THE ANTIMICROBIAL ACTIVITY OF FIVE FOOD SPICES WHEN TESTED AGAINST VARIOUS GRAM-POSITIVE –AND GRAM-NEGATIVE MICROORGANISMS

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Abstract

The discovery of antibiotics by Alexander Fleming in the early nineteen hundreds not only created an enormous breakthrough in medical treatment but along with it introduced the emergence of new and now what is considered an ever increasing number of multi-drug resistant pathogens. Like antibiotics, herbs and spices have been used traditionally by many, for the treatment of various aliments ranging from stomach indigestion, lesions of the skin to beauty therapy. At present it is estimated that about 80% of the world population rely on botanical preparations as medicines to meet their health needs as opposed to treatment by conventional medicine with spices creating a shelf of its own in the global medical cabinet.

In this study, the antimicrobial potential of five spices (commonly known as ginger, cinnamon, turmeric, nutmeg and chilli) was analysed against various Gram positive- and Gram negative microorganisms namely, ***Bacillus subtilis***, ***Escherichia coli***, ***Klebsiella pneumoniae***, ***Salmonella spp***, ***Shigella spp*** and ***Staphylococcus aureus***. Analysis of the results of sensitivity tests (disc and agar well diffusion assays) indicated each of the microorganisms to be completely inhibited, intermediately inhibited or completely resistant towards a particular spice extract. The formation of zones of inhibition present where inhibition had occurred indicated that the spice tested was effective as an antimicrobial agent when screened. Zones of absolute inhibition (greater than fifteen millimetres in diameter) were obtained during positive agar well and disc diffusion assaying with neomycin used as the antimicrobial agent of choice. Inhibition zones

observed to be in the upper limit range (pertaining to the study) of 20 mm – 27 mm in diameter. Comparative studies using the test spices indicated that chilli, turmeric, nutmeg, cinnamon and ginger each demonstrated zones of inhibition within this limit at one or more laboratory testing. Chilli was the most active antimicrobial agent when tested and in some instances demonstrated antimicrobial effectiveness greater than that exhibited by the positive control neomycin. Turmeric, nutmeg, cinnamon and ginger however each demonstrated inhibition within the same range as that of neomycin. The observations of such inhibition amongst the spices were comparatively significant and demonstrated the potential use of these spices as antimicrobial agents with an efficacy that can be compared to that of the already recognized and widely used antibiotic, neomycin.

The minimum inhibitory concentration (MIC) was successfully determined for each of the spice extracts. The reactions observed during MIC determination were confirmatory of the antimicrobial activity present in the extracts of each spice. Analyses of the results conclude that the active compounds present in the selected spices were effective against certain microbial species. This observation demonstrated that spice can and may be used in the treatment of bacterial infections. This could in the future be an alternative treatment to antibiotics for one or all of the microbial species investigated and in so doing allow the healing powers of spices to be acknowledged.

DECLARATION

I declare that this dissertation hereby submitted to the University of Zululand for the degree of Master of Science has not been previously submitted by me for a degree at this or any other University, that it is my own work in design and execution, and that all material contained therein has been duly acknowledged.

……

Signature

……

Date

DEDICATION

This work is dedicated to the precious, joyous memories of my beloved Buttons

(2004 – 2008).

ACKNOWLEDGEMENTS

To God Almighty for sustaining me and always making your presence felt in my times of difficulties, I am thankful to and honor Your precious hand upon my life

To my ever encouraging parents, brothers, sister-in-law and Rishi. Thank you all for always finding the right things to say when I needed to hear them the most. The patience and emotional support which you have given to me was and continues to be a fundamental strengthening agent in my life.

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My sincere gratitude goes to the NRF for the bursary which they awarded me. To me like many other students it helped shed the load of financial stress.

Last but not least to Prof. Opoku, I do not have enough words to thank you for the support and wisdom which you so willing gave to me, know that you will always be greatly appreciated and respected by me.

May God richly and abundantly bless each of you.

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Table 4.4 Inhibition zones resulting from disc diffusion assay of Gram-negative microorganisms against extracts of the spice turmeric (mm).

Bacteria → Extracts ↓	***E. coli*** ATCC 11775				***E. coli*** U15055				***E. coli*** U16403				***E. coli*** U16406			
Acetone	14	10	13	15	11	11	0	9	11	9	9.5	10	15	13	10	11
Ethanol	13	15	18	12	0	0	10	11	20	23	19	18	15	14	13	14
Methanol	18	21	20	16	10	0	0	0	11	13	12.5	10	10	10.5	11	11
Cold water	11	12	9	9	0	0	0	0	9	10	10.5	10	10	11	11	9
Hot water	16	10	11	11	0	0	0	0	10	10	10.5	12	9	10	10	11

During disc diffusion assay, three of the four stains of ***E.coli*** were observed to be greatly inhibited by both aqueous (hot and cold water) and organic (acetone, ethanol and methanol) extracts of turmeric exhibiting zones of inhibitions predominantly in the range of 9mm – 23mm in diameter. The organic extracts, ethanol and methanol were observed to be the most effective antimicrobial agents, demonstrating absolute inhibition for the ***E. coli*** ATCC 11775 and ***E. coli*** U16403 strains. However, ***E. coli*** U15055 was observed to be the most resistant strain to both organic and inorganic extract, exhibiting only intermediate inhibition in the range of 10mm – 11mm in diameter for the extracts of acetone and ethanol. The aqueous extracts were noticeably active as intermediate inhibitors (9mm – 16mm) for all of the ***E. coli*** strains except for that of ***E. coli*** U15055 which was resistant when tested against these extracts.

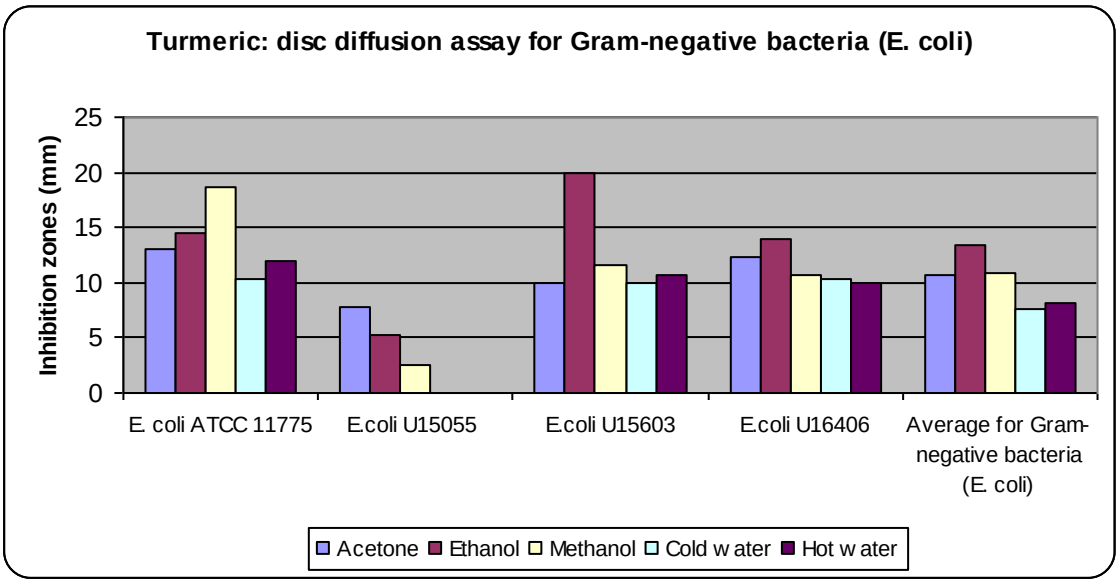

Figure 4.1 Average inhibition zone data of Gram-negative bacteria based on the disc diffusion assays for the various extracts of turmeric.

The average of the quadruplet testing for each extract as shown in Figure 4.1 indicated that all the extracts had an inhibiting effect on all the test microorganisms (Figure 4.1; average inhibition zones greater than 10 mm). ***E. coli*** U15055 was the completely resistant when tested against both aqueous extracts with average inhibition below 10 mm when tested against the various organic extracts.

Figure 4.1 demonstrated that the average inhibition action of turmeric against the Gram-negative microorganisms was the most effective when the organic extracts were tested with ethanol exhibiting the greatest activity followed by acetone and methanol. In the case of aqueous extracts, both were found to possess significant antimicrobial activity and were capable to inhibit all of test microorganisms except ***E. coli*** U15055 with the hot water extract exhibiting greater activity than the cold water extract.

Table 4.5 Results of agar well diffusion assay of Gram-negative microorganisms against extracts of the spice turmeric (mm).

Bacteria→ Extracts ↓	***E. coli*** ATCC 11775				***E. coli*** U15055				***E. coli*** U16403				***E. coli*** U16406			
Acetone	12	10	11	0	0	11	13	10	11	12	14	12	16	14	14	11
Ethanol	17	13	12	11.5	0	0	0	0	11	11	13	12	13	15	12	14
Methanol	14	9	10	12	0	0	0	0	12	10	10.5	11	13	12	12	10
Cold water	10	10	11.5	9	0	0	0	0	10	9	10	11	10	11	12	11
Hot water	12	12	10.5	9	0	0	0	0	11	12	13	11	14	12	12	11

As in the disc diffusion assay, the microbial strain ***E. coli*** U15055 was observed to be resistant to four of the five test extracts of turmeric. The exception was the organic extract acetone that was noted as an intermediate inhibitory agent for the strain ***E. coli*** U15055. For the remaining three strains, ATCC 11775 strain of ***E. coli***, ***E. coli*** U16403 and ***E. coli*** U16406 the organic extracts were observed as noticeable intermediate inhibitory agents with zones of inhibition in the range of 9mm – 17mm in diameter. Aqueous extracts were observed as effective antimicrobial agents with inhibition in the range of 9mm – 14mm.

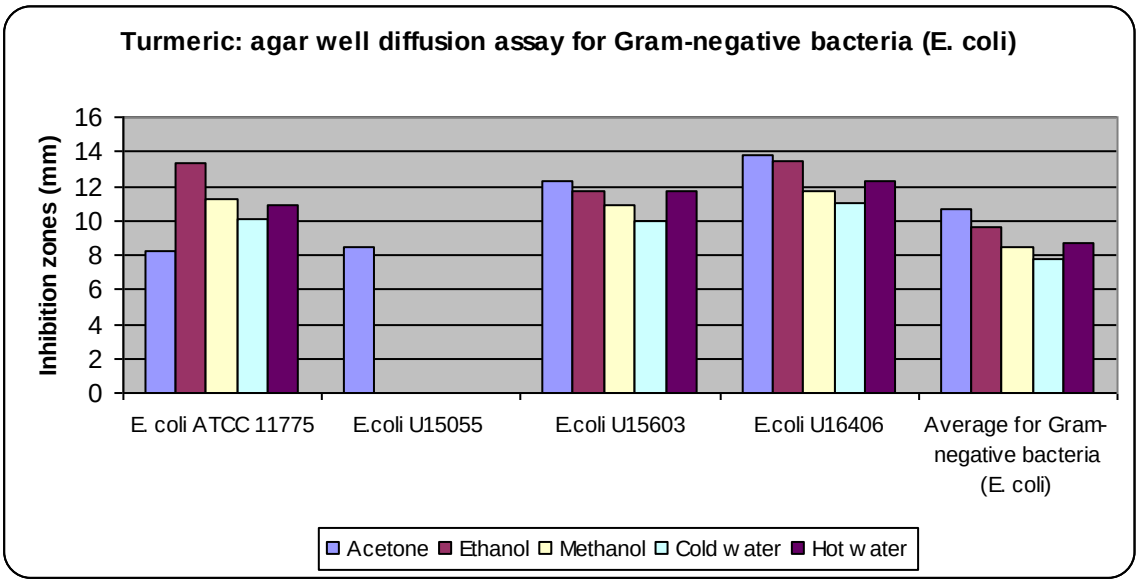

Figure 4.2 Average inhibition zone data of Gram-negative bacteria based on agar well diffusion assay for the various extracts of turmeric.

All of the Gram-negative microorganisms were found to be inhibited by all the extracts of turmeric, with the exception to ***E. coli*** U15055 which was only susceptible when tested against the acetone extract. Of the organic extracts, acetone and ethanol exhibited the greatest antimicrobial activity (greater than 10 mm) followed closely by the aqueous extract, hot water.

Average of all the inhibition zones for the agar well assay indicated that of the organic extracts, acetone was the most effective antimicrobial agent, followed by ethanol and then methanol. Hot water was the more effective of the two aqueous extracts. Figure 4.2 demonstrated, with the exception of ***E. coli*** U15055, that each of the various extracts had significant potential as an antimicrobial agent.

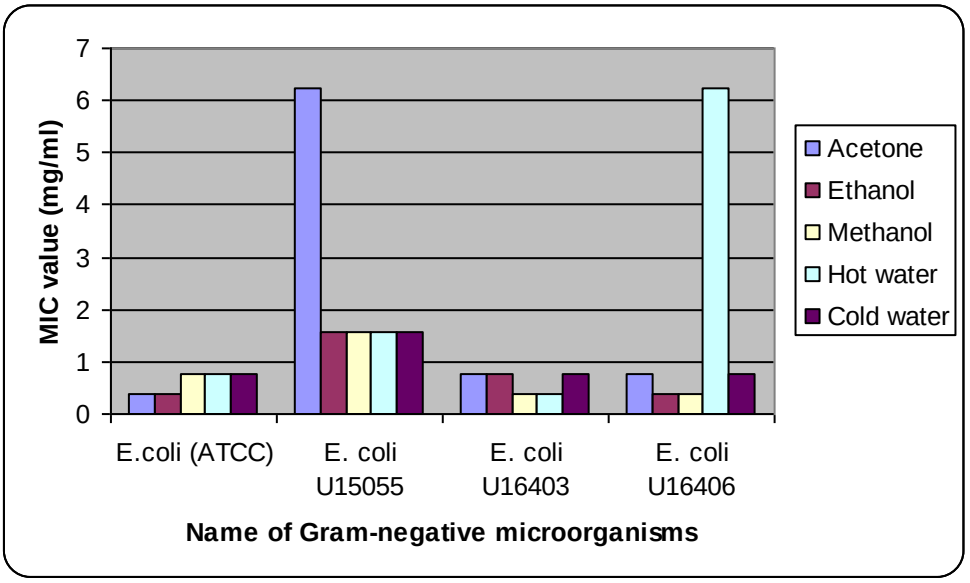

Figure 4.3 The MIC results of four Gram-negative strains of ***E. coli*** namely, ***E. coli*** 11775 (ATCC), ***E. coli*** U15055 and ***E. coli*** U16403 and ***E. coli*** U16406 when tested against five extracts of the spice turmeric (continued **Fig 4.6**).

The MIC determination for the above mentioned microorganisms indicated turmeric to be most effective when tested against the Gram-negative microorganisms where it was observed that a minimal concentration (0.39 mg/ml – 0.78 mg/ml) of turmeric extracts can be effective as an antimicrobial agent. However, Gram-negative ***E. coli*** U15055 demonstrated higher MIC values predominately in the range of 1.56 mg/ml – 6.25 mg/ml. ***E. coli*** U16406 was also observed to have an MIC value of 6.25mg/ml for the aqueous extract of hot water.

Table 4.6 Inhibition zones resulting from the disc diffusion assay of Gram-negative microorganisms against extracts of the spice turmeric (mm).

Bacteria → Extracts ↓	***K. pneumoniae*** ATCC 13883				***Salmonella spp.***				***Shigella flexneri***				***Shigella sonnei***			
Acetone	21	12	13	19	12	9	9.5	11	10	11	9	11	10	10	10.5	9
Ethanol	22	20	18	19	9	8.5	9	11	13	11	12	9	10	10	9	9
Methanol	16	14	9	9	11	14	12	10.5	9	9	10.5	10	0	9	11	10.5
Cold water	0	0	0	0	11	9	11.5	10	9	10	9	10	9	10	9	11
Hot water	14	18	12	10	9	9.5	11	12	9	9	9	9	0	11	9	8.5

K. pneumoniae ATCC 13883 strain was observed as resistant against the aqueous cold water extract but demonstrated both intermediate and absolute inhibition when tested against the organic extracts (acetone, ethanol and methanol) as well as the aqueous hot water extract. Inhibition zones were in the range of 9mm – 22mm in diameter. ***Salmonella spp.*** demonstrated intermediate inhibition against all extracts of turmeric in the range of 8.5mm – 14mm. All extracts were effective antimicrobial agents when tested against ***Shigella flexneri*** and ***Shigella sonnei*** resulting in intermediate inhibition in the range of 9mm – 13mm in diameter.

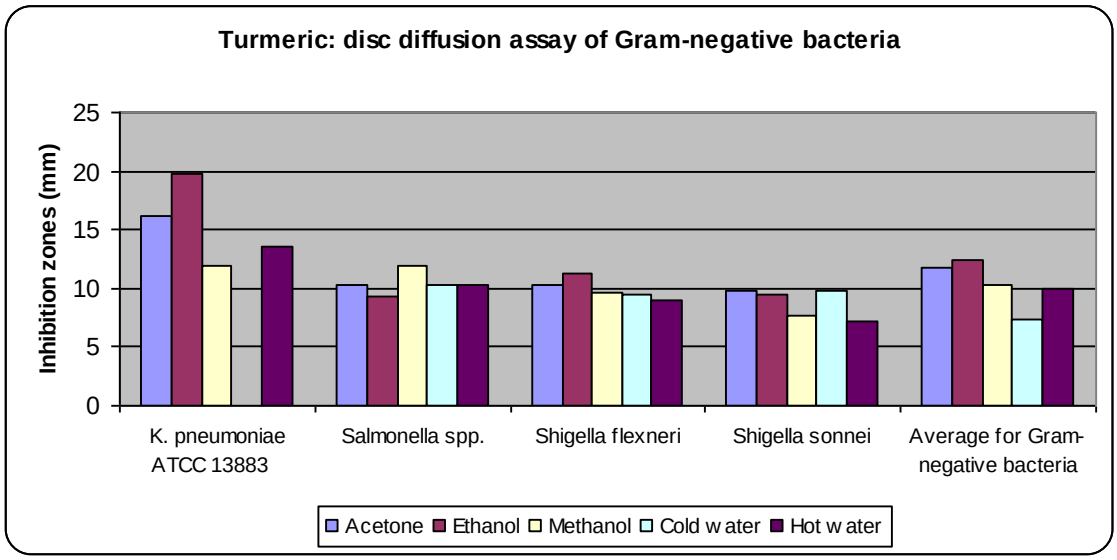

Figure 4.4 Average inhibition zone data of Gram-negative bacteria based on disc diffusion assay for the various extracts of turmeric.

Figure 4.4 demonstrated that all of the extracts tested were significantly effective as antimicrobial agents. ***K. pneumoniae*** ATCC 13883 exhibited the greatest susceptibility (average inhibition zones greater than 10mm) when against all extracts of turmeric except cold water. Acetone, ethanol, methanol and hot water extracts demonstrated consistent effectiveness against all of the microorganisms, being the most effective against ***K. pneumoniae*** ATCC 13883.

The average inhibition zone for the remaining three Gram-negative microbes clearly indicated that although the average inhibition zone for each quadruplet screening was between 8 mm – 13 mm, the effective of the various extracts as antimicrobial agents was pronounced and consistent throughout the investigation.

The average inhibition exhibited by each extract for each of the Gram-negative strains was also compared and indicated that the antimicrobial effectiveness of the extracts in the following order; ethanol, acetone, methanol and hot water, followed by cold water.

Table 4.7 Inhibition zones resulting from the agar well diffusion assay of Gram-negative microorganisms against extracts of the spice turmeric (mm).

Bacteria → Extracts ↓	***K. pneumoniae*** ATCC 13883				***Salmonella spp.***				***Shigella flexneri***				***Shigella sonnei***			
Acetone	13	16	10	10	13	15	13	11	10	12	12	10	10	11.5	10	12
Ethanol	9	10	12	14	10	12	10.5	9	0	0	11	9	13	11	9	11
Methanol	14	12	11.5	12	13	11	11.5	10	10	10	9	10	10	11	12	10
Cold water	0	0	0	0	9	8.5	10	9.5	12	10	11	13	0	0	10	0
Hot water	15	12	13	10	12	11.5	10	12	0	9	0	0	0	11	15	0

K. pneumoniae ATCC 13883 was once again observed to be resistant against cold water extract of turmeric but demonstrated intermediate susceptibility when tested against hot water and organic extracts of turmeric with zones of inhibitions in the range of 9mm – 16mm in diameter. Whilst ***Salmonella spp.*** was observed to be intermediately inhibited by all of the five extracts with an inhibition zone in the range of 8.5mm – 15mm in diameter. ***Shigella flexneri*** was initially observed to be resistant against the extracts of methanol and hot water but upon repetitive assaying exhibited intermediate susceptibility when tested against the same extracts with inhibition zones in the range of 9mm – 11mm. Acetone, methanol, and cold water extracts were all intermediate inhibitors in the range of 9.5mm – 13mm.

Shigella sonnei was initially resistant when tested against the aqueous extracts of turmeric but upon further testing observed to show intermediate inhibition whilst the organic extracts were all observed to be intermediate inhibitors with inhibition zones in the range of 9mm – 13mm in diameter.

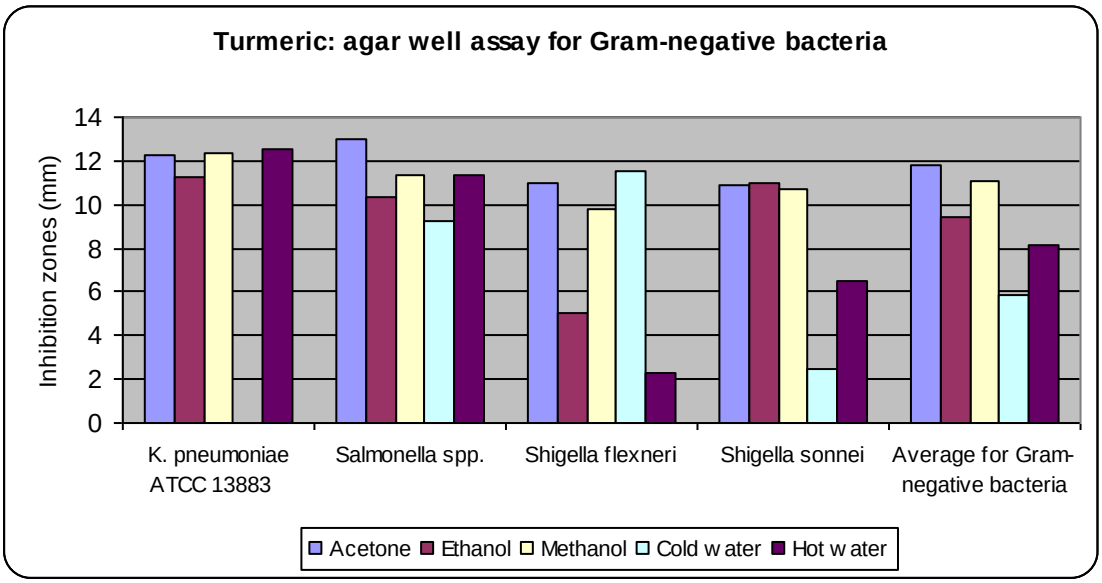

Figure 4.5 Average inhibition zone data of Gram-negative bacteria based on agar well diffusion assay for the various extracts of turmeric.

Figure 4.5 once again demonstrate that the cold water extracts was ineffective against ***K. pneumoniae*** ATCC 13883. However it was clearly exhibited from the figure above that all of the extracts of turmeric are highly capable as antimicrobial agents when tested.

Comparison of the average inhibition zones demonstrated that of the five test extracts, acetone was the most effective (11 mm), whilst cold water was noted as the least effective with an average inhibition of approximately 5mm.

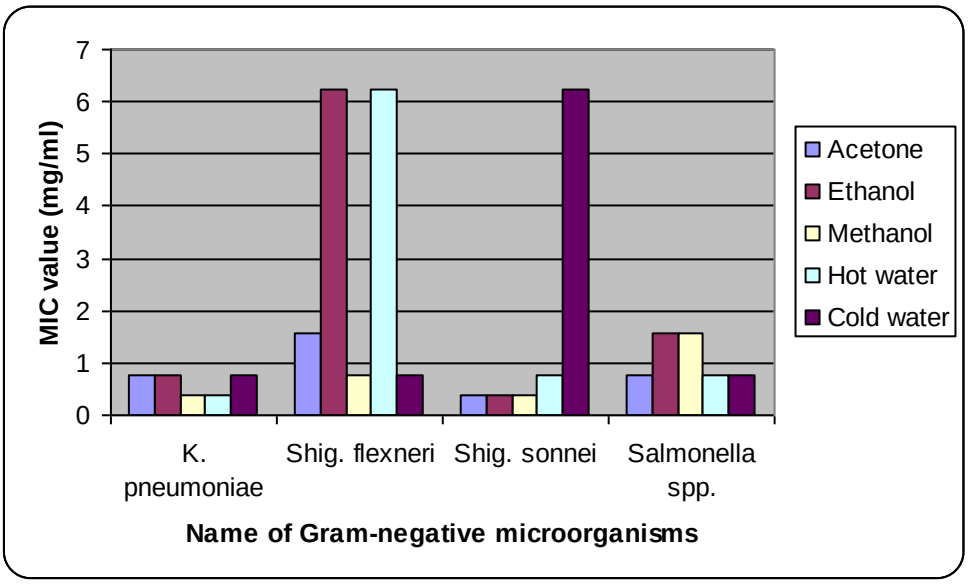

Figure 4.6 The MIC results of four Gram-negative microorganisms when tested against five extracts of the spice turmeric.

The extracts of ethanol, hot and cold water for the Gram-negative microorganisms were observed to have greater MIC value (6.25 mg/ml) when tested against ***Shigella*** species. However the effectiveness of turmeric against Gram-negative microorganisms was noticeable by the uniformly low MIC values (0.39 mg/ml – 1.56 mg/ml) obtained during testing.

Table 4.8 Inhibition zones resulting from the disc diffusion assay of Gram-positive microorganisms for extracts of the spice turmeric (mm).

Bacteria → Extracts ↓	***B. subtilis*** ATCC 6051				***S. aureus*** ATCC 12600				***S. aureus*** T1266				***S. aureus*** P4790			
Acetone	24	15	15	13	19	14	20	11	14	16	12	13	14	13	13	11
Ethanol	14	23	20	21	20	14	18	14	11	12	13	18	17	22	14	15
Methanol	17	18	16	15	19	16	12	10	16	15	15	12	9	10	9	12
Cold water	0	0	0	0	13	12	13	11	10	9	13	9	12	9	11	9
Hot water	11	10	11	9	12	11	11	11	11	11	0	9	10	11	11	9

B. subtilis ATCC 6051 showed predominately absolute inhibition against organic extracts in the range of 15mm – 24mm, intermediate inhibition against hot water extract and resistant when tested against the cold water extract of turmeric. ***S. aureus*** ATCC, 12600 showed both absolute and intermediate inhibition when tested against the organic and aqueous extracts with inhibition zones in the range of 10mm – 20mm in diameter. Similarly ***S. aureus*** T1266 and ***S. aureus*** P4790 were noted to exhibit zones of inhibition in the range of 11mm – 17mm for organic extracts and 9mm – 13mm for the aqueous extracts of turmeric.

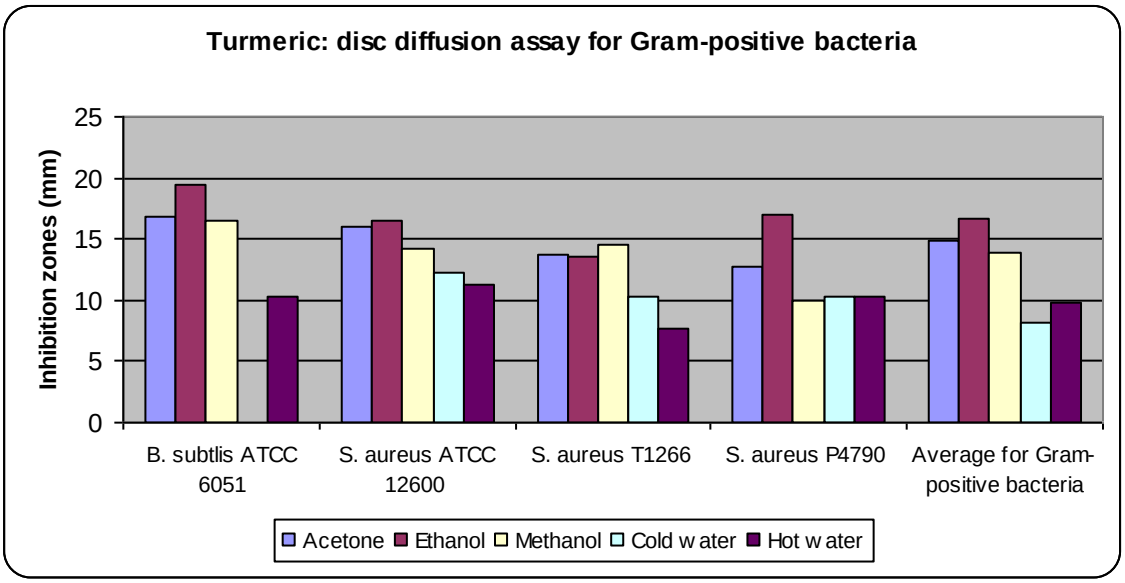

Figure 4.7 Average inhibition zone data of Gram-positive bacteria based on disc diffusion assay for the various extracts of turmeric.

B. subtilis ATCC 6051 was noted as resistant when tested against the cold water extract of turmeric whilst the same Gram-positive microbe was found to exhibit zones of inhibition greater than 10 mm when tested against the remaining four extracts.

The overall analysis showed that all of the organic extracts of turmeric were more effective (Figure 4.7; inhibition zones greater than 10mm) than the aqueous extracts. Ethanol possessed the greatest activity amongst the organic extracts with an average inhibition greater than 15 mm. A comparison of the average inhibition sizes of the Gram-positive microorganisms exhibited that all extract were highly effective in its antimicrobial activity and demonstrated that Gram-positive microorganisms were more susceptible to the extracts of turmeric than the Gram-negative (Figure 4.1 – Figure 4.5).

Table 4.9 Inhibition zones resulting from the agar well diffusion assay of Gram-positive microorganisms for extracts of the spice turmeric (mm).

Bacteria → Extracts ↓	***B. subtilis*** ATCC 6051				***S. aureus*** ATCC 12600				***S. aureus*** T1266				***S. aureus*** P4790			
Acetone	9	11	9	10.5	15	12	12	10	9	0	11	13	11	15	13	13
Ethanol	13	11	15	10	13	18	14	10	10	11	11	10	12	12	10	10.5
Methanol	16	12	10	12.5	0	15	0	12	10	12	13	10	9	11	11	11
Cold water	0	0	0	0	14	11	12	10	10	10	11	11	11	14	11	12
Hot water	10	13	9.5	13	10	9	11	14	13	13	12	11	9	10	11	12

The ATCC strains of ***B. subtilis*** 6051 and ***S. aureus*** 12600 exhibited predominately intermediate inhibition in the range of 9mm – 18mm for all extracts of turmeric except for ***B. subtilis*** 6051 which was observed to be resistant when tested against cold water extract. However ***S. aureus*** T1266 and ***S. aureus*** P4790 were both observed to exhibit intermediate inhibition when tested against all of the five extracts of turmeric with inhibition zones in the range of 9mm – 15mm in diameter.

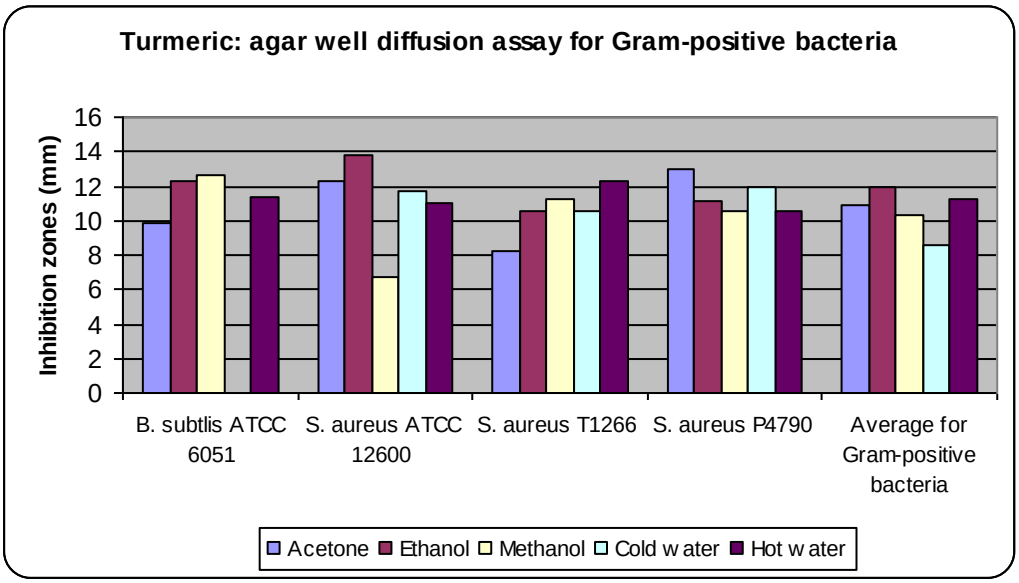

Figure 4.8 Average inhibition zone data of Gram-positive bacteria based on agar well assay for the various extracts of turmeric.

All of the Gram-positive microorganisms were significantly inhibited by all of the extracts with zones of inhibition predominately greater than 10 mm. ***B. subtilis*** ATCC 6051 was once again found to be resistant against the cold water extract whilst all strains of ***S. aureus*** were completely susceptible.

When average inhibition zone sizes were compared it was concluded that the effectiveness of turmeric as an antimicrobial agent was unquestionable. Turmeric as depicted in Figure 4.8 was capable of microbial inhibition on a commendable scale and this may hopefully be an indication of its potential future as part of antimicrobial strategy.

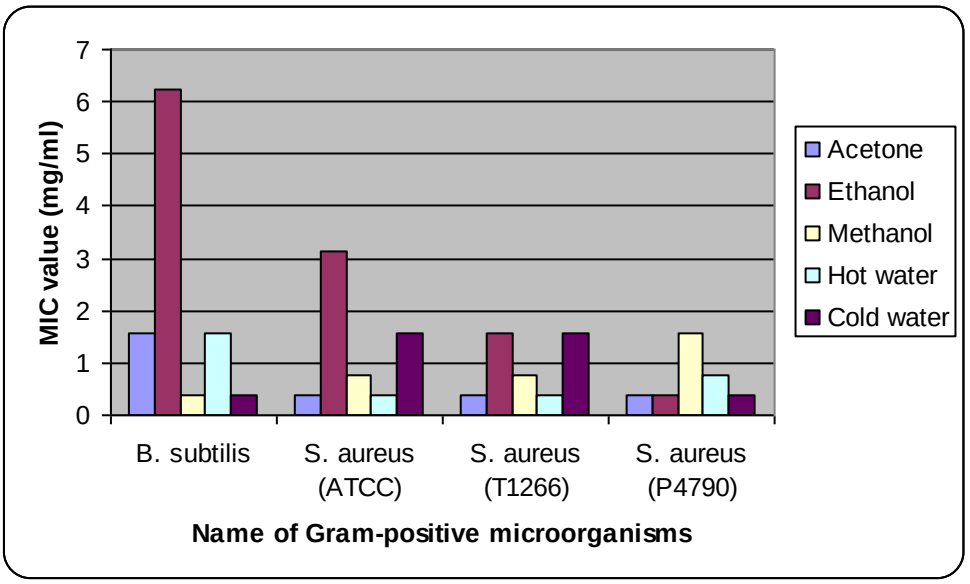

Figure 4.9 The MIC results of four Gram-positive microorganisms namely, ***B. subtilis*** ATCC, 65051 ***S. aureus*** ATCC, 12600 ***S. aureus*** T1266 and ***S. aureus*** T4790 when tested against five extracts of the spice turmeric.

All of the above Gram-positive microorganisms were observed to be highly susceptible to all of the five extracts of turmeric with the exception of ethanol and inorganic hot water extracts. MIC values were determined to be predominately in the lower range i.e. between 0.39 mg/ml – 1.56 mg/ml. The low MIC values indicate the potential use of turmeric as an effective antimicrobial agent against the microorganisms tested above.

Table 4.10 Inhibition zones resulting from the disc diffusion assay of Gram-negative microorganisms for extracts of the spice nutmeg (mm).

Bacteria → Extracts ↓	***E. coli*** ATCC 11775				***E. coli*** U15055				***E. coli*** U16403				***E. coli*** U16406			
Acetone	11	12.5	14.5	9	10	12	0	11	11	11	14	15	14	16	12	13
Ethanol	14	8	11	14	9	9	10.5	10	9	9	11	10	14	10	10	10
Methanol	9	9.5	11.5	13	13	9	10	12	11	9	9	10	19	13	12	15
Cold water	23.5	27	21.5	20.5	0	0	0	0	12	10	10	10.5	9	13	11	10
Hot water	12.5	11	8	8	0	0	0	0	8.5	10	9	10	11	11	9.5	12

E. coli ATCC 11775 exhibited noticeably greater zones of inhibition when tested against cold water extract of nutmeg than any of the other extracts (20.5mm – 27mm). The organic and hot water were all active in the inhibition of ***E. coli*** ATCC, 11775 with zones of inhibition in the range of 8mm – 14.5mm in diameter. ***E. coli*** U16403 and ***E. coli*** U16406 both exhibited predominately intermediate inhibition when tested against all of the five extracts of nutmeg with zones of inhibition in the range of 8.5mm – 15mm in diameter. ***E. coli*** U15055 was resistant to the aqueous extracts and demonstrated intermediate inhibition when tested against all of the three organic extracts of nutmeg.

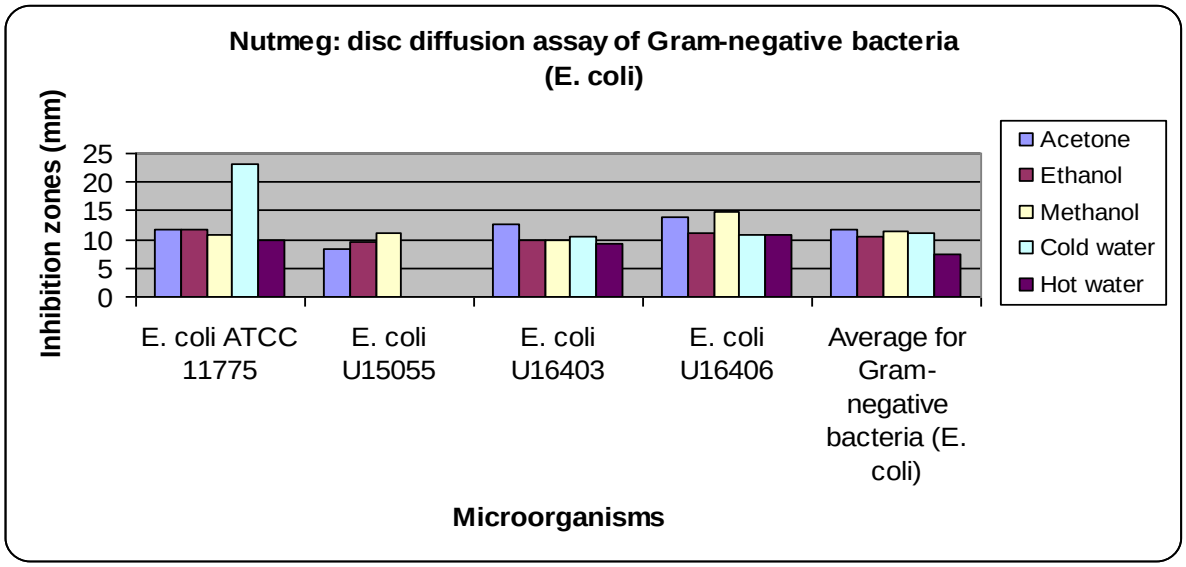

Figure 4.10 Average inhibition zone data of Gram-negative bacteria based on disc diffusion assay for the various extracts of nutmeg.

With the exception to ***E. coli*** U15055 (susceptibility exhibited only when tested against the organic extracts of nutmeg) all of the Gram-negative microorganisms exhibited inhibition when tested against all of the extracts of nutmeg. ***E. coli*** ATCC 11775 was found to be highly sensitive when testes against the cold water extract with the average inhibition size of approximately 23 mm. Figure 4.10 indicated that with the exception to hot water, each extract was capable of an average inhibition of 10 mm.

The comparison of the average inhibition zone sizes showed that acetone (13 mm) was the most effective as a microbial agent followed by the effectiveness of methanol (12 mm), cold water (11 mm), ethanol (10 mm), and hot water (less than 10mm). From Figure 4.10 it was clear that the spice nutmeg did possess significant antimicrobial properties to inhibit the growth of the all the Gram-positive microbes tested.

Table 4.11 Results of agar well diffusion assay of Gram-negative microorganisms against extracts of the spice nutmeg (mm).

Bacteria→ Extracts ↓	***E. coli*** ATCC 11775				***E. coli*** U15055				***E. coli*** U16403				***E. coli*** U16406			
Acetone	15	11	13	11	0	0	0	0	10.5	11	10	10	14	11	10	10
Ethanol	14	9	9	8.5	10	0	11	12	10	11	10.5	10.5	9	11	10.5	10
Methanol	9.5	0	7	7	14	12	11	9	12	13	10	9	12	10	10	10
Cold water	8	11	11	9	0	0	0	0	9	11	10	9	12	10	13	11
Hot water	10	14	13	15.5	0	0	0	0	11	11	10	13	10	10	9	11

E.coli ATCC 11775 predominately exhibited zones of intermediate inhibition in the range of 8mm – 15mm in diameter. ***E. coli*** U16403 and ***E. coli*** U16406 were also noted to exhibit intermediate inhibition in the range of 9mm – 14mm against all extracts of nutmeg. However, ***E. coli*** U15055 strain demonstrated resistance against both aqueous extracts of nutmeg as well as the organic extract of acetone and was intermediately inhibited by extracts of ethanol and methanol with inhibition zones in the range of 9mm – 14mm in diameter.

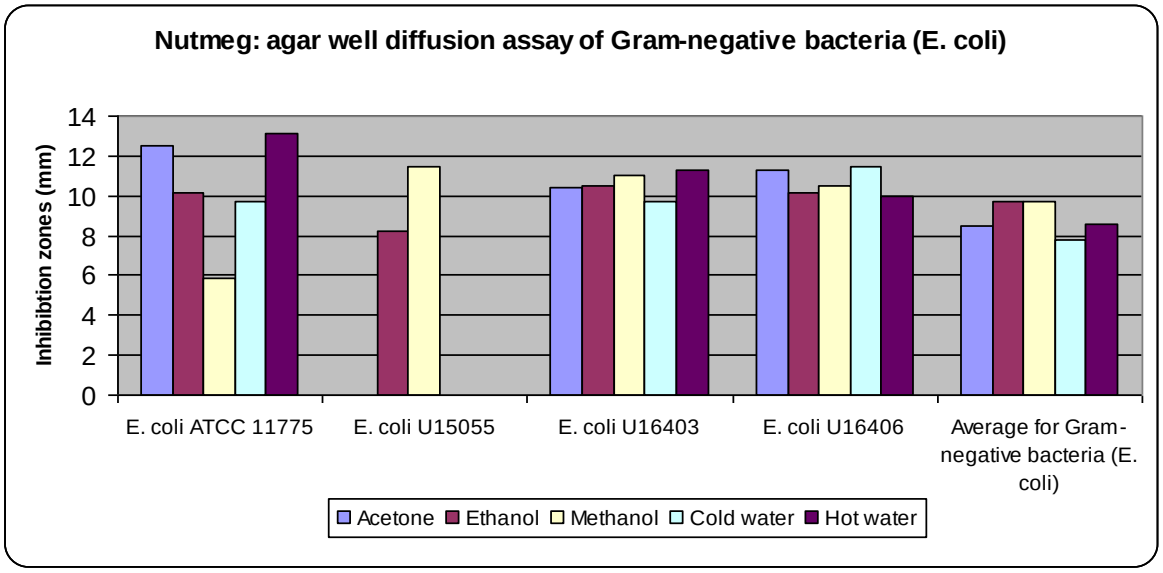

Figure 4.11 Average inhibition zone data of Gram-negative bacteria based on agar well diffusion assay for the various extracts of nutmeg.

Agar well screening were conducted and Figure 4.11 confirmed that all the extracts had inhibitory effect on all of the Gram-negative microbes with varying extents when tested. ***E. coli*** U15055 was susceptible only when tested against methanol and ethanol. ***E. coli*** ATCC 11775 was the most effected with hot water extract being the most effective of the five extracts. ***E. coli*** U16403 and ***E. coli*** U16406 were both significantly inhibited by all five extracts with inhibition zone sizes of 10mm and greater in all but one extract (***E. coli*** U16403, cold water). The average inhibition zone sizes indicated that of the five extracts, ethanol and methanol had the most inhibitory effect, followed by acetone and hot water, then cold water.

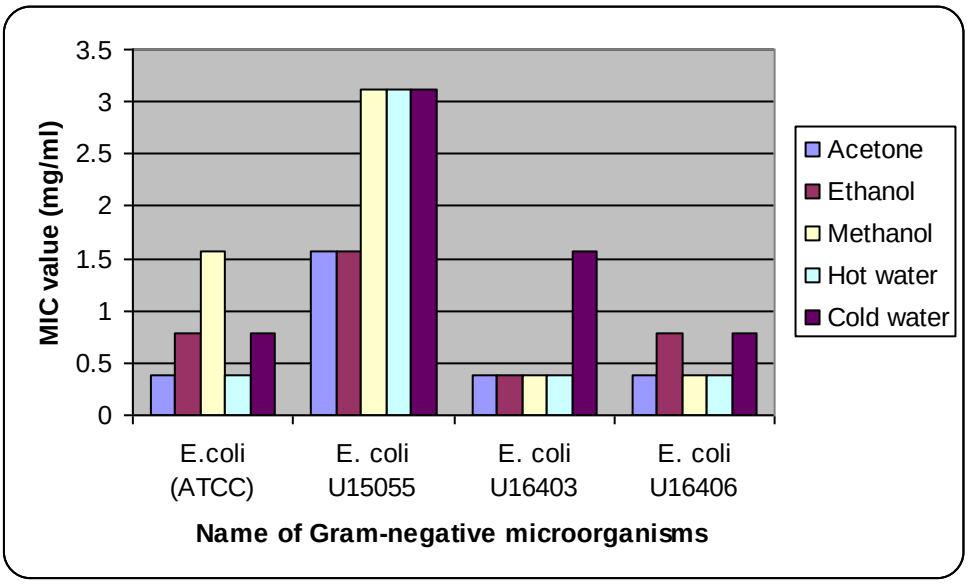

Figure 4.12 The MIC results of four Gram-negative strains of microorganisms namely, ***E.coli,*** ATCC 11775, ***E. coli*** U15055, ***E. coli*** U16403 and ***E. coli*** U16406 when tested against five extracts of the spice nutmeg (continued **Fig 4.5**).

Gram-negative E. coli U15055 exhibited an MIC value in the range of 1.56 mg/ml – 3.12 mg/ml, whilst the remaining Gram-negative microorganisms were observed to have MIC values in the range of 0.39 g/ml – 0.78 mg/ml. The low concentrations observed for the Gram-negative microbes demonstrate the effectiveness of the extracts of nutmeg as an antimicrobial agent against the above Gram-negative microorganisms.

Table 4.12 Inhibition zones resulting from the disc diffusion assay of Gram-negative microorganisms against extracts of the spice nutmeg (mm).

Bacteria → Extracts ↓	***K. pneumoniae*** ATCC 13883				***Salmonella spp.***				***Shigella flexneri***				***Shigella sonnei***			
Acetone	12	16	17.5	16	13	12	12	13	9	9.5	10	10	0	9	10	10
Ethanol	11	13.5	15	11	16	13	15	12	10	10	8.5	9	0	9	9.5	10
Methanol	9	13	15	13.5	13	11	11	12	11	11	0	9	0	0	9	9
Cold water	10	12	10.5	9	9	9	13	10.5	10	10	11.5	9	11	11.5	9.5	11
Hot water	7	11	8	9	11	16	12	15	9	9	10	10	0	8.5	0	0

K. pneumoniae 13883 exhibited zones if inhibition against all extracts of nutmeg in the range of 7mm – 17mm in diameter. ***Salmonella spp.*** was also intermediately susceptible to extracts of nutmeg with zones in the range of 9mm – 16mm. ***Shigella flexneri*** exhibited mostly intermediate inhibition in the range of 8.5mm – 11.5mm for all the extracts, however one test against the organic methanol did exhibit resistance.

Shigella sonnei was observed to be initially resistant against all except the cold water (9.5mm – 11.5mm) extract of nutmeg. Upon repetitive testing it was then observed that the microorganism exhibited intermediate inhibition in the range of 8.5mm – 10mm in diameter against the extracts that initially did not produce any inhibition (i.e. acetone, ethanol, methanol and hot water).

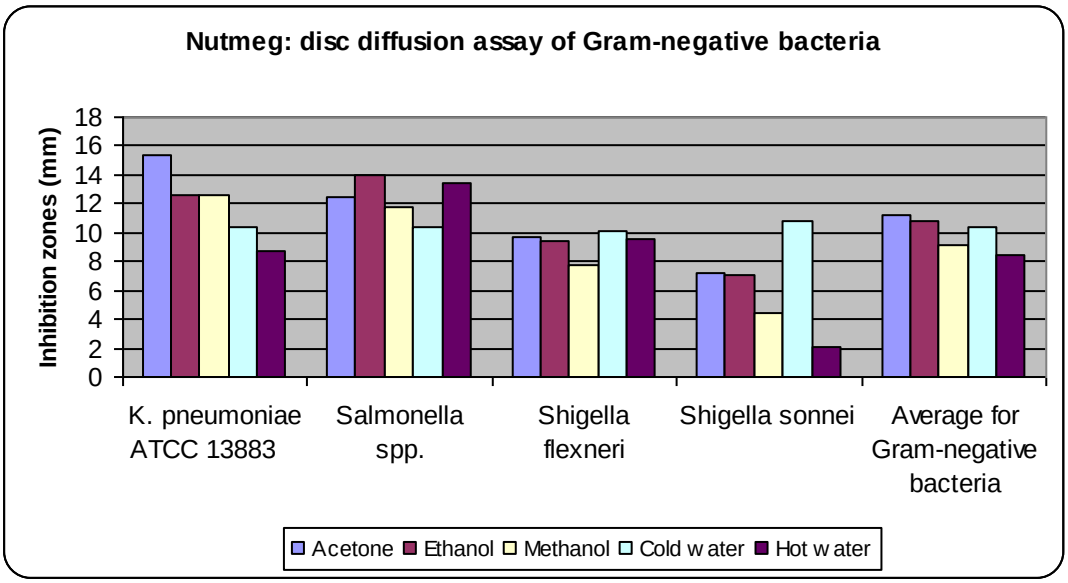

Figure 4.13 Average inhibition zone data of Gram-negative bacteria based on disc diffusion assay for the various extracts of nutmeg.

Results in Figure 4.13 indicated that all of the five extracts tested had inhibitory effects on all four of the Gram-negative microbe tested. ***Salmonella spp*** was found to be the most susceptible with acetone having had the greatest antimicrobial effect (15 mm) and hot water extracts exhibiting the least. ***K. pneumoniae*** ATCC 13883 followed as the second most affected, in that instance, ethanol was the most effective whilst the cold water extract was the least effective. Both ***Shigella*** species were also inhibited by all extracts of nutmeg with the cold water extracts having had the greatest effectiveness.

A comparison of the average inhibition zone sizes indicated that nutmeg has had significant effectiveness as antimicrobial agent against all of the Gram-negative microbes with the greatest effectivity having been exhibited by the organic extract, acetone, followed by ethanol, cold water (aqueous extract), methanol and then hot water.

Table 4.13 Inhibition zones resulting from the agar well diffusion assay of Gram-negative microorganisms against extracts of the spice nutmeg (mm).

Bacteria → Extracts ↓	***K. pneumoniae*** ATCC 13883				***Salmonella spp.***				***Shigella flexneri***				***Shigella sonnei***			
Acetone	10	11.5	10	7	10	11	9.5	10	0	0	9	11	11	12	12	10
Ethanol	9	9	8.5	9	11	10	11.5	9	0	0	0	13	14	12	12	9
Methanol	10.5	10.5	9	8	11	9	9	13	11	10	13	11	12	12	13	11.5
Cold water	10	11.5	9	10	15	13	13	11	16	14	14	0	15	13	11	13
Hot water	8	0	9.5	11	13	11	11	10	12	9	11	14	11	13	10.5	10

K. pneumoniae 13883 exhibited a single test result of resistance when tested against the hot water extract but upon further testing was observed to be predominately intermediately inhibited by all extracts of nutmeg in the range of 7mm -11.5mm in diameter. ***Salmonella spp.*** demonstrated susceptibility to each of the five extracts of nutmeg exhibiting inhibition zones in the range of 9.5mm – 13mm. ***Shigella sonnei*** was intermediately susceptible to all five extracts of nutmeg exhibiting zones of inhibition between 9mm – 15mm in diameter. ***Shigella flexneri*** on the other hand demonstrated some resistance when tested against the organic extracts of acetone and ethanol but upon further testing was observed to be intermediately inhibited in inhibition zones between 9mm – 13mm. Organic methanol as well as both aqueous extracts (hot and cold water) was effective as intermediate inhibitors of ***Shigella flexneri***.

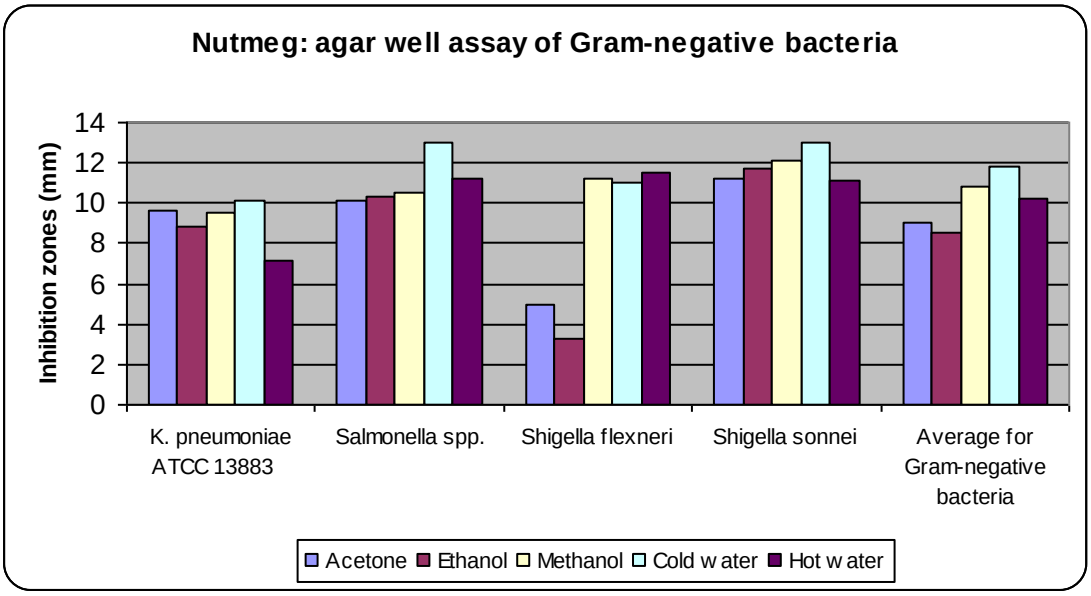

Figure 4.14 Average inhibition zone data of Gram-positive bacteria based on agar well assay for the various extracts of nutmeg.

Results of agar well (Figure 4.14) demonstrated that all of the Gram-negative microorganisms tested were profoundly inhibited by all of the extracts of nutmeg. In all instances, the aqueous extract, cold water was found to have been the most effective antimicrobial agent with zones of inhibition sizes of 10 mm and above. From the average inhibition zone sizes indicated, Figure 4.14 concluded that aqueous extracts of nutmeg (cold then hot water) was more effective than the organic extracts in which case acetone was found to be the most effective followed by ethanol and methanol.

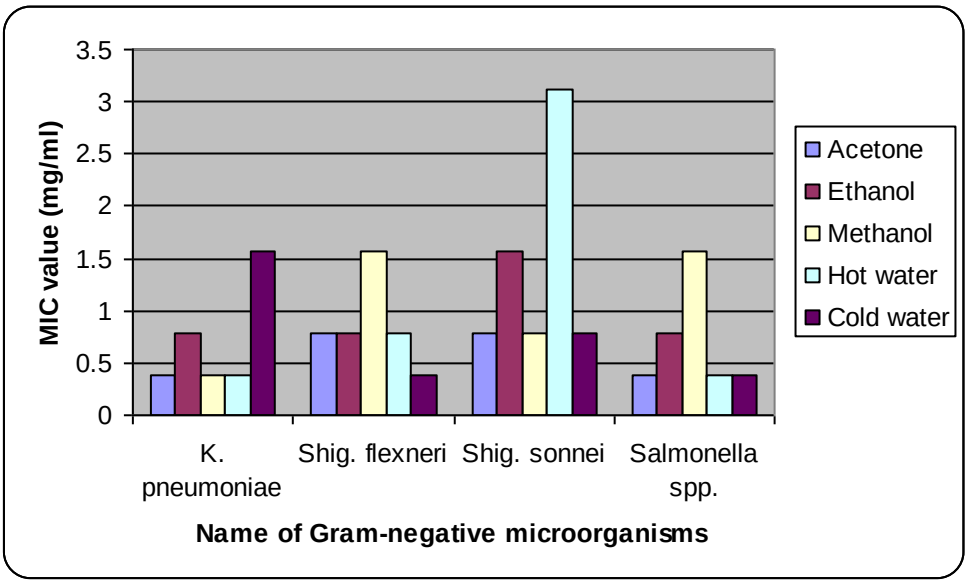

Figure 4.15 The MIC results of four Gram-negative microorganisms when tested against five extracts of the spice nutmeg.

MIC values for the above Gram-negative microorganisms were observed to be in the range of 0.78 mg/ml – 1.56 mg/ml with ***Shigella sonnei*** demonstrating a higher value of 3.12 mg/ml. From the MIC testing it was also observed that the extracts of nutmeg possess sufficient antimicrobial activity to inhibit the growth of the Gram-negative microorganisms.

Table 4.14 Inhibition zones resulting from the disc diffusion assay of Gram-positive microorganisms against extracts of the spice nutmeg (mm).

Bacteria → Extracts ↓	***B. subtilis*** ATCC 6051				***S. aureus*** ATCC 12600				***S. aureus*** T1266				***S. aureus*** P4790			
Acetone	8	6	9.5	9	8	12	14	9.5	11	9	12	11	8.5	10	10	11
Ethanol	7	12	14	0	15	13.5	17	12.5	10	10	11	10	11	13	11	11
Methanol	0	0	11.5	9	11	11.5	10.5	9	15	12	13	13.5	10	10	13	10
Cold water	6.5	8	7	9	10.5	8	8	8.5	12	15	14	13	11	10	9	9
Hot water	0	6.5	0	0	11	12.5	15.5	10.5	9	13	10	11	11	10	11	12

B. subtilis ATCC, 6051 demonstrated resistance when tested against organic ethanol and extract of hot water. Overall intermediate inhibition was apparent for testing conducted using all five extracts of nutmeg with inhibition zones in the range of 6mm – 14mm in diameter. It was observed that the organic and aqueous extracts of nutmeg were effective as antimicrobial agents when tested against each of the ***S. aureus*** strain of microorganism, exhibiting zones of inhibition in the range of 8mm -17mm in diameter.

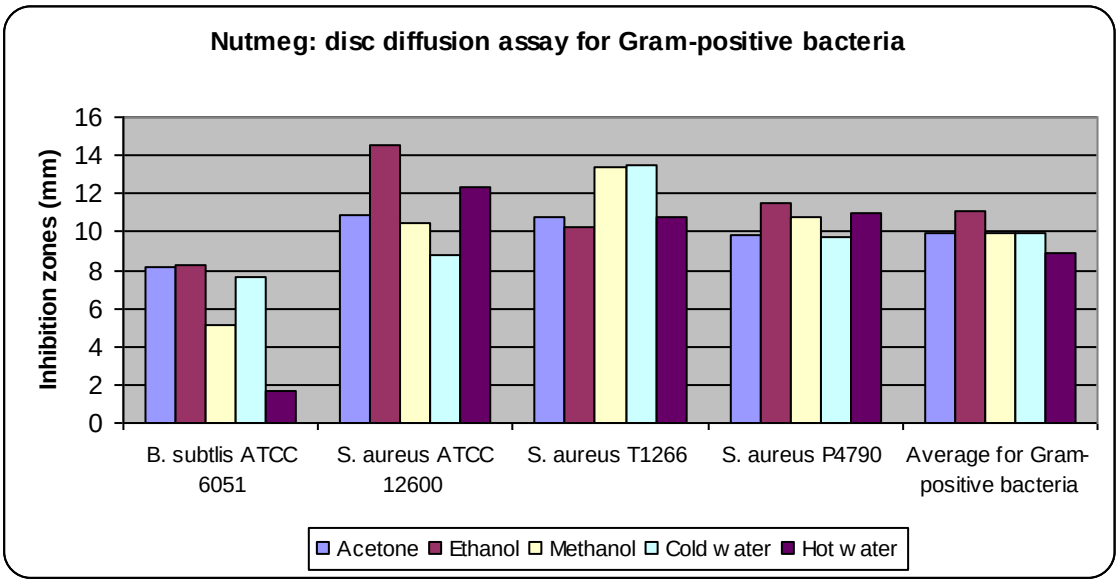

Figure 4.16 Average inhibition zone data of Gram-positive bacteria based on disc diffusion assay for the various extracts of nutmeg.

S. aureus strains were the more affected of the Gram-positive microorganisms tested. The general trend indicated that the ethanol extract had the greatest inhibitory effect amongst the ***S. aureus*** strains (Figure 4.16; inhibition zone sizes equal to or greater than 10 mm), followed by hot water extract (greater than 10 mm), methanol, acetone and then cold water. The ***B. subtilis*** ATCC 6051 stain was also found to be inhibited by extracts of nutmeg, with the organic extracts having had a greater inhibitory effect than the aqueous extracts.

The average inhibition zone sizes for all of the Gram-positive microorganisms indicated that ethanol was the most effective of the five extracts tested followed by acetone, methanol and cold water all having exhibited an average inhibition of 10 mm, and then the least effective of the five extracts, hot water.

Table 4.15 Inhibition zones resulting from the agar well diffusion assay of Gram-positive microorganisms against extracts of the spice nutmeg (mm).

Bacteria → Extracts ↓	***B. subtilis*** ATCC 6051				***S. aureus*** ATCC 12600				***S. aureus*** T1266				***S. aureus*** P4790			
Acetone	11	9	11.5	11	11	11	11	13	11	11.5	13	12	11	9	9	11
Ethanol	13	9	0	0	14	12.5	10	13	13	12	11	11	15	12	11.5	12
Methanol	16	10	12	12	10	11.5	9	9	14	11	10	14	12	12.5	10	13
Cold water	0	0	0	0	8	11	9	10.5	14	15	12	9	13	9	11	10
Hot water	9	11	7	13	11	13	14	13.5	11	9	9	12	15	12	14	15

B. subtilis ATCC 6051 demonstrated some resistance against ethanol extract and was observed as completely resistant to the cold water extract of nutmeg. However, the organic extracts were observed to be effective intermediate inhibitors of ***B. subtilis*** 6051 ATCC with inhibition zones in the range of 9mm – 16mm. Hot water extract was an effective aqueous inhibitor producing inhibition zones between 7mm – 13mm. As with the disc diffusion assay, all extracts of nutmeg were effective antimicrobial agents when tested against each strain of ***S. aureus***. The range of inhibition zones against the various strains of ***S. aureus*** was observed to be between 8mm – 15mm in diameter.

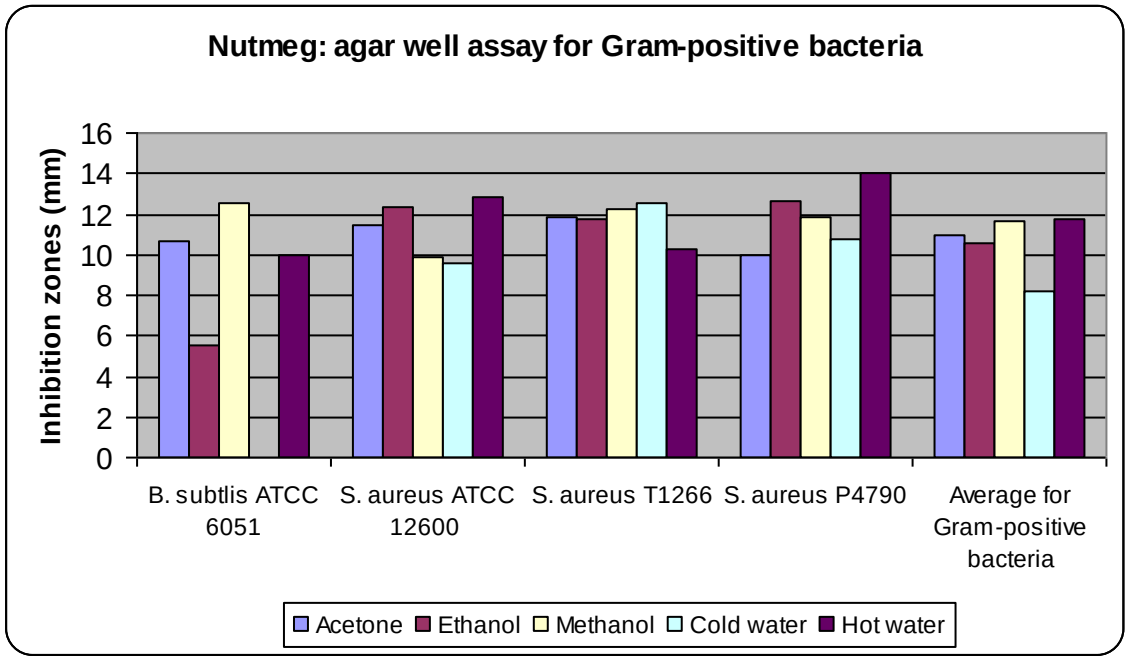

Figure 4.17 Average inhibition zone data of Gram-positive bacteria based on agar well assay for the various extracts of nutmeg.

Figure 4.17 indicated that methanol was most effective of the organic extracts when tested against ***B. subtilis*** ATCC 6051, whilst the cold water extract was found to have no effect on this microorganism at all. The overall effect showed that all strain of ***S. aureus*** were significantly inhibited by each of the five extracts of nutmeg with inhibition zone sizes predominately greater than 10 mm. The general overview indicated that amongst the ***S. aureus*** strains, ethanol was the most effective followed by hot water, acetone, and methanol and then cold water extracts. From the average of the inhibition zone sizes it was concluded that all extracts of were effective as antimicrobial agents against all of the Gram-positive microorganisms with methanol and hot water having had greatest effectiveness, followed by acetone, ethanol and finally, the cold water extract.

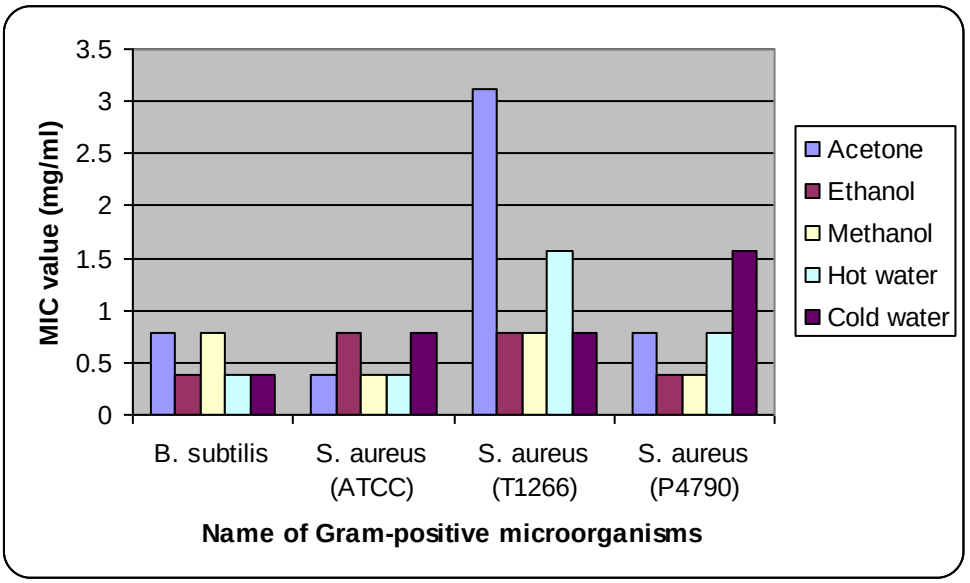

Figure 4.18 The MIC results of four Gram-positive strains of microorganisms namely, ***B. subtilis*** ATCC 6051, ***S. aureus*** ATCC 12600, ***S. aureus*** T1266 and ***S. aureus*** P4790 when tested against five extracts of the spice nutmeg.

The low concentration of extract required to inhibit the microorganism demonstrates once again the effectiveness of nutmeg as an antimicrobial agent. The Gram-positive microbes were inhibited in the range of 0.78 mg/ml – 1.56 mg/ml, a slightly higher concentration with the extracts at this concentration successful in the inhibition of microbial growth.

Table 4.16 Inhibition zones resulting from the disc diffusion assay of Gram-negative microorganisms against extracts of the spice chilli (mm).

Bacteria → Extracts ↓	***E. coli*** ATCC 11775				***E. coli*** U15055				***E. coli*** U16403				***E. coli*** U16406			
Acetone	27	22	19	24	0	0	0	0	20	16	15	11	9	10.5	9	11
Ethanol	16	24	25.5	19	0	0	0	0	11	11.5	13	11	9	10	11	13
Methanol	15	19	19	23	11	15	15	13	9	11	10	10	15	13	14	11
Cold water	20.5	23	19	20	12	9	10	9.5	9.5	9	10	9.5	22	18	20	25
Hot water	28	23	16	19	10	10.5	9	10	9.5	12	10	9	12	11	9.5	13

E. coli ATCC 11775 was observed to be highly susceptible to all extracts of chilli with absolute zones of inhibition in the range of 16mm -28mm in diameter. ***E. coli*** U15055 was resistant to the organic extracts acetone and ethanol but exhibited intermediate inhibition when tested against methanol and both aqueous extracts. ***E. coli*** U16403 and ***E. coli*** U16406 both showed intermediate inhibition when tested against all five extracts of the chilli with inhibition zones in the range of 9mm – 25mm.

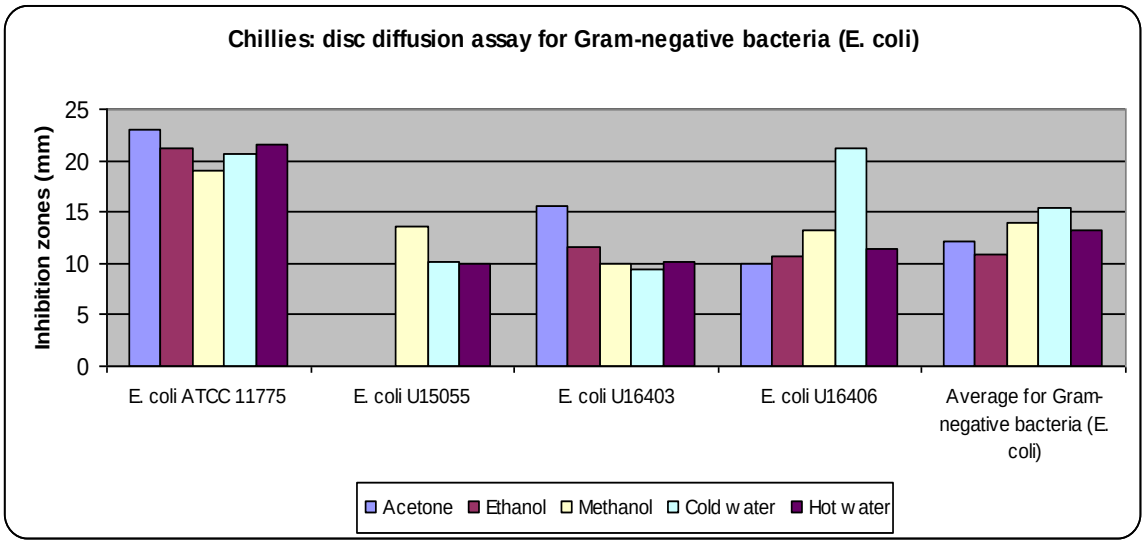

Figure 4.19 Average inhibition zone data of Gram-negative bacteria based on the disc diffusion assays for the various extracts of chilli.

E. coli U15055 was found to be the only microorganism the showed resistance when tested against two of the organic extracts (acetone and ethanol) of chilli but exhibited inhibition zones of greater than 10 mm when tested against the organic extract, methanol (Figure 4.19). In the case of the aqueous extracts, both were found to have inhibitory effects of approximately 10 mm throughout the investigation.

The results of Figure 4.19 clearly indicated that for the remaining three Gram-negative strains (***E. coli*** ATCC 11775, ***E. coli*** U16403 and ***E. coli*** U16406) tested, all of the five extracts of chilli exhibited profound inhibitory effects with inhibition zone sizes generally above 10 mm. Comparison of the average inhibition zone sizes further substantiated that the extracts of chilli did produce inhibition amongst all of the Gram-negative microorganisms (greater than 10 mm) and that these extracts did in fact prove to be highly effective antimicrobial agents for the purpose of the investigation. The order of

effective for the extracts was as follows; cold water, methanol, hot water, acetone and ethanol.

Table 4.17 Results of agar well diffusion assay of Gram-negative microorganisms against extracts of the spice chilli (mm).

Bacteria→ Extracts ↓	***E. coli*** ATCC 11775				***E. coli*** U15055				***E. coli*** U16403				***E. coli*** U16406			
Acetone	16	18	21	19	0	0	0	0	12	13	11.5	12	11	10.5	10	10
Ethanol	23	16	20	16.5	0	0	0	0	19	27	16	18	18	14	16	25
Methanol	18	19.5	19	16	19	21	16	18	20	16	15	14.5	23	26	21	17
Cold water	28	27.5	24	24	0	0	0	0	13	16	12	17	0	0	0	0
Hot water	22	26	19	20	0	0	0	0	12	10	11.5	11	13	12	10	11

As in the disc diffusion assay, ***E. coli*** ATCC 11775 was observed as highly susceptible to all extracts of chilli. The effectiveness of chilli as an antimicrobial agent was observed to be within the range of 16mm – 28mm in diameter. ***E. coli*** U15055 demonstrated resistance when tested against extracts of acetone, ethanol, cold and hot water. ***E. coli*** U15055 exhibited intermediate inhibition when tested against methanol extract of chilli (range of 16mm – 21mm). ***E. coli*** U16406 was resistant to cold water extract but demonstrated susceptibility when tested against hot water and all of the organic extracts. ***E. coli*** U16403 was observed to be greatly inhibited by all extracts of chilli with inhibition zones in the range of 10mm – 27mm in diameter.

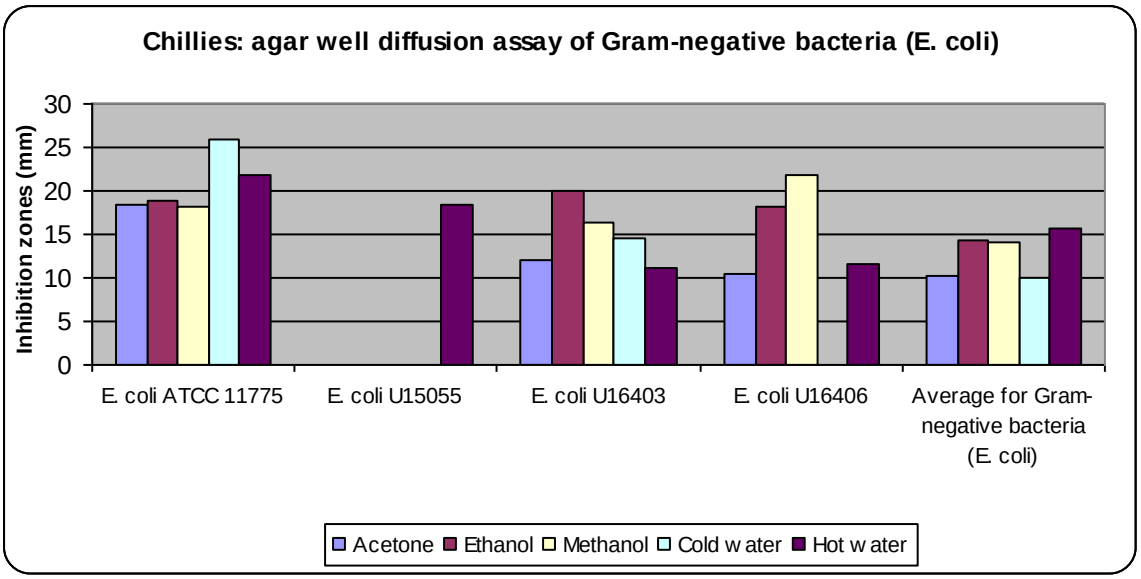

Figure 4.20 Average inhibition zone data of Gram-negative bacteria based on the agar well assays for the various extracts of chilli.

The result of agar well assay was always considered as the confirmatory measure for disc diffusion assaying. Figure 4.20 supports the conclusions of Figure 4.19 indicating that of the four ***E. coli*** strains tested; ***E. coli*** U 15055 was the most resistant showing only selective susceptibility during both screenings. However Figure 4.20 indicated that aside from the ***E. coli*** U15055, all of the Gram-negative were profoundly inhibited by all of the extracts of chilli with inhibition zone sizes greater than 10 mm. The comparison of the average inhibition zone sizes indicated that chilli was a powerful agent in its antimicrobial action against each of the microbe tested with hot water having had the greatest effect, followed by ethanol, methanol, acetone and cold water extracts (inhibition all greater than 10 mm).

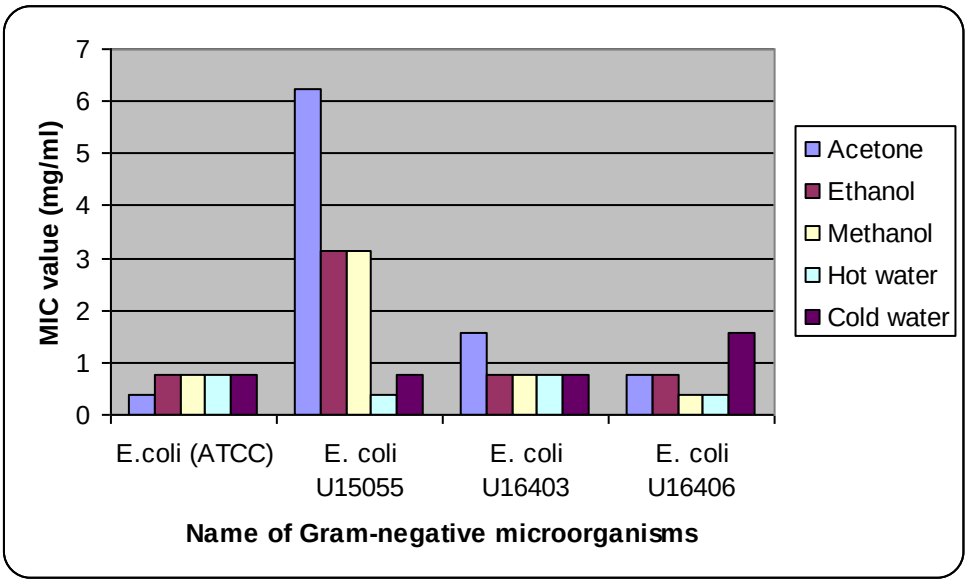

Figure 4.21 The MIC results of four Gram-negative strains of ***E.coli*** tested against five extracts of the spice chilli (continued **Fig 4.8**).

E. coli U15055 was observed to have the highest MIC values predominantly in the range of 3.12 mg/ml – 6.25 mg/ml. High MIC values are indication of possible resistance that may easily develop within this particular strain of microorganism. Gram-negative ***E. coli*** ATCC 11775, ***E. coli*** U16403 and ***E. coli*** U16406 was observed to have low MIC values between 0.39mg/ml – 1.56mg/ml. These low values can be considered indicators of the effectiveness of the active principles in the chilli spice that are capable of causing inhibition of microbial growth which may lead to microbial death.

Table 4.18 Inhibition zones resulting from the disc diffusion assay of Gram-negative microorganisms against extracts of the spice chilli (mm).

Bacteria → Extracts ↓	***K. pneumoniae*** ATCC 13883				***Salmonella spp.***				***Shigella flexneri***				***Shigella sonnei***			
Acetone	22	26.5	24	19	9	10	12	12.5	16	17	17.5	14	13	15	15	13
Ethanol	14	16	19	13	12	10	9.5	11	15	15	11	13	9	11	11	12
Methanol	16	19	17	20	10	13	9	14	19.5	15	16	14	13	14	12	12
Cold water	22	18	17	24	10	11	13	13.5	11	14	9	10.5	10	9	12	10
Hot water	17	14.5	18	21	17	16	12	13	13	10	10	10	9	10	11	13

It was observed from the large sizes of the inhibition zones for ***K. pneumoniae*** ATCC 13883 that the extracts of chilli were effective antimicrobial agents with absolute zones of inhibition in the range of 16mm – 26.5mm for the extracts acetone, methanol, hot and cold water. The organic extract of ethanol was observed to demonstrate an inhibition zone in the range of 13mm – 19mm. ***Salmonella spp***, ***Shigella flexneri*** and ***Shigella sonnei*** were observed to demonstrate inhibition with an overall range of 9mm – 19.5mm in diameter.

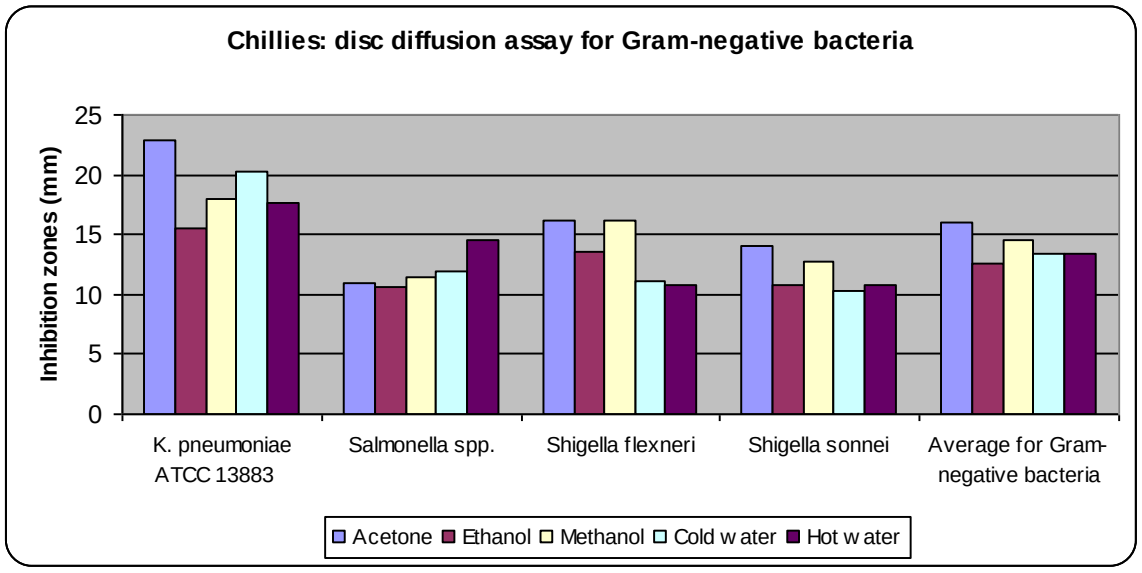

Figure 4.22 Average inhibition zone data of Gram-negative bacteria based on disc diffusion assays for the various extracts of chilli.

Figure 4.22 indicated that all of the Gram-negative microorganisms tested were significantly inhibited by each of the five extracts of chilli. The inhibitory action of the extracts was all found to be greater than 10 mm, this was indicative that the antimicrobial activity of the chilli extracts was clearly uniform and profound. Form the general analysis of effectiveness for each extract, it was clear that acetone had the greatest effect whilst the ethanol was found to be the extract with least effectiveness (with inhibition zone sizes still above 10 mm).

The overall comparison of the average inhibition zone sizes once again confirmed that the extracts of chilli are all capable of causing inhibition amongst the Gram-negative microorganisms with the organic extracts having had the more effective than the aqueous extracts. However in both cases the effective of the extracts were still noted to be greater than 10 mm, an indication of the effective of chilli as an antimicrobial agent.

Table 4.19 Inhibition zones resulting from the agar well diffusion assay of Gram-negative microorganisms against extracts of the spice chilli (mm).

Bacteria → Extracts ↓	***K. pneumoniae*** ATCC 13883				***Salmonella spp.***				***Shigella flexneri***				***Shigella sonnei***			
Acetone	22	20	21	19	15	11	12	11	10	9	11	10	10	15	13	13
Ethanol	21	18	17	19	0	0	0	0	9	9	9	11	12	15	13	12
Methanol	20	21	17	18	0	0	0	0	10	11	11	13	11	13	12	11
Cold water	20.5	21	20	19	15	11	0	12	10	11	10	12	11	10	10	12
Hot water	20	20.5	24	25	0	0	0	0	11	14	10	10.5	15	13	11	13

As in disc diffusion ***K. pnuemoniae*** ATCC 13883 exhibited the highest susceptibility to all of the extracts of chilli. Zones of inhibition were noted in the range of 17mm – 25mm. ***Shigella flexneri*** and ***Shigella sonnei*** both demonstrated intermediate inhibition zones within the range 9mm – 15mm. ***Salmonella spp.*** was observed to exhibit intermediate inhibition against organic acetone and inorganic cold water extracts (11mm – 15mm). Ethanol, methanol and hot water extracts were however ineffective as antimicrobial agents against ***Salmonella spp.***

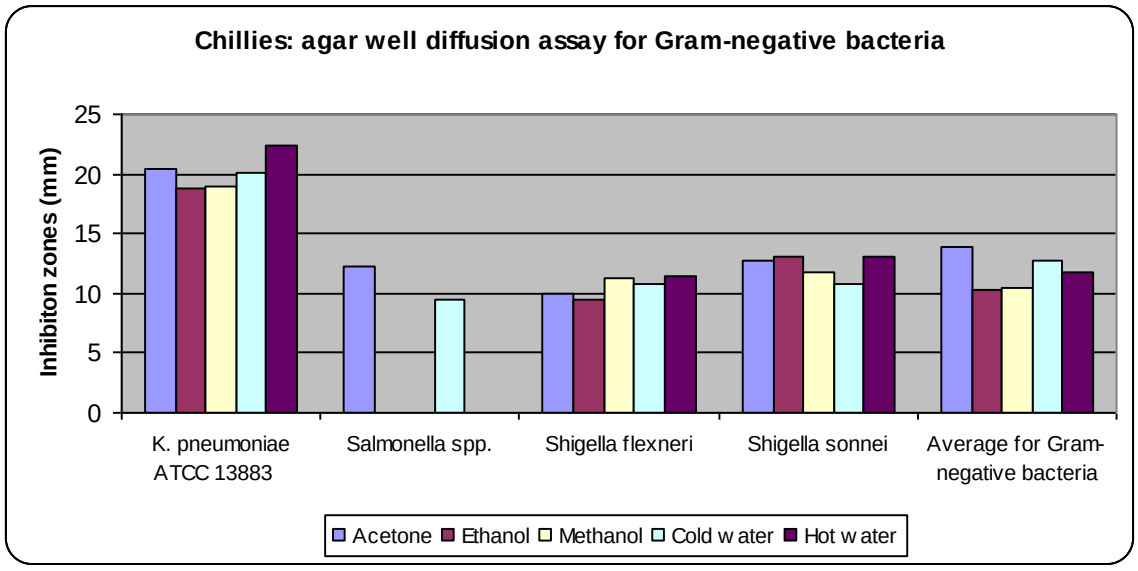

Figure 4.23 Average inhibition zone data of Gram-negative bacteria based on agar well assays for the various extracts of chilli.

Salmonella spp was found to be susceptible only when tested against the acetone and cold water extracts of chilli with acetone showing an inhibition greater than 10 mm. ***Shigella spp*** were susceptible to all extracts of chilli with ***Shigella sonnei*** being more susceptible than the ***Shigella flexneri***. In both cases the hot water extract was found to be highly effective in its antimicrobial activity producing inhibition zones greater than 10mm.

The result of the average analysis for all on the Gram-negative microorganisms indicated that each extract was capable of inhibitory effect amongst the different microbes producing significant inhibition zone sizes of greater than 10 mm in all instances. The average inhibition zone sizes further indicated that the orders of effectiveness (most effective to least effective) for the extracts are as follows; acetone, hot water, cold water, followed by methanol and ethanol. The overall result also indicated that the aqueous extracts were more effective than the organic extracts.

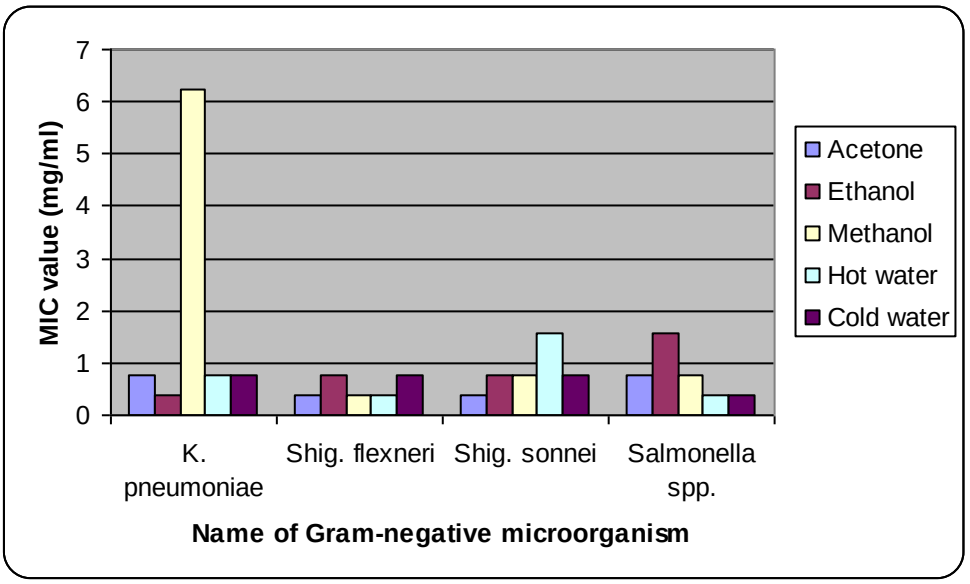

Figure 4.24 The MIC results of four Gram-negative microorganisms namely, ***K. pneumoniae*** ATCC 13883, ***Shigella flexneri, Shigella sonnei*** and ***Salmonella sp***. when tested against five extracts of the spice chilli.

Observation from the above graph indicates the high efficiency of chilli as an antimicrobial agent when tested against Gram-negative microorganisms. From the low MIC values (0.78 mg/ml – 1.56 mg/ml) obtained the potential use of chilli for antimicrobial therapy may be possible due to low but effective dose concentrations required for inhibition.

Table 4.20 Inhibition zones resulting from the disc diffusion assay of Gram-positive microorganisms against extracts of the spice chilli (mm).

Bacteria → Extracts ↓	***B. subtilis*** ATCC 6051				***S. aureus*** ATCC 12600				***S. aureus*** T1266				***S. aureus*** P4790			
Acetone	23	29	17	20	19	23	27	18	11	9	14	13	14	13	14	12
Ethanol	11	21	19	26	15	15	20	17	15	17	15	12	9	11	14	15
Methanol	27	16	18	16.5	15	17	13	17	12	11	14	12	11	11	9	10
Cold water	19	15.5	19	19	21	23	23	22.5	16	13	13	14	9	11	10	11
Hot water	19	20	13	16	20	19	19.5	19	9	9.5	12	10	9	12	11	12

B. subtilis ATCC 6051 and ***S. aureus*** ATCC 12600 were both highly susceptible to all extracts of chilli, the large inhibition zones noted indicated the effectiveness of the chilli extracts as antimicrobial agents with inhibition zones in the range of 11mm -29mm. ***S. aureus*** T1266 and ***S. aureus*** P4790 were also observed to be inhibited by all extracts of chilli with inhibition zones in the range of 9mm – 17mm. It was observed however that the antimicrobial activity of the chilli extracts was reduced in effectiveness when compared to the inhibition zones of ***B. subtilis*** ATCC 6051 and ***S. aureus*** ATCC 12600.

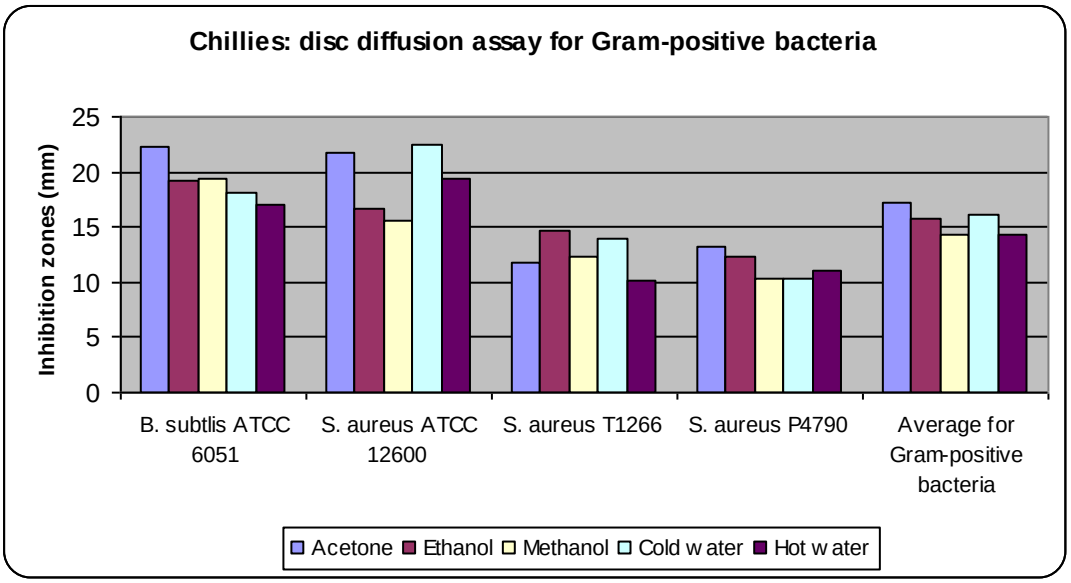

Figure 4.25 Average inhibition zone data of Gram-positive bacteria based on the disc diffusion assays for the various extracts of chilli.

Figure 4.25 clear indicated the profound effects all of extracts as antimicrobial agents when tested against all of the Gram-positive microorganisms. ***B. subtilis*** ATCC 6051 was noted to have been the most affected of the Gram-positive microbe, being more sensitive to the organic extracts than the aqueous. ***B. subtilis*** was inhibited by all extracts with zone sizes greater than 15 mm (acetone; greater than 20 mm). ***S. aureus*** ATCC 12600 was found to be the most sensitive of the three strains tested, with cold water producing the greatest inhibition. Amongst the ***S. aureus*** strains, it was clear that all extracts were capable of substantial inhibition with inhibition zone sizes all greater than 10 mm.

The average sizes of inhibition zones (all approximately 15mm or greater) concluded that extracts of chilli were all capable of distinct inhibition as an antimicrobial agent.

Table 4.21 Inhibition zones resulting from the agar well diffusion assay of Gram-positive microorganisms against extracts of the spice chilli (mm).

Bacteria → Extracts ↓	***B. subtilis*** ATCC 6051				***S. aureus*** ATCC 12600				***S. aureus*** T1266				***S. aureus*** P4790			
Acetone	23	21	27	27	19	23	25	21	9	9	11	11.5	11	11	12.5	12
Ethanol	19	16	21	21.5	22	21	20	19	12	9	10	10	12	13	12	12
Methanol	25	21	26	24	19	19	18	20.5	0	0	12	10	9	9	10	9
Cold water	20	21	18	15	19	19	22	18	9	10	0	9	9	11	9	12
Hot water	25	23	21	19	23.5	20.5	20	21	13	11	11	13	12	12	11	12

As with disc diffusion assaying the ATCC strains, ***B. subtilis*** ATCC 6051 and ***S.aureus*** ATCC 12600 were once again observed to be most susceptible when tested against all five extracts of chilli exhibiting inhibition zone sin the range of 16mm – 27mm in diameter. ***S. aureus*** T1266 and ***S. aureus*** P4790 also similar to the results of disc diffusion assaying, were observed to be intermediately inhibited by the extracts of chilli (9mm – 13mm). The strains were observed to show some resistance to the organic methanol and the extract of cold water.

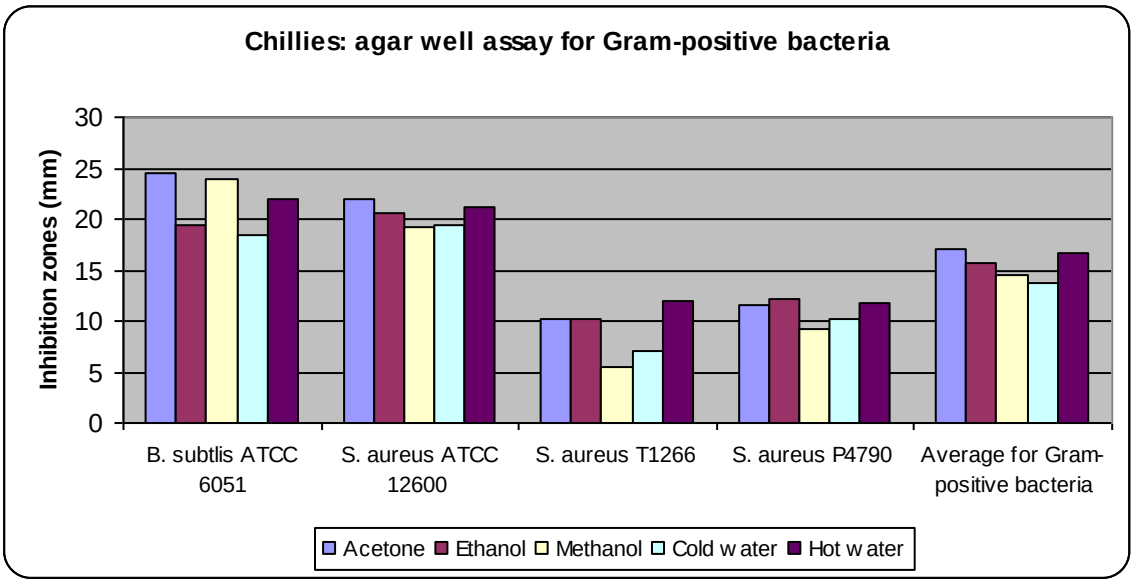

Figure 4.26 Average inhibition zone data of Gram-positive bacteria based on the agar well assays for the various extracts of chilli.

The results of Figure 4.26 indicated that ***B. subtilis*** was again the most sensitive of the Gram-positive microbes with inhibition zones greater than 15 mm for all extracts. Acetone was the most effect of the organic extracts with hot water extract being more effect of the two aqueous extracts. ***S. aureus*** ATCC 12600 was again the found to be the most inhibited of the ***S. aureus*** strains, producing inhibition zone well above 15 mm for all extracts of chilli.

When the average inhibition zone sizes for all extracts were compared, it was deduced that the organic extracts were generally more effective than the aqueous extracts. Acetone was the most effective organic extract with hot water being the more effective of the two aqueous extracts.

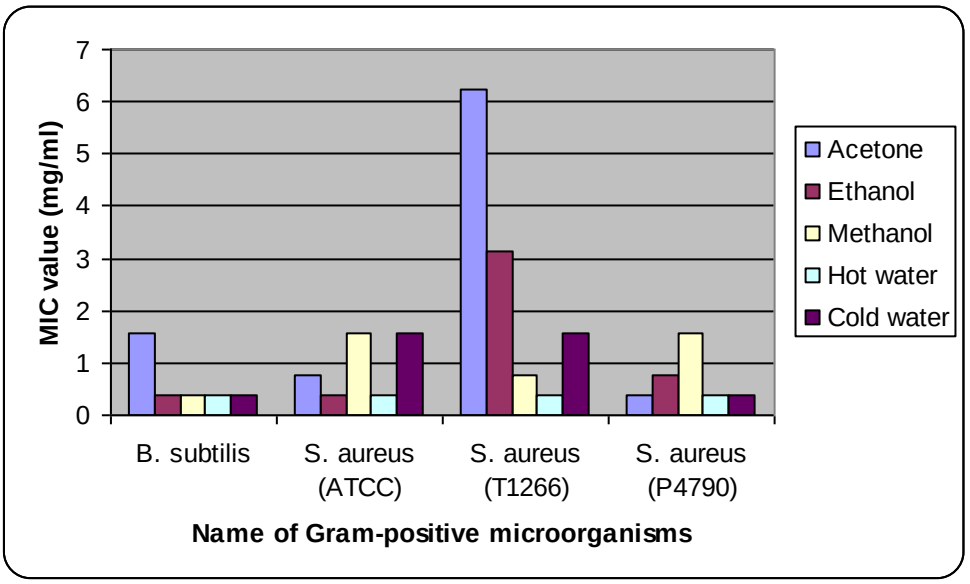

Figure 4.27 The MIC results of four Gram-positive microorganisms namely, ***B. subtilis*** ATCC 6051, ***S. aureus*** ATCC 12600, ***S. aureus*** T1266 and ***S. aureus*** T4790 and Gram-negative ***E. coli*** U16406 when tested against five extracts of the spice chilli.

All of the Gram-positive microorganisms tested were observed to have MIC values predominately in the range of 0.78 mg/ml – 1.56 mg/ml with Gram-positive ***S. aureus*** strains possessing significantly higher MIC values of up to 6.26 mg/ml. However the low concentrations of extracts capable of having antimicrobial effects on the various microorganisms are indicative of the effectiveness of the chilli spice as antimicrobial agent for the above mentioned microorganisms.

Table 4.22 Inhibition zones resulting from the disc diffusion assay of Gram-negative microorganisms against extracts of the spice ginger (mm).

Bacteria → Extracts ↓	***E. coli*** ATCC 11775				***E. coli*** U15055				***E. coli*** U16403				***E. coli*** U16406			
Acetone	10	0	0	9.5	0	0	0	0	10	9	8.5	9	10	11	10	9
Ethanol	13	15	15	12	0	0	0	0	10	9	11	9.5	9	12	15	13
Methanol	11	13	11	9	0	0	0	0	10	9	11	9.5	9	12	9	8.5
Cold water	8	11	9	7	0	0	0	0	9	10.5	10	10	16	11	13	11
Hot water	13	13.5	9	11	0	0	0	0	12	9	10	10.5	9	9	11	10

E. coli U15055 was observed to be completely resistant to all test extracts of ginger. This demonstrated the inefficacy of the ginger extracts as an antimicrobial agent. The remaining three strains of ***E. coli*** were each observed to be predominately intermediately inhibited (6mm – 16mm) by all of the five extracts of ginger, exhibiting the efficacy of the ginger extracts as potential antimicrobial agents against these strains of Gram-negative microorganisms.

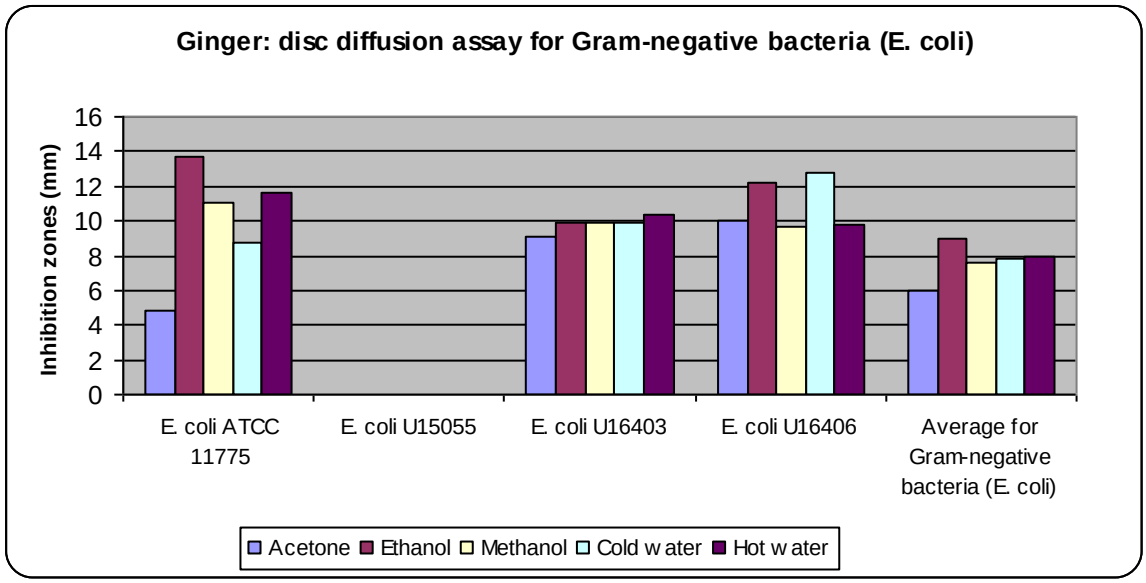

Figure 4.28 Average inhibition zone data of Gram-negative bacteria based on the disc diffusion assays for the various extracts of ginger.

E. coli U15055 was found to be completely resistant when tested against all the extracts of ginger. ***E. coli*** ATCC 11775, ***E. coli*** U16403 and ***E. coli*** U16406 were all inhibited by all of the extracts of ginger. Ethanol was the most effective extract with inhibition zones of 10 mm and greater.

The average inhibition zone sizes (Figure 4.28) for all Gram-negative extracts indicated that the aside form ***E. coli*** U15055, all extracts of ginger were capable of inducing inhibition. Overall, ethanol was the most effective, followed by the aqueous extracts, and the methanol and hot water.

Table 4.23 Results of agar well diffusion assay of Gram-negative microorganisms against extracts of the spice ginger (mm).

Bacteria→ Extracts ↓	***E. coli*** ATCC 11775				***E. coli*** U15055				***E. coli*** U16403				***E. coli*** U16406			
Acetone	9	8.5	13	10	0	0	0	0	10	10	11	12.5	12	10	10.5	11
Ethanol	11	9	11.5	10	0	0	0	0	11	12	9	10.5	11	13	11.5	12
Methanol	12	11.5	10	8	0	0	0	0	11	12	11.5	10	10	10	11	14
Cold water	11	11	0	9	0	0	0	0	11	14	14	12	18	21	17	14
Hot water	0	11	12	9	0	0	0	0	0	0	0	0	0	0	0	0

E. coli U15055 showed the same results as observed in disc diffusion assaying. The ATCC 11775 strain of ***E. coli*** was observed to be intermediately inhibited by the various extracts of ginger with inhibition zones in the range of 8.5mm – 12mm. Both ***E. coli*** U16403 and ***E. coli*** U16404 demonstrated predominately intermediate inhibition in the range of 9mm – 21mm for all but one of the ginger extracts namely, hot water to which these microorganisms were observed to be resistant.

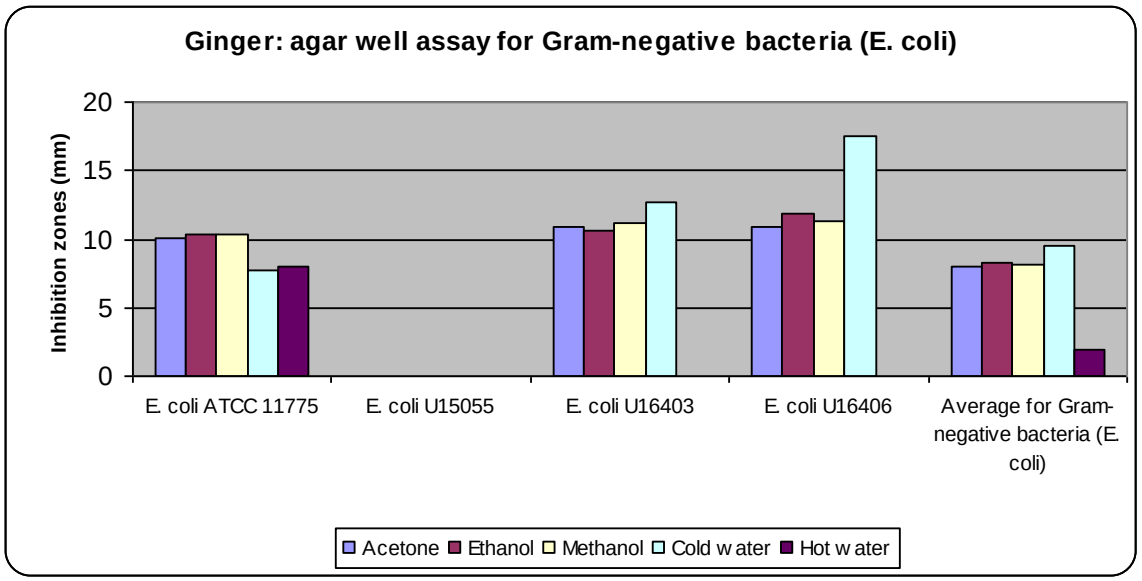

Figure 4.29 Average inhibition zone data of Gram-negative bacteria based on the agar well assays for the various extracts of ginger.

The ginger extracts were once again found to be ineffective against ***E. coli*** U15055. However ***E. coli*** ATCC 11775, ***E. coli*** U16403 and ***E. coli*** U16406 was found to be inhibited by the extracts of ginger. The organic extracts for ***E. coli*** ATCC 11775 were found to have inhibition zone sizes greater than 10 mm whilst the aqueous extracts had a general effect less than 10 mm. ***E. coli*** U16403 and ***E. coli*** U16406 were both resistant when tested against the hot water extract of ginger.

Comparison of the average inhibition zones indicate that the extracts of ginger were capable of inhibition of the Gram-negative microbes with hot water having had the least inhibitory effect of the microorganisms, whilst cold water was found to be the most effective.

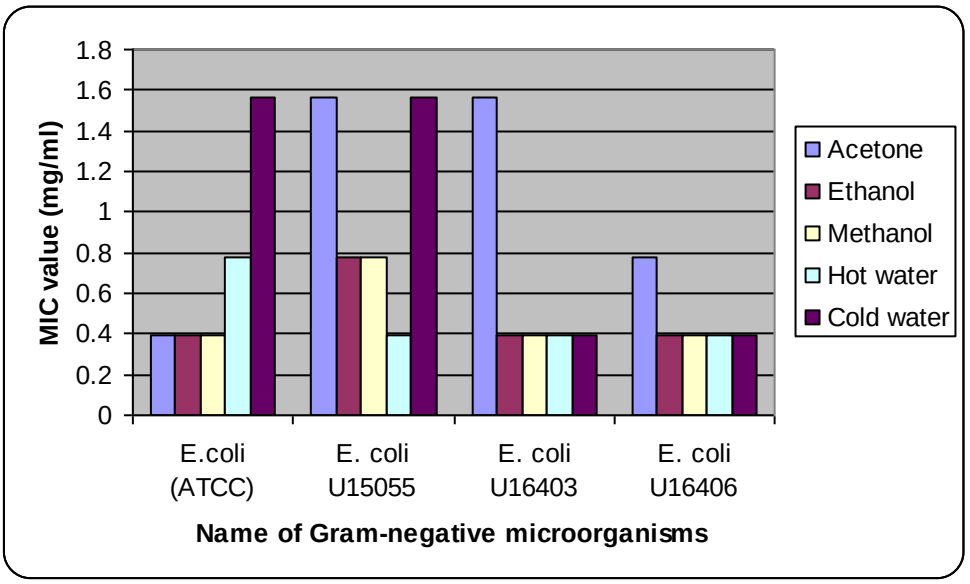

Figure 4.30 The MIC results of four Gram-negative microorganisms namely ***E. coli*** ATCC 11775, ***E. coli*** U15055, ***E. coli*** U16403 and ***E. coli*** U16406 when tested against five extracts of the spice ginger (continued **Fig 4.11**).

The low MIC values (all predominately within the range of 0.78 mg/ml – 1.56 mg/ml) for the various Gram-negative strains of ***E. coli*** microorganisms are indicative of the high efficiency of ginger as an antimicrobial agent. However it must be noted that although ***E. coli*** U15055 did not demonstrate considerable inhibition during either of the assaying techniques, the relatively low MIC values obtained contradict the ineffectiveness of the ginger against ***E. coli*** U15055 implying that the extracts may posses antimicrobial activity capable of inhibiting these microorganisms.

Table 4.24 Inhibition zones resulting from the disc diffusion assay of Gram-negative microorganisms for extracts of the spice ginger (mm).

Bacteria → Extracts ↓	***K. pneumoniae*** ATCC 13883				***Salmonella spp.***				***Shigella flexneri***				***Shigella sonnei***			
Acetone	8	8.5	8	8	9	10.5	9.5	11	0	9	9	11	0	9.5	11	10
Ethanol	0	0	0	0	16	12	14	12	9	9	10	11	0	10	0	11
Methanol	9	9.5	9	11	0	0	0	0	10	9	13	11	9	9.5	10	9
Cold water	9	0	0	0	11	11	11.5	9.5	10	9	10	10	0	9	9.5	9
Hot water	0	10	9	7	14	10.5	9	11	9	10	10	12	12	10	9	10

K. pneumoniae ATCC 13883 was observed to be resistant when tested against the organic extracts of ethanol and predominantly resistant when tested against the extract of cold water. However, ***K. pneumoniae*** ATCC 13883 was observed to be intermediately inhibited upon a single testing with an inhibition diameter of 9mm. ***Salmonella spp.*** exhibited resistance against methanol extracts of ginger but demonstrated intermediate inhibition in the range of 9mm – 14mm for the remaining four extracts of ginger. ***Shigella flexneri*** and ***Shigella sonnei*** were both intermediately inhibited by all extracts of ginger within the range of 9mm – 13mm in diameter.

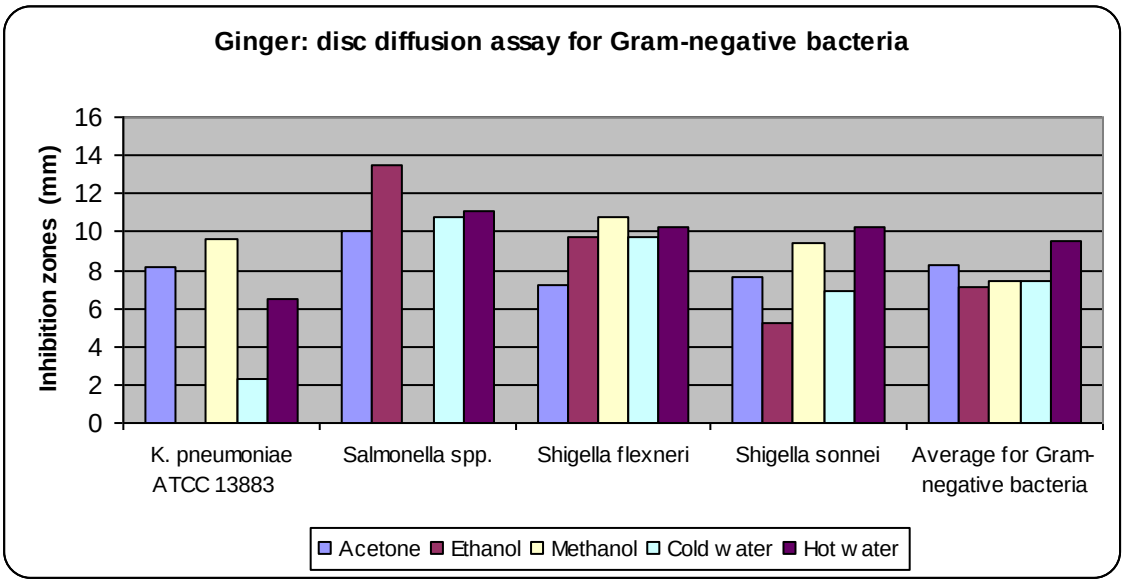

Figure 4.31 Average inhibition zone data of Gram-negative bacteria based on disc diffusion assays for the various extracts of ginger.

Results in Figure 4.31 indicated that ***K. pneumoniae*** and ***Salmonella spp*** were resistant when tested against the ethanol and methanol extracts respectively. Ethanol was found the be most effect (13mm) against the ***Salmonella spp***, with acetone, hot and cold water all having had inhibitory effect greater than 10 mm. ***Shigella*** strains were both susceptible to all extracts of ginger, with hot water and methanol having had the greatest inhibitory effect.

When the average sizes of e inhibition zones were compared, hot water was found to be the most effect against the Gram-negative strains tested, followed by acetone, methanol and cold water and then ethanol.

Table 4.25 Inhibition zones resulting from the agar well diffusion assay of Gram-negative microorganisms for extracts of the spice ginger (mm).

Bacteria → Extracts ↓	***K. pneumoniae*** ATCC 13883				***Salmonella spp.***				***Shigella flexneri***				***Shigella sonnei***			
Acetone	9	10.5	8	8.5	13	10	9	11	10	9	11	9	10	11	10.5	10.5
Ethanol	10	12	9.5	10	21	16	14	17	0	0	10	12	0	13	10	10
Methanol	0	0	8	0	13	10	11	12	13	11	11	12	12	12	10	10
Cold water	8	9.5	8	7.5	10	8	9	8.5	11	11.5	12	11	13	12	0	11
Hot water	11	10	11.5	10	0	10	12	0	10	11	10	10	2	10	10	13

K. pneumoniae ATCC 13883 exhibited a susceptibility to the methanol extract of ginger (8mm) but was intermediately inhibited by the other four extracts (acetone, ethanol, hot and cold water) with zones in the range of 7.5mm – 11.5mm in diameter. ***Salmonella spp.*** was observed to be intermediately inhibited by all extracts of ginger demonstrating susceptibility within the range of 8.5mm – 21mm in diameter. ***Shigella flexneri*** and ***Shigella sonnei*** both demonstrated initial resistance to the organic extract ethanol but upon further testing susceptibility to ethanol was noted. The organic extracts acetone, methanol, and both aqueous extracts were observed as being intermediate inhibitors of ***Shigella flexneri*** and ***Shigella sonnei***.

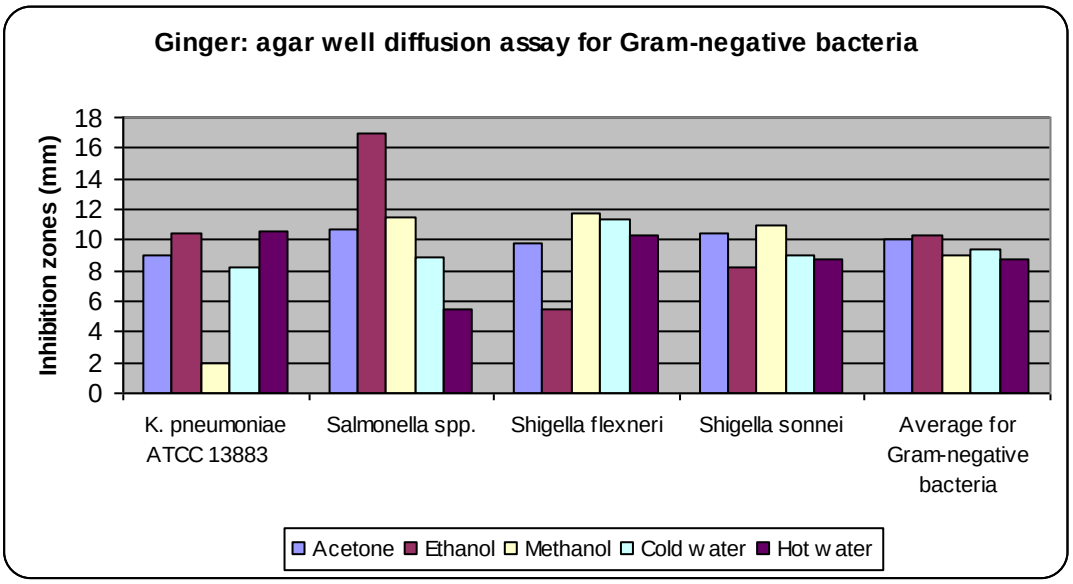

Figure 4.32 Average inhibition zone data of Gram-negative bacteria based on agar well assays for the various extracts of ginger.

Results of Figure 4.32 indicated that of the extracts did have and inhibitory effect on the Gram-negative microbes tested. The organic ethanol extract as found to be the most effective as an antimicrobial agent with the greatest susceptibility against ***Salmonella spp*** with inhibition zones sizes above 15 mm. Acetone was identified as having a constant and profound effect on all of the Gram-negative microbes, whilst organic extract methanol was found to have been very effective (10 mm) in its antimicrobial action, with the exception to ***K. pneumoniae***, in which case the activity was found to be reduced.

The comparison of the average inhibition zone sizes however indicated that the antimicrobial effectiveness of the ginger extracts was clearly evident and that the spice, did contain significant amounts of antimicrobial activity to inhibit the Gram-negative microorganisms being investigated.

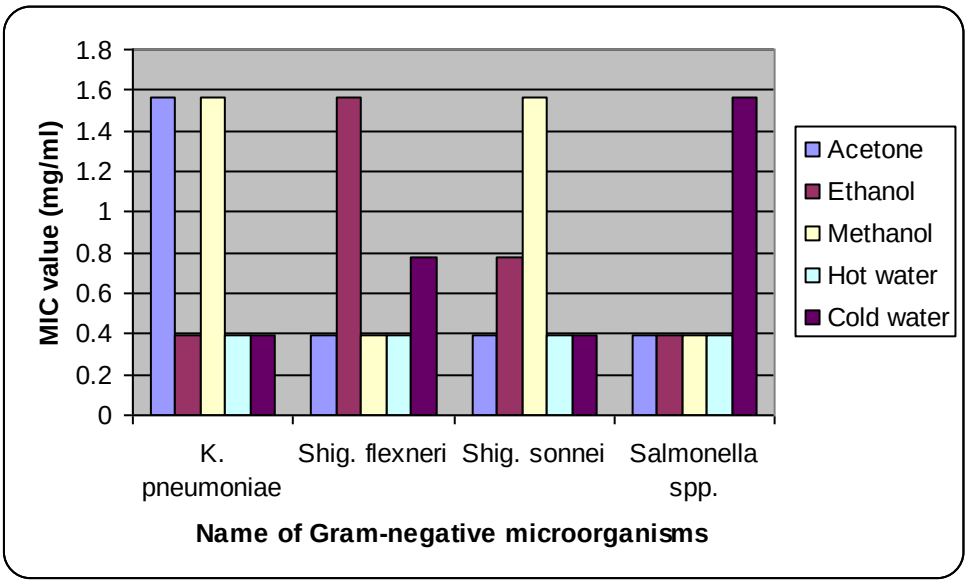

Figure 4.33 The MIC results of four Gram-negative microorganisms when tested against five extracts of the spice ginger.

The organic extracts acetone, methanol, and inorganic hot and cold water extracts were observed to have MIC values of 1.56 mg/ml when tested against the four Gram-negative microorganisms. The MIC values of the Gram-negative microorganisms against the various spice extracts were observed to be as low as 0.39 mg/ml. The high efficacy of ginger extracts at relatively low concentrations was once again an indicator for the potential use of ginger as an antimicrobial agent.

Table 4.26 Inhibition zones resulting from the disc diffusion assay of Gram-positive microorganisms against extracts of the spice ginger (mm).

Bacteria → Extracts ↓	***B. subtilis*** ATCC 6051				***S. aureus*** ATCC 12600				***S. aureus*** T1266				***S. aureus*** P4790			
Acetone	15	11	13	9	8.5	10	9.5	8	10	11	11	9	10	9	11	10
Ethanol	11.5	9	11	16	10	9.5	11	8	10	13	10	11	9.5	10.5	11	13
Methanol	11.5	8	10	11	8	8.5	9	8	12	10.5	10	10	11	10.5	11	13
Cold water	10	9	8	10	16	22.5	20.5	24	11	13	10	12	9	10	10.5	9.5
Hot water	9	10	9.5	9.5	9	9.5	9	8	10	12	14	11	12	10	11	11

During disc diffusion assay it was observed that all of the Gram-positive strains of microbes tested were susceptible to all the organic and aqueous extracts of ginger. ***S. aureus*** ATCC 12600 however was noticeably most susceptible when tested against the cold water extract demonstrating absolute inhibition zones in the range of 16mm – 24mm in diameter. ***B. subtilis*** ATCC 6051, ***S.aureus*** T1266 and ***S. aureus*** P4790 were inhibited by all of the five extracts of ginger with inhibition zones in the range of 8mm – 15mm in diameter.

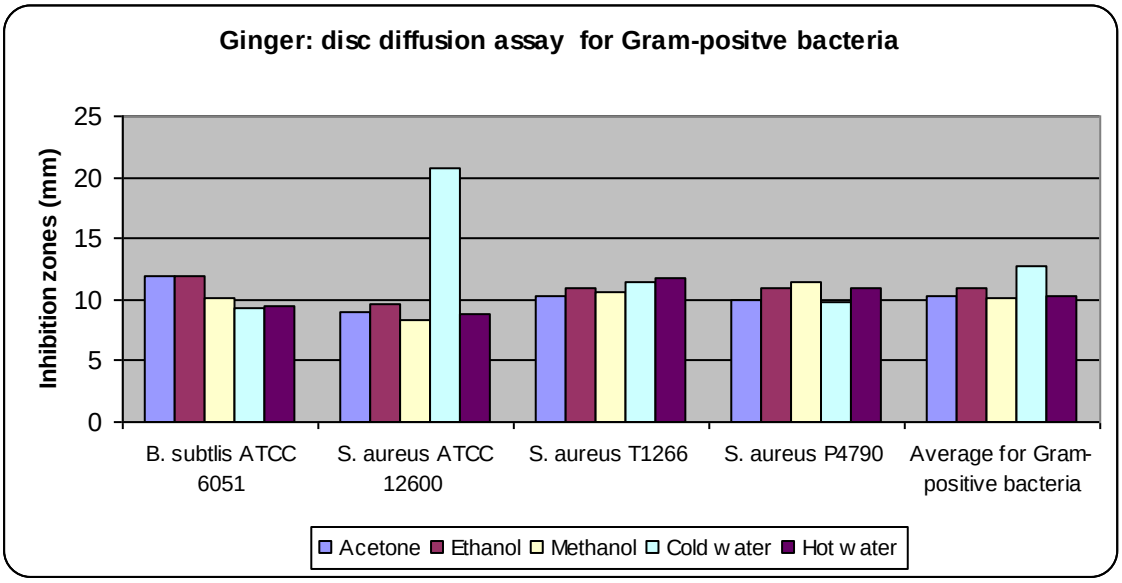

Figure 4.34 Average inhibition zone data of Gram-positive bacteria based on disc diffusion assays for the various extracts of ginger.

Figure 4.34 showed that all on the extracts of nutmeg had inhibitory effects on all of the Gram-positive microbes. The organic extracts were found to be more effective on ***B. subtilis*** than the aqueous extracts, with inhibition zones of 10 mm and above. ***S. aureus*** ATCC 12600 exhibited the greatest susceptibility when tested against the cold water extract (Figure 4.34; greater than 20 mm). ***S. aureus*** T1266 and ***S. aureus*** P4790 exhibited inhibition with zone sizes greater than 10 mm.

The average inhibition zone sizes were also compared and it was concluded that ginger had inhibitory effect greater than 10mm when tested against all of the Gram-positive microbes and was effectively capable in its potential as an antimicrobial agent.

Table 4.27 Inhibition zones resulting from the agar well diffusion assay of Gram-positive microorganisms against extracts of the spice ginger (mm).

Bacteria → Extracts ↓	***B. subtilis*** ATCC 6051				***S. aureus*** ATCC 12600				***S. aureus*** T1266				***S. aureus*** P4790			
Acetone	11	13	10	11.5	11	12.5	13	15	12	11	11	10	11	14	11.5	12
Ethanol	13	14.5	16	12	17	13	11	10	12	12.5	11	12	10	9	13	11
Methanol	13	11	9	11	11	9.5	9	13	10	11	11	9	11	10	10.5	12
Cold water	14	12	9	10.5	9.5	7	8	8.5	11	11	13	16	13	16	15	13
Hot water	11.5	10	14	25	8	9	8.5	8.5	11	11.5	10	12	14	14	12	13

As in disc diffusion assay, each Gram-positive test microorganism was inhibited when tested against all of the ginger extracts. The uniform occurrence of inhibition throughout the assay for Gram-positive microorganisms was indicative that the test extracts of ginger were effective as antimicrobial agents producing an overall result of inhibition zones in the range of 7mm – 17mm in diameter.

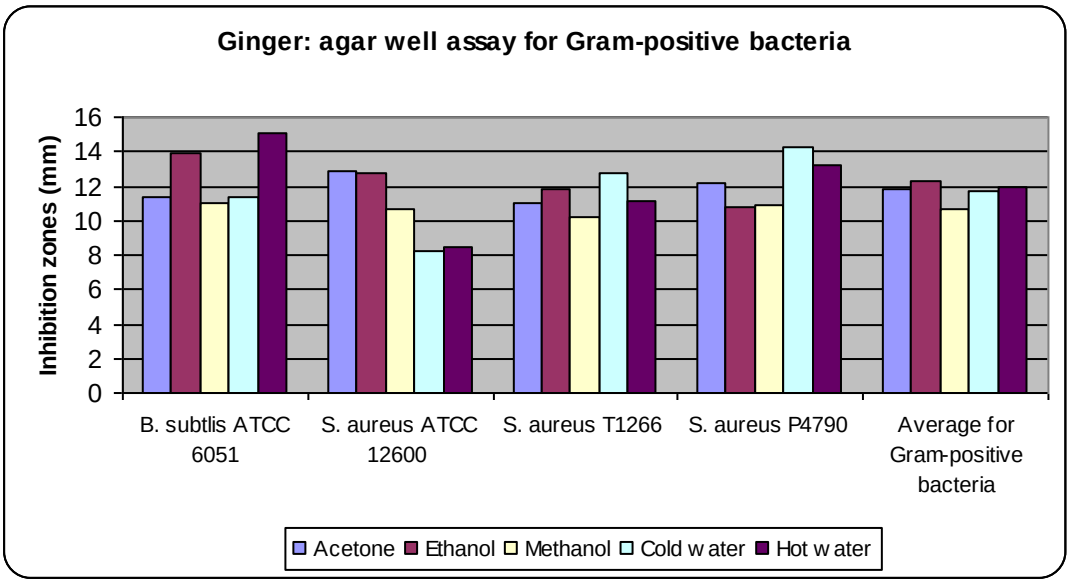

Figure 4.35 Average inhibition zone data of Gram-positive bacteria based on agar well assays for the various extracts of ginger.

The results of Figure 4.35 showed the profound effect of all the extracts of ginger an all of the Gram-positive microorganisms (inhibition zones generally greater than 10 mm). The average inhibition zone sizes indicated that the Gram-positive were most strongly inhibited by the organic extracts of ethanol, followed by; acetone and hot water, cold water and then methanol. Figure 4.35 demonstrated that the antimicrobial effect of ginger was generally greater than 10 mm in all instances and that this indicated that ginger did possess significant antimicrobial effectivity when investigated.

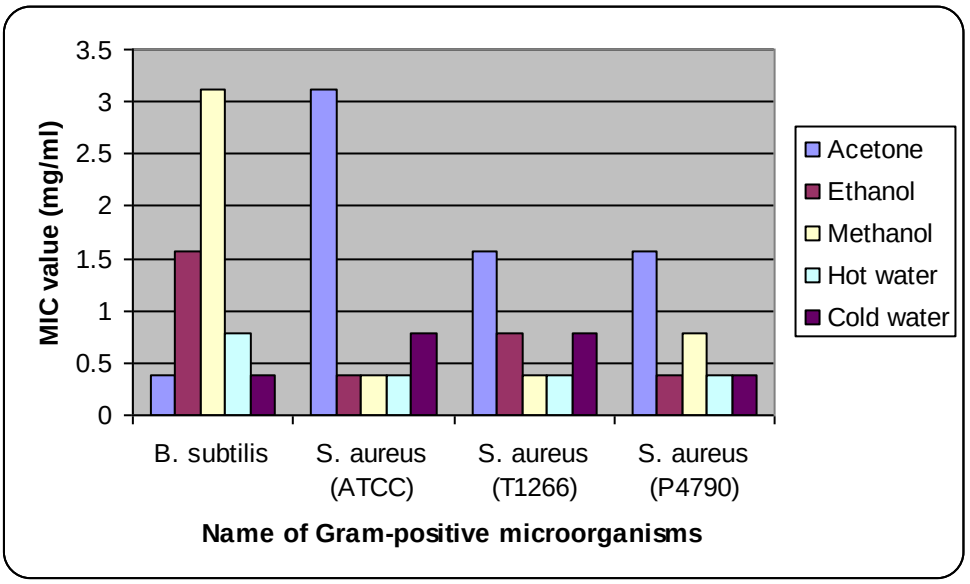

Figure 4.36 The MIC results of four Gram-positive microorganisms namely, ***B. subtilis*** ATCC 6051, ***S. aureus*** ATCC 12600, ***S. aureus*** T1266 and ***S. aureus*** T4790 when tested against five extracts of the spice ginger.

All of the microorganisms were observed to have MIC values in the range of 0.78 mg/ml – 3.12 mg/ml. The low MIC values demonstrated the effect of all the extracts of ginger against the microorganisms that were tested above and also indicated that even low concentrations of the extracts demonstrate antimicrobial activity that is capable of inhibiting microbial growth. It was also observed for the various strains of ***S. aureus*** that methanol, ethanol, hot and cold water extracts were more effective as antimicrobial agents during MIC determination when compared to the activity of acetone. The higher MIC values for acetone may be indicative of a reduced antimicrobial potential possibly caused by the organic extract acetone itself.

Table 4.28 Inhibition zones resulting from the disc diffusion assay of Gram-negative microorganisms against extracts of the spice cinnamon (mm).

Bacteria → Extracts ↓	***E. coli*** ATCC 11775				***E. coli*** U15055				***E. coli*** U16403				***E. coli*** U16406			
Acetone	11	9	12	12	12	12.5	10	13	9	11	11	11	11	14	12	10.5
Ethanol	0	11	9	9.5	15	15	13	12	12	10	10	11	9	10.5	10	12
Methanol	0	0	0	0	0	11	9	0	9	9.5	11	9	10	10	11.5	10
Cold water	13	9	8	11	13	13	10	11	12	9	10	11	11	0	12	13.5
Hot water	0	0	0	0	11	9	9	13	9	13	10	11	9	10.5	10	10

E. coli ATCC 11775 was observed to be resistant to methanol and hot water extracts whilst demonstrating intermediate inhibition when tested against the extracts of acetone, ethanol and cold water with inhibition zones in the range of 8mm – 13mm in diameter. However ***E. coli*** U15055, ***E. coli*** U16403 and ***E. coli*** U16406 all demonstrated intermediate inhibition when tested against each of the five extracts of cinnamon. The zone of inhibition was noted to be in the range of 9mm – 15mm in diameter.

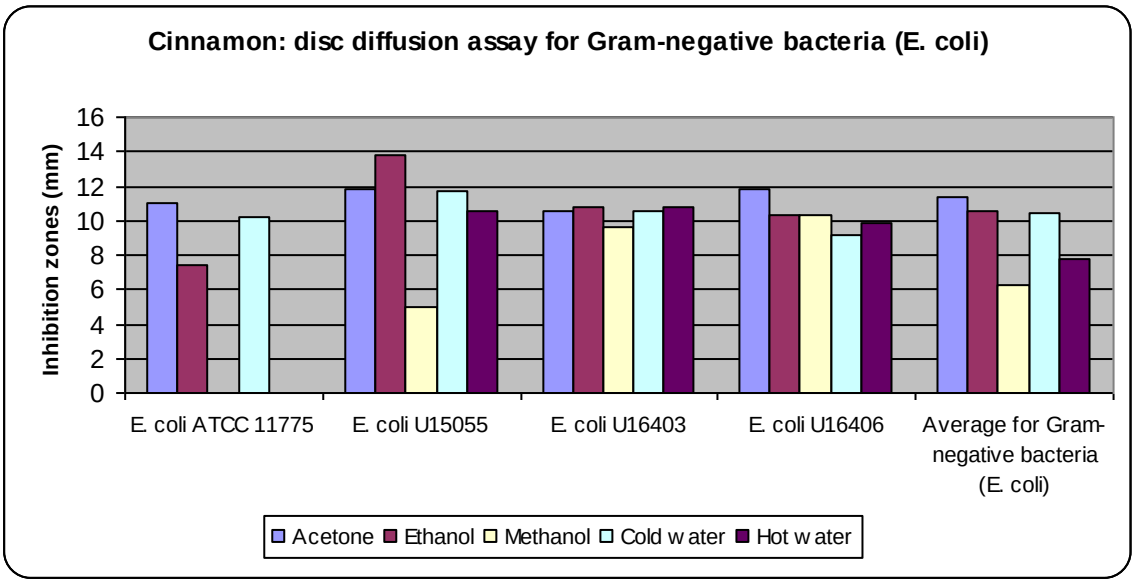

Figure 4.37 Average inhibition zone data of Gram-negative bacteria based on the disc diffusion assays for the various extracts of cinnamon.

Figure 4.37 indicated that ***E. coli*** ATCC 11775 displayed some resistance when tested against the extracts of cinnamon (resistant to methanol, and hot water extracts). In all instances, acetone extract was observed to have had the inhibitoriest effect on all of the Gram-negative microbes tested with inhibition zone sizes all greater than 10 mm. ***E. coli*** U15055 was the most susceptible to the ethanol extract with an average inhibition greater than 10 mm. ***E. coli*** U16406 was the most susceptible to the methanol extract than all the other Gram-negative strains.

Comparison of the average zone of inhibition sizes indicated that the extracts of cinnamon all have general effectivity as antimicrobial agents when tested against the various Gram-negative organisms. The order of effectiveness in descending order was found to be the following; acetone, ethanol, cold water, hot water, and then methanol extract.

Table 4.29 Results of agar well diffusion assay of Gram-negative microorganisms against extracts of the spice cinnamon (mm).

Bacteria→ Extracts ↓	***E. coli*** ATCC 11775				***E. coli*** U15055				***E. coli*** U16403				***E. coli*** U16406			
Acetone	9	13	12	12	9	10	10	12	12	9	9	10	10	12	13	10
Ethanol	12	11	11	13	18	20	15	19	0	11	0	13	14	14.5	12	12
Methanol	0	0	0	0	0	0	0	0	0	0	11	0	9	0	11	9.5
Cold water	12	9	10	12	12	11	14	10	12.5	0	0	11	11	12	11	10.5
Hot water	0	0	0	0	0	0	0	0	12	10.5	12	13	16	12	11	14

E. coli ATCC 11775 and ***E. coli*** U15055 were once again observed to be resistant against the extracts of methanol and hot water and intermediately inhibited by acetone, ethanol and cold water extracts of cinnamon (9mm – 13mm). ***E. coli*** U16403 and ***E. coli*** U16406 were both observed to be intermediately inhibited (range of 9mm – 14.5mm) by all extracts of cinnamon during agar well assaying.

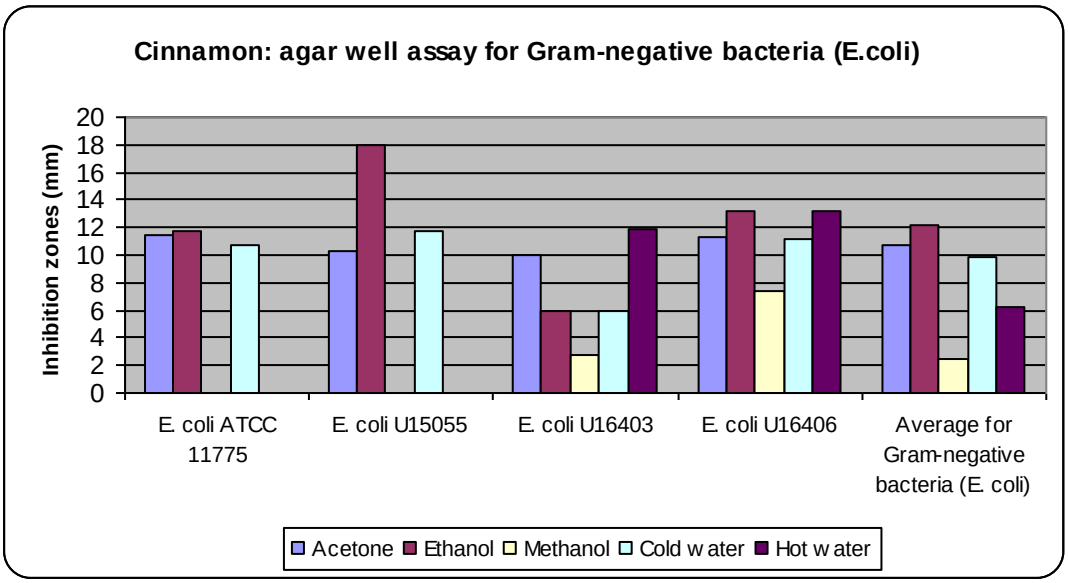

Figure 4.38 Average inhibition zone data of Gram-negative bacteria based on the agar well assays for the various extracts of cinnamon.

Results of Figure 4.38 clearly indicated that ***E. coli*** ATCC 11775 and ***E. coli*** U15055 were both resistant when tested against the methanol and cold water extracts of cinnamon. Of the two microorganisms, ***E. coli*** U15055 showed more susceptibility when tested against the ethanol and cold water extracts, whilst ***E. coli*** ATCC 11775 was found to be more susceptible when tested against acetone. In instances of susceptibility, inhibition zone sizes were greater than 10 mm. ***E. coli*** U16403 and ***E. coli*** U16406 was observed to be susceptible when tested against all extracts of cinnamon.

Comparison of the average inhibition zones sizes indicated the on the overall, cinnamon did possess antimicrobial activity that was capable of inhibition of the various strains of ***E. coli***. The descending order of effectiveness was as follows; ethanol, acetone, cold water, hot water, and then methanol extract of cinnamon.

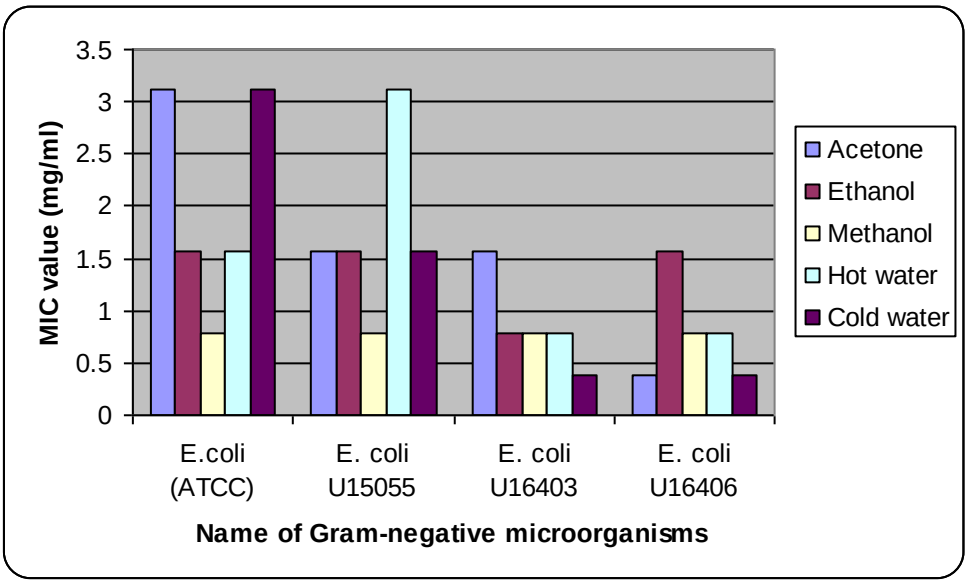

Figure 4.39 The MIC results of four Gram-negative microorganisms namely ***E. coli*** ATCC 11775, ***E. coli*** U15055, ***E. coli*** U16403 and ***E. coli*** U16406 when tested against five extracts of the spice cinnamon (continued **Fig 4.14**).

E.coli ATCC 11775 and ***E. coli*** U15055 of the Gram-negative microorganisms were observed to have MIC values predominately in the range of 1.56 mg/ml – 3.12 mg/ml. Gram-negative ***E. coli*** U16406 was observed to have low MIC values in the range of 0.39 mg/ml – 1.56 mg/ml. This demonstrates the antimicrobial effectiveness of the cinnamon extracts on ***E. coli***.

Table 4.30 Inhibition zones resulting from the disc diffusion assay of Gram-negative microorganisms against extracts of the spice cinnamon (mm).

Bacteria → Extracts ↓	***K. pneumoniae*** ATCC 13883				***Salmonella spp.***				***Shigella flexneri***				***Shigella sonnei***			
Acetone	10	10	6	12	11	11	12	10	10	9	11	10	10	11	10	9
Ethanol	12	11	11.5	11	16	15	11	13	12	11	14	13	11	10	10	9
Methanol	9	9.5	8	10.5	14	12	12	0	11	11	13	9	9	11	10	13
Cold water	8	11	10	12	12	12	12	14	12	12	10	10	9	10	10	10.5
Hot water	9	9	8.5	6	11	12	11.5	13	12	10	9	9	9	9	11	9.5

It was observed that each of the Gram-negative microorganisms demonstrated intermediate inhibition when tested against each of the five extracts of cinnamon. Zones of inhibition were noticeably uniform during all disc diffusion test procedures.

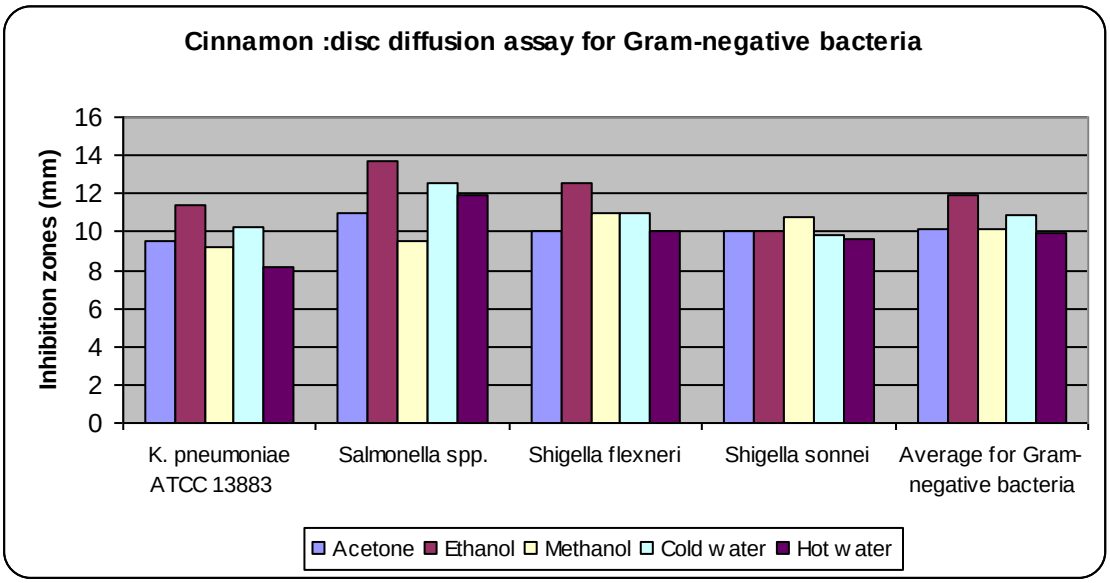

Figure 4.40 Average inhibition zone data of Gram-negative bacteria based on the disc diffusion assays for the various extracts of cinnamon.

In Figure 4.40, it was observed that all of the extracts of cinnamon were effective in inhibiting all of the Gram-negative strains tested. Ethanol was observed to have the greatest effect of all the extracts, with inhibition zones size greater than 10 mm in all instances.

The average inhibition zone sizes for all of the Gram-negative microorganisms was compared and the results indicated that each of the extracts produced inhibition zone sizes equal to or greater than 10 mm. The average inhibition zones sizes also confirmed that the most effective extract was ethanol, followed by cold water (greater than 10 mm), and then the extracts of acetone and methanol having had the same effect (10 mm inhibition zones), and then hot water. The general effectiveness of cinnamon was observed to have produced inhibition zones greater than 10 mm.

Table 4.31 Inhibition zones resulting from the agar well diffusion assay of Gram-negative microorganisms against extracts of the spice cinnamon (mm).

Bacteria → Extracts ↓	***K. pneumoniae*** ATCC 13883				***Salmonella spp.***				***Shigella flexneri***				***Shigella sonnei***			
Acetone	14	12.5	12	12	12	11	11	10.5	12	10	10	11	13	13	12	11
Ethanol	10.5	11.5	9	11	0	9	13	10.5	15	12	12	17	14	13	11	11
Methanol	11	14	11	9	13	11	0	16	17	23	16	19	11	10	10.5	11
Cold water	13	11	11	9	13	12	13	15	18	15	15	12	9	0	11	9
Hot water	9	0	13	11	0	13	0	0	9	10	10	10	9	10	10	9

Similar to disc diffusion assaying, all of the Gram-negative microorganisms exhibited intermediate inhibition when tested against the various organic and aqueous extracts of cinnamon. ***Shigella flexneri*** was also noted to have exhibited absolute inhibition when tested against the methanol extract with zones of inhibition in the range of 16mm – 23mm in diameter.

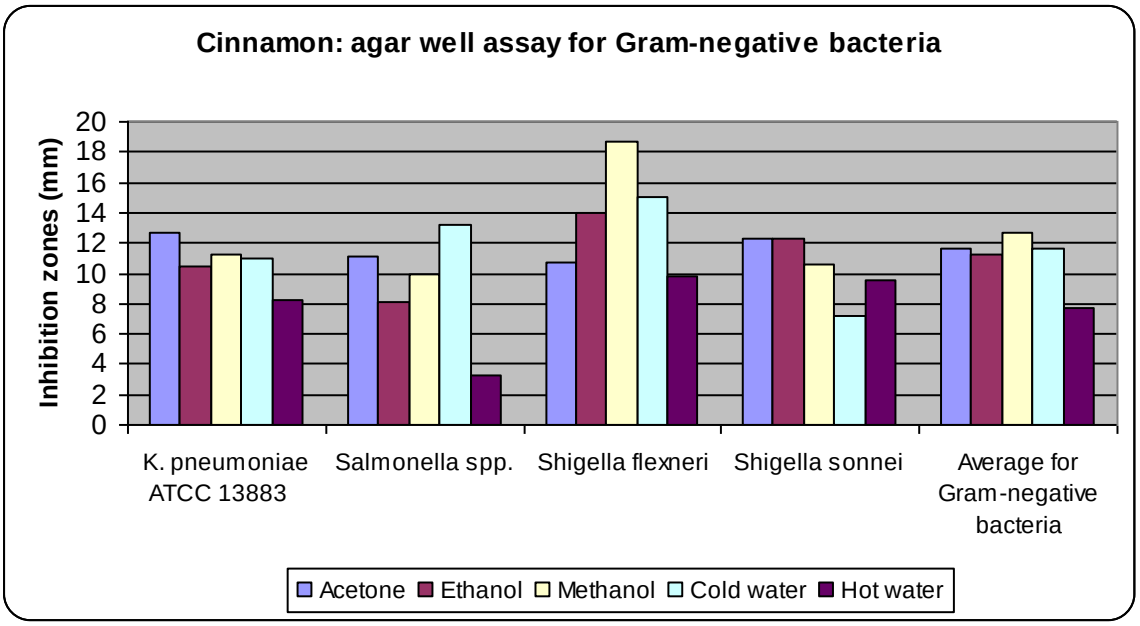

Figure 4.41 Average inhibition zone data of Gram-negative bacteria based on the agar well assays for the various extracts of cinnamon.

K. pneumoniae was observed to susceptible to all extracts of cinnamon with inhibition zone sizes greater than 10 mm with the exception to the hot water extract which produced an average inhibitory effect below 10 mm. Acetone was the most effective of the extracts with average zone size greater than 12 mm. ***Salmonella spp*** was inhibited by all extracts of cinnamon with hot water once again being observed as the least effective whilst the cold water extracts was found to most effective. ***Shigella flexneri*** and ***Shigella sonnei*** were both found to be profoundly inhibited by the all the extracts of cinnamon with inhibitory effects greater than 10 mm in all the aqueous extracts against ***Shigella sonnei***.

Figure 4.41 indicated that the extracts of cinnamon all produced inhibition zones with sizes predominately greater than 12 mm. The uniform inhibition zones sizes produced was indicative of the antimicrobial potential of cinnamon as an antimicrobial agent.

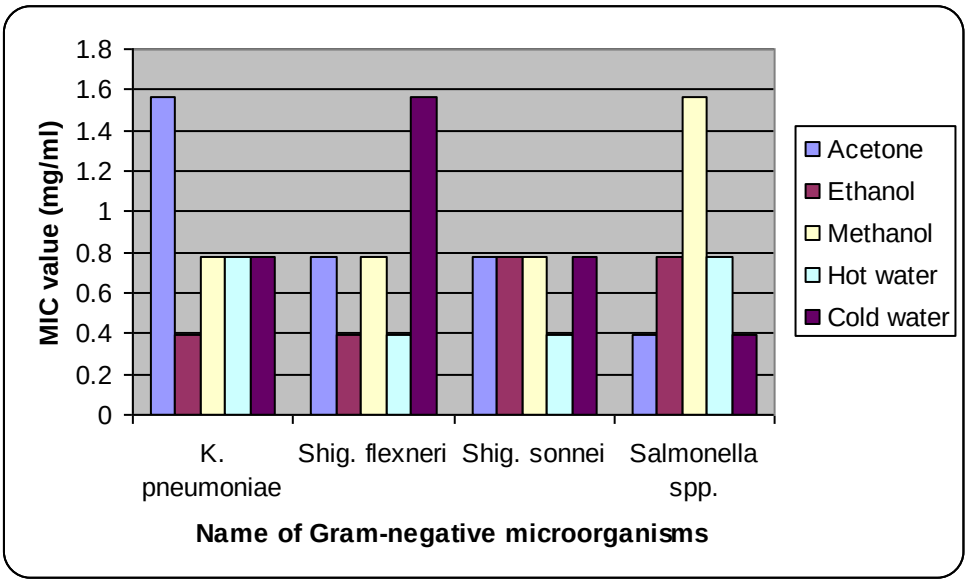

Figure 4.42 The MIC results of four Gram-negative microorganisms namely, ***K. pneumoniae*** ATCC 13883***, Shigella flexneri, Shigella sonnei*** and ***Salmonella spp***. when tested against five extracts of the spice cinnamon.

For the above Gram-negative microorganisms, MIC values were all predominantly in the range of 0.39 mg/ml – 1.56 mg/ml. The noticeably low concentration of cinnamon extracts required to inhibit the microorganisms tested demonstrated the high effectiveness of cinnamon as an antimicrobial agent especially against the species tested.

Table 4.32 Inhibition zones resulting from the disc diffusion assay of Gram-positive microorganisms against extracts of the spice cinnamon (mm).

Bacteria → Extracts ↓	***B. subtilis*** ATCC 6051				***S. aureus*** ATCC 12600				***S. aureus*** T1266				***S. aureus*** P4790			
Acetone	13	15	12	13	10	9.5	11	9	10	11	12	11	9	9	10	10
Ethanol	16	13	12	14	8	11.5	9.5	10	11	11	10	9	9	11	10	10
Methanol	10	12.5	11	10.5	11	9.5	8	0	9	10	10	9	10	9	9.5	9
Cold water	12	9	8	11	8	9	9.5	7.5	10	9	9	9	13	13	13.5	12
Hot water	11	11	9	11	11	9	12	10.5	9	11	9	10	9	11	9	10

Cinnamon extracts were observed to cause intermediate inhibition when tested against each of the Gram-positive microorganisms. ***B. subtilis*** ATCC 12600 was noticeably more susceptible when tested against acetone and ethanol extracts with inhibition zones in the range of 12mm – 16mm in diameter. All of the strains of ***S. aureus*** were observed to demonstrate inhibition zones in the range of 7.5mm – 13.5mm in diameter.

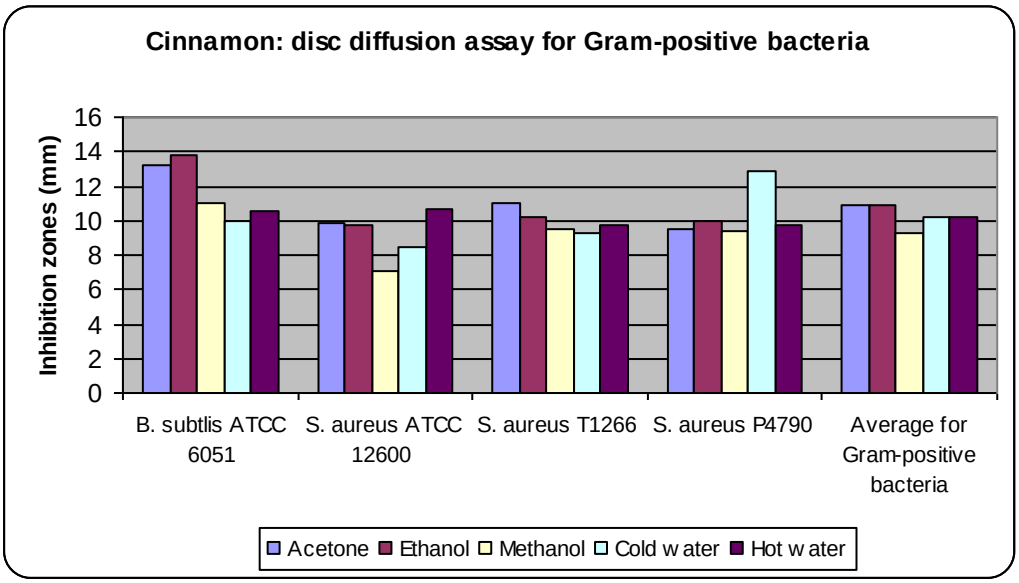

Figure 4.43 Average inhibition zone data of Gram-positive bacteria based on the disc diffusion assays for the various extracts of cinnamon.

Figure 4.43 demonstrated the profound effectiveness of all the extracts of cinnamon when tested against all of the Gram-positive microorganisms. ***B. subtilis*** was observed to display inhibitory effect greater than 10 mm in all instances of testing with ethanol being the most effective and old water the least effective. Gram-positive ***S. aureus*** ATCC 12600 was most susceptible toward the hot water extract (Figure 4.43 inhibition zone sizes greater than 10 mm) and the least effective observed to be methanol. ***S. aureus*** T1266 was observed to be more susceptible towards the organic extracts than the aqueous of cinnamon, with acetone being the most effective of the organic extracts, and hot water of the two aqueous extracts. ***S. aureus*** P4790 showed the greatest susceptibility when tested against the cold water extract of cinnamon and was uniformly inhibited by the four other extracts of cinnamon.

The comparison of the average inhibition zone sizes indicated that acetone and ethanol were most effective followed by hot and cold water extracts, then followed by the organic extract, methanol.

Table 4.33 Inhibition zones resulting from the agar well diffusion assay of Gram-positive microorganisms against extracts of the spice cinnamon (mm).

Bacteria → Extracts ↓	***B. subtilis*** ATCC 6051				***S. aureus*** ATCC 12600				***S. aureus*** T1266				***S. aureus*** P4790			
Acetone	19	16	15.5	19.5	0	10	11.5	0	14	13	14	15	15	13	16	16
Ethanol	13	12	9	12	10	13	12	11	11	9	9	10	15	16	13	13
Methanol	12	12	9	14	15	14	11	17	13	13	12	13	12	12	13	11
Cold water	16	13.5	11	12	10	11	9	15	13	11	11	9	11	11	12	10
Hot water	15	12	12	10	12	12	13	15	9	9.5	9	10	11	10	10	9

B. subtilis ATCC 6051 showed absolute inhibition when tested against the acetone extracts of cinnamon with zones on inhibition in the range of 15.5mm – 19mm, whilst a range of 9mm – 16mm was observed for the extracts of ethanol, methanol, hot and cold water. All of the strains of ***S. aureus*** were observed to be intermediately inhibited by each of the extracts of cinnamon, once again demonstrating the effectiveness of the extracts against the Gram-positive ***S. aureus*** strains of microorganisms.

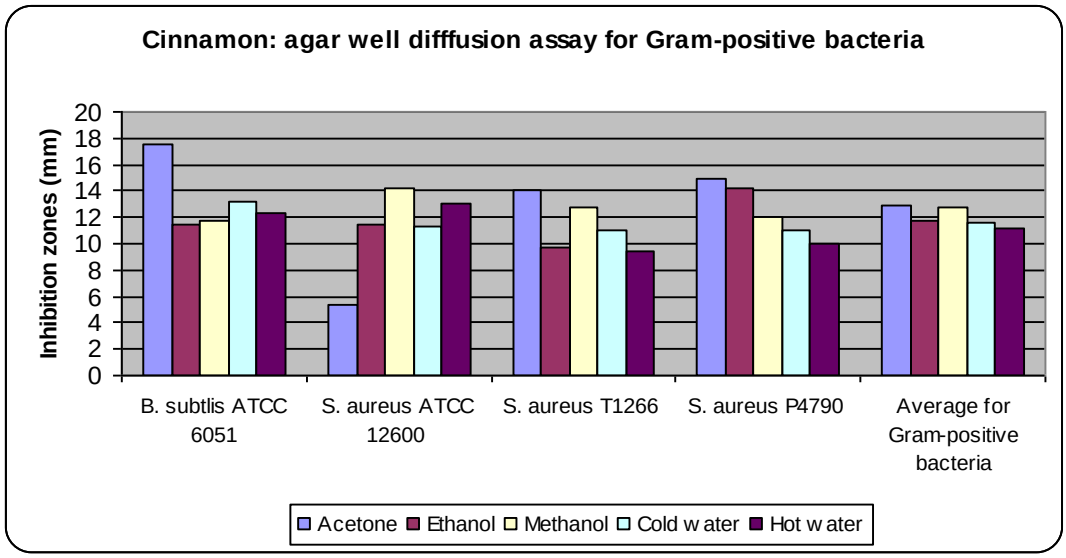

Figure 4.44 Average inhibition zone data of Gram-positive bacteria based on the agar well assays for the various extracts of cinnamon.

The results of Figure 4.44 clearly stated that all of the extracts of cinnamon were capable of inhibitory effect on all of the Gram-positive microorganisms. ***B .subtilis*** ATCC 6051 was observed to have been profoundly inhibited by all extracts with inhibition zone sizes greater the 10 mm in all instance. Acetone was found to be the most effective against this Gram-positive microbe. ***S. aureus*** P4790 was the second most inhibited of the Gram-positive microbes with inhibition zones also, all above 10 mm and acetone once again being the most effect of the five extracts tested. ***S. aureus*** ATCC 12600 and ***S .aureus*** T1266 were both observed to have inhibitory effects generally greater than 10 mm. Predominate effectivity of cinnamon against these two Gram-positive microbes indicated their susceptibility when tested against the extracts of cinnamon and the effectiveness of the extracts as antimicrobial agents. Average inhibition zone sizes (all greater than 10mm) further concluded that the spice was capable of significant inhibitory effects when tested Gram-positive microorganisms.

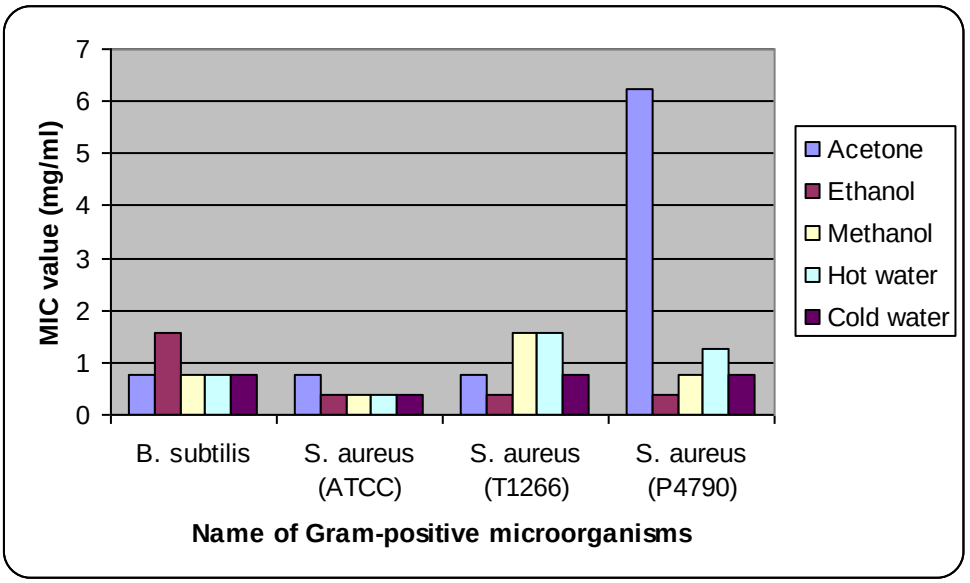

Figure 4.45 The MIC results of four Gram-positive microorganisms namely ***B.subtilis*** ATCC 6051, ***S. aureus*** ATCC 12600, ***S. aureus*** T1266 and ***S. aureus*** T4790 when tested against five extracts of the spice cinnamon.

The microorganism ***B. subtilis*** ATCC 6051 was observed to have an MIC value of 0.78 mg/ml for all extracts of cinnamon except for ethanol which had an MIC value of 1.56 mg/ml. Gram-positive microorganisms of the ***S. aureus*** strain were observed to have significantly low MIC values, 0.39 mg/ml – 1.56 mg/ml for extracts of cinnamon. The exception to this was observed for ***S. aureus*** T4790 which had an MIC value of 6.25 mg/ml.

The significantly low MIC value observed using the extracts of cinnamon was once again indicative of the potential effectiveness of these extracts as antimicrobial agents against the four Gram-positive test microorganisms.

4.2 Analysis of Variance (ANOVA system)

In tables 4.34 – Table 4.36, the ANOVA system of analysis (obtained from the web site: **stat.tamu.edu/sts30x/notes/node126**) was used to compare the various groups of data (inhibition zone sizes) obtained during disc diffusion and agar well assaying.

The function of the ANOVA system was the analysis of data and the prediction of differences that occurred between the different groups being compared. The difference was indicated by the Sigma value (P-value). When the P-value was less than 0.05, it indicated that the difference in the diameter of the inhibition zones (data analyzed) did occur when then the individual groups of data were compared against each other i.e.

1) Comparison of microbes against the average resultant inhibition zones to determine the susceptibility of the various microbes.
2) Comparison of extracts against the resultant inhibition zone sizes to determine the effectivity of each extract.
3) Comparison of each spice and the average resultant inhibition zone sizes that occurred to determine the order of effectivity amongst the five spices investigation.

Table 4.34 Total Analysis of Variance (ANOVA) for twelve microbial species when tested against the various extracts five selected spices.

ANOVA

Resultant inhibition zones (mm)

Method used		Sum of Squares	degree of freedom	Mean Square	Frequency	Sigma P-value
Disc diffusion	Between Groups	7020.655	11	638.241	33.208	0.0
	Within Groups	28983.092	1508	19.220		
	Total	36003.747	1519			
Agar well diffusion assay	Between Groups	7825.984	11	711.453	21.707	0.0
	Within Groups	46804.077	1428	32.776		
	Total	54630.061	1439			

The P-value for both, disc diffusion and agar well 0.0. This indicated that there was a difference between the various microorganisms used and suggested the degree of susceptibility exhibited by each microorganism. Figure 4.46 and Figure 4.47 indicated the differences that occurred during both assaying methods.

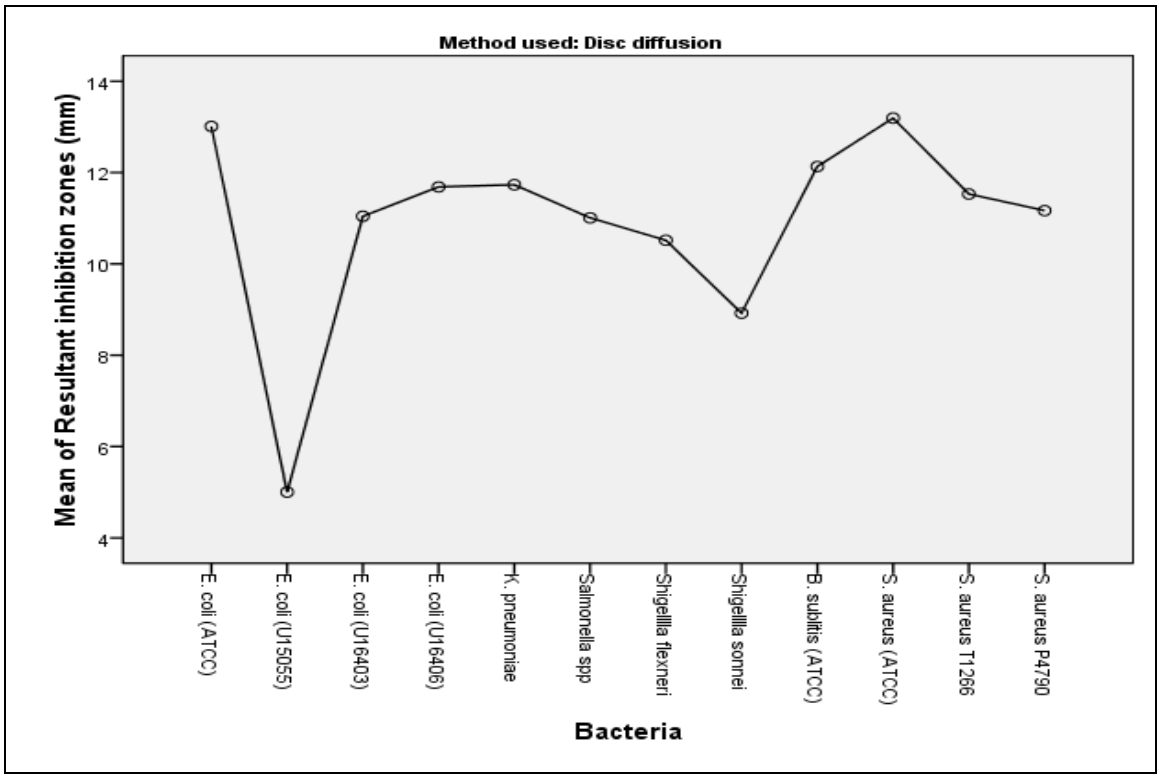

Figure 4.46 Zones of inhibition exhibited by the various microorganisms during disc diffusion assaying.

Results indicated in Figure 4.46 indicated that the most susceptible microorganism with an average resultant mean of inhibition of greater than 12 mm was found to be the ATCC strain of ***S. aureus*** 12600. The most resistant microbe was found to be the Gram-negative strain of ***E. coli*** U15055. However, ***E. coli*** U16403 and ***E. coli*** U16406 were both found to have susceptibility greater than 10 mm. Figure 4.46 clearly demonstrated that during the investigation all of the Gram-positive microbes were found to be more inhibited when tested against the various spices and extracts. The analysis concluded that the order of susceptibility in descending order as found as follows; ***S. aureus*** ATCC 12600, ***E. coli*** ATCC 11775, ***B. subtilis***, ***K. pneumoniae***, ***E. coli*** U16406, ***S. aureus*** T1266, ***S. aureus*** P4790, ***E. coli*** U16403, ***Salmonella spp***, ***Shigella flexneri***, ***Shigella sonnei*** and ***E. coli*** U15055.

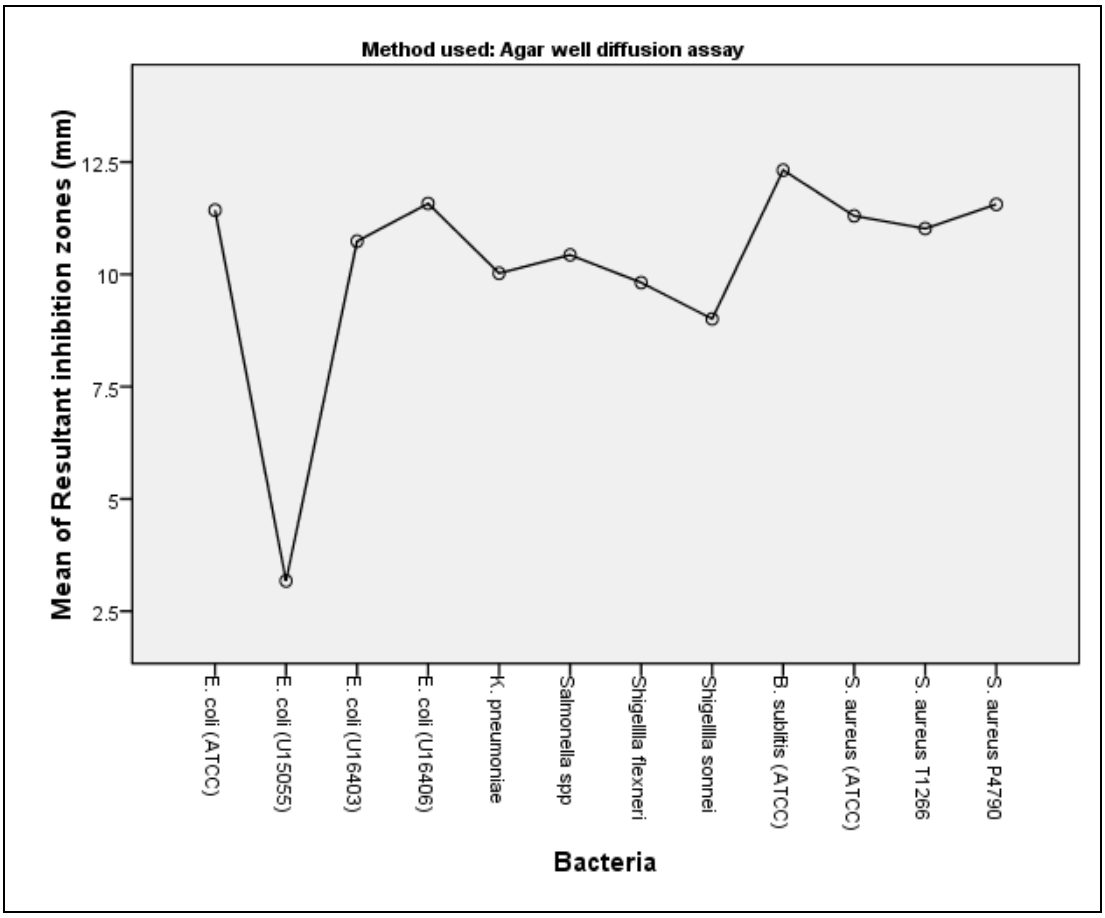

Figure 4.47 Zones of inhibition exhibited by the various microorganisms during agar well assaying.

Figure 4.47 clearly indicated that the Gram-positive microorganisms were the most susceptible during the investigation. ***B. subtilis*** was found to exhibit the greatest susceptibility whilst ***E. coli*** U15055 was once again was confirmed to be the most resistant microbe tested. Figure 4.47 also demonstrated that two strains of ***E. coli*** (namely, ***E. coli*** U16406 and ***E. coli*** 11775) were found to be intermediately placed amongst the top five most susceptible microbes. However, the overall analysis of the mean resultant inhibition zones showed that the Gram-positive microbes were more susceptible to the various extracts of the different spices.

Table 4.35 Total Analysis of Variance (ANOVA) of inhibition zones for the various spice extracts.

ANOVA

Resultant inhibition zones (mm)

Method used		Sum of Squares	Degree of freedom	Mean Square	Frequency	Sigma/ P-value
Disc diffusion	Between Groups	1082.302	4	270.575	11.738	0.0
	Within Groups	34921.446	1515	23.050		
	Total	36003.747	1519			
Agar well diffusion assay	Between Groups	850.468	4	212.617	5.673	0.0
	Within Groups	53779.593	1435	37.477		
	Total	54630.061	1439			

From the ANOVA system, the P-value for both disc diffusion and agar well assay are both less than 0.05 (i.e. 0.0), indicative of differences that has occurred between the various extracts of each spice. Figure 4.48 and Figure 4.49 represent the difference that has occurred between the different groups of extracts throughout the investigation. The graphs also indicate the efficacy of each spice by comparing the various sizes of inhibition zones that occurred during the investigation and plotting them against the various extracts used.

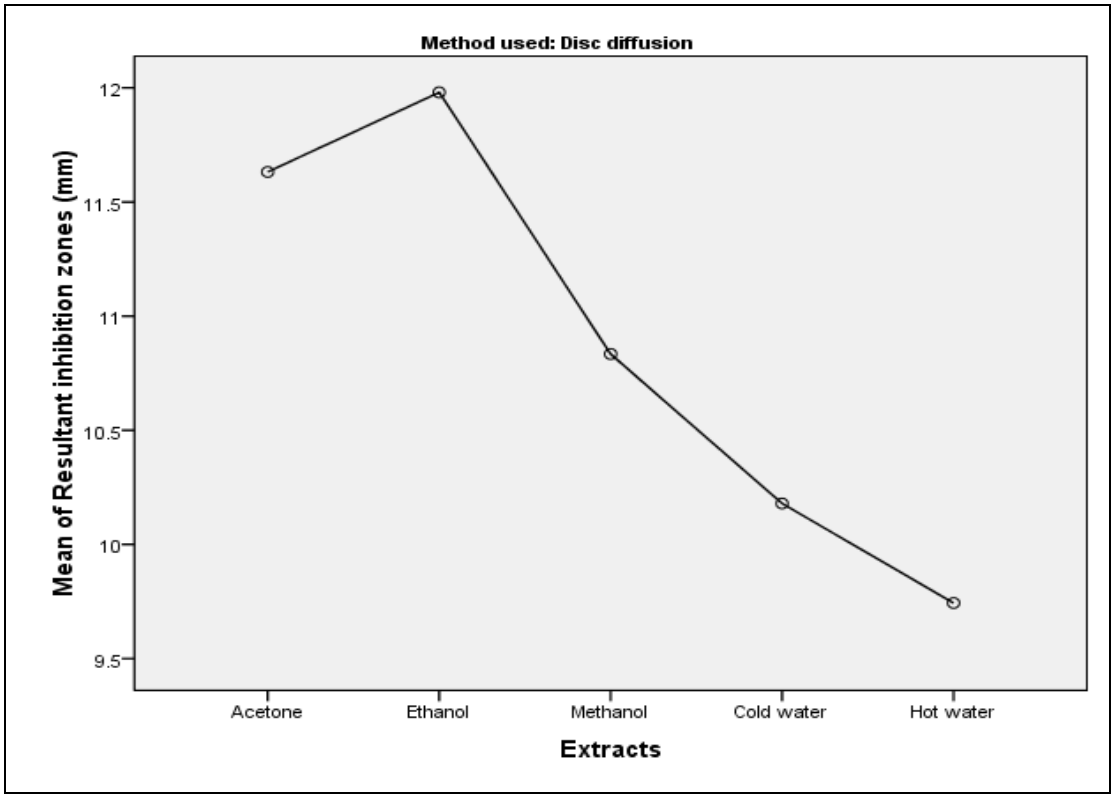

Figure 4.48 The efficacy of spice extracts by comparing the various sizes of inhibition zones during disc diffusion assay.

The analysis of the average inhibition zone sizes were compared and it was determined that the extract which produced the greatest inhibition when tested against the various microbes after being used in a homogenous mixture was found to be ethanol, with a mean resultant inhibition zone size of approximately 12 mm. The order of effectivity indicated that ethanol was the most effective, followed by acetone, methanol, cold water and then hot water extract. As a result it was concluded that the organic extracts produced a greater inhibitory effect on the microorganisms than the aqueous extracts.

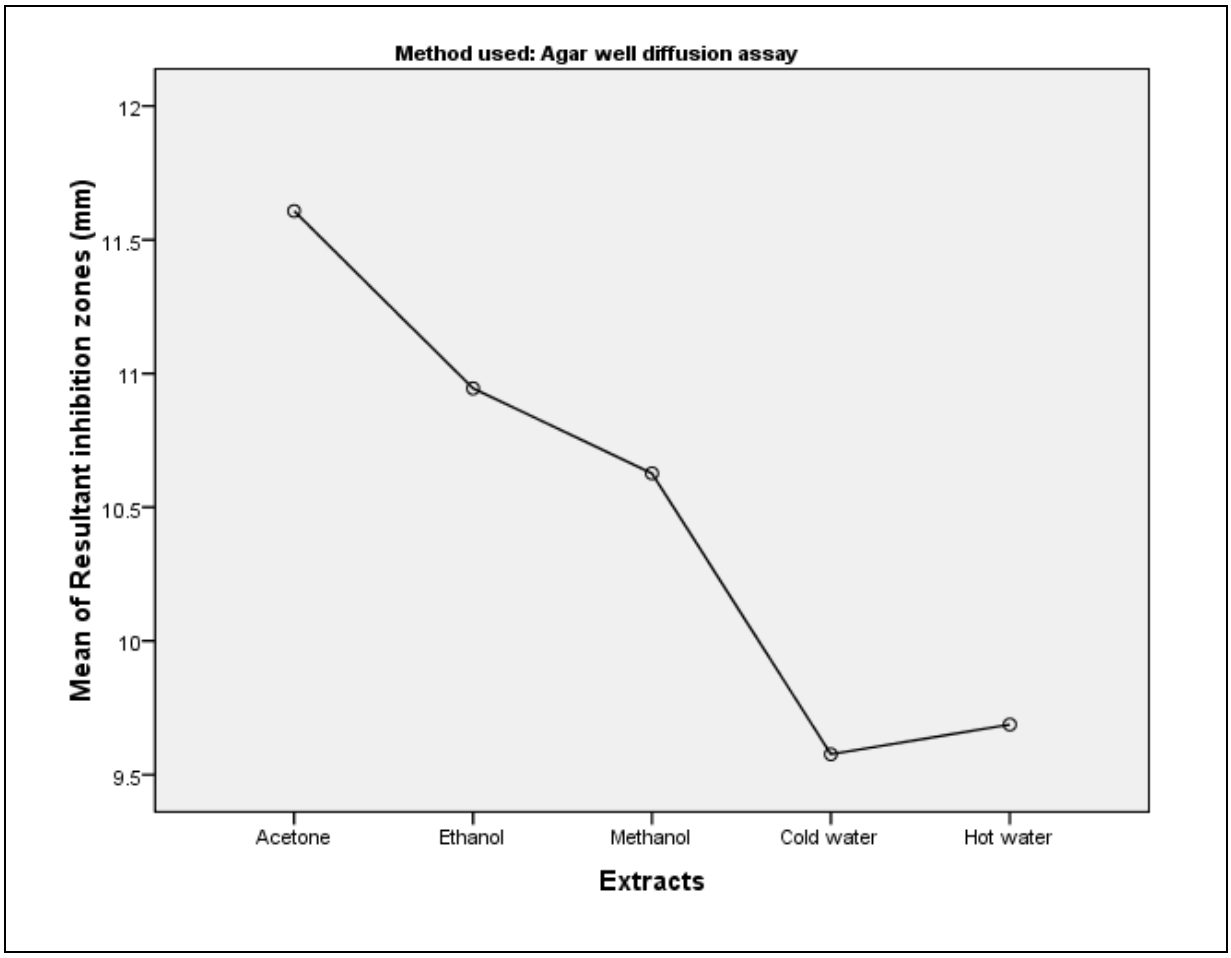

Figure 4.49 The efficacy of spice extracts by comparing the various sizes of inhibition zones during agar well assay.

During agar well assaying, acetone was found to be the most effective extract. The extracts of acetone, ethanol and methanol all displayed an average inhibition zone size greater than 10 mm. Once again the organic extracts were noticed to be more effective than the aqueous extracts.

Table 4.36 Total Analysis of Variance (ANOVA) of inhibition zones for the various spices.

ANOVA

Resultant inhibition zones (mm)

Method used		Sum of Squares	Degree of freedom	Mean Square	Frequency	Sigma/ P-value
Disc diffusion	Between Groups	4004.207	4	1001.052	47.394	0.0
	Within Groups	31999.541	1515	21.122		
	Total	36003.747	1519			
Agar well diffusion assay	Between Groups	2673.310	4	668.327	18.459	0.0
	Within Groups	51956.751	1435	36.207		
	Total	54630.061	1439			

The P-value as obtained from the AVONA system was once again less than 0.05. The difference of activity between the various groups can be observed on Figure 4.50 and Figure 4.51. From these graphs, the activity of the five selected spices was noted as well the degree of antimicrobial activity exhibited by each spice. These graphs represent the overall efficacy of each as antimicrobial agents and the order of activity which was consistent with the results observed during disc diffusion and agar well assaying throughout the investigation.

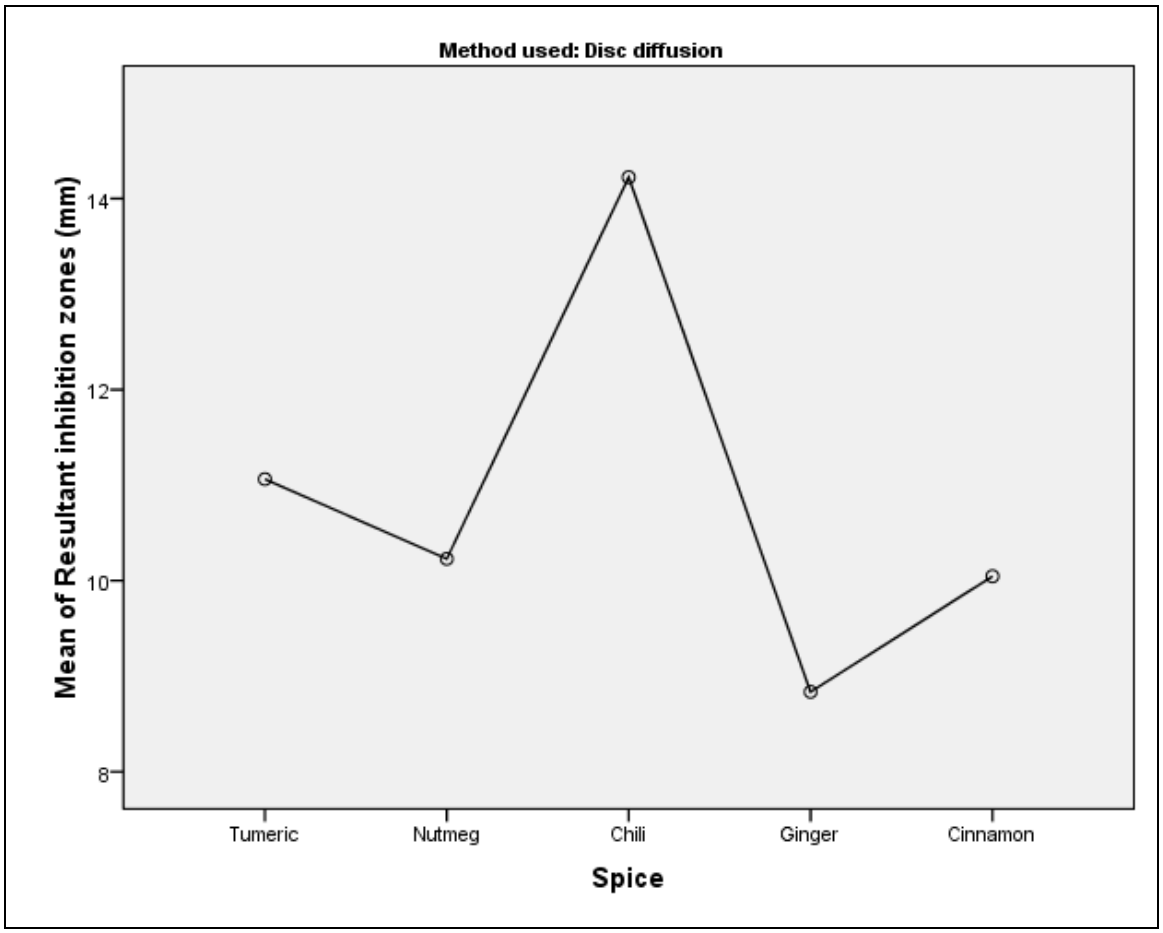

Figure 4.50 The overall antimicrobial activity of the five selected spices and the average inhibition exhibited by each spice during disc diffusion assaying.

Figure 4.50 used the mean average of the resultant inhibition zone sizes for the various extracts obtained from each of the five spices. Comparison of the five spices indicated that chilli produced the greatest inhibitory effects when tested against the various Gram-positive- and Gram-negative microorganisms, followed by turmeric, nutmeg, cinnamon, and ginger.

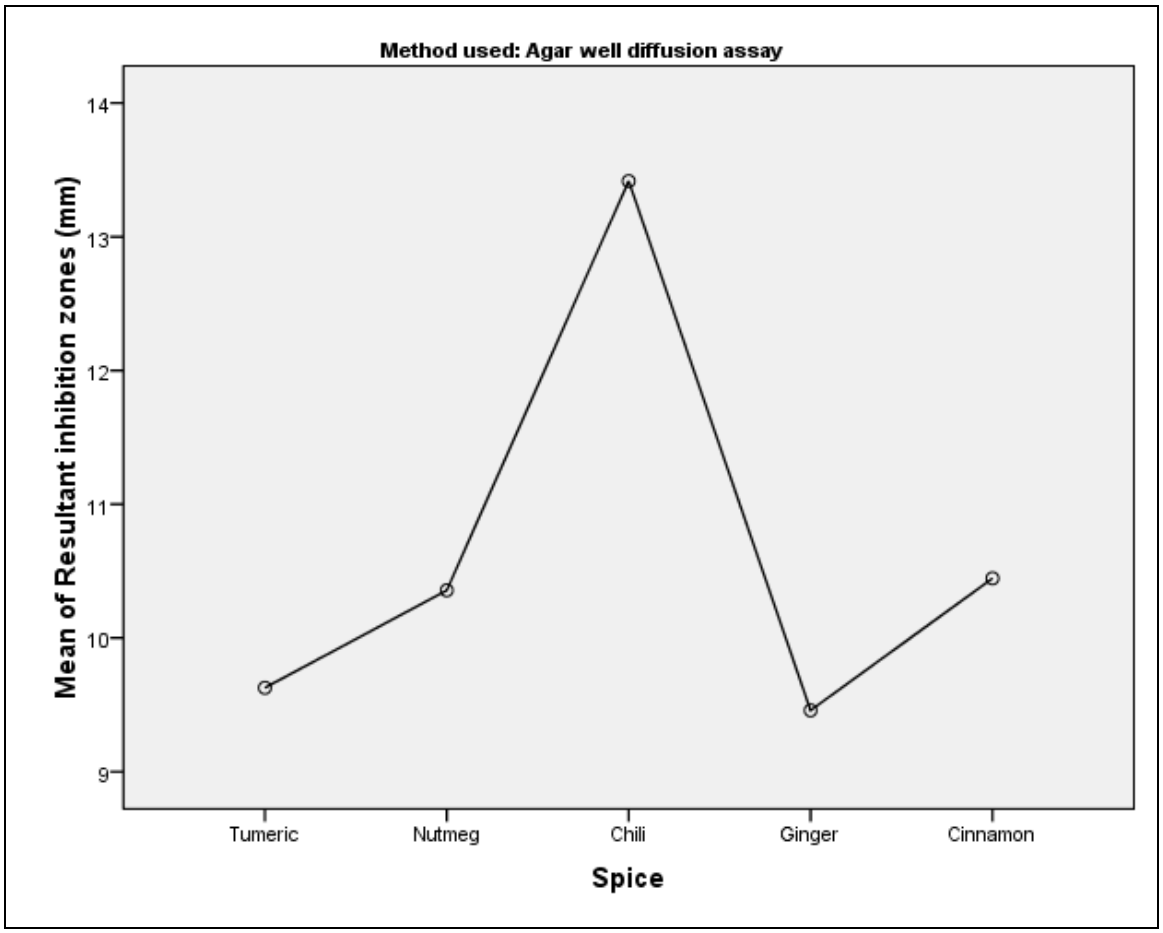

Figure 4.51 The overall antimicrobial activity of the five selected spices and the average inhibition exhibited by each spice during agar well assaying.

Analysis of the agar well assay showed that chilli still remained the most effective spice with cinnamon being the second most effective. The antimicrobial potential of each of the five spices were clearly evident from Figure 4.50 and Figure 4.51.

Chapter five

Discussion

5.1 Introduction

Spices have since ancient times been an important group of agricultural commodities being used by many civilizations all over the world adding flavor and nutritional values to a wide variety of food. However such spices are no longer used for only their culinary effects. Scientists are now forming an impressive body of data that suggests a successful story of microbial chemocontrol, which lies in the continuous search for antimicrobial agents of spices (Notermans and Hoogenboon-Verdegaal, 1992). Gould (1995) proposed the "natural antibiotic system" that emphasized the use of spices and their derivatives as possible alternatives for mainstream medicine. This system expanded on the synergistic effect of antimicrobial compounds extracted from plants and spices that was expressed in physical testing procedures in the attempt to create inhospitable living conditions for microorganisms.

The present study was aimed at investigating the potential of five extensively used culinary spices commonly known as turmeric, nutmeg, chilli, ginger, and cinnamon as effective antimicrobial agents that could possibly contribute to the "natural antibiotic system." Six microbial species including Gram-positive (***B. subtilus*** ATCC 6051, ***S. aureus*** ATCC 12600, ***S. aureus*** T1266 and ***S. aureus*** T4790) and Gram-negative (***E. coli*** ATCC 11775, ***E. coli*** U15505, ***E. coli*** U16403 and ***E. coli*** U16406, ***K. pneumoniae*** ATCC 13883, ***Shigella flexneri*** and ***Salmonella spp***) strains subjected to sensitivity testing, their average inhibition zone sizes determined when tested against various

extracts of the test spices and the minimum inhibitory concentration (MIC) value of each extract was also determined.

Sensitivity and MIC testing indicated that all five spices possess a significant amount of antimicrobial activity against eleven of the twelve microbial strains tested with ***E. coli*** U15055 exhibiting the greatest amount of resistance against the different spice extracts.

5.2 Turmeric: antimicrobial sensitivity and resistance

In the disc and agar well diffusion assay for the spice turmeric, analysis of Gram-negative microorganisms indicated that ***E. coli*** U15055 was the most resistant of microbial strains with Gram-positive ***B. subtilis*** ATCC 6051 (Table 4.8 and Table 4.9) proving to be the most susceptible strain. The organic extracts of turmeric showed the greatest amount of inhibition during disc diffusion assay against the ATCC strains of ***E. coli*** 11775 (Table 4.4) and ***B. subtilis*** 12600 (Table 4.8). Resistance exhibited by ***E. coli*** U15055 may be attributed to the differences in the structural integrity of the cell wall that is, the lack of "binding material" and hence interaction between the cellular lipoproteins (present in the peptidoglycan dense cell walls of Gram-positive microorganisms providing a greater target surface for the active components to attach to and initiate its antimicrobial action) and the active compounds present in turmeric extracts.

Table 4.8 demonstrated the profound susceptibility of ***S. aureus*** T1266 and ***S. aureus*** P4790 when tested against all extracts of turmeric during disc diffusion assay. This indicated that the antimicrobial ability of the active components in turmeric, contrary to

the scientific reasoning that the Gram-negative microbes display greater susceptibility due to a weak cell wall integrity (when compared to that of Gram-positive microbes), was found to be indiscriminate in its antimicrobial activity against both Gram-positive- and Gram-negative microorganisms. The thick structural constituents of Gram-positive microbes in this instance can be held responsible for the increase in the interaction between the active compound, curcumin and the structural lipoproteins. This increased interaction being detrimental factor for the inhibition of the Gram-positive microbes.

During the agar well diffusion assays all except three of the eight Gram- negative strains were observed to be intermediately inhibited by the extracts of turmeric. ***K. pneumoniae*** ATCC 13883 and ***Shigella spp*** demonstrated resistance when tested against the aqueous extracts of turmeric (Table 4.7) with ***K. pneumoniae*** particularly resistant when tested against the cold water extract of turmeric. Microbial resistance was observed most frequently amongst the aqueous extracts raising the question of whether or not these mediums were effective enough to allow the release and activation of the active compounds present in turmeric, or could it just have been an instance of an insufficient concentration of the extract being used to induce an inhibitory effect. A possible answer to the latter question was in the observation of significantly low MIC values, including those of the aqueous extracts that (Figure 4.1 and Figure 4.2) indicated otherwise.

Predominately low MIC was confirmation that the active compounds of turmeric were in-fact active and as a result caused inhibition of microbial growth in dilution concentrations as low as 0.39 mg/ml with an average inhibition zone size of 2.7mm-13mm

demonstrating an overall resistance exhibited by the microorganisms that can be attributed to the insufficient concentration of extract being used to bring about microbial inhibition.

Once again the assumption of Gram-positive microorganisms, being structurally more stable and therefore more resistant than Gram-negative microorganisms due to their cell wall composition was not applicable. Greater susceptibility observed in the Gram-positive microbes may be related to the bioactive polyphenolic phytochemical curcumin that is found in turmeric. This active compound is known to be the derivative of a wide group of phenylpropane compounds in the highest state of oxidation that are also known be effective against bacteria, fungi and viruses (Xu ***et al***., 1996). It is the hydroxylated groups of the phenolic compounds that are thought to be related to the relative toxicity of turmeric against various microorganisms (Geissman. 1963).

Many researchers have found that phenolic toxicity may be induced as a result of enzyme inhibition by the oxidized compounds, possibly through reaction with one or more non-specific interactions with proteins within the microbial walls and the cells themselves. Given that Gram-positive microorganisms are composed of a greater amount of structural protein than the Gram-negative, implies that there may have been increased interaction between the structural proteins and the toxic hydroxylated groups that may have been the detrimental factor for the cause of cellular inhibition and death (Manson and Wasserman., 1987).

5.3 Nutmeg: antimicrobial sensitivity and resistance

In the investigation assay for the extracts of the spice nutmeg, it was observed that Gram-positive ***B. subtilis*** ATCC 6051 displayed some resistance when tested against the aqueous extracts of nutmeg during disc (Table 4.14) and agar well (Table 4.15) diffusion assay respectively. Conversely to the susceptibility of the Gram-positive microbes observed in the testing of turmeric extracts, here the resistance of the Gram-positive microorganisms be attributed to the structural complexity of their cell walls making it difficult for the active components of the extract to enter the cell wall of the microbes and cause inhibition. A possible future investigation may reveal that if the concentrations of the extracts used are increased, the structural components of these microbes may be weakened inducing cellular malfunctioning, inhibition and possible cell death. Agar well diffusion all of the Gram-positive strains of ***S. aureus*** were observed to be inhibited by the extracts of nutmeg with inhibition zones in the range of 8mm – 15mm (Table 4.15) which further demonstrated that the active ingredients of nutmeg, like turmeric was independent on whether or not the microorganisms were Gram-positive- or Gram-negative when carrying out its inhibitory action.

Disc diffusion assay of the Gram-negative microorganisms indicated that all microorganisms were inhibited by all the extracts of nutmeg. ***Shigella sonnei*** of the Gram-negative microorganisms exhibited some resistance when tested against all but the hot water extract of nutmeg (Table 4.12). Repetitive screening confirmed that the microbes did exhibit sensitivity to these extracts. ***Shigella flexneri*** was susceptible when tested against all extracts of nutmeg (Table 4.12) Gram-negative ***E. coli*** U15055 was

observed to be resistant when tested against cold and hot water extracts of nutmeg during disc diffusion assaying (Table 4.7) and the acetone, cold, and hot water extracts during agar well assaying (Table 4.11). However the profound antimicrobial activity of the extracts against the various Gram-negative microbes indicated once again that the peptidoglycan lacking cell walls allows for cell wall penetration and destruction leading to growth inhibition (Chan ***et al***., 1993).

The susceptibility exhibited by these microorganisms can be related to research conducted on the volatile oils of the spice. Phytochemical studies show that the antimicrobial activity of nutmeg stems from a volatile essential oil that contains myristin and myristic acid. Nutmeg yields 5-15% of this volatile oil that has also been found to contain pinene, myristicin, eugenol, elemian, safole, lignas and neolignas (Ahmad ***et al***., 2005). In support of this study, additional chemical constituents have also been reported by Nakatani (2003) which claim that the monoterpens present in nutmeg showed promising antimicrobial activity. This claim by Nakatani (2003) was to a great extent validated by the antimicrobial activity observed during the investigation.

The inhibiting action of the essential oils in nutmeg and their chemical constituents has been hypothesized to sensitize the phospholipid bilayer of the microbial cytoplasmic membrane causing increased permeability, reducing the availability of vital intracellular substances thereby depriving the cell of nutrients which led to impaired bacterial enzyme function and eventual overall cellular collapse and death (Juven ***et al***., 1994; Kim ***et al***., 1995).

To further substantiate these finding, reports by Lattaoui and Tantaoui (1994) state that essential oils containing eugenol (as in the case of nutmeg) posses significant antimicrobial performing ability. Adequate production of the essential oil leads to increased antimicrobial activity. This dependency relies on the hydrophobicity and partitioning in the microbial plasmatic membrane. The penetration of the essential oil molecules into the plasmatic membrane affects the proton motive force, intracellular adenosine-triphosphate (ATP) content and the overall functioning of cellular activity including turgor pressure control, solute transport and metabolic regulation (Lanciotto ***et al***., 2004). Prolonged or irreversible failure in one or more of these systems was detrimental to living cells. It was thereby concluded that the weak cellular stability of Gram-negative microorganisms resulted in an increase amount of eugenol molecules penetrating the cell leading to cellular malfunctioning and eventual death.

The effectiveness of nutmeg extracts against the different microbial strains indicated the potential of nutmeg for treatment involving the successful inhibition of these microbial strains. The minimal concentrations of extracts required for inhibition of Gram-positive microbes were predominately in the lower concentration range of 0.39 mg/ml – 0.78 mg/ml (Figure 4.6) whilst the MIC values of Gram-negative microbes were in the range of 0.39 mg/ml – 3.12 mg/ml (Figure 4.4 - Figure 4.5). These low concentration ranges of both Gram-positive- and Gram-negative microbes indicated effectiveness by minute concentrations of the extract on microbial growth thereby demonstrating the antimicrobial potential of nutmeg for reasons given and further confirmed that Gram-

positive microbes were susceptible than Gram-negative microbes as indicated by the analysis of the mean resultant inhibition zone size in Figure 4.46.

5.4 Chilli: antimicrobial sensitivity and resistance

In the investigation of chilli, the ATCC 11775 strain of ***E. coli*** was noted to be the most susceptible of the Gram-negative microorganisms when tested against the various extracts (Table 4.16 and Table 4.17; Figure 4.19 and Figure 4.20). ATCC 6051 strain of ***B. subtilis*** (Table 4.4.17), ***S. aureus*** 12600 and ***K. pneumoniae*** ATCC 13883 (Table 4.20) were observed to exhibit the greatest zones of inhibition during both assays for the spice chilli. Analysis of the average inhibition zone sizes indicated that the Gram-negative microorganisms, ***E. coli*** U15055 and ***Salmonella spp*** exhibited most resistance whilst Gram-negative ***E. coli*** 17755, ***E. coli*** U16403, ***E. coli*** U16404, ***K. pneumoniae***, ***Salmonella*** and ***Shigella*** species exhibited distinct zones of inhibition in the range of 9mm – 26mm. The Gram-positive microbes, ***S .aureus*** T1266 and ***S. aureus*** P4790 were also observed to be susceptible predominately of intermediated inhibition (1mm – 15mm). For the duration of the investigation, the appearance of intermediate zones of inhibition were considered to have one of two possible explanations, that is; a) that those microbes exhibiting intermediate inhibition may indicate that if the concentrations of extract used was reduced, the same microbe could develop resistance to the same extracts against which they were initially intermediately inhibited or b) when concentration of the same spice extract is increased the microorganisms may exhibit complete inhibition (this may be confirmed upon potential future research).

According to antimicrobial research conducted by Outara ***et al***. (1997), the antimicrobial effectiveness of spices can be classified as strong, medium or weak. Similarly in this investigation, the antimicrobial activities of the various extracts were classified by the measurement of the visual zones of inhibition during the testing procedures. This technique exhibited that Gram-negative microorganisms were over ally more susceptible to the active substances in chilli than the Gram-positive (table 4.16 and Table 4.21) with the exception of ***B. subtilis*** and ***S. aureus*** (ATCC) in which case both strains were strongly inhibited by the extracts of chilli.

Similar to the conclusion that the resultant inhibitions of turmeric and nutmeg extracts were based on the differences within the cell wall structural integrity of Gram-positive and Gram-negative microorganisms, so to was the conclusion of chilli. In the case of chilli, the dense cell wall in Gram-positive composed of a peptidoglycan matrix responsible for the greater resistance to the antimicrobial activities of the chilli extracts. The cell wall serves to protect the intracellular functional components of the cells thereby exhibiting cellular resistance that was observed as intermediate inhibition (partial resistance) and no inhibition (complete resistance). Gram-negative microorganisms on the other hand due to the lack of the thick protective peptidoglycan cell wall component are structurally weaker cells increasing their susceptibility to the antimicrobial activities of the chilli extracts. The weaker cell wall allows for easy access by the antimicrobial agent into the intracellular component of the bacterial cell and the induction of microbial inhibiting activity leads to cell destruction and the appearance of zones of complete inhibition, in both instances leading to cellular inhibition and death (Chan ***et al***., 1993).

Furthermore, although the exact mechanism of antibacterial action of many spices has not yet been identified (Lanciotti ***et al***., 2004) many scientists hypothesise that other factors may play a vital role in the antimicrobial effectiveness of spices. Such factors include the hydrophobic and hydrogen bonding of phenolic compounds to the membrane proteins, followed by the perturbation of the lipid bilayer (Juven ***et al***., 1994). Membrane inhibition then occurs as a result of increased membrane permeability and fluid influx (Cox ***et al***., 2000), membrane destruction, destruction of the electron transport system and cell wall perturbation then lead to the death of the microbial cells (Evandro ***et al***., 2005).

MIC tests conducted on all Gram-positive microorganisms indicated that the concentration of extracts required for a reaction to occur was uniformly higher than those of the Gram-negative microorganisms with ***S. aureus*** 12600 and ***S. aureus*** T1266 exhibiting MIC values in the range of 0.39 mg/ml – 6.26 mg/ml (Figure 4.21). The remaining Gram-positive microorganisms demonstrated relatively lower MIC values in the range of 0.39 mg/ml – 1.56 mg/ml. Gram-negative ***E. coli*** U15055 exhibited uniformly higher MIC values than all other Gram-negative microorganisms (Figure 4.7), this was consistent with the high resistance exhibited during susceptibility testing (Table 4.17 and Table 4.17). The remaining Gram-negative microbes, MIC values exhibited within the lower concentration range of 0.39 mg/ml - 0.78 mg/ml with an exception to ***K. pneumoniae*** ATCC 13883 which had an MIC value for methanol extract of chilli of 6.25 mg/ml (Fig 4.8). The low MIC values observed were confirmation of the effectiveness of the active ingredients in chilli against the various Gram-positive- and Gram-negative microorganisms.

The resultant analysis if the average inhibition zone sizes for disc diffusion assaying it can be concluded that all of the extracts of chilli exhibited significant amounts of antimicrobial activity and that the active terpenoid compound, capsaicin found in chilli (Cowan. 1999) must be duly noted as a phytochemical whose effectiveness as an antimicrobial potential cannot be ignored. Terpenoids, which are synthesized from acetate units within the spices contain extensive branching and are cyclic. Terpenes are active against bacteria and although the mechanism of action is not fully understood, it is speculated that disruption of the cell membrane by terpenoids is the destructive force of antimicrobial activity in chilli (Chaurasia and Vyas., 1997).

5.5 Ginger: antimicrobial sensitivity and resistance

Disc diffusion assay for the spice ginger demonstrated complete inhibition by the Gram-positive ATCC microorganisms of ***B. subtilis*** and ***S. aureus*** when tested against the all organic extracts and intermediate inhibition when tested against the aqueous extracts. The Gram-positive microorganisms all predominately exhibited intermediate zones of inhibition with ***S. aureus*** T1266 and ***S. aureus*** P4790 (Table 4.26 and Table 4.27) also exhibiting absolute inhibition when tested against the aqueous extracts once again thereby demonstrating the antimicrobial effectiveness of the ginger extracts on the Gram-positive microorganisms.

E. coli U15055 of the Gram-negative microbes was also completely resistant against all extracts of the ginger during disc diffusion assay (Table 4.22 and Table 4.23). ***K. pneumoniae*** ATCC 13883 was observed to show both extremes during testing, i.e.

absolute inhibition and resistance when tested against extracts of ginger whilst ***Salmonella spp***. also showed resistance when tested against the methanol extract of ginger. ***Shigella flexneri*** and ***Shigella sonnei*** were both intermediately inhibited by the extracts of ginger. Confirmation of sensitivity testing was achieved by the results of the agar well diffusion indicated that of all the Gram-positive- and Gram-negative microorganisms present, ***E. coli*** U15055 was the only microbe that exhibited complete resistance when tested against all extracts of ginger.

The question that prompted an answer was; how was it possible for the active ingredients present in ginger to possess a broad spectrum of antimicrobial activity in both Gram-positive- and Gram-negative microorganisms?

The answer may lie herein; ginger like turmeric belongs to the botanical family ***Zingiberaceae***. The antimicrobial action of ginger is due to the phenolic derivatives present in ginger. The hydroxylated group of gingerole (active principle found in ginger) is known to be responsible for destabilization of the phospholipid bilayer thereby causing increase cellular permeability and cellular malfunctioning (Lanciotti ***et al***., 2004).

Furthermore, it has been reported that the essential oils of ginger also contain eugenol (Karapinar and Aktug., 1987) and carvacrol (Lattaoui and Tantaoui-Elaraki., 1994) that are responsible for intracellular metabolic interruptions bringing about malfunctioning of the microbial cells and cellular death (Lanciotti ***et al***., 2004). One or a combination of

these actions was responsible for the different extents of antimicrobial activity of ginger proving that the aim of the study was once again achieved.

MIC values (Figure 4.30, Figure 4.33 and Figure 4.36) were mostly in the range of 0.39 mg/ml -1.56 mg/ml. This noticeably low concentration of the ginger extract required for microbial inhibition once again demonstrated the antimicrobial effectiveness of the extracts on both Gram-positive- and Gram-negative microorganisms.

5.6 Cinnamon: antimicrobial sensitivity and resistance

And finally, sensitivity and MIC value determinations were conducted for the spice extracts of cinnamon where it was observed that the ATCC 11775 strain of ***E. coli*** exhibited resistance when tested against the extracts of methanol and hot water (Table 4.28). The possible reason for this could once again lie in any one or a combination of the three potential explanations, 1) the structural integrity of the microbial cell wall did not permit the antimicrobial agent to penetrate and cause inhibition, 2) the dilution medium was not effective in the release or may have destroyed the active principles of the spice, or 3) by simply increasing the concentration of the extract used, greater antimicrobial activity of the particular extract used may be permitted. In addition to this, the research more often than not indicated that cinnamaldehyde, a non-phenolic compound present in cinnamon is capable of antimicrobial efficiency by inhibiting amino acid decarboxylation activity within the cells. This inhibitory action leads to energy deprivation within the microbial cell and death (Wendakoon and Sakaguchi., 1995).

Observation during the investigation showed that all of the ***S. aureus*** microbial strains and ***B. subtilis*** demonstrated either absolute or intermediate inhibition when tested against the five extracts of cinnamon (Table 4.33). The sensitivity of these microorganisms indicated that despite the structural complexity of the Gram-positive microbes, cinnamon has the potential to penetrate and cause inhibition within the cells. This inhibitory action is evidence that cinnamon does posses good antimicrobial properties and that its future use as an antimicrobial agent is promising.

The variability in the extent of inhibition within the various microbial strains may ultimately be related to the Gram- reaction of the microbes themselves and cellular destruction as described earlier in the discussion. Chemical constituents of essential oils found in cinnamon include ethyl cinnamate, eugenol and chavicol (Alan. 1988). As previously stated, these are phenolic compounds that are capable of further cellular destruction and inhibition by establishing the hydrophobic and hydrogen bonding of these degradative phenolic compounds to membrane proteins resulting in portioning of the lipid bilayer (Juven ***et al***., 1994), perturbation of membrane permeability leading to inhibition of embedded enzymes (Cox ***et al***., 2002), membrane destruction, destruction of electron transport system (Tassou ***et al***., 1995), and cell wall perturbation (Odhav ***et al***., 2002) leading to cellular inhibition and death. This can be observed from the results, both Gram-positive- and Gram-negative microorganisms profound exhibited susceptibility when tested against the various extracts of cinnamon demonstrating the versatility of the active ingredients found in cinnamon as effective antimicrobial agents.

5.7 Comparative controls and MIC tests conducted

For control measures, a positive control test using synthetic neomycin was also conducted. Gram-positive- and Gram-negative microorganisms (Table 4.1 and Table 4.2) were completely inhibited by the control antibiotic which has known antimicrobial effects. Similar findings (zones sizes) in Gram-positive- and Gram-negative microbes was observed and served to reinforce the conclusion demonstrated by the sensitivity exhibited by each the spice. This was an indication of susceptibility exhibited by both Gram-positive- and Gram-negative microorganisms and antimicrobial potential contained within the spices themselves. DMSO that was used as a negative control demonstrated no effect as an antimicrobial agent. This synthetic compound (Ellis ***et al***., 1996) was used and provided visible results which demonstrated that no inhibition had occurred. This aided in the comparison of extracts with antimicrobial activity thereby proving that these extracts did in fact posses activity far greater than that of the negative inhibition of DMSO.

Furthermore the MIC values of these synthetic compounds were also determined as the positive and negative controls. Neomycin was observed to have value in the lower concentration range of 0.46 mg/ml – 1.8mg/ml proving to be highly effective as an antibiotic against the various Gram-positive- and Gram-negative microorganisms. The results for the negative control using DMSO were recorded as negative (Table 4.3).

For the purpose of this investigation the positive control tests provided a measure of comparison for the effectiveness displayed by the various spice extracts when tested. The

similarly low MIC values observed for the various spice extracts during testing once again confirmed the these extracts are capable of antimicrobial inhibition that was induced by the medically known and used antibiotic, neomycin. This implied that selected spices were successful as antimicrobial agents and may in the future find themselves as part of an international medical system for the treatment of the one or any of the various microbial strains tested during the investigation.

Chapter six

Conclusion

The emergence of antimicrobial resistance is becoming a world wide problem facing many if not all health professions. Technology has yet to put a halt to the evolutionary tactics of many new and old pathogens. The attempt to discover new natural therapeutics, has enticed scientists all over the world to embark on a search into the field of ethnobotany, within which may lay vast and important ethno-pharmacological remedies.

This study has evaluated the antimicrobial activity of some well known culinary spices (turmeric, ginger, cinnamon, nutmeg and chilli) with emphasis on their application as effective antimicrobial agents, thereby enhancing the extent of ethno-botanical medicine.

Results obtained it was observed that the active ingredients curcumin, eugenol, capsaicin, cinnamaldehyde and chavicol contained within the selected spices are effective antimicrobial agents that can inhibit and cause microbial death of various microorganisms.

From the total AVONA results of the five spices, twelve microbial species and the various spice extracts tested, the investigation concluded that Gram-positive- and Gram-negative microorganisms exhibited successful inhibition during testing. The assumption of Gram-positive microbes being less susceptible than Gram-negative was disproved and graphically represented in Graphs 4.46 – Graph 4.47. The overall analysis clearly indicated that ***E. coli*** U15055 was the most resistant of the twelve microbes with ATTC,

B. subtilis, ***E. coli*** and ***S. aureus spp*** exhibiting greatest susceptibility with the overall conclusion that ethanol was determined as the most effective extracts followed by acetone, methanol, cold and then hot water (Graph 4.48 and Graph 4.49).

The ability of these spices to interact with the lipid bilayer of the cell wall inducing cellular inhibition and death coupled with other carefully monitored factors such as the type of spices used, the composition and concentration of spice extract and the resident microbial occurrence on the spice itself, has all played an important role in their function as antimicrobial agents. The varying ability of each spice extract to exhibit antimicrobial activity suggests that the culinary spices often used as normal ingredients in our everyday cooking can to some extent act as agents providing protection against certain known pathogens with chilli, turmeric, cinnamon, nutmeg and ginger providing these optimal inhibitory effect in descending order (Graph 4.50).

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APPENDIX A

Table A.1 Mass of extract and volume of DMSO added for the spice turmeric.

Solvent	Actual mass of extract (g)	Amount of DMSO added (ml)
Ethanol	0.71g	7.1ml
Methanol	1.27g	12.7ml
Acetone	0.91g	9.1ml
Hot water	0.60g	6ml
Cold water	0.44g	4.4ml

Table A.2 Mass of extract and volume of DMSO added for the spice nutmeg.

Solvent	Actual mass of extract (g)	Amount of DMSO added (ml)
Ethanol	1.5g	15ml
Methanol	1.33g	13.3ml
Acetone	1.29g	12.9ml
Hot water	0.29g	2.9ml
Cold water	0.34g	3.4ml

Table A.3 Mass of extract and volume of DMSO added for the spice chilli.

Solvent	Actual mass of extract (g)	Amount of DMSO added (ml)
Ethanol	2.42g	24.2ml
Methanol	2.57g	25.7ml
Acetone	1.1g	11ml
Hot water	2,53g	25.3ml
Cold water	1.85g	18.5ml

Table A.4 Mass of extract and volume of DMSO added for the spice ginger.

Solvent	Actual mass of extract (g)	Amount of DMSO added (ml)
Ethanol	0.96g	9.6ml
Methanol	0.87g	8.7ml
Acetone	0.35g	3.5ml
Hot water	1.12g	11.2ml
Cold water	1.05g	10.5ml

Table A.5 Mass of extract and volume of DMSO added for the spice cinnamon.

Solvent	Actual mass of extract	Amount of DMSO added
Ethanol	0.83g	8.3ml
Methanol	1.05g	10.5ml
Acetone	0.68g	6.8ml
Hot water	2.61g	26.1ml
Cold water	1.95g	19.5ml

APPENDIX B

Table B. 1 – Table B. 12 tabulate the descriptive statistical analysis for each microbial strain tested against each extract of turmeric. The range, mean (and the standard error), standard deviation and variance are represented:

Table B.1 Descriptive statistics of *E. coli* ATCC 11775 for extracts of turmeric.

	N	Range	Mean		Std. Deviation	Variance
	Statistic	Statistic	Statistic	Std. Error	Statistic	Statistic
Acetone	4	5.00	13.00	1.08	2.16	4.66
Ethanol	4	6.00	14.50	1.32	2.64	7.00
Methanol	4	5.00	18.75	1.10	2.21	4.91
Hot water	4	3.00	10.25	0.75	1.50	2.25
Cold water	4	6.00	12.00	1.35	2.70	7.33

Table B. 2 Descriptive statistics of *E. coli* U15055 for extracts of turmeric.

	N	Range	Mean		Std. Deviation	Variance
	Statistic	Statistic	Statistic	Std. Error	Statistic	Statistic
Acetone	4	11.00	5.50	3.17	6.35	40.33
Ethanol	4	11.00	5.25	3.03	6.07	36.91
Methanol	4	10.00	2.50	2.50	5.00	25.00
Hot water	4	0.00	0.00	0.00	0.00	0.00
Cold water	4	0.00	0.00	0.00	0.00	0.00

Table B. 3 Descriptive statistics of *E. coli* U16403 for extracts of turmeric.

	N	Range	Mean		Std. Deviation	Variance
	Statistic	Statistic	Statistic	Std. Error	Statistic	Statistic
Acetone	4	2.00	9.87	0.42	0.85	0.72
Ethanol	4	10.00	18.50	2.10	4.20	17.66
Methanol	4	3.00	11.62	0.68	1.37	1.89
Hot water	4	1.50	9.87	0.31	0.62	0.39
Cold water	4	2.00	10.62	0.47	0.94	0.89

Table B. 4 Descriptive statistics of *E. coli* U16406 for extracts of turmeric.

	N	Range	Mean		Std. Deviation	Variance
	Statistic	Statistic	Statistic	Std. Error	Statistic	Statistic
Acetone	4	5.00	12.25	1.10	2.21	4.91
Ethanol	4	2.00	14.00	0.40	0.81	0.66
Methanol	4	1.00	10.62	0.23	0.47	0.22
Hot water	4	2.00	10.25	0.47	0.95	0.91
Cold water	4	2.00	10.00	0.40	0.81	0.66

Table B. 5 Descriptive statistics of *K. pnuemoniae* ATCC 13883 for extracts of turmeric.

	N	Range	Mean		Std. Deviation	Variance
	Statistic	Statistic	Statistic	Std. Error	Statistic	Statistic
Acetone	4	9.00	16.25	2.21	4.42	19.58
Ethanol	4	4.00	19.75	0.85	1.70	2.91
Methanol	4	7.00	12.00	1.77	3.55	12.66
Hot water	4	0.00	0.00	0.00	0.00	0.00
Cold water	4	8.00	13.50	1.70	3.41	11.66

Table B. 6 Descriptive statistics of *Salmonella spp.* for extracts of turmeric.

	N	Range	Mean		Std. Deviation	Variance
	Statistic	Statistic	Statistic	Std. Error	Statistic	Statistic
Acetone	4	3.00	10.37	0.68	1.37	1.89
Ethanol	4	2.50	9.37	0.55	1.10	1.22
Methanol	4	3.50	11.87	0.77	1.54	2.39
Hot water	4	2.50	10.37	0.55	1.10	1.22
Cold water	4	3.00	10.37	0.68	1.37	1.89

Table B. 7 Descriptive statistics of *Shigella flexneri* for extracts of turmeric.

	N	Range	Mean		Std. Deviation	Variance
	Statistic	Statistic	Statistic	Std. Error	Statistic	Statistic
Acetone	4	2.00	10.25	0.47	0.95	0.91
Ethanol	4	4.00	11.25	0.85	1.70	2.91
Methanol	4	1.50	9.62	0.37	0.75	0.56
Hot water	4	1.00	9.50	0.28	0.57	0.33
Cold water	4	0.00	9.00	0.00	0.00	0.00

Table B. 8 Descriptive statistics of *Shigella sonnei* for extracts of turmeric.

	N	Range	Mean		Std. Deviation	Variance
	Statistic	Statistic	Statistic	Std. Error	Statistic	Statistic
Acetone	4	1.50	9.87	0.31	0.62	0.39
Ethanol	4	1.00	9.50	0.28	0.57	0.33
Methanol	4	11.00	7.62	2.57	5.15	26.56
Hot water	4	2.00	9.75	0.47	0.95	0.91
Cold water	4	11.00	7.12	2.43	4.87	23.72

Table B. 9 Descriptive statistics of *B. subtilis ATCC 6051* for extracts of turmeric.

	N	Range	Mean		Std. Deviation	Variance
	Statistic	Statistic	Statistic	Std. Error	Statistic	Statistic
Acetone	4	11.00	16.75	2.46	4.92	24.25
Ethanol	4	9.00	19.50	1.93	3.87	15.00
Methanol	4	3.00	16.50	0.64	1.29	1.66
Cold water	4	0.00	0.00	0.00	0.00	0.00
Hot water	4	2.00	10.25	0.47	0.95	0.91

Table B. 10 Descriptive statistics of *S. aureus* ATCC 12600 for extracts of turmeric.

	N	Range	Mean		Std. Deviation	Variance
	Statistic	Statistic	Statistic	Std. Error	Statistic	Statistic
Acetone	4	9.00	16.00	2.12	4.24	18.00
Ethanol	4	6.00	16.50	1.50	3.00	9.00
Methanol	4	9.00	14.25	2.01	4.03	16.25
Cold water	4	2.00	12.25	0.47	0.95	0.91
Hot water	4	1.00	11.25	0.25	0.50	0.25

Table B. 11 Descriptive statistics of *S. aureus* T1266 for extracts of turmeric.

	N	Range	Mean		Std. Deviation	Variance
	Statistic	Statistic	Statistic	Std. Error	Statistic	Statistic
Acetone	4	4.00	13.75	0.85	1.70	2.91
Ethanol	4	7.00	13.50	1.55	3.10	9.66
Methanol	4	4.00	14.50	0.86	1.73	3.00
Cold water	4	4.00	10.25	0.94	1.89	3.58
Hot water	4	11.00	7.75	2.62	5.25	27.58

Table B. 12 Descriptive statistics of *S. aureus* P4790 for extracts of turmeric.

	N	Range	Mean		Std. Deviation	Variance
	Statistic	Statistic	Statistic	Std. Error	Statistic	Statistic
Acetone	4	3.00	12.75	0.62	1.25	1.58
Ethanol	4	8.00	17.00	1.77	3.55	12.66
Methanol	4	3.00	10.00	0.70	1.41	2.00
Cold water	4	3.00	10.25	0.75	1.50	2.25
Hot water	4	2.00	10.25	0.47	0.95	0.91

Tables B.13 – Table B.24 tabulate the descriptive statistical analysis for each microbial strain tested against each extract of nutmeg. The range, mean (and the standard error), standard deviation and variance are represented.

Table B. 13 Descriptive statistics of *E. coli* ATCC 11775 for extracts of nutmeg.

	N	Range	Mean		Std. Deviation	Variance
	Statistic	Statistic	Statistic	Std. Error	Statistic	Statistic
Acetone	4	5.50	11.75	1.16	2.32	5.41
Ethanol	4	6.00	11.75	1.43	2.87	8.25
Methanol	4	4.00	10.75	0.92	1.84	3.41
Cold water	4	6.50	23.00	1.48	2.97	8.83
Hot water	4	4.50	9.87	1.12	2.25	5.06

Table B. 14 Descriptive statistics of *E. coli* U15055 for extracts of nutmeg.

	N	Range	Mean		Std. Deviation	Variance
	Statistic	Statistic	Statistic	Std. Error	Statistic	Statistic
Acetone	4	12.00	8.25	2.78	5.56	30.91
Ethanol	4	1.50	9.62	0.37	0.75	0.56
Methanol	4	4.00	11.00	0.91	1.82	3.33
Cold water	4	0.00	0.00	0.00	0.00	0.00
Hot water	4	0.00	0.00	0.00	0.00	0.00

Table B. 15 Descriptive statistics of *E. coli* U16403 for extracts of nutmeg.

	N	Range	Mean		Std. Deviation	Variance
	Statistic	Statistic	Statistic	Std. Error	Statistic	Statistic
Acetone	4	4.00	12.75	1.03	2.06	4.25
Ethanol	4	2.00	9.75	0.47	0.95	0.91
Methanol	4	2.00	9.75	0.47	0.95	0.91
Cold water	4	2.00	10.62	0.47	0.94	0.89
Hot water	4	1.50	9.37	0.37	0.75	0.56

Table B. 16 Descriptive statistics of *E. coli* U16406 for extracts of nutmeg.

	N	Range	Mean		Std. Deviation	Variance
	Statistic	Statistic	Statistic	Std. Error	Statistic	Statistic
Acetone	4	4.00	13.75	0.85	1.70	2.91
Ethanol	4	4.00	11.00	1.00	2.00	4.00
Methanol	4	7.00	14.75	1.54	3.09	9.58
Cold water	4	4.00	10.75	0.85	1.70	2.91
Hot water	4	2.50	10.87	0.51	1.030	1.06

Table B. 17 Descriptive statistics of *K. pnuemoniae* ATCC 13883 for extracts of nutmeg.

	N	Range	Mean		Std. Deviation	Variance
	Statistic	Statistic	Statistic	Std. Error	Statistic	Statistic
Acetone	4	5.50	15.37	1.17	2.35	5.56
Ethanol	4	4.00	12.62	0.98	1.97	3.89
Methanol	4	6.00	12.62	1.28	2.56	6.56
Cold water	4	3.00	10.37	0.62	1.25	1.56
Hot water	4	4.00	8.75	0.85	1.70	2.91

Table B. 18 Descriptive statistics of *Salmonella spp.* for extracts of nutmeg.

	N	Range	Mean		Std. Deviation	Variance
	Statistic	Statistic	Statistic	Std. Error	Statistic	Statistic
Acetone	4	1.00	12.50	0.28	0.57	0.33
Ethanol	4	4.00	14.00	0.91	1.82	3.33
Methanol	4	2.00	11.75	0.47	0.95	0.91
Cold water	4	4.00	10.37	0.94	1.88	3.56
Hot water	4	5.00	13.50	1.19	2.38	5.66

Table B. 19 Descriptive statistics of *Shigella flexneri* for extracts of nutmeg.

	N	Range	Mean		Std. Deviation	Variance
	Statistic	Statistic	Statistic	Std. Error	Statistic	Statistic
Acetone	4	0.50	9.75	0.14	0.28	0.08
Ethanol	4	1.50	9.37	0.37	0.75	0.56
Methanol	4	11.00	7.75	2.62	5.25	27.58
Cold water	4	2.50	10.12	0.51	1.03	1.06
Hot water	4	1.00	9.50	0.28	0.57	0.33

Table B. 20 Descriptive statistics of *Shigella sonnei* for extracts of nutmeg

	N	Range	Mean		Std. Deviation	Variance
	Statistic	Statistic	Statistic	Std. Error	Statistic	Statistic
Acetone	4	10.00	7.25	2.42	4.85	23.58
Ethanol	4	10.00	7.12	2.38	4.76	22.72
Methanol	4	9.00	4.50	2.59	5.19	27.00
Cold water	4	2.00	10.75	0.43	0.86	0.75
Hot water	4	8.50	2.12	2.12	4.25	18.06

Table B. 21 Descriptive statistics of *B. subtilis* ATCC 6051 for extracts of nutmeg.

	N	Range	Mean		Std. Deviation	Variance
	Statistic	Statistic	Statistic	Std. Error	Statistic	Statistic
Acetone	4	3.50	8.12	0.77	1.54	2.39
Ethanol	4	14.00	8.25	3.11	6.23	38.91
Methanol	4	11.50	5.12	3.00	6.00	36.06
Cold water	4	2.50	7.62	0.55	1.10	1.22
Hot water	4	6.50	1.62	1.62	3.25	10.56

Table B. 22 Descriptive statistics of *S. aureus* ATCC 12600 for extracts of nutmeg.

	N	Range	Mean		Std. Deviation	Variance
	Statistic	Statistic	Statistic	Std. Error	Statistic	Statistic
Acetone	4	6.00	10.87	1.32	2.65	7.06
Ethanol	4	4.50	14.50	0.97	1.95	3.83
Methanol	4	2.50	10.50	0.54	1.08	1.16
Cold water	4	2.50	8.75	0.59	1.19	1.41
Hot water	4	5.00	12.37	1.12	2.25	5.06

Table B. 23 Descriptive statistics of *S. aureus* T1266 for extracts of nutmeg.

	N	Range	Mean		Std. Deviation	Variance
	Statistic	Statistic	Statistic	Std. Error	Statistic	Statistic
Acetone	4	3.00	10.75	0.62	1.25	1.58
Ethanol	4	1.00	10.25	0.25	0.50	0.25
Methanol	4	3.00	13.37	0.62	1.25	1.56
Cold water	4	3.00	13.50	0.64	1.29	1.66
Hot water	4	4.00	10.75	0.85	1.70	2.91

Table B. 24 Descriptive statistics of *S. aureus* P4790 for extracts of nutmeg.

	N	Range	Mean		Std. Deviation	Variance
	Statistic	Statistic	Statistic	Std. Error	Statistic	Statistic
Acetone	4	2.50	9.87	0.51	1.03	1.06
Ethanol	4	2.00	11.50	0.50	1.00	1.00
Methanol	4	3.00	10.75	0.75	1.50	2.25
Cold water	4	2.00	9.75	0.47	0.95	0.91
Hot water	4	2.00	11.00	0.40	0.81	0.66

Table B. 25 – Table B. 36 tabulate the descriptive statistical analysis for each microbial strain tested against each extract of chilli. The range, mean (and the standard error), standard deviation and variance are represented.

Table B. 25 Descriptive statistics of *E. coli* ATCC 11775 for extracts of chilli.

	N	Range	Mean		Std. Deviation	Variance
	Statistic	Statistic	Statistic	Std. Error	Statistic	Statistic
Acetone	4	8.00	23.00	1.68	3.36	11.33
Ethanol	4	9.50	21.12	2.20	4.40	19.39
Methanol	4	8.00	19.00	1.63	3.26	10.66
Cold water	4	4.00	20.62	0.85	1.70	2.89
Hot water	4	12.00	21.50	2.56	5.19	27.00

Table B. 26 Descriptive statistics of *E. coli* U15055 for extracts of chilli.

	N	Range	Mean		Std. Deviation	Variance
	Statistic	Statistic	Statistic	Std. Error	Statistic	Statistic
Acetone	4	0.00	0.00	0.00	0.00	0.00
Ethanol	4	0.00	0.00	0.00	0.00	0.00
Methanol	4	4.00	12.50	0.95	1.91	3.66
Cold water	4	3.00	10.12	0.65	1.31	1.72
Hot water	4	1.50	9.87	0.31	0.62	0.39

Table B. 27 Descriptive statistics of *E. coli* U16403 for extracts of chilli.

	N	Range	Mean		Std. Deviation	Variance
	Statistic	Statistic	Statistic	Std. Error	Statistic	Statistic
Acetone	4	9.00	15.50	1.84	3.69	13.66
Ethanol	4	2.00	11.62	0.47	0.94	0.89
Methanol	4	2.00	10.00	0.40	0.81	0.66
Cold water	4	1.00	9.50	0.20	0.40	0.16
Hot water	4	3.00	10.12	0.65	1.31	1.72

Table B. 28 Descriptive statistics of *E. coli* U16406 for extracts of chilli.

	N	Range	Mean		Std. Deviation	Variance
	Statistic	Statistic	Statistic	Std. Error	Statistic	Statistic
Acetone	4	2.00	9.87	0.51	1.03	1.06
Ethanol	4	4.00	10.75	0.85	1.70	2.91
Methanol	4	4.00	13.25	0.85	1.70	2.91
Cold water	4	7.00	21.25	1.49	2.98	8.91
Hot water	4	3.50	11.37	0.74	1.49	2.22

Table B. 29 Descriptive statistics of *K. pneumoniae* ATCC 13883 for extracts of chilli.

	N	Range	Mean		Std. Deviation	Variance
	Statistic	Statistic	Statistic	Std. Error	Statistic	Statistic
Acetone	4	7.50	22.87	1.58	3.17	10.06
Ethanol	4	6.00	15.50	1.32	2.64	7.00
Methanol	4	4.00	18.00	0.91	1.82	3.33
Cold water	4	7.00	20.25	1.65	3.30	10.91
Hot water	4	6.50	17.62	1.34	2.68	7.22

Table B. 30 Descriptive statistics of *Salmonella spp.* for extracts of chilli.

	N	Range	Mean		Std. Deviation	Variance
	Statistic	Statistic	Statistic	Std. Error	Statistic	Statistic
Acetone	4	3.50	10.87	0.82	1.65	2.72
Ethanol	4	2.50	10.62	0.55	1.10	1.22
Methanol	4	5.00	11.50	1.19	2.38	5.66
Cold water	4	3.50	11.87	0.82	1.65	2.72
Hot water	4	5.00	14.50	1.19	2.38	5.66

Table B. 31 Descriptive statistics of *Shigella flexneri* for the extracts of chilli.

	N	Range	Mean		Std. Deviation	Variance
	Statistic	Statistic	Statistic	Std. Error	Statistic	Statistic
Acetone	4	3.50	16.12	0.77	1.54	2.39
Ethanol	4	4.00	13.50	0.95	1.91	3.66
Methanol	4	5.50	16.12	1.19	2.39	5.72
Cold water	4	5.00	11.12	1.04	2.09	4.39
Hot water	4	3.00	10.75	0.75	1.50	2.25

Table B. 32 Descriptive statistics of *Shigella sonnei* for the extracts of chilli.

	N	Range	Mean		Std. Deviation	Variance
	Statistic	Statistic	Statistic	Std. Error	Statistic	Statistic
Acetone	4	2.00	14.00	0.57	1.15	1.33
Ethanol	4	3.00	10.75	0.62	1.25	1.58
Methanol	4	2.00	12.75	0.47	0.95	0.91
Cold water	4	3.00	10.25	0.62	1.25	1.58
Hot water	4	4.00	10.75	0.85	1.70	2.91

Table B. 33 Descriptive statistics of *B. subtilis ATCC* 6051 *for* the extracts of chilli.

	N	Range	Mean		Std. Deviation	Variance
	Statistic	Statistic	Statistic	Std. Error	Statistic	Statistic
Acetone	4	12.00	22.25	2.56	5.12	26.25
Ethanol	4	15.00	19.25	3.11	6.23	38.91
Methanol	4	11.00	19.37	2.57	5.15	26.56
Cold water	4	3.50	18.12	0.87	1.75	3.06
Hot water	4	7.00	17.00	1.58	3.16	10.00

Table B. 34 Descriptive statistics of *S. aureus* ATCC 12600 for the extracts of chilli.

	N	Range	Mean		Std. Deviation	Variance
	Statistic	Statistic	Statistic	Std. Error	Statistic	Statistic
Acetone	4	9.00	21.75	2.05	4.11	16.91
Ethanol	4	5.00	16.75	1.18	2.36	5.58
Methanol	4	4.00	15.50	0.95	1.91	3.66
Cold water	4	2.00	22.37	0.47	0.94	0.89
Hot water	4	1.00	19.37	0.23	0.47	0.22

Table B. 35 Descriptive statistics of *S. aureus* T1266 for the extracts of chilli.

	N	Range	Mean		Std. Deviation	Variance
	Statistic	Statistic	Statistic	Std. Error	Statistic	Statistic
Acetone	4	5.00	11.75	1.10	2.21	4.91
Ethanol	4	5.00	14.75	1.03	2.06	4.25
Methanol	4	3.00	12.25	0.62	1.25	1.58
Cold water	4	3.00	14.00	0.70	1.41	2.00
Hot water	4	3.00	10.12	0.65	1.31	1.72

Table B. 36 Descriptive statistics of *S. aureus* P4790 for the extracts of chilli.

	N	Range	Mean		Std. Deviation	Variance
	Statistic	Statistic	Statistic	Std. Error	Statistic	Statistic
Acetone	4	2.00	13.25	0.47	0.95	0.91
Ethanol	4	6.00	12.25	1.37	2.75	7.58
Methanol	4	2.00	10.25	0.47	0.95	0.91
Cold water	4	2.00	10.25	0.47	0.95	0.91
Hot water	4	3.00	11.00	0.70	1.41	2.00

Table B.37 – Table B.48 tabulate the descriptive statistical analysis for each microbial strain tested against each extract of ginger. The range, mean (and the standard error), standard deviation and variance are represented.

Table B. 37 Descriptive statistics of *E. coli* ATCC 11775 for the extracts of ginger.

	N	Range	Mean		Std. Deviation	Variance
	Statistic	Statistic	Statistic	Std. Error	Statistic	Statistic
Acetone	4	10.00	4.87	2.81	5.63	31.72
Ethanol	4	3.00	13.75	0.75	1.50	2.25
Methanol	4	4.00	11.00	0.81	1.63	2.66
Cold water	4	4.00	8.75	0.85	1.70	2.91
Hot water	4	4.50	11.62	1.02	2.05	4.22

Table B. 38 Descriptive statistics of *E. coli* U15055 for the extracts of ginger.

	N	Range	Mean		Std. Deviation	Variance
	Statistic	Statistic	Statistic	Std. Error	Statistic	Statistic
Acetone	4	0.00	0.00	0.00	0.00	0.00
Ethanol	4	0.00	0.00	0.00	0.00	0.00
Methanol	4	0.00	0.00	0.00	0.00	0.00
Cold water	4	0.00	0.00	0.00	0.00	0.00
Hot water	4	0.00	0.00	0.00	0.00	0.00

Table B. 39 Descriptive statistics of *E. coli* U16403 for the extracts of ginger.

	N	Range	Mean		Std. Deviation	Variance
	Statistic	Statistic	Statistic	Std. Error	Statistic	Statistic
Acetone	4	1.50	9.12	0.31	0.62	0.39
Ethanol	4	2.00	9.87	0.42	0.85	0.72
Methanol	4	2.00	9.87	0.42	0.85	0.72
Cold water	4	1.50	9.87	0.31	0.62	0.39
Hot water	4	3.00	10.37	0.62	1.25	1.56

Table B. 40 Descriptive statistics of *E. coli* U16406 for the extracts of ginger.

	N	Range	Mean		Std. Deviation	Variance
	Statistic	Statistic	Statistic	Std. Error	Statistic	Statistic
Acetone	4	2.00	10.00	0.40	0.81	0.66
Ethanol	4	6.00	12.25	1.25	2.50	6.25
Methanol	4	3.50	9.62	0.80	1.60	2.56
Cold water	4	5.00	12.75	1.18	2.36	5.58
Hot water	4	2.00	9.75	0.47	0.95	0.91

Table B. 41 Descriptive statistics of *K. pneumoniae* ATCC 13833 for the extracts of ginger.

	N	Range	Mean		Std. Deviation	Variance
	Statistic	Statistic	Statistic	Std. Error	Statistic	Statistic
Acetone	4	0.50	8.12	0.12	0.25	0.06
Ethanol	4	0.00	0.00	0.00	0.00	0.00
Methanol	4	2.00	9.62	0.47	0.94	0.89
Cold water	4	9.00	2.25	2.25	4.50	20.25
Hot water	4	10.00	6.50	2.25	4.50	20.33

Table B. 42 Descriptive statistics of *Salmonella spp.* for the extracts of ginger.

	N	Range	Mean		Std. Deviation	Variance
	Statistic	Statistic	Statistic	Std. Error	Statistic	Statistic
Acetone	4	2.00	10.00	0.45	0.91	0.83
Ethanol	4	4.00	13.50	0.95	1.91	3.66
Methanol	4	0.00	0.00	0.00	0.00	0.00
Cold water	4	2.00	10.75	0.43	0.86	0.75
Hot water	4	5.00	11.12	1.04	2.09	4.39

Table B. 43 Descriptive statistics of *Shigella flexneri* for the extracts of ginger.

	N	Range	Mean		Std. Deviation	Variance
	Statistic	Statistic	Statistic	Std. Error	Statistic	Statistic
Acetone	4	11.00	7.25	2.46	4.92	24.25
Ethanol	4	2.00	9.75	0.47	0.95	0.91
Methanol	4	4.00	10.75	0.85	1.70	2.91
Cold water	4	1.00	9.75	0.25	0.50	0.25
Hot water	4	3.00	10.25	0.62	1.25	1.58

Table B. 44 Descriptive statistics of *Shigella sonnei* for the extracts of ginger.

	N	Range	Mean		Std. Deviation	Variance
	Statistic	Statistic	Statistic	Std. Error	Statistic	Statistic
Acetone	4	11.00	7.62	2.56	5.12	26.22
Ethanol	4	11.00	5.25	3.03	6.07	36.91
Methanol	4	1.00	9.37	0.23	0.47	0.22
Cold water	4	9.50	6.87	2.29	4.58	21.06
Hot water	4	3.00	10.25	0.62	1.25	1.58

Table B. 45 Descriptive statistics of *B. subtilis* ATCC 6051 for the extracts of ginger.

	N	Range	Mean		Std. Deviation	Variance
	Statistic	Statistic	Statistic	Std. Error	Statistic	Statistic
Acetone	4	6.00	12.00	1.29	2.58	6.66
Ethanol	4	7.00	11.87	1.47	2.95	8.72
Methanol	4	3.50	10.12	0.77	1.54	2.39
Cold water	4	2.00	9.25	0.47	0.95	0.91
Hot water	4	1.00	9.50	0.20	0.40	0.16

Table B. 46 Descriptive statistics of *S. aureus* ATCC 12600 for the extracts of ginger.

	N	Range	Mean		Std. Deviation	Variance
	Statistic	Statistic	Statistic	Std. Error	Statistic	Statistic
Acetone	4	2.00	9.00	0.45	0.91	0.83
Ethanol	4	3.00	9.62	0.62	1.25	1.56
Methanol	4	1.00	8.37	0.23	0.47	0.22
Cold water	4	8.00	20.75	1.73	3.47	12.08
Hot water	4	1.50	8.87	0.31	0.62	0.39

Table B. 47 Descriptive statistics of *S. aureus* T1266 for the extracts of ginger.

	N	Range	Mean		Std. Deviation	Variance
	Statistic	Statistic	Statistic	Std. Error	Statistic	Statistic
Acetone	4	2.00	10.25	0.47	0.95	0.91
Ethanol	4	3.00	11.00	0.70	1.41	2.00
Methanol	4	2.00	10.62	0.47	0.94	0.89
Cold water	4	3.00	11.50	0.64	1.29	1.66
Hot water	4	4.00	11.75	0.85	1.70	2.91

Table B. 48 Descriptive statistics of *S. aureus* P4790 for the extracts of ginger.

	N	Range	Mean		Std. Deviation	Variance
	Statistic	Statistic	Statistic	Std. Error	Statistic	Statistic
Acetone	4	2.00	10.00	0.40	0.81	0.66
Ethanol	4	3.50	11.00	0.73	1.47	2.16
Methanol	4	2.50	11.37	0.55	1.10	1.22
Cold water	4	1.50	9.75	0.32	0.64	0.41
Hot water	4	2.00	11.00	0.40	0.81	0.66

Table B. 49 – Table B.60 tabulate the descriptive statistical analysis for each microbial strain tested against each extract cinnamon. The range, mean (and the standard error), standard deviation and variance are represented.

Table B. 49 Descriptive statistics of *E. coli* ATCC 11775 for the extracts of cinnamon.

	N	Range	Mean		Std. Deviation	Variance
	Statistic	Statistic	Statistic	Std. Error	Statistic	Statistic
Acetone	4	3.00	11.00	0.70	1.41	2.00
Ethanol	4	11.00	7.37	2.49	4.98	24.89
Methanol	4	0.00	0.00	0.00	0.00	0.00
Cold water	4	5.00	10.25	1.10	2.21	4.91
Hot water	4	0.00	0.00	0.00	0.00	0.00

Table B. 50 Descriptive statistics of *E. coli* U15055 for the extracts of cinnamon.

	N	Range	Mean		Std. Deviation	Variance
	Statistic	Statistic	Statistic	Std. Error	Statistic	Statistic
Acetone	4	3.00	11.87	0.65	1.31	1.72
Ethanol	4	3.00	13.75	0.75	1.50	2.25
Methanol	4	11.00	5.00	2.91	5.83	34.00
Cold water	4	3.00	11.75	0.75	1.50	2.25
Hot water	4	4.00	10.50	0.95	1.91	3.66

Table B. 51 Descriptive statistics of *E. coli* U16403 for the extracts of cinnamon.

	N	Range	Mean		Std. Deviation	Variance
	Statistic	Statistic	Statistic	Std. Error	Statistic	Statistic
Acetone	4	2.00	10.50	0.50	1.00	1.00
Ethanol	4	2.00	10.75	0.47	0.95	0.91
Methanol	4	2.00	9.62	0.47	0.94	0.89
Cold water	4	3.00	10.50	0.64	1.29	1.66
Hot water	4	4.00	10.75	0.85	1.70	2.91

Table B. 52 Descriptive statistics of *E. coli* U16406 for the extracts of cinnamon.

	N	Range	Mean		Std. Deviation	Variance
	Statistic	Statistic	Statistic	Std. Error	Statistic	Statistic
Acetone	4	3.50	11.87	0.77	1.54	2.39
Ethanol	4	3.00	10.37	0.62	1.25	1.56
Methanol	4	1.50	10.37	0.37	0.75	0.56
Cold water	4	13.50	9.12	3.08	6.16	38.06
Hot water	4	1.50	9.87	0.31	0.62	0.39

Table B. 53 Descriptive statistics of *K. pneumoniae* ATCC 13883 for the extracts of cinnamon.

	N	Range	Mean		Std. Deviation	Variance
	Statistic	Statistic	Statistic	Std. Error	Statistic	Statistic
Acetone	4	6.00	9.50	1.25	2.51	6.33
Ethanol	4	1.00	11.37	0.23	0.47	0.22
Methanol	4	2.50	9.25	0.52	1.04	1.08
Cold water	4	4.00	10.25	0.85	1.70	2.91
Hot water	4	3.00	8.12	0.71	1.43	2.06

Table B. 54 Descriptive statistics of *Salmonella spp.* for the extracts of cinnamon.

	N	Range	Mean		Std. Deviation	Variance
	Statistic	Statistic	Statistic	Std. Error	Statistic	Statistic
Acetone	4	2.00	11.00	0.40	0.81	0.66
Ethanol	4	5.00	13.75	1.10	2.21	4.91
Methanol	4	14.00	9.50	3.20	6.40	41.00
Cold water	4	2.00	12.50	0.50	1.00	1.00
Hot water	4	2.00	11.87	0.42	0.85	0.72

Table B. 55 Descriptive statistics of *Shigella flexneri* for the extracts of cinnamon.

	N	Range	Mean		Std. Deviation	Variance
	Statistic	Statistic	Statistic	Std. Error	Statistic	Statistic
Acetone	4	2.00	10.00	0.40	0.81	0.66
Ethanol	4	3.00	12.50	0.64	1.29	1.66
Methanol	4	4.00	11.00	0.81	1.63	2.66
Cold water	4	2.00	11.00	0.57	1.15	1.33
Hot water	4	3.00	10.00	0.70	1.41	2.00

Table B. 56 Descriptive statistics of *Shigella sonnei* for the extracts of cinnamon.

	N	Range	Mean		Std. Deviation	Variance
	Statistic	Statistic	Statistic	Std. Error	Statistic	Statistic
Acetone	4	2.00	10.00	0.40	0.81	0.66
Ethanol	4	2.00	10.00	0.40	0.81	0.66
Methanol	4	4.00	10.75	0.85	1.70	2.91
Cold water	4	1.50	9.87	0.31	0.62	0.39
Hot water	4	2.00	9.62	0.47	0.94	0.89

Table B. 57 Descriptive statistics of *B. subtilis* ATCC *6051* for the extracts of cinnamon.

	N	Range	Mean		Std. Deviation	Variance
	Statistic	Statistic	Statistic	Std. Error	Statistic	Statistic
Acetone	4	3.00	13.25	0.62	1.25	1.58
Ethanol	4	4.00	13.75	0.85	1.70	2.91
Methanol	4	2.50	11.00	0.54	1.08	1.16
Cold water	4	4.00	10.00	0.91	1.82	3.33
Hot water	4	2.00	10.50	0.50	1.00	1.00

Table B. 58 Descriptive statistics of *S. aureus* ATCC12600 for the extracts of cinnamon.

	N	Range	Mean		Std. Deviation	Variance
	Statistic	Statistic	Statistic	Std. Error	Statistic	Statistic
Acetone	4	2.00	9.87	0.426	0.85	0.72
Ethanol	4	3.50	9.75	0.72	1.44	2.08
Methanol	4	11.00	7.12	2.45	4.90	24.06
Cold water	4	2.00	8.50	0.45	0.91	0.83
Hot water	4	3.00	10.62	0.62	1.25	1.56

Table B. 59 Descriptive statistics of *S. aureus* T1266 for the extracts of cinnamon.

	N	Range	Mean		Std. Deviation	Variance
	Statistic	Statistic	Statistic	Std. Error	Statistic	Statistic
Acetone	4	2.00	11.00	0.40	0.81	0.66
Ethanol	4	2.00	10.25	0.47	0.95	0.91
Methanol	4	1.00	9.50	0.28	0.57	0.33
Cold water	4	1.00	9.25	0.25	0.50	0.25
Hot water	4	2.00	9.75	0.47	0.95	0.91

Table B. 60 Descriptive statistics of *S. aureus* P4790 for the extracts of cinnamon.

	N	Range	Mean		Std. Deviation	Variance
	Statistic	Statistic	Statistic	Std. Error	Statistic	Statistic
Acetone	4	1.00	9.50	0.28	0.57	0.33
Ethanol	4	2.00	10.00	0.40	0.81	0.66
Methanol	4	1.00	9.37	0.23	0.47	0.22
Cold water	4	1.50	12.87	0.31	0.62	0.39
Hot water	4	2.00	9.75	0.47	0.95	0.91

APPENDIX C

1. Preparation of Mueller-Hinton Agar

1.1 Application: it is a media used by Mueller and Hinton (1941) for testing the sensitivity of clinically important pathogens towards antibiotics or sulphonamides.

1.2 Typical Composition (grams per litre):

Meat infusion	5.0
Casein hydrolysate	17.5
Soluble starch	1.5
Agar	14.0

pH = 7.4 (±0.2) at 25°C.

1.3 Mode of Action:

The composition of the media provides favorable growth conditions. The media is almost totally devoid of sulphonamide antagonists.

In order to improve the growth of fastidious microorganisms, blood can be added to the Mueller-Hinton agar, but for the purpose of this investigation the addition of blood was not necessary. According to Jenkins ***et al.*** (1985), this may lead to false results when testing the susceptibility of enterococci to aminoglycosides.

1.4 Preparation:

38g of the agar powder was suspended in 1 litre of demineralised water and allowed to stand for 15 minutes. This was the heated until boiling whilst stirring until completely dissolved.

This was followed by autoclaving at 121°C for 15 minutes. Caution was taken not to overheat the media. Once autoclaved, the media was cooled to 45-50°C. It was mixed well/shaken and poured in Petri-plates. The plates were clear to opalescent in colour. The plates were then either immediately used for making spread plates or refrigerated and used at a later stage (Thrupp ***et al***., 1998) (www.merck.co.za).

2. The "Four-way streak" (Streak plate) method for inoculation:

The streak plate technique provides a simple and practical procedure to isolate pure cultures. It is essentially a dilution technique that spreads a loopful of culture over the surface of an agar plate. Although there are many ways to streak a plate to isolate colonies, the "four-way method" is simple, convenient and fast (***Chan et al.,*** 1993).

2.1 Procedure used to make a Spread Plate

- Spread the broth inoculum (aliquot) on top of a prepoured nutrient agar plate and spread with a sterile glass spreader.
- Leave the inoculated plate to stand for approximately three to five minute in an incubator preset with a temperature of 25°C for 48 hours in an inverted position to

prevent condensation from falling onto the surface and interfering with discrete colony formation (***Chan et al.,*** 1993).

3. **96-Well Plate Method for Antibacterial Testing** (Eickhoff, 1972).

DAY 1:

a. Autoclave distilled water, nutrient broth, nutrient broth in culture flasks, pipette tips.

b. Prepare cultures of bacteria and incubate overnight at 37°C.

DAY 2:

i) Dilute bacterial cultures 50:50 ratio with nutrient broth

ii) Make up 100mg/100ml stock solution of the test compound

iii) Add 25µl sterile water to all the wells

iv) Add 25µl of stock extract/compound to the wells in row A and mix well

v) Remove 25µl from wells in row A and transfer it to row B. mix well

vi) Repeat v) up to wells in row H

vii) Discard 25µl from wells in row H

viii) NB: each well now has 25µl of water and extract

ix) Add 25µl of the bacteria culture to all wells (Wells in row A now have 25% of the initial concentration of the stock, Wells in row B, 50% of A, etc…)

x) Cover the micro-plate and incubate overnight at 37°C.

DAY 3:

i) Make up the *P*-Iodonitrotetrazolium (INT) solution (0.2mg/ml)

ii) Add 10µl of INT to all wells

iii) Incubate at 37°C for 10-30 minutes

iv) INT is reduced to a reddish/pink coloured product by the mitochondrial dehydrogenase, and thus indicates living bacteria. A colourless well indicates inhibition in that well.

NB: If 100mg/100ml was the initial concentration of the stock solution, the final concentrations in the wells are:

A = 25 mg/ml

B = 12.5 mg/ml

C = 6.25 mg/ml

D = 3.125 mg/ml

E = 1.56 mg/ml

F = 0.78 mg/ml

G = 0.39 mg/ml

H = 0,195 mg/ml

For the determination of MIC values of controls the initial concentration of antibiotic and DMSO used positive and negative controls respectively was thirty microlitres. The final concentration in well for well A to well H were as follows:

A = 30 mg/ml

B = 15 mg/ml

C = 7.5 mg/ml

D = 3.75 mg/ml

E = 1.8 mg/ml

F = 0.93 mg/ml

G = 0.46 mg/ml

H = 0.23 mg/ml

4. Reagents used for Spice Extraction

- Methanol 215 – super purity solvent CH_3OH, ROMIL Chemicals Limited, 63 Ashley ,Shepted, Loughborough, Leics, LE12. 9Bs
- Absolute ethanol. Saarchem, Merck Chaemicals. 259 Davidson Road, Wadeville, Guateng, South Africa.
- Acetone. Merck Lab Supplies ,Fedstone Park, Unit 11, Tonnetti Street, Midrand, Guateng ,1685.
- Pure distilled water (heated and cooled water).

5. Antibiotic used for positive control testing

- Neomycin powder – 1 gram. POT: 100%

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