recorded for the treatment of 19 different gynaecological and obstetric disorders. Sixteen new plant species were found to be recorded for the first time in literature to treat gynaecological and obstetric disorders, and twelve new Zulu vernacular names were documented. The three most treated gynaecological conditions were dysmenorrhoea by 21 plant species, infertility (14 species) and menorrhagia (10 species), whereas blood purification (14 species), to ease labour (9 species) and to induce abortion (2 species) were the most mentioned for obstetric conditions. Bridelia cathartica was the most cited plant species for treating both gynaecological and obstetric problems. This wealth of new knowledge gained with the current survey reinforces the importance of documenting lay people's indigenous medicinal plant knowledge in rural communities.

Appendix B: National conference presentation abstract

Ethnobotany and mutagenicity of some medicinal plants used by women in northern Maputaland to treat gynaecology ans obstetrics compaints

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Majority of the women in Maputaland still rely on medicinal plants to treat gynaecology and obstetrics complaints. In most cases these plants are perceived as safe based on their long-term use with no signs of immediate toxicity. However, some plants may cause adverse effects such as gene mutation, which is among the highest causes of cancer. This study explores indigenous knowledge and investigated the potential mutagenicity of plants used for women problems in Maputaland. The aqueous and methanol-dichloromethane plant extracts were prepared from 51 plant parts sourced from 35 different plant species. The mutagenicity test was conducted using the Ames test by exposing the mutant bacteria, *Salmonella typhimurium* TA98 and TA100 strains to the extracts. Results from this study indicated that 8% aqueous and 27% organic extracts were mutagenic against the TA98, while it was 35% aqueous and 69% organic extracts that were mutagenic against TA100 strain. *Cassytha filiformis, Hypoxis hemerocallidea* and *Opuntia stricta* demonstrated the most mutagenic activity in both TA98 and TA100 strains. *Bridelia cathartica* is among the most common medicinal plants for different gynaecology and pregnancy problems, however, it was found to be mutagenic. This poses a risk to the martenal mother who may pass on genetic defects on to the foetus. Such studies are needed warrant caution and dispel the belief that all medicinal plants are safe.

Appendix C: Manusript published

South African Journal of Botany 94 (2014) 129–139

Contents lists available at ScienceDirect

South African Journal of Botany

journal homepage: www.elsevier.com/locate/sajb

Traditional herbal remedies used by women in a rural community in northern Maputaland (South Africa) for the treatment of gynaecology and obstetric complaints

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ARTICLE INFO

Article history:
Received 15 April 2014
Received in revised form 12 June 2014
Accepted 17 June 2014
Available online xxxx

Edited by: V Steenkamp

Keywords:
Medicinal plants
Gynaecology
Obstetrics
Lay people
Northern Maputaland
South Africa

ABSTRACT

According to the World Health Organization, there are annually 350,000 maternal deaths in sub-Saharan Africa, with obstetric haemorrhage the most common cause of death. Although maternal mortality can be reduced by health-care interventions such as the provision of family planning, maternity care and access to safe abortion practice, it is not happening in rural areas. Previous studies in a rural community in northern Maputaland had indicated the importance of medicinal plants in their primary health care-system. However, no survey has been done in this region to document the medicinal plants used to treat various gynaecological and obstetric problems. The aim of this study was to conduct an ethnobotanical survey, focussing on lay people's knowledge on plants used to treat gynaecological and obstetric complaints. A total of 70 lay people (all female) were purposively interviewed using structured questionnaires. The focus was on plants used for the treatment of gynaecological and obstetric conditions and information was collected regarding vernacular plant names, plant parts used, preparation and application methods. Thirty two plant species from 21 families were recorded for the treatment of 19 different gynaecological and obstetric disorders. When searching the most frequently used scientific databases (ScienceDirect, Scopus and Pubmed), 16 plant species (*Acacia burkei, Acanthospermum glabratum, Commiphora neglecta, Crotalaria monteiroi* var. *galpinii, Cyperus natalensis, Doispyros villosa, Erythrina humeana, Hermannia boraginiflora, Hypoxis cf. longifolia, Opuntia stricta, Ozoroa engleri, Ranunculus multifidus, Sapium integerrimum, Searsia nebulosa, Senecio deltoideus, Senecio serratuloides*) were found to be recorded for the first time in literature to treat gynaecological and obstetric disorders, and twelve new Zulu vernacular names were documented. The three most treated gynaecological conditions were dysmenorrhoea by 21 plant species, infertility (14 species) and menorrhagia (10 species), whereas blood purification (14 species), to ease labour (9 species) and to induce abortion (2 species) were the most mentioned for obstetric conditions. *Bridelia cathartica* was the most cited plant species (18 times) for treating both gynaecological and obstetric problems. The modes of plant preparations were mostly concoctions which were taken orally. This wealth of new knowledge gained with the current survey reinforces the importance of documenting lay people's indigenous medicinal plant knowledge in rural communities. Results also strongly suggest that the availability of plants is not the only criteria for usage; cultural influence may play a pertinent role in the choice of plant species. Literature indicates that there is very little research done on assessing the safety and efficacy of botanical remedies taken during pregnancy and lactation. Pregnant women should therefore be made aware of the risks they take when consuming herbal remedies.

1. Introduction

In developing countries, pregnancy and childbearing have been major contributors to death and disability amongst women. Thus a woman in Africa may face death during pregnancy and the mortality rate of children during birth is as high as one in 26, compared with only one death in 7300 births in developed countries (WHO, 2009). In South Africa the maternal mortality is 300 deaths per 100,000 live births as estimated in 2010 by the WHO (2013). Pregnancy and childbirth deaths can be reduced by health-care interventions such as the provision of family planning and maternity care and access to safe abortion practices. According to WHO (2009), the poorer and less educated women and those living in rural areas are far less likely to give birth in the presence of a skilled health worker than better educated women who live in wealthier households or urban areas. Reasons for this include distance and expenses to reach health-care centres, but also inappropriate sociocultural practices. Another maternal health problem causing significant proportions of deaths is unsafe abortions. In

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http://dx.doi.org/10.1016/j.sajb.2014.06.009
0254-6299/

Appendix D: Ethical Clearance Certificate

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

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ETHICAL CLEARANCE CERTIFICATE

Certificate Number	**UZREC 171110-030 PGM 2017/443**							
Project Title	**A Toxicological Evaluation of Plants Used by Women in Northern Maputaland**							
Principal Researcher/ Investigator	SC Ngubane							
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-01 July 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)
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