Safety and efficacy of traditional medicinal plant combinations for the treatment of sexually transmitted infections in Northern Maputaland, South Africa

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Declaration

I, Deshnee Naidoo, declare that this dissertation is my own work. It is being submitted in fulfilment for the degree of Master of Pharmacy at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at this or any other University.

Deshnee Naidoo

Date

Dedication

To my mother, sister and granny, Anjini, Yagashnie and ma, thank you for all the guidance and strength you have all instilled in me.

To my supervisor and co-supervisors, without you all I would never have been able to get the confidence to complete this project.

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Abstract

Sexually transmitted infections (STIs) are a global concern and more specifically southern Africa has seen a tremendous upsurge in infection rates. KwaZulu-Natal is the province found to have the highest Human Immunodeficiency Virus and STI infection rates. From an ethnobotanical study conducted specifically in northern Maputaland (Mabibi, Tshongwe, Mseleni and Mbazwana), it was found that the lay people most often used plants in various combinations for the treatment of STI related symptoms. The use of these plant combinations were thus antimicrobially investigated and the toxicity properties determined.

The dichloromethane: methanol (organic) and aqueous extracts were prepared for each plant *in situ* using collected ground dried plant material. The plants (individually and in combination) were investigated for toxic potential using the 3-[4,5-dimethyl-2-thiazol-yl]-2,5-diphenyl-2H-tetrazolium bromide (MTT) cellular viability assay on the human kidney epithelial (Graham) cell line. The antimicrobial activities for each sample, as well as for each combination, were then further investigated using the minimum inhibitory concentration (MIC) assay. The six STI pathogens investigated in this study were *Candida albicans* (ATCC 10321), *Ureaplasma urealyticum* (clinical strain), *Oligella ureolytica* (ATCC 43534), *Gardnerella vaginalis* (ATCC 14018), *Trichomonas vaginalis* (clinical strain) and *Neisseria gonnorhoeae* (ATCC 19424).

When investigating individual efficacies, *Ureaplasma urealyticum* was the most sensitive of the six test organisms, with the aqueous extract of *Ranunculus multifidus* (0.02 mg/ml) and the organic extract of *Peltophorum africanum* (0.04 mg/ml) found to be the most antimicrobially active plant species studied. *Sclerocarya birrea* and *Syzygium cordatum* were found to have the broadest spectrum of activity. The only plant to exhibit some toxicity against the kidney epithelial cell line was *Kigelia africana* (22% for aqueous extract) and 16% (organic extract) cell death) at a concentration of 10 mg/ml.

For the two plant combination studies, fractional inhibitory concentrations (ΣFICs) values of the seven combinations were calculated from the MIC data. The most synergistic interactions (ΣFIC

values > 0.50) for the two plant combinations were the aqueous extract of *Albizia adianthifolia* with *Trichilia dregeana* (ΣFIC = 0.15) and the organic extract of *Aloe marlothii* with *Senecio serratuloides* (ΣFIC = 0.31) against *O. ureolytica*. The only increase in toxicity was noted for the 1:1 combination of *S. birrea* and *S. cordatum* (aqueous extract: 25%, organic extract: 21% cell death).

For studies on varied ratio combinations, *A. adianthifolia* with *T. dregeana* and *S. birrea* with *S. cordatum* were found to be the most interesting. Synergistic interactions were found regardless of the ratios when either *A. adianthifolia* or *T. dregeana* (aqueous extract) were combined and tested against *O. ureolytica*. When *S. birrea* was combined with *S. cordatum* (aqueous extract) select ratios, *S. birrea*: *S. cordatum* (6:4, 3:7), were found to interact synergistically against *O. ureolytica* and found to be non-toxic against the kidney epithelial cell line.

For the poly-herbal combinations, no toxicity was found against the kidney epithelial cells. The most significant combinations *C. papaya*, *S. serratuloides* and *H. hemerocallidea* (organic extract; MIC = 0.25 mg/ml) and *Euphorbia hypericifolia*, *S. serratuloides*, *H. hemerocallidea* and *Ozoroa engleri* (aqueous extract; 0.50 mg/ml and organic extract 0.25 mg/ml) were most effective against *U. urealyticum*. Whereas the combination of *Bidens pilosa*, *R. multifidus*, *Sarcophyte sanguinea* and *Clematis brachiata* was most effective (MIC = 0.63 mg/ml) against *U. urealyticum*. These poly-herbal combinations exhibited no toxicity against the kidney epithelial cells.

The combination of *Carica papaya*, *S. serratuloides* and *H. hemerocallidea* was selected (using MODDE 9.1® software) to optimize the antimicrobial activity. The software was used to calculate the best ratios to combine the plants in order to obtain the lowest MIC value whereby there was a correlation for 71.4% of the experiments therefore demonstrating MODDE 9.1® software is beneficial to use.

Despite popular belief that by combining plants the antimicrobial efficacy would increase this was not substantiated with the tested poly-herbal combinations. However, it is possible that some plants have antimicrobial efficacy, whilst the others contribute toward symptomatic relief. It was encouraging to find that little toxicity is evident in the plants for this study area.

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Abbreviations

AIDS	Acquired immunodeficiency syndrome
ATCC	American type culture collection
CFU	Colony forming units
°C	Degree celsius
DMEM	Dulbeco's modified eagles medium
DMSO	Dimethyl sulfoxide
DoE	Design of Experiment
e.g.	Example
ΣFIC	Sum of fractional inhibitory concentration
g	Gram
HBI	Heart and brain infusion
HIV	Human Immunodeficiency Virus
GC	Gonnococcal
INT	*p*-Iodonitrotetrazolium violet
kPa	Kilo Pascals
L	Litre
LD_{50}	Lethal Dose 50%
MDR	Multidrug resistant
mg	Milligram
MIC	Minimum inhibitory concentration
MIMS	Monthly Index of Medical Specialities
ml	Millilitre

MTT	3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide
NCCLS	National Committee for Clinical Laboratory Standards
N/D	Not Determined
NHLS	National Health Laboratory Services
nm	Nano-metres
PBS	Phosphate buffer saline
PID	Pelvic inflammatory disease
RPM	Revolutions per minute
SAMF	South African Medicines Formulary
STI	Sexually transmitted infections
TB	Tuberculosis
TSA	Tryptone soya agar
TSB	Tryptone soya broth
UV	Ultra Violet
WHO	World Health Organisation
µl	Micro litres
µm	Micro metres

Chapter 1

Introduction

1.1 The sexually transmitted infection burden, globally and in southern Africa

According to the World Health Organisation (WHO) approximately 444 million global cases of curable sexually transmitted infections (STIs) (syphilis, gonorrhoea, chlamydia and trichomoniasis) are reported annually; whereby 111 million individuals infected with STIs are under the age of 25 years old (WHO, 2011). Furthermore, there is a staggering 42 million individuals infected with human immunodeficiency virus (HIV) worldwide. These infections have become a global concern. Pertaining to southern Africa, in 2008, there is reported an estimate of 5.2 million people infected with HIV (UNAIDS, 2002; UNAIDS, 2009). The WHO has also stated that the sub-Saharan African region has the highest STI rate in the world for those under the age of 25 years old (WHO, 2001). Furthermore, in 2000 STI infections were responsible for up to 26% of all deaths within South Africa (Johnson, 2008).

The human immunodeficiency virus and STIs are interchangeably related as the human immunodeficiency virus depletes an individuals' immune system therefore allowing opportunistic pathogens to invade the human body at a phenomenal rate. STIs rupture the genital membrane thereby increasing the risk of other STIs as well as the transmission of AIDS (Aquired Immunodeficiency Syndrome) (Laga et al., 1993; Cohen et al., 1997). Intense community campaigns have started to educate high school scholars to encourage safe sex practices, however, despite these attempts the STI rate continues to increase. Most STIs are contracted via sexual intercourse (e.g. gonorrhoea), however, there are many cases where the STI is contracted via close proximity (e.g. herpes) and lack of good hygiene (e.g. candidiasis). The route of entry for all STIs are via the male and female genital tracts. STIs are not limited to a specific age group, gender, creed or race group. In fact on the contrary, according to Bodley-Tickell et al., (2008), STIs affect infants, the younger generation ($\leq$ 25 years of age), as well as the older generations ($\geq$ 50 years of age).

The STI infection rate is increasing at a tremendous rate daily and this problem is compounded with the development of resistant strains. The treatment of these antibiotic resistant bacteria is of major concern to both healthcare workers and the public which is one possible reason why people are seeking other curative therapies such as herbal alternatives (Mugisha et al., 2008).

1.2 Pathogens associated with STIs

There are various STIs that present in the form of bacterial (e.g. gonorrhoea), fungal (e.g. candidiasis), protozoal (e.g. trichomoniasis) and viral (e.g. herpes) infections. The most common STI pathogens that are seen in southern African government clinics are *Neisseria gonorrhoeae*, *Trichomonas vaginalis*, *Ureaplasma urealyticum*, *Oligella ureolytica*, *Candida albicans*, *Gardnerella vaginalis*, *Chlamydia trachomatis*, *Treponema pallidum* and *Herpes simplex* (WHO, 2001; Donovan, 2004; UNAID, 2009). This study takes into account six of these pathogens when considering infections of the urogenital tract.

Neisseria gonorrhoeae; with an incubation period of 2-14 days is the disease causing agent for gonorrhoea and it is commonly referred to as ‘dropsy. *Neisseria gonorrhoeae* thrives in moist, dark, warm areas in the body. Clinical features in men are a continuous whitish-yellow membrane that is secreted from the penis. If severe, pelvic inflammatory disease (PID) may develop. In women, the infection manifests as a thick white membranous secretion that is discharged from the vaginal area and the endocervical canal. Both men and women experience burning on micturition (Hay and Ugwumadu, 2009). There is an odour associated with this infection in both men and women. An infection can be contracted by children if proper hygiene is not implemented by the parents, as well as infants born to mothers infected with gonorrhoea at the time of birth (Kumar and Clark, 2005). The antibiotic of choice is ciprofloxacin (SAMF, 2011).

Candida albicans manifests as a yeast infection called candidiasis. This condition presents following an incubation period of approximately 1 week on the skin and mucous membranes (mouth, vagina and rectum), and may disseminate into the blood stream if the infection is severe and wide spread. *Candida* infections are usually identified by a creamy white ‘cottage cheese’

growth that is inflamed. *C. albicans* is the cause of at least 80% of all vaginal candidiasis, but can also cause blisters on the penis (Richardson and Warnock, 2003). *Candida* infections are characterized by a yellow discharge with inflammation which will cause intense burning and an itching sensation. Topically applied clotrimoxazole or amphoteracin B are the treatment of choice, while for systemic infections fluconazole or itraconazole are used (SAMF, 2011).

Gardnerella vaginalis is a bacterial infection that is responsible for the condition known as bacterial vaginosis. It is easy to diagnose based on its common name since this infection affects females in the vaginal region. It is characterized by a grey or yellowish discharge with a very distinguishing 'fish odour' originating from the vaginal region (Hay and Ugwumadu, 2009). *Gardnerella vaginalis* has an incubation period of one week. The antibiotic of choice is metronidazole (SAMF, 2011).

Trichomonas vaginalis is classified as a flagellated protozoal pathogen (Kumar and Clark, 2005) and gives rise to an estimated 250 million cases worldwide of trichomoniasis (Mundodi et al., 2006). Women often experience urethral discharge which is foul smelling and dysuria is present. Complications include urethritis, cervitis, pelvic inflammatory disease and infertility (Kumar and Clark, 2005; Calzada et al., 2007). The incubation period for such an infection is one to two weeks. The antibiotic of choice is metronidazole (SAMF, 2011).

Oligella ureolytica is one of the causes of vaginal urethritis. Following an incubation period of 1-5 weeks the infection is characterized by a discharge, dysuria and a crusting membrane on the underwear in early mornings (Kumar and Clark, 2005). The antibiotic of choice is usually a stat dose of tetracycline or azithromycin (SAMF, 2011).

Ureaplasma urealyticum cause infections that lead to urethritis which may impair male fertility (Ochsendorf, 2008). Dysuria, inflammation of the vagina and burning upon micturition are also symptoms, following an incubation period of 2 to 3 weeks. An antibiotic that is effective for the treatment of the infections is ciprofloxacin (SAMF, 2011).

1.3 Background of study

KwaZulu-Natal is the province with the highest STI and HIV infection rate in South Africa (WHO, 2001). In a previous study by De Wet et al., (2012), four regions were selected in northern Maputaland (Mabibi, Tshongwe, Mseleni and Mabazwana) whereby the lay people were surveyed to gain information on their treatments of STIs (Figure 1.1). The surveys were conducted in the native language (isiZulu) with a translator present. Within these communities it was found that STIs are seen on a daily basis, where the economic status is low and hospitals/clinics are sparsely located. The low socio-economic status, with approximately 60% unemployment (Fyhrquist et al., 2002), plays a major role in the rural peoples' attitudes to modern healthcare as often healthcare becomes secondary to providing food for their families. The most commonly reported STIs treated by the lay people were urethritis, causative pathogens *U. urealyticum* and *O. ureolytica* as well as *N. gonorrhoeae*.

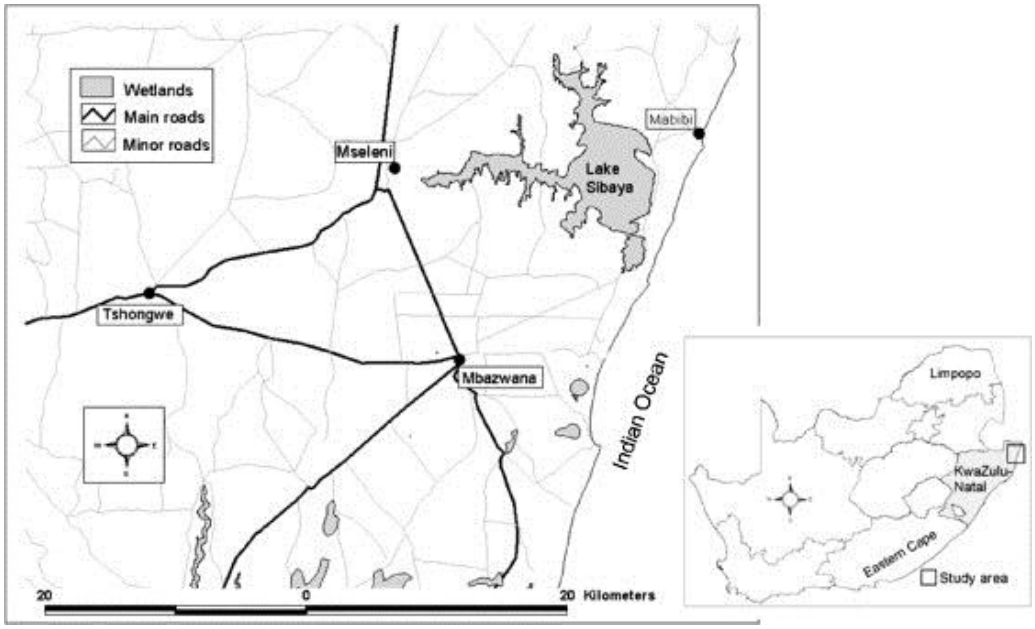


Figure 1.1 Map of the study area (De Wet et al., 2012).

Traditional therapies are seen as convenient and are entrusted by communities as remedies have been passed on from the older generations. The communities were extremely forthcoming with the relevant information especially with plant names, preparation of remedies and the dosages. From the raw data it was found that 33 plant species were commonly used to treat STIs (De Wet et al., 2012). The documented plants were collected from the four regions (Mabibi, Tshongwe, Mseleni and Mabazwana) where plant availability had to be taken into consideration.

The raw data also revealed that in many instances plants were being combined to form poly-herbal remedies. The plant combinations consisted of two plant combinations (e.g. *Ximenia caffra* with *Tabernaemontana elegans*), three plant combinations (e.g. *Carica papaya*, *Senecio serratuloides* and *Hypoxis hemerocallidea*), and even multiple combinations (e.g. *Euphorbia hypericifolia*, *Senecio serratuloides*, *Hypoxis hemerocallidea* and *Ozoroa engleri*).

Twenty plants from the study undertaken by De Wet et al., (2012) were considered for *in vitro* toxicity and antimicrobial evaluation. These plants together with their reported STI use are provided in Table 1.1.

1.4 Toxicological studies and the relevance to this study

Plants have been well documented as herbal remedies to date, however, this is not so for the adverse effects and toxicity levels of the plants (Elgorashi et al., 2003). Where toxicology is defined as the study of adverse effects of chemicals on living organisms (Katzung et al., 2011). Despite the fact that approximately 80% of individuals in South Africa use herbal therapy to treat various infections, there is little knowledge of the correct distribution between effective and fatal dosage (Green, 1997). The use of herbal therapies has increased and scientists are now gaining better ethnobotanical insight into plants. But the human poisoning information is still not well documented (Botha and Penrith, 2008). Plant toxicity may be caused intentionally or accidentally, but nevertheless it is so serious that it is estimated that 15% of people in southern Africa will die if poisoned by a medicinal plant compared to 2% fatality by a non-plant induced poisoning (Gaillard and Paquin, 1999). To avoid such incidents from occurring it is highly

Table 1.1 Medicinal plants from northern Maputaland and their STI uses as documented in De Wet et al., (2012).

Plant name	Administration
Albizia adianthifolia (Schumach.) W. Wight	(a) Chopped bark is boiled in 2 L of water with a handful of *Trichilia dregeana* leaf. Half a cup of the decoction is taken as an enema once a day to treat syphilis.
Aloe marlothii A. Berger	(a) *Aloe marlothii* with *Hypoxis hemerocallidea* (corm) and *Senecio serratuloides* (leaves) boiled in 2 L water. Half a cup is taken orally three times a day to cure internal and external sores ("cauliflower"). Half a cup once a day can be taken as an enema. (b) *Aloe marlothii* in two cups of water and *Hypoxis hemerocallidea*, *S. serratuloides*, *S. birrea* and *S. cordatum*. Drink half a cup of the decoction four times a day or use one cup once a day as an enema to treat sores cause by STIs.
Bidens pilosa L.	(a) The stem/leaves with *Clematis brachiata* (stem and leaves), *Ranunculus multifidus* (stem and leaves) and *Sarcophyte sanguinea* stem and boil it in water. It is taken orally to treat "cauliflower" genital sores and warts.
Carica papaya L.	(a) Boil the leaves in 4 L of water with *Senecio serratuloides* leaves and *Hypoxis hemerocallidea* corm. Half a cup is taken twice a day to treat gonorrhoea infection. (b) The leaves, with *Senecio serratuloides* leaves boiled 1L water. One table spoon taken orally three times daily to treat gonorrhoea/ shingles. (c) Boil the roots in water for 10 to15 minutes together with *Hypoxis hemerocallidea* corm and leaves of *Senecio serratuloides*. Three quarters of a cup is taken orally twice a day to treat internal sores caused by STIs.

Table 1.1: Continued medicinal plants from northern Maputaland and their uses as documented in De Wet et al., (2012).

Plant name	Administration
Cassipourea malosana L.	(a) Boil the bark in 2 L of water with *Hypoxis hemerocallidea*. Drink half a cup of the concoction twice a day to cure gonorrhoea infection.
Clematis brachiata Thunb	(a) The stem and leaves with *Bidens pilosa* (stem and leaves), *Ranunculus multifidus* (stem and leaves) and *Sarcophyte sanguinea* stem and boil it in water. It is taken orally to treat "cauliflower" genital sores and warts.
Euphorbia hypericifolia L.	(a) The leaves, boil it in 10 L of water for 1 hour with *Tabernaemontana elegans*, *Ozoroa engleri* leaves and *Hypoxis hemerocallidea* corm. *Ozoroa engleri* roots are apparently more effective than its leaves. Take 2 sips of the decoction twice a day to treat gonorrhoea.
Hypoxis hemerocallidea L.	a) Boil *Hypoxis hemerocallidea* corm and *Senecio serratuloides* in 2 L of water for 30 minutes. Drink half a cup of the decoction three times a day to treat internal and external sores caused by STIs. (b) Boil one corm and one handful of *Senecio serratuloides* leaves in 2 L of water. Drink half a glass of the concoction three times a day to treat gonorrhoea. (c) Boil one corm, one handful of leaves of *Senecio serratuloides* and *Ranunculus multifidus* in 1.5 L of water for 10 minutes. Drink one table spoon of the decoction three times a day to treat shingles and internal sores.

Table 1.1: Continued medicinal plants from northern Maputaland and their uses as documented in De Wet et al., (2012).

Plant name	Administration
Hypoxis hemerocallidea L.	(d) Boil the roots in 2.5 L of water for 10-15 minutes with *Hypoxis hemerocallidea* corm and *Senecio serratuloides*. Three quarters of a cup is taken orally twice a day to treat internal sores caused by STIs. (e) It is use in combination with *Euphorbia hypericifolia, Tabernaemontana elegans* and *Ozoroa engleri.* (f) Mix one handful of *Musa acuminata* roots with *Senecio serratuloides* leaves and *Hypoxis hemerocallidea* corm, add 2 L of water and boil. Drink one cup of this concoction three times a day to cure HIV/AIDS related infections, especially internal and external sores.
Kigelia africana (Lam.) Benth	(a) One mature fruit with the corm of *Hypoxis hemerocallidea*, add 2 L water and bring to boil. Half a cup of this decoction is taken three times a day to cure sores.
Musa acuminata Colla	(a) Mix one handful of *Musa acuminata* roots with *Senecio serratuloides* leaves and *Hypoxis hemerocallidea* corm. Add 2 L of water and boil. Drink one cup of this concoction three times a day to cure HIV/AIDS related infections, especially internal and external sores.
Ozoroa engleri R. Fern. & A. Fern.	(a) Mix one handful of *Ozoroa engleri* leaves with *Euphorbia hypericifolia* stem and *Tabernaemontana elegans* leaves and *Hypoxis hemerocallidea* corm. Add 10 L of water and boil for one hour. Take 2 sips of this concoction twice a day to treat gonorrhoea.

Table1.1: Continued medicinal plants from northern Maputaland and their uses as documented in De Wet et al., (2012).

Plant name	Administration
Peltophorum africanum Sond	(a) Boil one handful of *Peltophorum africanum*, *S. serratuloides*, *H. hemerocallidea*, *S. serratuloides* and *K. africana* roots in 2 L of water. Drink one cup of this concoction three times a day to treat sores cause by STIs or used half a cup once a day as an enema.
Ranunculus multifidus Forssk	(a) The stem and leaves combined with *Clematis brachiata* (stem and leaves), *Bidens pilosa* (leaf) and *Sarcophyte sanguinea* stem and boil it in water. Dosage depends on the seriousness of the infection. It is taken orally to treat "cauliflower" genital sores and warts.
Sarcophyte sanguinea Sparrm.	(a) The stem and leaves combined with *Clematis brachiata* (stem and leaves), *Runnunculus multifidus* (stem and leaves) and *Bidens pilosa* (leaf) and boil it in water. Dosage depends on the seriousness of the infection. It is taken orally to treat "cauliflower" genital sores and warts.
Sclerocarya birrea (A. Rich.) Hochst	(a) Boil the of bark of *Sclerocarya birrea* and *Syzygium cordatum* in 2 L of water for 15 minutes (if it tasted very strong, dilute 1 L of the above concoction with 1 L of water). Drink two tablespoons once or twice a day to treat gonorrhoea (drop).
Senecio serratuloides DC.	(a) Boil one handful of *Senecio serratuloides* leaves in 1 L of water. (b) *Aloe marlothii* with *Hypoxis hemerocallidea* (corm) and *Senecio serratuloides* (leaves) boiled in 2 L of water. Half a cup is taken orally three times a day to cure internal and external sores ("cauliflower").

Table 1.1: Continued medicinal plants from northern Maputaland and their uses as documented in De Wet et al., (2012).

Plant name	Administration
Senecio serratuloides DC.	(c) The leaves *Carica papaya* leaves and *Hypoxis hemerocallidea* corm. Half a cup is taken twice a day to treat gonorrhoea infection. (d) *Musa acuminata* roots with *Senecio serratuloides* leaves and *Hypoxis hemerocallidea* corm. Add 2 L of water and boil. Drink one cup of this concoction three times a day to cure HIV/AIDS related infections, especially internal and external sores.
Syzygium cordatum Hochst. ex C. Krauss	(a) Boil the bark of *Sclerocarya birrea* and *Syzygium cordatum* in 2 L of water for 15 minutes. Drink two tablespoons once or twice a day to treat gonorrhoea.
Tabernaemontana elegans Stapf	(a) It is used in combination with *Euphorbia hypericifolia* leaves, *Ozoroa engleri* leaves and *Hypoxis hemerocallidea* corm to treat gonorrhoea. (b) Mix half a handful of *Ximenia caffra* roots with *Tabernaemontana elegans* roots and boil in 1 L of water. Drink one table spoon of the concoction three times a day to cure gonorrhoea.
Trichilia dregeana Sond.	(a) A handful of bark is boiled in 2 L of water in combination with a handful of *Trichilia dregeana* leaf. Used to treat syphilis.
Ximenia caffra Sond.	(a) Mix half a handful of *Ximenia caffra* roots with *Tabernaemontana elegans* roots and boil it in 1 L of water. Drink one tablespoon of the concoction three times a day to cure gonorrhoea.

recommended that before entering into long term herbal therapy, toxicological screenings should be performed (Elgorashi et al., 2003).

Poisonous plants affect various organs (e.g. cardio-, nephro- or -hepato-toxic) and some plants have several toxic components that affect different systems, the degree of effect depends on a few factors such as the growth stage of the plant, seasonal variation of the plant, the plant part consumed, the route of administration, amount consumed and the susceptibility of the consumer (Botha and Penrith, 2008). An outline of some systems that are most effected by toxic plants are provided as follows:

- **Nervous system**: When plant compounds attack the nervous system, often an individuals' experiences will differ dependent on the plant species. Peripheral neuropathy and spastic muscle is seen with *Lathyrus sativa* (Tor-Agbidye et al., 1999), blindness and tremors can be experienced with *Datura stramonium* (Steenkamp et al., 2002), whereas seizures and even coma with toxic *Boophane disticha* (Steenkamp, 2005).

- **Cardiovascular system**: Many garden plants (e.g. *Digitalis purpurea*, *Nerium oleander*) possess glycosides which have a cardiac effect. This is important to note as this is how accidental toxicity may occur. Cardio-toxicity results in the heart to slow and eventually lead to death (Botha and Penrith, 2008).

- **Hepatotoxicity**: One of the contributing factors that cause liver cancer and cirrhosis of the liver is exposure to plants that have the active compound such as pyrrolizidine alkaloids (Steenkamp et al., 2000). Often plants such as fruits from cycads once ingested turn into a by-product (methyl azoxymethanol) which is toxic to the liver and carcinogenic (Spartz et al., 1967). Such hepatotoxic plants include *Callilepsis laureola* and the *Senecio* species.

- **Digestive system**: Toxic effects occur mainly as diarrhoea, vomiting and nausea, which is often the body's way of rejecting the toxin. Some examples are ripe berries from *Melia azedarach* which cause vomiting, dyspnea and diarrhoea in children (Van Wyk et al.,

2002) or green vegetables like potatoes and tomatoes (family Solanaceae) which irritate the stomach (Botha and Penrith, 2008).

- **Skin and adnexa**: Skin irritation is often caused by plants that are applied topically and manifests as allergic dermatitis, itchiness and inflammation (Van Wyk et al., 2002). This occurs with plants such as *Smodingium argutum* (poison ivy) and *Eurphorbia triculli*.

Herbal products have always been perceived and assumed to be safe (Fennell et al., 2004). However, there are various plants that have potential medicinal properties but have been found to be toxic at certain levels to the human body such as *Digitalis purpurea* (from which digoxin, a cardiac glycoside was extracted) or *Callilepsis laureola* (used for indigestion) (Wainwright, 1977; Botha and Penrith, 2008). It is important to note that even toxic plants that are lethal at high dosages can be considered safe at lower dosages and often effective like the *Digitalis* plant which when used correctly is an effective heart medication. These plants therefore cannot be discarded due to the high toxicity levels, but must be administered with extreme caution (Botha and Penrith, 2008). Since medicinal plants are widely utilized throughout Africa, the efficacy and potential toxicity of the remedies need to be scientifically evaluated (Baker et al., 1995; Muñoz and Suvain, 2002; Sowemimo et al., 2007; Botha and Penrith, 2008; Bussmann et al., 2011). There have been numerous studies conducted worldwide, spanning from northern Peru to Mexico that have acknowledged the importance of herbal remedies and identified the importance in determining plant safety (Kassie et al., 1996; De Sá Ferreira and Ferrão Vargas, 1999; Kirira et al., 2006, Déciga-Campos et al., 2007, Sowemimo et al., 2007). Acute poisoning by traditional herbal medicines is not uncommon in South Africa, and with the data available, it is estimated that approximately 8,000 to 20,000 cases are presented per annum (Thompson, 2000).

Toxicological studies are required to assist the regulatory systems of herbal therapies to ascertain the safety and efficacy of the plants. Poisonings often occur due to incorrect-administrations, incorrect plant identification, inappropriate dosages and incorrect preparation (Steward et al., 1998). Achieving therapeutic optimum potential is what every therapy, be it traditional remedies or pharmaceutical formulations, should aim to achieve. Toxicology studies provide a better

understanding of the requirements necessary to reduce potential fatalities (e.g. optimal dosage, administration route).

A lack of knowledge on the dosage has led to fatalities of thousands. According to Thompson (2000) the mortality rate due to plant toxicity is between 10,000-20,000 individuals per annum. This statistic is believed to be a small portion of individuals as the study was based on reported cases at a hospital, but there is still a large population that do not seek healthcare from government sources.

Further analysis of the literature revealed that of the 20 plants selected for this study, toxicity data could be found for approximately half of these plants (Table 1.2), however, no studies were found for the 13 plant combinations. Thus became evident that this study needed to investigate the unexplored area of toxicity in combinations.

1.5 Traditional healing and impact on STIs

Up to 80% of the population in developing countries depend on plants for their medicinal needs. This indicates the trust people have for plant remedies (Farnsworth, 1998; WHO, 2008). Africa has a wide diversity of culture and indigenous flora and fauna, and for generations native people have used natural sources to treat various ailments. Traditional therapies (muthi) have become an integral part of each ethnic group (Van Vuuren, 2008). Indigenous people of southern Africa and in all developing countries have for centuries been using herbal medicines for all aspects of primary health care and more frequently for the treatment of STIs (Grierson and Afolyan, 1999; Sokmen et al., 1999). Furthermore, according to Jäger (2005), traditional therapy is thought to be a more holistic approach and not solely scientific. Very often the traditional healer is focused on the patients' wellbeing and not only on symptomatic treatment. According to Ndubani (1997), the traditional healer utilizes the abundant indigenous plants known to him to allow for the treatment of many illnesses including STIs. Individuals also view the herbal approach to be safe, with minimal side effects (Vermani and Garg, 2002). One of the most common reasons that individuals seek treatment from a traditional healer is due to STI infections (e.g. syphilis,

Table 1.2 Plant species used for the treatment of STIs, with relevant ethnopharmacological and toxicological information.

Plant and family name	**Traditional STI reported use** (adapted from De Wet et al., 2012)	**Related antimicrobial, toxicity and other therapeutic *in vitro* studies**
Albizia adianthifolia (Fabaceae)	Gonnorhoeae (Van Puyvelde et al., 1983)	Activity against *Mycobacterium aurum* (Eldeen and Van Staden, 2007), Prostaglandin synthesis inhibitor (Jäger et al., 1996), Toxicity (Prozesky et al., 2001; Haddad et al., 2004), Antibacterial (McGaw et al., 2000), Antimicrobial (Boily and Van Puyvelde, 1986)
Aloe marlothii (Asphodelaceae)	Unspecified sexually transmitted infections (Turner, 2001)	Antimalarial activity (Van Zyl and Viljoen, 2002; Clarkson et al., 2004; Pillay et al., 2008), Antibacterial (McGaw et al., 2000), Antimicrobial activity (Okamura et al., 1996)
Bidens pilosa (Asteraceae)	Syphilis (Hutchings et al., 1996)	Not toxic if used short term (Ngogang et al., 2008), Anti-malarial activity (Brãndao et al., 1997; Clarkson et al., 2004; Pillay et al., 2008), Prostaglandin synthesis inhibitor/ anti-inflammatory (Jäger et al., 1996; Pereira et al., 1999), Toxicity (Towers et al., 1977; Mirvish, 1979; Yuan et al., 2008), Uterine contraction (Kamatenesi-Mugisha, 2004; Kamatenesi-Mugisha and Oryem-Origa, 2007; Nikolajsen et al., 2011), Antifungal (Motsei et al., 2003; Deba et al., 2008; Abdou et al., 2010, Rybalchenko et al., 2010), Antiviral (Chiang et al., 2003), Antibacterial (Rabe and Van Staden, 1997; McGaw et al., 2000; Mathabe et al., 2006; Deba et al., 2008; Bussmann et al., 2011), Antimicrobial (Boily and Van Puyvelde, 1986; Khan et al., 2001)

Table 1.2 Plant species used for the treatment of STIs, with relevant ethnopharmacological and toxicological information.

Plant and family name	**Traditional STI reported use** (adapted from DeWet et al., 2012)	**Related antimicrobial, toxicity and other therapeutic *in vitro* studies**
Carica papaya (Caricaceae)	Gonorrhoeae (Abbiw, 1990; Chomnawang et al., 2009), Venereal infections (Arnold and Gullumiam, 1984), Sexually transmitted infections (Ndubani and Höjer, 1999), *Candida* infections (Runyaro et al., 2006)	Unripe fruit is toxic (Runyaro et al., 2006), Tuberculosis (TB) (Green et al., 2010), Antimicrobial (Osato et al., 1993), Wound healing (Starley et al., 1999; Azarkan et al., 2004; Gurung and Ŝkalko-Basnet, 2009), Antimalarial (Cẻline et al., 2009), Anti-inflammatory and analgesic (Akah et al., 2002; Amazu, 2010), Bacteriostatic activity (Osato et al., 1993), Antibacterial (Tona et al., 1999), Male contraception (Chinoy et al., 1994; Lohiya et al., 1999), Uterine contractions (Cherian, 2000; Adebiyi et al., 2003; Sarma and Mahanta, 2000; Kamatenesi-Mugisha, 2004; Kamatenesi-Mugisha and Oryem-Origa, 2007)
Cassipourea malosana (Rhizophoraceae)	No previous records	No previous reports found
Clematis brachiata (Ranunculaceae)	Syphilis (Chhabra et al., 1991)	Leaf was non-toxic (Pooley, 1998; Koch et al., 2005), Antimalarial activity (Clarkson et al., 2004; Koch et al., 2005; Muthaura et al., 2007; Pillay et al., 2008), Prostaglandin synthesis inhibitor (Jäger et al., 1996)
Euphorbia hypericifolia (Euphorbiaceae)	STI treatment.	No previous reports found
Hypoxis hemerocallidea (Hypoxidaceae)	Anti- HIV (Pooley, 2005), Related uninary tract infections (Van Wyk, 2009)	The leaf is non-toxic (Verschaeve and Van Staden, 2008, Elgorashi et al., 2003), Antimicrobial (Aremu et al., 2010; Ncube et al., 2012), Treating wound infections (De Wet et al., 2008), Anti-inflammatory (Ojewole, 2006; Steenkamp et al., 2006), Antiviral and antifungal (Drewes et al., 2008), Antibacterial (Gaidamashvili and Van Staden, 2002; Steenkamp et al., 2006)

Table 1.2 Plant species used for the treatment of STIs, with relevant ethnopharmacological and toxicological information.

Plant and family name	**Traditional STI resported use** (adapted from DeWet et al., 2011)	**Related antimicrobial, toxicity and other therapeutic *in vitro* studies**
Kigelia africana (Bignoniaceae)	Syphilis and sores (Watt and Breyer-Brandwijk, 1962; Abbiw, 1990; Van Wyk, 2009), Gonorrhoeae (Watt and Breyer-Brandwijk, 1962), Venereal infections (Akunyili et al., 1991)	The bark was toxic (Nyarko et al., 2005, Eldeen and Van Staden, 2007), Toxicity of unripe fruit (Palmer and Pitman, 1972), Non-genotoxicity for flower (Elgorashi et al., 2003), Antimalarial activity (Clarkson et al., 2004; Muthaura et al., 2007; Pillay et al., 2008), Toxicity (Fennell et al., 2004; Fouche et al., 2008; Shai et al., 2008; Kamuhabwa et al., 2000), Antibacterial activity (McGaw et al., 2000; Owolabi et al., 2007; Maregesi et al., 2008), Antimicrobial (Akunyili et al., 1991), Antiviral (Maregesi et al., 2008), Antifungal (Owolabi et al., 2007; Maregesi et al., 2008)
Musa acuminata (Musaceae)	Sores on genital parts (Ndubani and Höjer, 1999), Sexual transmitted infections (Kambizi and Afolayan, 2001)	Antimicrobial activity (Richter and Vore, 1989; Debnath et al., 2011), Drug-resistant TB (Camacho-Corona et al., 2008), Uterine contractions (Kamatenesi-Mugisha and Oryem-Origa, 2007), Antibacterial (Maïkere-Faniyo et al., 1989), Antifungal properties (Leone et al., 2006)
Ozoroa engleri (Anacardiaceae)	Venereal diseases (Hutchings et al., 1996; Prozesky et al., 2001)	Antimalarial activity (Pillay et al., 2008)
Peltophorum africanum (Fabaceae)	Venereal diseases (Arnold and Gulumiam, 1984; Mabogo, 1990; Mulaudzi et al., 2011), Syphilis (Maroyi, 2011)	TB (Green et al., 2010), Schistosomiasis (Mølgaard et al., 2001), Anti-viral (Bessong et al., 2005), Antibacterial (Samie et al., 2005; Steenkamp et al., 2007), No antifungal activity detected (Steenkamp et al., 2007)
Ranunculus multifidus (Ranunculaceae)	Syphilis (Hutchings, 1989	Antimalarial activity (Clarkson et al., 2004; Pillay et al., 2008)
Sarcophyte sanguinea (Balanophoraceae)	No previous records	Treat wound infections and antibacterial (De Wet et al., 2008)

Table 1.2 Plant species used for the treatment of STIs, with relevant ethnopharmacological and toxicological information.

Plant and family name	**Traditional STI reported use** (adapted from DeWet et al., 2012)	**Related antimicrobial, toxicity and other therapeutic *in vitro* studies**
Sclerocarya birrea (Anacardiaceae)	*Candida* infections (Runyaro et al., 2006)	Found to be genotoxic at high doses (Fennell et al., 2004), TB (Green et al., 2010), Antimalarial activity (Pillay et al., 2008), Antimicrobial and cyclooxygenase enzyme inhibitory (Moyo et al., 2011), Anti-inflammatory (Ojewole, 2003), Antibacterial (McGaw et al., 2000; Eloff, 2001; Mathabe et al., 2006; Dimo et al., 2007), Antimicrobial (Njume et al., 2011), Toxicity (Loughlin et al., 2008)
Senecio serratuloides (Asteraceae)	Syphilis (Githens, 1949)	Leaf was found to be toxic at high doses (Elgorashi et al., 2003), Antibacterial (Kelmanson et al., 2000), Treat wound infections (De Wet et al., 2008), Anti-inflammatory (Fawole et al., 2010)
Syzygium cordatum (Myrtaceae)	General STIs (Van Vuuren and Naidoo, 2010)	Antibacterial (Mathabe et al., 2006; Steenkamp et al., 2007; Sibandze et al., 2010), Antimalarial activity (Clarkson et al., 2004; Pillay et al., 2008)
Tabernaemontana elegans (Apiaceae)	Venereal diseases (Arnold and Gulumiam, 1984; Mabogo, 1990)	Antifungal (Steenkamp et al., 2007), Cytotoxicity (Pallant and Steenkamp, 2008), Antibacterial, antimycobacterial and cytotoxicity (Steenkamp et al., 2007; Luo et al., 2011; Pallant et al., 2012), Toxicity (Loughlin et al., 2008; Luo et al., 2011)
Trichilia dregeana (Meliaceae)	Gonorrhoea and syphilis (Arnold and Gulumiam, 1984; Mabogo, 1990; Desta, 1993)	Prostaglandin synthesis inhibitor (Jäger et al., 1996), Anti-inflammatory and antibacterial (Eldeen et al., 2005; Eldeen et al., 2007), Antimicrobial (Hutchings et al., 1996)
Ximenia caffra (Olacaceae)	Venereal diseases (Arnold and Gulumiam, 1984; Mabogo, 1990; Maroyi, 2011), STIs (Ndubani and Höjer, 1999; Kambizi and Afolayan, 2001; Mulaudzi et al., 2011)	Antibacterial (Fabrey et al., 1998; Mathabe et al., 2006; Steenkamp et al., 2007), Antimalarial (Clarkson et al., 2004), Antimicrobial (Mulaudzi et al., 2010)

gonorrhoea, urethritis, bacterial vaginosis), and this is the main reason why most cases are never reported to the health authorities (Green, 1992).

Globally there have been numerous antimicrobial studies with a STI focus, that have been conducted over the years, some of which have investigated one or two STI pathogens that are relevant to this study (Ndubani and Höjer, 1999; Kambizi and Afolayan, 2001; Silva et al., 2002; Okoli and Iroegbu, 2004; Hamza et al., 2006; Al-Heali and Rahemo, 2006; Shokeen et al., 2009; Chomnawang et al., 2009). Furthermore, in the South African context venereal infections have been investigated in various studies (Vermani and Garg, 2002; Motsei et al., 2003; Tshikalange et al., 2005; Buwa and Van Staden, 2006; Steenkamp et al., 2007; Kambizi and Afolayan, 2008; Van Vuuren and Naidoo, 2010; Mulaudzi et al., 2011). However, there are very few publications that address the investigation of multiple STI pathogens within the global and South African context. Studies have commonly focused on *Candida albicans* (Motsei et al., 2003; Buwa and Van Staden, 2006; Kambizi and Afolayan, 2008; Mulaudzi et al., 2011; Ncube et al., 2012), *Neisseria gonorrhoeae* (Silva et al., 2002; Kambizi and Afolayan, 2008; Shokeen et al., 2009; Mulaudzi et al., 2011) and *Trichomonas vaginalis* (Calzada et al., 2007; Arthan et al., 2008; Moo-Puc et al., 2008; Tiwari et al., 2008; De Villiers et al., 2010). These studies have shown some antimicrobial efficacy, therefore encouraging further studies on other relevant pathogens.

People often have a preconception that plants used in combination may have a greater overall effect than when used singularly. It is known that traditional healers rarely use single plants as remedies and more often concoct multiple plant regimens. Traditional healers combine various plants parts and even different plant species (Van Vuuren and Viljoen, 2008). There have been previous studies conducted specific to STI treatment in India and Mexico whereby poly-herbal preparations were investigated (Bourne et al., 1999; Talwar et al., 2000; Bagga et al., 2006; Bhengraj et al., 2008). These poly-herbal preparations have not only been investigated for STI inhibitory properties, but also as topical microbicides to aid in the prevention of STIs. The poly-herbal treatments have been investigated on a broad-spectrum of micro-organisms; however, the micro-organisms only consisted of one or two of the pathogens relevant to this study. This

prompted further investigation (as presented here) into evaluating the antimicrobial activity of plant combinations against STI pathogens in rural Maputaland.

1.6 Aims and objectives of the study

Taking into consideration the ethnobotanical study published by De Wet et al., (2012) and the lack of scientific and toxicological data validating the traditional use of plants for the treatment of STIs in northern Maputaland, a study was designed to address these areas of concern.

Despite numerous antimicrobial studies conducted on South African plants with STI relevance, many have not focused on bacterial pathogens associated with sexually transmitted infections (Van Vuuren and Naidoo, 2010). The aim of the study was therefore to scientifically validate the antimicrobial STI use of the twenty plants, (individually and in combination). Furthermore, the aim of the study was to assess the safety of the plants used when tested against the human kidney epithelial cell line. A further breakdown of the objectives (Figure 1.2) of the study is as follows:

- Review the data from an ethnobotanical study done by Nzama (2009) and confirm the use of plants used in combination to treat sexually transmitted infections.
- Collect and prepare extracts (aqueous and organic) of all the relevant plant material from KwaZulu-Natal.
- Perform toxicity studies on the plant extracts (aqueous and organic), for both the individual and combined plants used, using the human kidney epithelial (Graham, HEK-293) cell line.
- Test for the antimicrobial efficacy using the minimum inhibitory concentration (MIC) assay against six pathogens associated with the urogenital tract.
- Assess toxicity interactions between the plants in 1:1 combination using ΣFIC and isobolograms.
- Assess the interaction between the plants in combination using the sum of the plants fractional inhibitory concentration (ΣFIC) and isobolograms.
- To optimize, using MODDE 9.1® software, the best possible combination where three plants are used in combination.

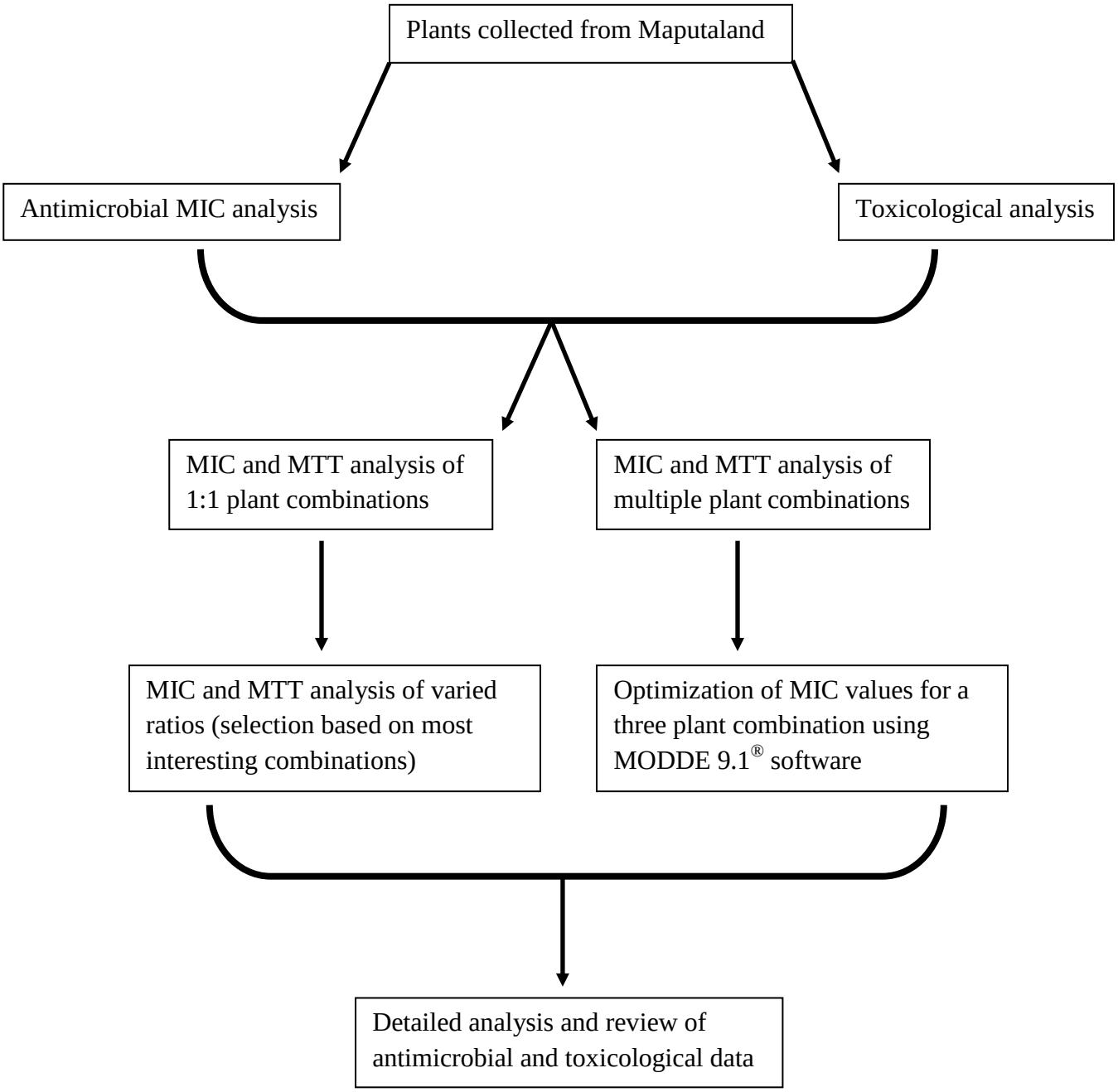


Figure 1.2 Flow diagram which describes in a stepwise manner how this research study was conducted.

Chapter 2

Materials and Methods

2.1 Review of raw data and collection of plant material

From the raw data's (interview questionnaires conducted in selected areas of Maputaland) original list, 33 plant species were documented of which 20 plants were collected due to plant availability. By examining the raw data and in consolidation with the overview conducted by De Wet et al., (2012) 13 combinations of two or more plants per herbal remedy were considered for further investigation. All plant species (Table 2.1) were collected from four regions of northern Maputaland, KwaZulu-Natal (Mabibi, Mseleni, Tshongwe, Mabazwana). The collection of plant material occurred in March 2010 to May 2010.

The plant species obtained from the raw data were collected from the homesteads in which the interviews were conducted and voucher specimens were prepared on site (Figure 2.1). The voucher specimens were then prepared (Figure 2.1) and deposited in the herbarium of the Botany Department at the University of Zululand. Botanical identification was made by Dr. De Wet and further confirmation of authenticity was undertaken by Mr. Ngwenya from the South African National Biodiversity Institute, KwaZulu-Natal Herbarium. The plant materials collected were then separated into the plant part that was commonly used by the lay people.

Figure 2.1 *In situ* collection of leaf material (Mabibi) and preparation of voucher specimens.

Table 2.1 Voucher specimens and yields of plant species used in the study.

Name of plant	Part used	Voucher specimen	Yield (%)	
			Aqueous	Organic
Albizia adianthifolia	Leaf	NZ3	12.6	1.09
Aloe marlothii	Leaf	NZ16	17.1	2.25
Bidens pilosa	Leaf	NZ14	9.36	0.22
Carica papaya	Roots	NZ21	5.71	0.67
Cassipourea malosana	Leaf	NZ5	10.8	1.15
Clematis brachiata	Leaf	NZ27	9.36	1.30
Euphorbia hypericifolia	Leaf	NZ37	12.5	1.57
Hypoxis hemerocallidea	Corm	NZ38	26.7	4.39
Kigelia africana	Leaf	NZ11	19.2	1.92
Musa acuminata	Leaf	NZ25	9.84	0.14
Ozoroa engleri	Leaf	NZ34	11.6	3.46
Peltophorum africanum	Leaf	NZ17	3.44	0.68
Ranunculus multifidus	Leaf	NZ36	20.0	1.54
Sarcophyte sanguinea	Stem	NZ29	9.93	1.09
Sclerocarya birrea	Bark	NZ22	13.4	1.92
Senecio serratuloides	Leaf	NZ10	16.0	3.58
Syzygium cordatum	Bark	NZ12	26.3	2.27
Tabernaemontana elegans	Bark	NZ35	6.65	1.09
Trichilia dregeana	Leaf	NZ23	8.60	1.57
Ximenia caffra	Leaf	NZ26	5.71	2.10

2.2 Preparation of the plant extracts

The 20 plants (bark, seeds, leaves and roots) collected were firstly dried before being macerated to a fine powder using various grinders (Sunbeam EMO400, Wellington, New Zealand and Moulinex, Johannesburg, South Africa, (Figure 2.2). The grinders were rinsed thoroughly with water between all sample grinding to eliminate cross contamination of the plants. Weighed ground plant material (approximately 40 g) was transferred into containers for extraction.

Figure 2.2 Apparatus for the maceration of plant material.

2.2.1 Aqueous extracts

Aqueous extracts were prepared in the same manner as the lay people of Maputaland, by using water as the common solvent for the preparation of the herbal remedies used to treat STIs. Macerated plant material (± 20 g) was added to distilled water (40 ml), enough to fully submerge the plant material. The beakers were sealed with parafilm, to prevent evaporation when incubating at a temperature of 37°C, prior to being placed into a platform shaking incubator (Labcon) for 24 hours. The plant extracts were stored in the -70°C freezer prior to lypholisation. The KBM lyophiliser (Bergbron) was used to freeze dry the plant extracts at a pressure of 4kPa and temperature of -70°C.

After seven days the completely dry samples were removed and the yields calculated (Table 2.1). Samples were placed under UV light (350-375 nm) (Esco) for 24 hours to reduce further contamination prior to antimicrobial analysis. Samples were stored in a fridge at -4°C.

2.2.2 Organic extracts

Macerated plant material (± 20 g) was saturated in 1:1 mixture of dichloromethane and methanol (Merck Chemicals), enough to submerge (± 40 ml) the plant material. The mixture was covered with foil (to prevent evaporation) and then placed in a platform shaking incubator at 37°C for 24 hours. The extracted samples were placed under the fume hood and monitored until the solvent evaporated, providing remnants of extract for which percentage yields were calculated (Table 2.1). Thereafter, samples were stored in a fridge at -4°C.

2.3 Toxicology studies

Numerous studies (Mosmann, 1983; Philips, 1996; Van Dyk et al., 2009; De Mesquita et al., 2009; Scherlie, 2011) have used the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) cell proliferation assay for the evaluation of plant extracts. This assay is used as a means of rapid toxicity characterization of new drug formulations and in this case for the use of plant remedies (Liu et al., 1997). The MTT assay was chosen not only for its reliability, but also on the basis of the assay being cost effective, its simple methodology and the fact that the assay bypasses labour intensive and time consuming steps (Wang et al., 2010). In order to undertake toxicity profiling, numerous cell lines would be required, however, for the screening process to gain an indication of the safety, only one cell line (human kidney epithelial cell line) was used. Interestingly, kidney epithelial cells contain a significant gene called the multidrug resistance gene (MDR_1) which is important for toxicity studies. This gene is also found in the liver, intestinal mucosa and blood brain barrier which links all organs to the toxicity study. Elimination of most drugs occurs via the kidneys therefore the kidney made for a vital organ to use for testing.

With regards to the toxicity assay, MTT is a yellow water soluble dye that can be reduced by metabolic enzymes to a water insoluble purple formazan crystal (Mosmann, 1983; Carmicheal et al., 1987; Morgan, 1998). The assay then quantitatively measures the reduction of MTT into an insoluble product by the mitochondria of viable cells. The crystals can be quantified spectrophotometrically by their dissolution in an organic solvent (Gabrielson et al., 2002; Tunney et al., 2004).

2.3.1 Preparation of media Dulbeco's Modified Eagles Medium

Dulbeco's Modified Eagles Medium (DMEM) (Highveld Biologicals) was the required media for optimal cell viability and was used in both the experimental and culture media. The ingredients required to constitute the media were 13.53 g/L DMEM and 3.7 g/L sodium hydrogen carbonate ($NaHCO_3$) (Merck chemicals). All components were dissolved using a thermo-stirrer (IKA-Combimag) before being filtered (0.22 µm). The sterility of the media was confirmed following incubation at 37°C for 48 hours.

2.3.2 Culturing of human kidney epithelial cells

The human kidney epithelial cells (Graham, HEK-293) which the ethics committee approved for the use of the toxicity study (Waiver number W-CJ-110202-5, Appendix B) were grown in 25 ml culture flasks. The experimental media consisted of DMEM, 10% foetal calf serum (Delta Bioproducts) and $NaHCO_3$; whilst the culture media also contained 10% foetal calf serum and 1% penicillin/streptomycin mixture (10000U penicillin/ml and 10000 µg streptomycin/ml), with the latter reducing the chance of contamination. The cell cultures were then incubated at 37°C in a humidified atmosphere of 5% CO_2 and 95% air. The culture medium was replenished every second day in order to optimize cell viability. Culture maintenance was performed in a laminar air flow unit to ensure sterility. Typsinization of confluent flasks was performed weekly.

2.3.3 Trypsinization of cell cultures

The cell cultures were routinely checked to confirm cell viability and confluency on a daily basis using an inverted microscope (Nikon; x1000 magnification). The flasks that were approximately

90% confluent were trypsinized. The cells were first washed with 3 ml phosphate buffer saline (PBS, pH 7.4; 8 g NaCl, 0.3g KCl, 0.73 g Na_2HPO_4. $2H_2O$, 0.2 g KH_2PO_4). Then trypsin-versene (3ml) was added. Once all the cells detached from the flask surface 20 ml culture media was added. The cell suspension was disrupted into a single cell suspension in order to perform a cell count. The single cell suspension was then centrifuged at 1500 rpm for 5 min and 10 ml of culture media was added to make up a new cell suspension. The new suspension (40 µl) was added to 0.40% Trypan blue (40 µl) in order to calculate the cell density and cell viability using a haemocytometer. The cell suspension was adjusted to 500, 000 cells/ml (Equation 2.1) and used for further toxicity studies; while the balance of cells was returned to the flask to maintain the culture for further assays (Section 2.3.2).

$$\text{Cell density} = \frac{0.5 \times 10^6 \text{ cells/ml}}{\text{Cells counted on haemocytometer} \times 10^4} \times 20\text{ml} \qquad \textbf{Equation 2.1}$$

2.3.4 The tetrazolium based cellular viability assay

The MTT assay is a colourimetric assay that measures the cell survival as a percentage of cell survival compared to untreated controls (Mosmann, 1983; Van Zyl and Viljoen, 2002). To maintain sterility this assay was performed in the laminar flow unit which was swabbed with 70% ethanol before and after the assay. Following (Section 2.3.3), 180 µl of this suspension was aliquoted per well, along with 20 µl plant extract (0.1-100 µg/ml) in triplicate wells. The micro-titre plates were then incubated for 44 hours at 37°C under humidified conditions in 5% CO_2.

When combining the plant species 50 µl of plant **A** (10 mg/ml) was combined to 50 µl of plant **B** (10 mg/ml) in order to make up a plant sample of 100 µl. For plant combinations containing more than two plant species, a stock solution was prepared with 100 µl of each plant sample whereby each plant in the final solution was combined in equal proportions.

For those plant combinations showing some degree of cytotoxicity, the plants were prepared in varying ratios, two plants were combined in nine ratios i.e. 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7,2:8, 1:9, to observe which ratio was the safest and most toxic (Section 2.5.2).

The MTT assay was used to test seven 1:1 combinations and six multiple combinations where more than two plants were mixed. All plant combinations were selected on the basis of traditional use (Table 2.2).

Table 2.2 Various combinations tested in this study.

Two plant combinations	Multiple plant combinations
Albizia adianthifolia + Trichilia dregeana	*Carica papaya + Senecio serratuloides + Hypoxis hemerocallidea*
Aloe marlothii + Senecio serratuloides	*Musa acuminata + Senecio serratuloides + Hypoxis hemerocallidea*
Aloe marlothii + Hypoxis hemerocallidea	*Bidens pilosa + Ranunculus multifidus + Sarcophyte sanguinea + Clematis brachiata*
Kigelia africana + Hypoxis hemerocallidea	*Euphorbia hypericifolia + Senecio serratuloides + Hypoxis hemerocallidea + Ozoroa engleri*
Sclerocarya birrea + Syzygium cordatum	*Peltophorum africanum + Senecio serratuloides + Hypoxis hemerocallidea + Kigelia africana*
Ximenia caffra + Tabernaemontana elegans	*Hypoxis hemerocallidea + Aloe marlothii + Senecio serratuloides + Sclerocarya birrea + Syzygium cordatum*
Cassipourea malosana + Hypoxis hemerocallidea	

The colour of the plant extract had the potential to interfere with the absorbance reading and interact with the MTT results; therefore before the addition of MTT solution, 100 µl of culture media/extract was discarded from each well and replaced with an equivalent volume of PBS (pH 7.4). Plates that contained plants very dark in colour such as *S. cordatum*, *S. birrea* and *C. papaya*, had to be washed two to three times with PBS before the next step could be conducted.

Thereafter, 150 µl of solution was removed from each well before adding 40 µl MTT solution (0.25 g MTT (Merck Chemicals) dissolved into 50 ml PBS (pH 7.4) and sterilised with a 0.22 µm filter) to all wells of the assay plate. The micro-titre plates were then re-incubated at 37°C in humidified atmosphere of 5% and CO_2 95% air in the water jacked incubator (Forma Scientific) for 4 hr. Thereafter, dimethyl sulphoxide (DMSO) (Merck Chemicals) was added to all the wells and mixed thoroughly, using a thermo-shaker (MRC) at 1040 rpm for 5 min, to dissolve the purple crystals. DMSO is the most suitable solvent for dissolution due to its high solubilizing efficiency and low volatility (Carmicheal et al., 1987; Morgan, 1998). Left for 5 min at ambient temperature to ensure that all crystals are dissolved, the micro-titre plates were then read on a Labsystems iEMS MF reader, using a test wavelength of 540 nm and a reference wavelength of 690 nm (Mosmann, 1983). Appropriate controls were adhered to, positive controls contained the plant-derived compound, quinine and the negative controls consisted of an extract/drug free well and an extract and cell-free control.

2.4 Antimicrobial studies

2.4.1 Culturing of pathogens

Pathogens associated with STI included in the current study were: *Trichomonas vaginalis* (clinical strain), *Candida albicans (*ATCC 10231), *Oligella ureolytica* (ATCC 43534), *Ureaplasma urealyticum* (clinical strain), *Neisseria gonorrhoeae* (ATCC 19424) and *Gardnerella vaginalis* (ATCC 14018). Clinical strains were used when no reference strains were available and were obtained from the STI Reference Centre, NHLS where positive identification was confirmed. All cultures were grown in appropriate media and incubated accordingly (Table 2.3). Standard protocols were adhered to for all pathogens with the exception of two pathogens for which the protocols were optimized.

G. vaginalis grew optimally in Müller Hinton broth (Oxoid, Hampshire England) supplemented with 5% sheep's blood, however, due to the fastidious nature of this organism an alternate method was followed as described by Muli and Struthers (1998). It was found that *G. vaginalis* grew well in Brain and Heart infusion (HBI, Oxoid LTD), which proved to be a better option and

improved the visibility when reading results. Brain and Heart infusion broth was made by dissolving 18.5 g HBI in 500 ml sterile water and then autoclaved and streaked onto blood agar plates to ensure sterility.

N. gonorrhoeae was grown on New York City agar plates (Quebact Laboratories) with selectavial (LCAT) supplement and thereafter inoculated into Müller-Hinton broth (Oxoid LTD, Hampshire, England) supplemented with GC (gonnococcal) selectavial growth media (Mast Diagnostics, Merseyside, U.K) and 5% GC selectavial supplement (Mast Diagnostics, Merseyside, U.K) (Kambizi and Afolayan, 2008). Incubation conditions were 37°C for 24 hours in a humidified CO_2 enriched atmosphere, created in a candle jar.

It was imperative to ensure the purity of the STI cultures and this was achieved by first streaking the pathogens onto agar plates (Table 2.3) in order to achieve single colony identification. The pathogens were thereafter streaked onto agar plates after each MIC assay, to ensure culture purity. If any sign of contamination was detected the contaminated culture was discarded and new cultures were re-innoculated. Identification and confirmation of culture purity was obtained by macroscopically viewing the size of the colony, colour, shape and the presence/absence of haemolysis or not (Table 2.3). Stock cultures were kept at -20°C.

2.4.2 Minimum inhibitory concentration assays

The aqueous and organic plant extracts were prepared in distilled water and acetone respectively, to a starting concentration of 64 mg/ml. Those samples that did not dissolve in acetone were placed in the Transsonic TS 540 sonicator (Labotec) for 3-5 min. The plants that needed to undergo sonification were *S. birrea, P. africanum, S. cordatum, K. africana* and *T. elegans*. After sonification the samples were stored at 4°C in sterile amber vials.

The micro-titre method was adapted from Eloff (1998). All the wells of a 96 well micro-titre plate (NUNC, Denmark) were filled with 100 µl of sterilised deionised water. Duplicates of the plant samples (100 µl) at starting concentrations of 64 mg/ml were added into the first row of the

Table 2.3 Relevant information on the selected STI associated pathogens used in this study.

Pathogen	Macroscopic identification	Agar plate for purity assessment	Media for MIC analysis	Incubation conditions	Pathogenesis
Candida albicans (ATCC 10321)	Colonies were small, white, smooth and circular	Tryptone soya agar	Tryptone soya broth	37°C, 48 hours INT added* and left to stand for 24 hours	Candidiasis
Gardnerella vaginalis (ATCC 14018)	Pin point to small size colonies (0.5-10mm), opaque, dome shaped and greyish white.	Blood agar- Haemolysis present	Brain and heart infusion	37°C, 24 hours INT added* and left to stand for 24 hours	Bacterial vaginosis
Ureaplasma urealyticum (Clinical strain)	Dark to golden brown colonies	Blood agar	U9 or Diamonds	37°C, 24 hours U9 - no INT needed	Urethritis Vaginitis
Oligella ureolytica (ATCC 43534)	White, opaque non-haemolytic, smooth, circular, tan colour	Blood agar	U9	37°C, 24 hours No INT needed	Urethritis
Trichomonas vaginalis (Clinical strain)	Colonies smooth, opaque, circular and dull cream colour about 0.5-10mm	Chocolate agar	Diamonds	37°C, 24 hours INT added* and left to stand for 24 hours	Trichomoniasis
Neisseria gonorrhoeae (ATCC 19424)	Brown on colour and larger than *Trichomonas vaginalis* colonies	Specialised New York City agar	Mueller Hinton Broth supplemented with 5% GC selectavial supplement and GC selectavial growth	37°C, 48 hours CO_2 INT added* and left to stand for 24 hours	Gonorrhoea

INT*- iodonitrotetrazolium violet indicator added after incubation.

allocated wells. Serial doubling dilutions were performed and lastly 100 µl of fresh broth inoculated with a pathogen (1:100) of an approximate innoculum size of 1×10^6 colony forming units (CFU) were prepared and added into each well. Micro-titre plates were then sealed with sterile adhesive film (NUNC, Denmark), to ensure no evaporation occurred upon incubation. Incubation was undertaken at optimum conditions for each specific pathogen (Table 2.3).

After appropriate incubation, 40 µl of 0.40 mg/ml iodonitrotetrazolium violet (INT, Sigma-Aldrich) indicator was added to each well in order to enhance visual identification of the growth of all pathogens. Inhibition of *C. albicans*, *G. vaginalis*, *T. vaginalis* and *N. gonorrhoeae* were monitored by a change in colour from clear to a pink/red (pink/red colour indicated growth of culture) compared to the positive and negative controls. *O. ureolytica* and *U. urealyticum* were cultured in U9 broth (STI Reference Centre, NHLS) which contained 1% phenol red which served as an indicator, therefore INT was not added.

Ciprofloxacin (a broad-spectrum antibiotic) at a starting concentration of 0.01 mg/ml was used as the positive control, as this antibiotic was effective in inhibiting all pathogens except *C. albicans* and *T. vaginalis*. For *C. albicans*, amphotericin B (Sigma-Aldrich) (an antibiotic that is specifically effective for the inhibition of fungi and yeasts) was used at a starting concentration of 0.10 mg/ml and for *T. vaginalis*, metronidazole (Sigma-Aldrich) was used at a starting concentration of 0.01 mg/ml. Two negative controls, solvent and media were added to monitor microbial growth and sterility. The plant dilutions were prepared in a laminar flow unit (Esco) to maintain sterility. Assays were performed in duplicate and triplicate where necessary to ensure accuracy.

2.5 Analysis of antimicrobial combination studies

Plants (concentrations 64 mg/ml) for the two plant combinations were combined in equal ratios to make up a plant sample of 100 µl (i.e. plant **A** (50 µl) and plant **B** (50 µl)). For the plant combinations with more than two plants, a stock solution was made and mixed plant samples (100 µl) whereby each plant in the mixture was combined in equal proportions were introduced

into the wells. The MIC assay (Section 2.4.2) was used to test all plant combinations selected on the basis of traditional use (Table 2.2).

2.5.1 Fractional inhibitory concentration

The FIC index (ΣFIC) was used to assess the interactive antimicrobial effects for the plant combinations and was used to determine the degree to which multiple plants are synergistic, additive, indifferent or antagonistic when mixed together. This was calculated according to Equation 2.2 and Equation 2.3 which was adapted from Berenbaum (1989):

$$FIC^{(i)} = \frac{\text{MIC (a) in combination with (b)}}{\text{MIC (a) independently}} \qquad \textbf{Equation 2.2}$$

$$FIC^{(ii)} = \frac{\text{MIC (b) in combination with (a)}}{\text{MIC (b) independently}} \qquad \textbf{Equation 2.3}$$

(i) and (ii) = in this study represents the different plants studies in combination

The sum of the FIC, known as the FIC index was thus calculated using Equation 2.4:

$$\Sigma FIC = FIC^{(i)} + FIC^{(ii)} \qquad \textbf{Equation 2.4}$$

The FIC index (Scheltz et al., 2006; Van Vuuren and Viljoen, 2011) was used to determine the correlation between the two plants and were classified as either synergistic if the ΣFIC is (≤0.5), additive (>0.5-1.0), indifferent (>1.0- ≤4.0) or antagonistic (>4.0).

2.5.2 Isobologram interpretation

Berenbaum (1989) noted that when introducing two samples of equal concentration into a mixture, it should not be assumed that both plants possess an equal antimicrobial effect. Thus, the study of varied ratio combinations (and representation as isobolograms) for the plants in this study was undertaken in order to determine if varied mixtures would produce an alternative result.

The isobole method is known to be one of the oldest methods of investigating interactive assessments (Van Vuuren and Viljoen, 2011). It is a graphical representation of the growth inhibition data for two samples used in combination at different ratios. This method was used to assess the MIC value of the plant sample determined individually and in comparison with the MIC value of the plant samples in combination (Van Vuuren and Viljoen, 2011). Where plant combinations show a synergistic interpretation from the 1:1 combinations, two plants were combined in nine ratios i.e. 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, 1:9. MIC values were determined for all the ratios as well as for the plant samples independently. Triplicate tests were performed and mean values were plotted to maintain accuracy. Ratios were then calculated using Equations 2.5 and 2.6.

$$X = \frac{\text{MIC value of combination of plant extract}}{\text{MIC value of plant extract}}$$ **Equation 2.5**

$$Y = \frac{\text{MIC value of combination of plant extract}}{\text{MIC value of plant extract}}$$ **Equation 2.6**

MIC values of plant extracts in combination relative to the independent MIC were plotted into an isobologram using Graphpad Prism 5® software. This allowed for a graphical representation of the interaction of various combinations (Figure 2.3).

Upon examination of the relevant isobologram data, there are three straight lines which represent critical points on the graph. These are 0.5:0.5, 1.0:1.0, 4.0:4.0. Interpretation of the data indicated points below or on the 0.5:0.5 solid line are synergy. Points between 0.5:0.5 to and including 1.0:1.0 indicated an additive effect. Points in between 1.0:1.0 and below the 4.0:4.0 indicated an indifferent effect. Points above the 4.0:4.0 line indicated antagonism. (Figure 2.3)

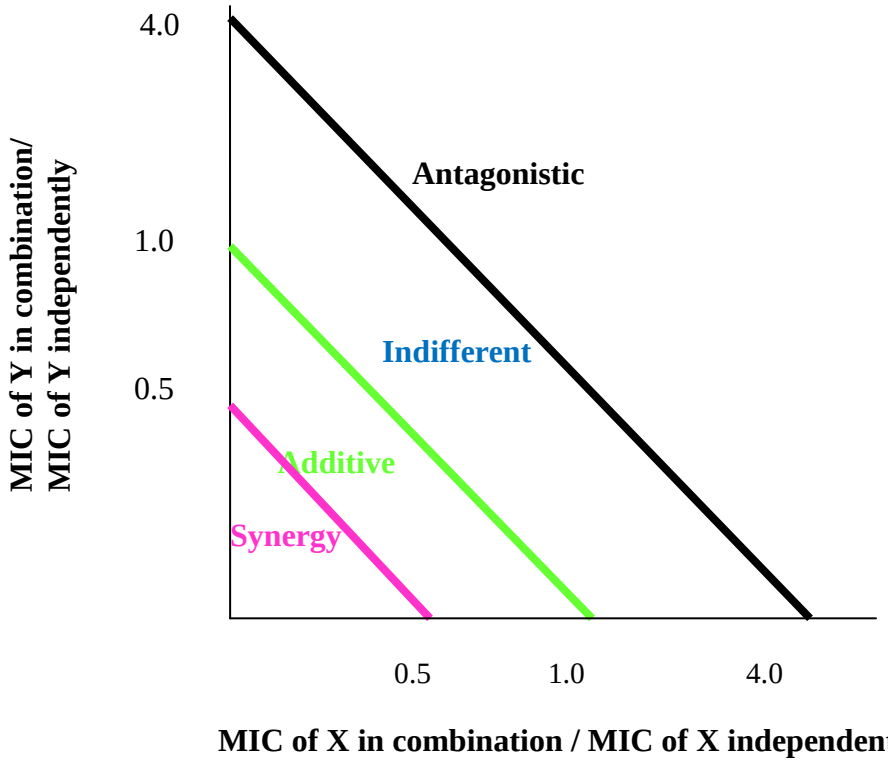


Figure 2.3 Interpretation of an isobologram showing synergy, additivity, indifference and antagonistic pharmacological interactions.

2.6 Optimization using MODDE 9.1® software analysis

The Design of Experiments (DoE) (MODDE 9.1®) software was used to identify and optimize selected three plant:plant combination. The DoE approach is an organised method that addresses both simple and complex experimental problems (Eriksson et al., 2008). In this study, the experimental objective was to optimize the MIC value of a three plant combination in order to obtain the lowest MIC value which would provide the best antimicrobial activity when all three plants are combined. The plants for further DoE analysis was *C. papaya*, *S. serratuloides* and *H. hemerocallidea* in combination. This three plant combination was selected due to the number of times the combination was referred to by the lay people (Table 2.2).

The response is the MIC value (mg/ml) and the factors are the plants within the combination. In order to formulate the optimized ratios a series of experiments formulated by MODDE® 9.1 software were provided in which the three plants are tested individually (1:00:0.00:0.00) and combined at various ratios (66.70: 16.70: 16.70; 0.50: 0.50: 0.00 and 33.33: 33.33: 33.33) and tested in the laboratory. The new ratios of experiments are included as well a standard reference experiment (all plants combined in equal ratios) which is also known as the 'center point' (Eriksson et al., 2008).

The factors and responses are entered into MODDE® 9.1 and various graphical representations of the experiments need to be interpreted before the final optimization. The analysis of the graphical representation is of utmost importance as the graphs indicate if the full factorial design is a suitable model to be used for analysis and if the model can reproduce reliable results. The graphs required for analysis are the replicate plot, the histogram of skewness, the summary of fit plot, the coefficients plot, the observed versus predicted plot and the response contour plot (Eriksson et al., 2008).

2.6.1 The replicate plot

The measured values (obtained in the laboratory by combining the various ratios) of a response (MIC values) are plotted against a unique number associated with each experiment (Errikson et al., 2008). The replicate plot is designed to understand how similar the MIC values obtained in the laboratory for all the triplicate experiments are to one another. Each experiment is assigned a number and if all the triplicate MIC values are the same, then the vertical lines will overlap one another. The replicate plot establishes if the experimental results vary too much and if so, then there could be an error in your method or plant sample. The centre points are depicted as point A on the replicate plot x axis (Figure 2.4). These points are experiments of the combination in ratio (0.33:0.33:0.33), it is essential for a good experiment design to have these points as close together as possible.

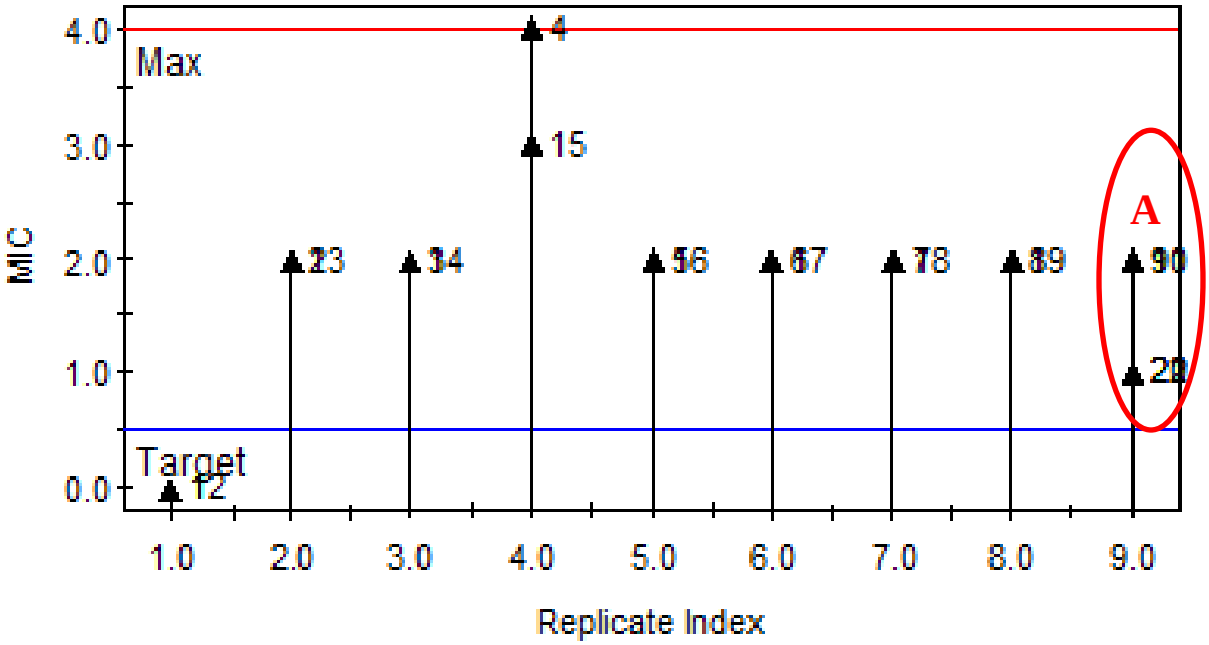


Figure 2.4 An ideal replicate plot with the centre points all closely plotted on the extreme right.

2.6.2 The histogram of skewness

The histogram of skewness is used to evaluate the statistical properties of the response data (i.e. MIC values) (Eriksson et al., 2008). The histogram indicates the distribution of a response variable (MIC values). The ideal histogram is representative of a mean predominant response (MIC value) which graphically is a 'bell shaped' histogram with one peak (Figure 2.5). This may not always be the outcome in which case a transformation, which is a variant statistical calculation which can configure the stabilisation of residuals and removes 'outliers' (background noise) from a non-normal distribution into a normal distribution ('bell shaped'), may occur. The transformations are performed as a negative log, positive log and exponential calculations.

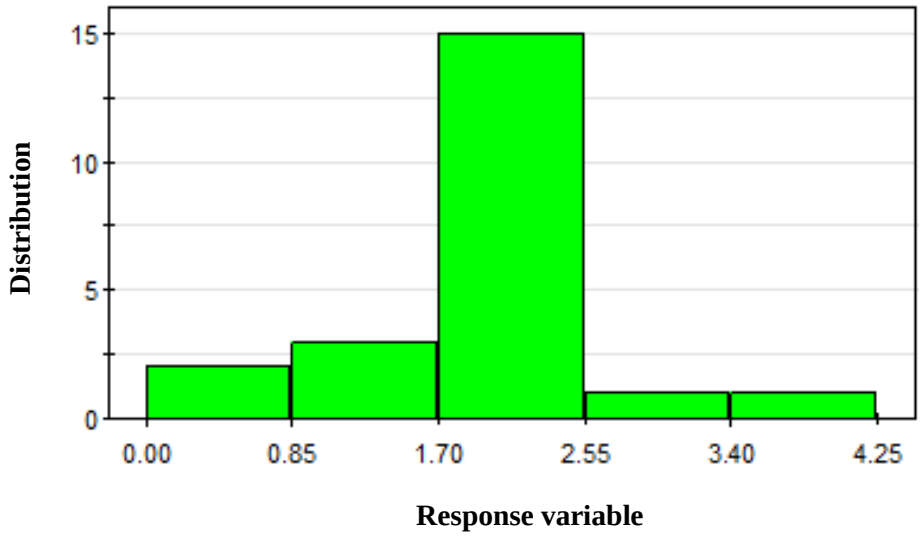


Figure 2.5 The bell shape histogram indicative of normal distribution.

2.6.3 The summary of fit plot

The summary of fit plot is a tool used to interpret if this software can optimize the MIC value (Eriksson et al., 2008). The summary of fit graph indicates if the factorial design is understandable, predictable, valid and reproducible. There are four performance indicators that need to be considered (Figure 2.6) should be as high as possible.

The performance indicators are the R_2 (green bar) value which measures how well the model is suited for the experiments and therefore how well the model is suitable for the predictability of MIC values (Eriksson et al., 2008) (Figure 2.6). The R_2 value must be greater than 0.70 (70.0%) which will indicate a good model whereas an R_2 value of 1.00 indicates a perfect model.

The performance indicator Q_2 (dark blue bar) value is used to determine how well the full factorial design can predict new experiments (Eriksson et al., 2008) (Figure 2.6). The Q_2 value must be greater the 0.40 (40.0%) which will indicate a good predictability model, whereas a Q_2 value greater than 0.90 indicates an excellent predictable model.

The Model Validity (yellow bar) is based on the model error over the pure error (Eriksson et al., 2008). Therefore a Model Validity value < 0.25 indicates a good model (Figure 2.6).

The Reproducibility value (light blue bar) must be over 0.50 (Eriksson et al., 2008). If the value is below 0.50 this indicates large pure error and poor control of the experimental procedure (Figure 2.6).

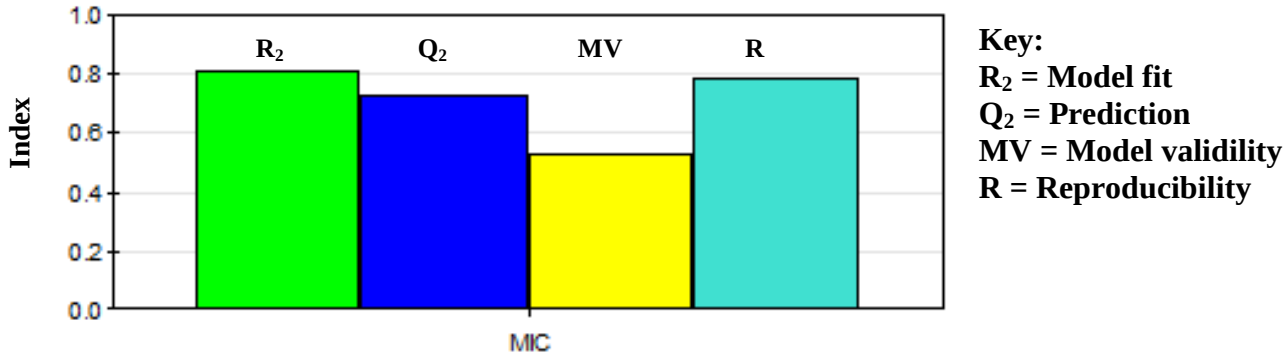


Figure 2.6 The summary of fit plot indicating the full factorial design reproducibility and predictability.

2.6.4 The coefficients plot

The coefficients plot displays the impact of the plants on the response variable (MIC value) (Eriksson et al., 2008). The coefficient plot allows for the software to interpret which plant has the best MIC value therefore the most antimicrobially dominant plant and then further if the combinations are significant or non-significant (irrelevant) is primarily calculated and based on biopharmaceutical formulations and analysis, therefore, the interpretation from a pharmaceutical perspective is different from an antimicrobial perspective. The difference in interpretation becomes crucial when interpreting the coefficient plot. The coefficient that extends' into the negative region is more effective than the coefficients that are in the positive region (Figure 2.7). This interpretation stems from the antimicrobial interpretation of a lower MIC value possessing stronger antimicrobial activity. The lines that extend through bar graphs are indicators of variables (plant species or combined plant species) that could be rendered insignificant to obtaining the best MIC values. In such a case the bar (variable) may be excluded so long as the

Q_2 value is not effected. In the example the variable (*C. papaya* with *S. serratuloides*) has been excluded (Figure 2.7).

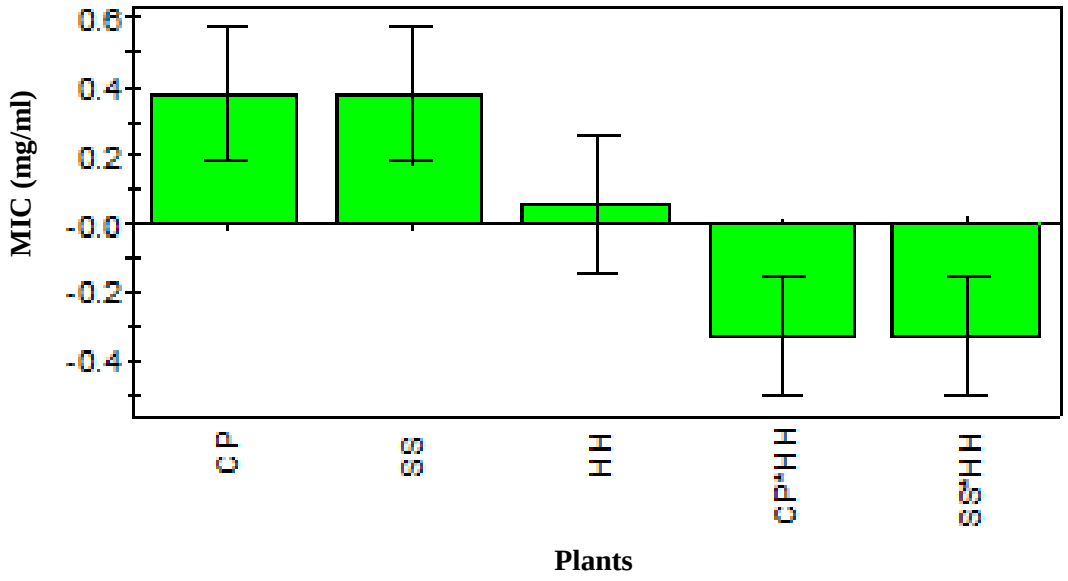


Figure 2.7 The coefficient plot indicating the influence of the variables on the response where CP = *C. papaya*, SS = *S. serratuloides* and HH = *H. hemerocallidea*.

2.6.5 The observed versus predicted plot

The observed versus predicted plot displays the relationship between the measure and calculated data (Eriksson et al., 2008). A straight line is plotted on the graph and all plot points should fall close to the straight line (Figure 2.8). If a specific area on the graph deviates from the line significantly then the full factorial design may need to be reconsidered as the model may not fit the response. If a few points on the graph are deviant the software does allow for the exclusion of those points, however, if majority of the points are deviant then the model should be reconsidered.

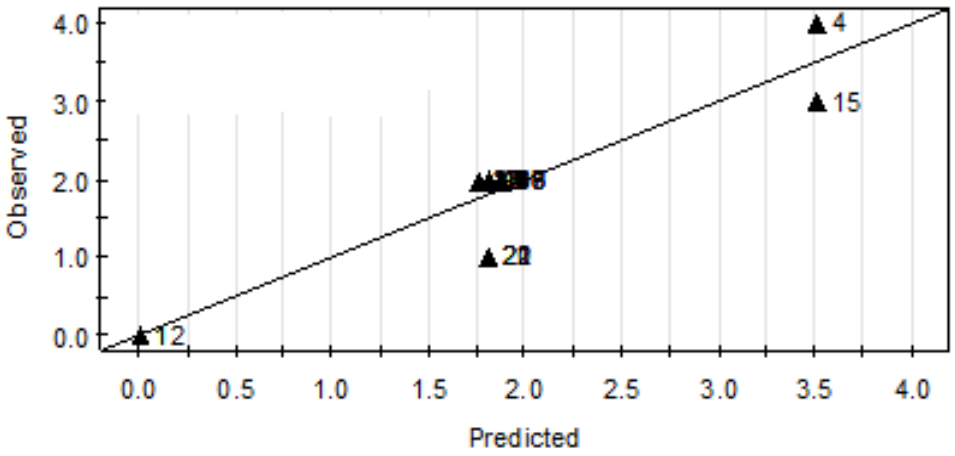


Figure 2.8 Observed versus predicted plot with no points deviating significantly from the centre line.

2.6.6 The response contour plot

The response contour plot indicates a maximum and minimum region (Figure 2.9). Specifically to MIC values which are needed to be low the green region is the main focus. The contour plot provides a region in which the three plants are allocated various ratios to be combined and a possible MIC value is provided. The contour plot therefore provides an optimal regression model and maximises the best possible combination to give the lowest MIC value (Eriksson et al., 2008).

The contour plot provides a ratio and the ratios then need to be calculated into a volume in order to combine the plant species to test in the laboratory (Section 2.4.2). Ratios *C. papaya* = a, *S. serratuloides* = b and *H. hemerocallidea* = c.

Y (%) = ratio a+ ratio b+ ratio c **Equation 2.7**

Volume of plant = ratio a /y (%) x 64 mg/ml **Equation 2.8**

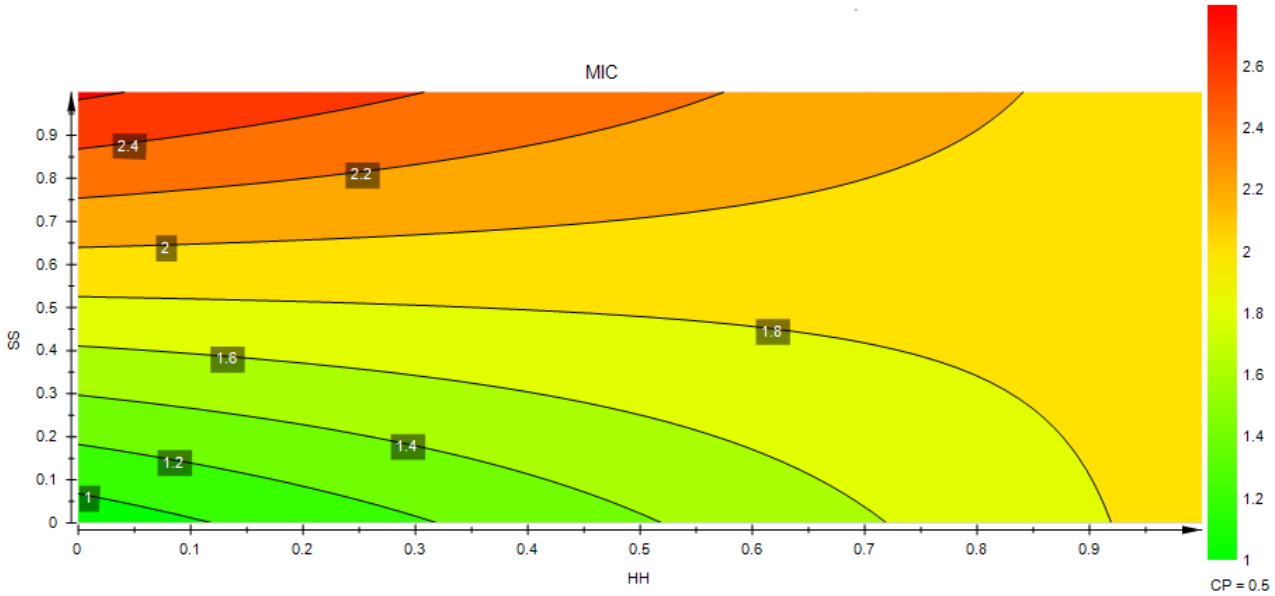


Figure 2.9 The contour plot indicates the ratios the three plants can be combined for a maximum and minimum MIC value. Where CP = *C. papaya*, SS = *S. serratuloides* and HH = *H. hemerocallidea.*

Chapter 3

Antimicrobial analysis and toxicity properties of plants used singularly to treat STIs in Maputaland

3.1 Introduction

A selection of 20 plants were indentified by the lay people of Maputaland to treat STIs. An overview of the plants and background of the study is provided in Chapter 1. The traditional use comprised mostly of various plant combinations. The antimicrobial efficacy of these plants were assessed singularly in order to determine whether the plants exhibit anti-STI activity. This was done to gain a better understanding as to why certain plants are preferred, for the treatment of STIs.

The aim of this chapter was to study the antimicrobial efficacy of the twenty medicinal plants identified as STI treatment regimens in Maputaland. Furthermore, the twenty plants (both aqueous and organic plant extracts) were tested against the human kidney epithelial cells to investigate potential toxicity. The toxicity and antimicrobial assays were all conducted concurrently, however, the toxicity results are reported first followed by the antimicrobial results in order to cross reference the resulting noteworthy antimicrobial activities with the observed toxicity results.

3.2 Results

3.2.1 Toxicity cellular viability assay

The cellular viability toxicity assay was conducted in order to gain insight into whether or not the plants used individually are toxic to the human kidney epithelial cells. The results from this study aids with alerting users of potential adverse effects which directly relate to the administration of the plants when supplied as a treatment for STIs. For this study the extract/drug-free control produced 100% cell viability. Percentage of cells that decreased more than 50% were considered to be toxic at 100 µg/ml against the kidney epithelial cells. If a plant causes an increase of cells

the plant could cause cell replication and stimulatory effects. Cell replication is not a negative feature of the plant in this circumstance.

The results indicate that only one of the twenty plants, namely *K. africana*, had potential for toxicity at 100 µg/ml and therefore dosages must be given with caution. The aqueous extract of *K. africana* resulted in 78% cell viability, which equates to 22% cell death, which was similar for the organic extract of *K. africana* where there was a 16% cell death (Table 3.1).

The results also indicated that both the aqueous and organic extracts of a few plants seemed to slightly enhance metabolism of the tetrazolium salt of the human kidney epithelial cells. These changes were observed in each triplicate experimental run and on more than experimental run, with all precautions taken to ensure no interaction occurred between the MTT and plant extracts or there was a colour interference. These plants were *A. adianthifolia*, *B. pilosa*, *E. hypericifolia* and *O. engleri* (Table 3.1).

Table 3.1 Toxic properties of individual plants where the values represent percentage (%) cell viability ± standard deviation for at least triplicate experiments.

Plant species	Aqueous extract	Organic extract
Albizia adianthifolia	110.2 ± 0.75	112.3 ± 0.65
Aloe marlothii	102.4 ± 0.70	99.4 ± 0.85
Bidens pilosa	112.1 ± 0.76	115.3 ± 0.82
Carica papaya	102.0 ± 0.74	115.4 ± 0.67
Cassipourea malosana	104.2 ± 0.78	116.1 ± 0.82
Clematis brachiata	100.5 ± 0.84	112.1 ± 0.76
Euphorbia hypericifolia	118.3 ± 0.77	120.3 ± 0.81
Hypoxis hemerocallidea	104.2 ± 0.79	102.4 ± 0.88
Kigelia africana	**78.2 ± 0.84**	**84.4 ± 0.85**

Table 3.1 Properties of individual plants where the values represent percentage (%) cell viability ± standard deviation for at least triplicate experiments.

Plant species	Aqueous extract	Organic extract
Musa acuminata	115.4 ± 0.76	100.3 ± 0.82
Ozoroa engleri	112.3 ± 0.78	120.5 ± 0.91
Peltophorum africanum	98.2 ± 0.76	99.4 ± 0.81
Ranunculus multifidus	102.2 ± 0.83	104.1 ± 0.75
Sarcophyte sanguinea	99.4 ± 0.89	102.2 ± 0.92
Sclerocarya birrea	102.3 ± 0.67	100.5 ± 0.89
Senecio serratuloides	112.4 ± 0.75	99.4 ± 0.78
Syzygium cordatum	104.1 ± 0.81	102.4 ± 0.82
Tabernaemontana elegans	99.0 ± 0.78	106.1 ± 0.76
Trichilia dregeana	100.2 ± 0.78	99.4 ± 0.87
Ximenia caffra	108.5 ± 0.81	102.3 ± 0.85

Extract/Drug-Free control: untreated control = 100% cell viability.
Positive control: Quinine IC_{50} = 141.34 ± 22.08 µg/ml.
Plant and values in bold indicate a decrease of cell viability indicative of toxic properties.

3.2.2 Antimicrobial MIC assay

For the antimicrobial MIC assays, the controls are provided in Table 3.2 to avoid repetition in graphs. The same controls were used for all tested samples. Antimicrobial investigations were undertaken to determine the antimicrobial efficacy when the plants are used singularly against the six STI pathogens. The MIC values are represented as bar graphs for each pathogen (Figure 3.1 – 3.6). The plants were assessed for antimicrobial efficacy using the following guideline: MIC values < 1.00 mg/ml = noteworthy antimicrobial activity; ≥ 1.00 mg/ml or < 4.00 mg/ml = moderately good antimicrobial activity; 4.00 mg/ml or < 8.00 mg/ml = moderately good

antimicrobial activity; ≥ 8.00 mg/ml = poor antimicrobial activity (Van Vuuren and Viljoen, 2011).

Table 3.2 Mean MIC values (µg/ml) for controls tested in the antimicrobial assay.

Pathogen	Antibiotic	MIC (µg/ml)
U. urealyticum (clinical strain)	Ciprofloxacin	0.20
O. ureolytica (ATCC 43534)		0.63
G. vaginalis (ATCC 14018)		0.39
T. vaginalis (clinical strain)		0.12
N. gonorrhoeae (ATCC 19424)		0.04
C. albicans (ATCC 10321)	Amphotericin B	2.50
Negative controls	Acetone	>2.50
	Broth	>2.50

3.2.2.1 Plants tested against *C. albicans*

S. cordatum (bark) was the only organic plant extract to achieve demonstrate antimicrobial activity against *C. albicans* with an MIC value of 0.50 mg/ml. It is also the plant most effective against *C. albicans* (Figure 3.1). Noteworthy antimicrobial activity was further seen with aqueous plant extracts of *O. engleri* (leaf) with an MIC value of 0.75 mg/ml, *S. cordatum* (bark) with an MIC value of 0.50 mg/ml and *T. elegans* (bark) with an MIC value of 0.38 mg/ml (lowest MIC value against *C. albicans*). The aqueous plant extracts of *H. hemerocallidea*, *O. engleri*, *P. africanum*, *S. serratuloides* and *T. elegans* proved to be more antimicrobially effective compared to the organic plant extracts (Figure 3.1). This result would be promising for the lay people as water is the solvent mostly used when preparing the herbal remedies.

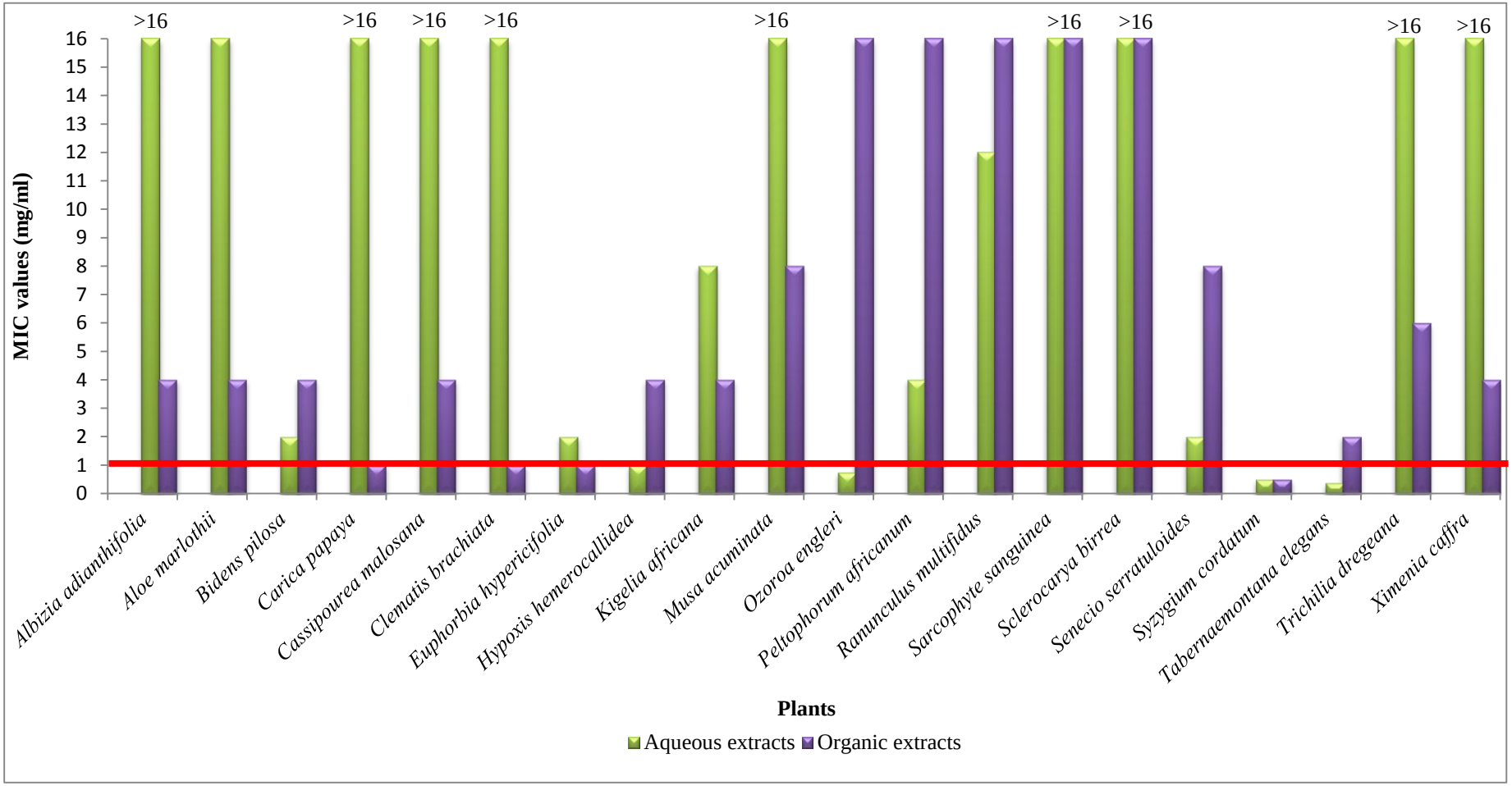


Figure 3.1 Antimicrobial activity for the individual plants (aqueous and organic extracts) against *C. albicans* (ATCC 10321).

3.2.2.2 Plants tested against *U. urealyticum*

Thirteen aqueous plant extracts possessed noteworthy antimicrobial activity against *U. urealyticum* (Figure 3.2). The aqueous plant extracts of *A. marlothii* (leaf), *C. papaya* (leaf) and *S. serratuloides* (leaf) obtained MIC values of 0.75 mg/ml, 0.38 mg/ml and 0.50 mg/ml respectively. A further ten aqueous plant extracts depicted in Figure 3.2 resulted in MIC values of 0.25 mg/ml. The lowest MIC value of 0.02 mg/ml was achieved by the aqueous extract of *R. multifidus* (leaf). Twelve organic plant extracts exhibited noteworthy antimicrobial activity against *U. urealyticum*. *C. malosana* (leaf), *M. acuminata* (root) and *S. serratuloides* (leaf) obtained MIC values of 0.75 mg/ml, 0.63 mg/ml and 0.63 mg/ml respectively. Furthermore, *B. pilosa* (leaf), *C. papaya* (leaf), *K. africana* (leaf) and *X. caffra* (leaf) obtained MIC values of 0.50 mg/ml with *A. adianthifolia* (bark), *R. multifidus* (leaf), *S. birrea* (bark) and *T. dregeana* (bark) obtaining MIC values of 0.25 mg/ml (Figure 3.2). The lowest MIC obtained from the organic extracts was from *P. africanum* with an MIC value of 0.04 mg/ml.

Upon comparison of the aqueous extracts with the organic plant extracts, the aqueous plant extracts of *C. malosana, H. hemerocallidea, R. multifidus, S. cordatum* and *T. elegans* were more antimicrobially efficacious (Figure 3.2). The plants possessing an overall noteworthy effect with both aqueous and organic extracts were the ten plants namely, *A. adianthifolia, B. pilosa, C. papaya, C. malosana, K. africana, R. multifidus, S. birrea, S. serratuloides, T. dregeana* and *X. caffra*.

3.2.2.3 Plants tested against *O. ureolytica*

The two plants (aqueous extracts) exhibiting noteworthy antimicrobial activity against *O. ureolytica* were *C. papaya* (leaf) and *A. marlothii* (leaf) resulting in MIC values of 0.50 mg/ml and 0.38 mg/ml respectively (Figure 3.3). The organic plant extract of *X. caffra* (leaf) possessed noteworthy antimicrobial activity against *O. ureolytica* with an MIC value of 0.75 mg/ml. Also, *C. papaya* (leaf), *E. hypericifolia* (leaf) and *O. engleri* (leaf) organic plant extracts achieved noteworthy antimicrobial activity with MIC values of 0.50 mg/ml (Figure 3.3).

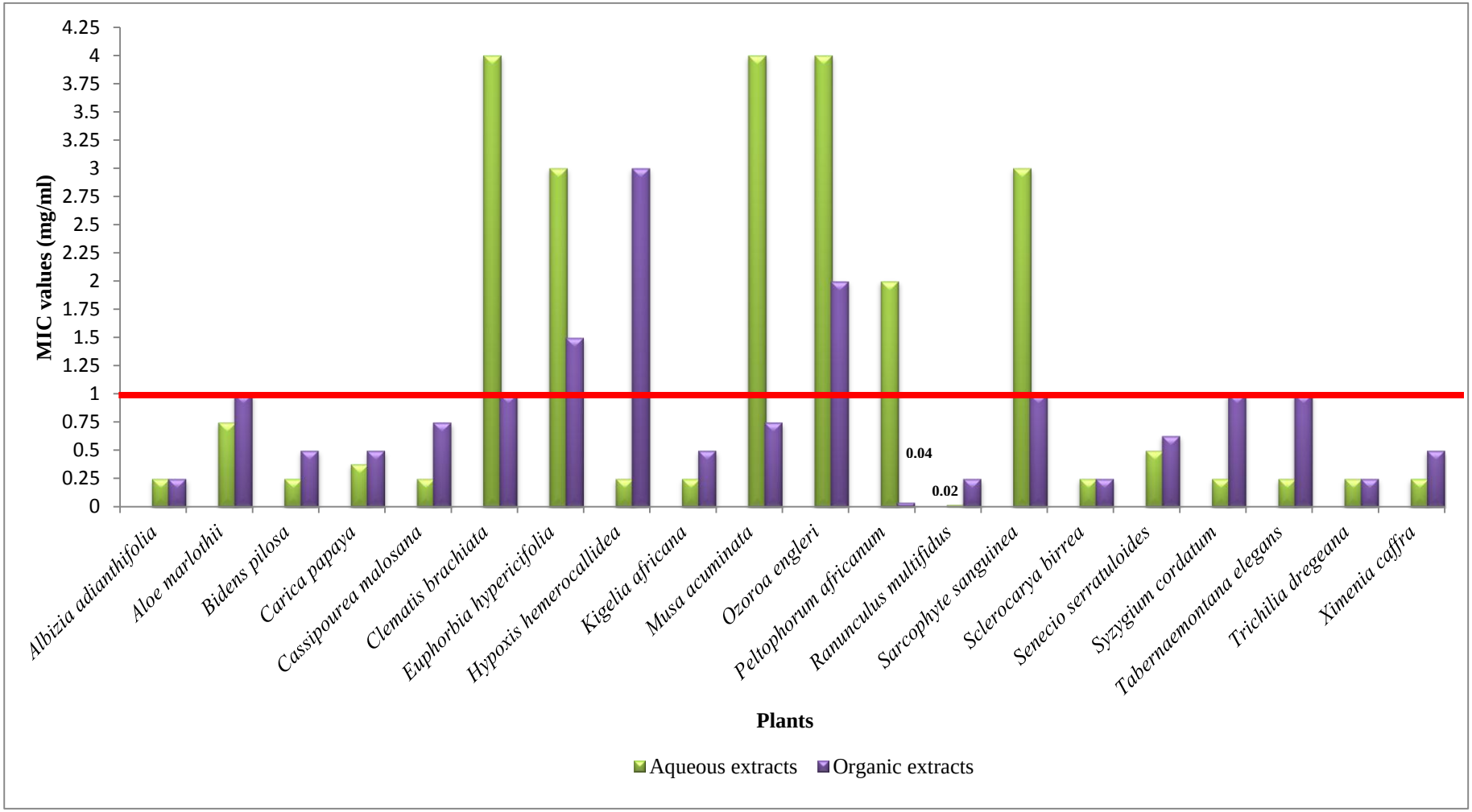


Figure 3.2 Antimicrobial activity for the individual plants (aqueous and organic extracts) against *U. urealyticum* (clinical strain).

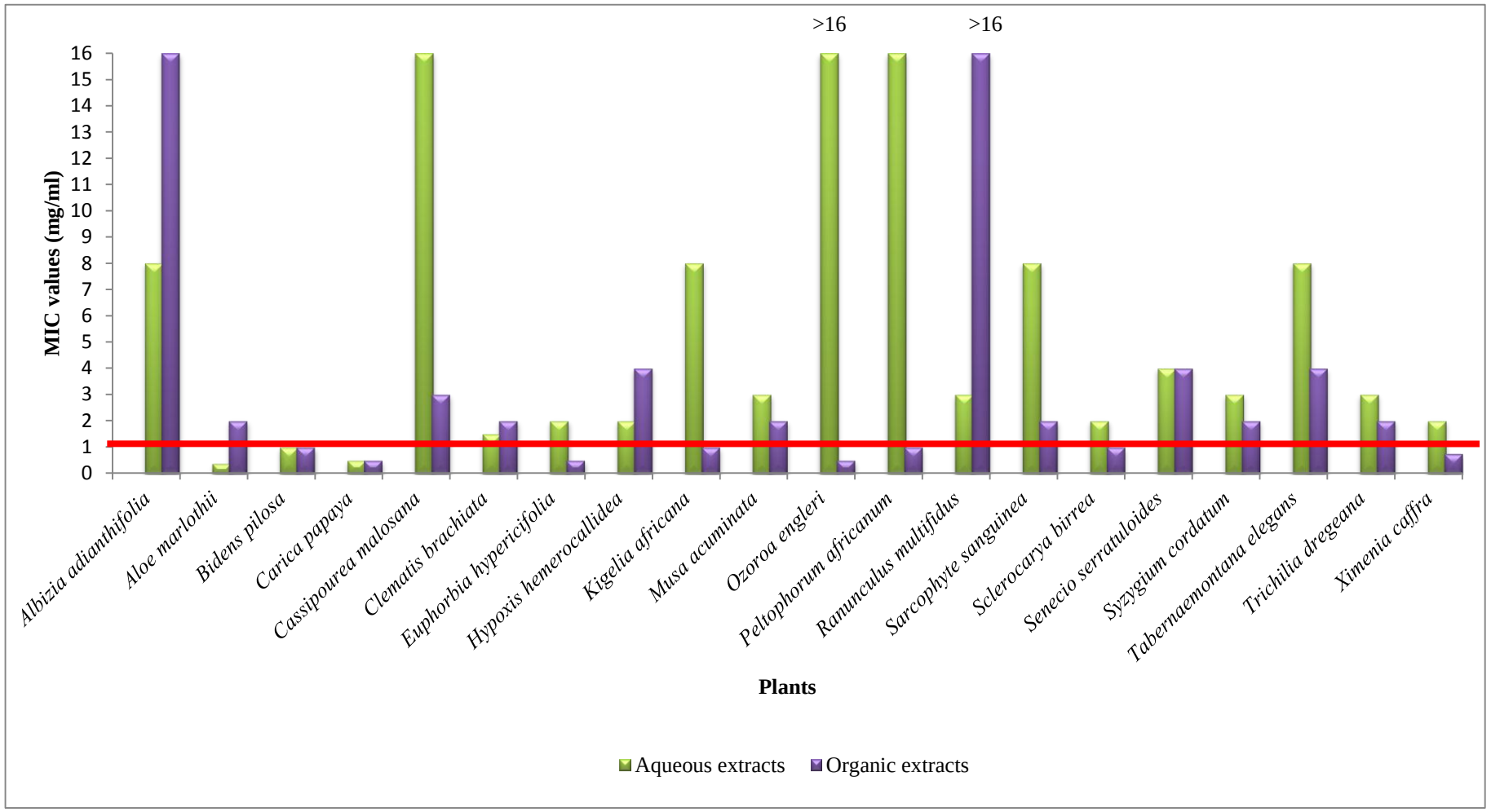


Figure 3.3 Antimicrobial activity for the individual plants (aqueous and organic extracts) against *O. ureolytica* (ATCC 43534).

The lowest MIC value overall when comparing the organic and aqueous extracts against *O. ureolytica* was, 0.38 mg/ml, which was obtained by the aqueous plant extract of *A. marlothii*. The aqueous plant extract of *R. multifidus* should be noted for the phenomenal MIC difference between the aqueous and organic plant extracts. In this case the aqueous plant extract possessed more than five fold better antimicrobial activity compared to the organic plant extract (Figure 3.3). *C. papaya* was the only plant to possess noteworthy antimicrobial activity for both aqueous and organic plant extracts with MIC value of 0.50 mg/ml for both extracts.

3.2.2.4 Plants tested against *T. vaginalis*

B. pilosa (leaf) was the only plant to have moderately good activity with the aqueous plant extract, MIC value 2.00 mg/ml against *T. vaginalis*. Greater antimicrobial effectiveness was seen with the organic plant extracts, whereby seventeen plants exhibited moderately good activity with the lowest MIC value of 1.00 mg/ml for *C. papaya* (leaf), *O. engleri* (leaf), *S. sanguinea* (stem), *S. cordatum* (bark) and *T. elegans* (bark). *A. marlothii* (leaf) possessed moderate antimicrobial activity 6.00 mg/ml (Figure 3.4). No noteworthy activity was obtainable from the aqueous or the organic plant extracts.

3.2.2.5 Plants tested against *G. vaginalis*

Noteworthy antimicrobial activity against *G. vaginalis* was observed with the aqueous plant extracts for *S. cordatum* (bark) and *P. africanum* (root) having MIC values of 0.75 mg/ml and 0.50 mg/ml respectively (Figure 3.5). With the organic plant extracts for *E. hypericifolia* (MIC value = 0.75 mg/ml), *S. sanguinea* (MIC value = 0.63 mg/ml), *S. birrea* (MIC value = 0.50 mg/ml), *P. africanum* (0.50 mg/ml) and *T. elegans* (MIC value = 0.25 mg/ml) noteworthy antimicrobial activity was demonstrated (Figure 3.5). The lowest MIC values obtained for the aqueous and organic extracts of *P. africanum* and *T. elegans* against *G. vaginalis* were 0.50 mg/ml and 0.25 mg/ml respectively. The aqueous extract of *X. caffra* demonstrated the most notable difference where a four fold improvement in the MIC value was observed with the aqueous extract compared to the organic plant extracts (Figure 3.5).

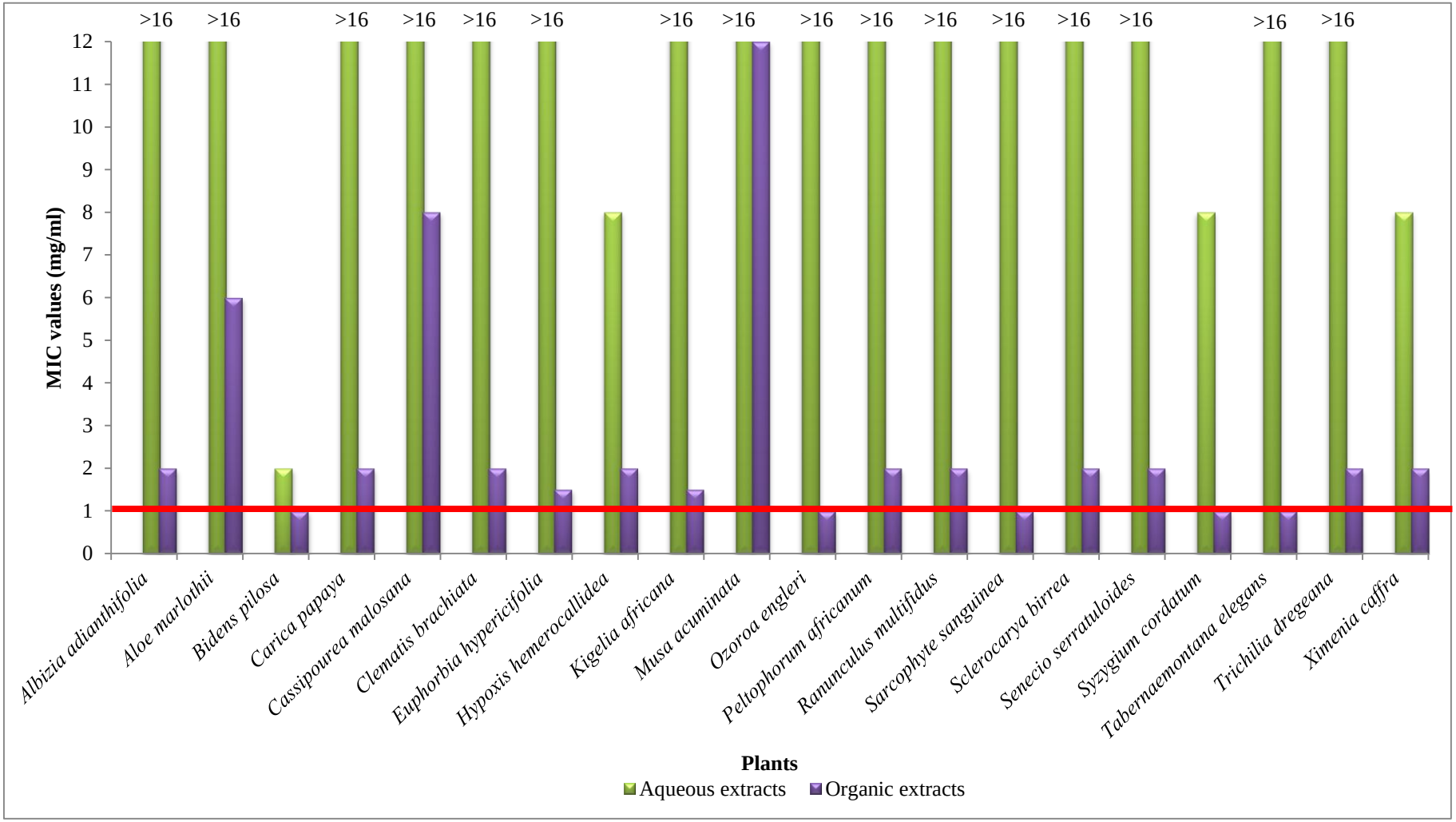


Figure 3.4 Antimicrobial activity for the individual plants (aqueous and organic extracts) against *T. vaginalis* (clinical strain).

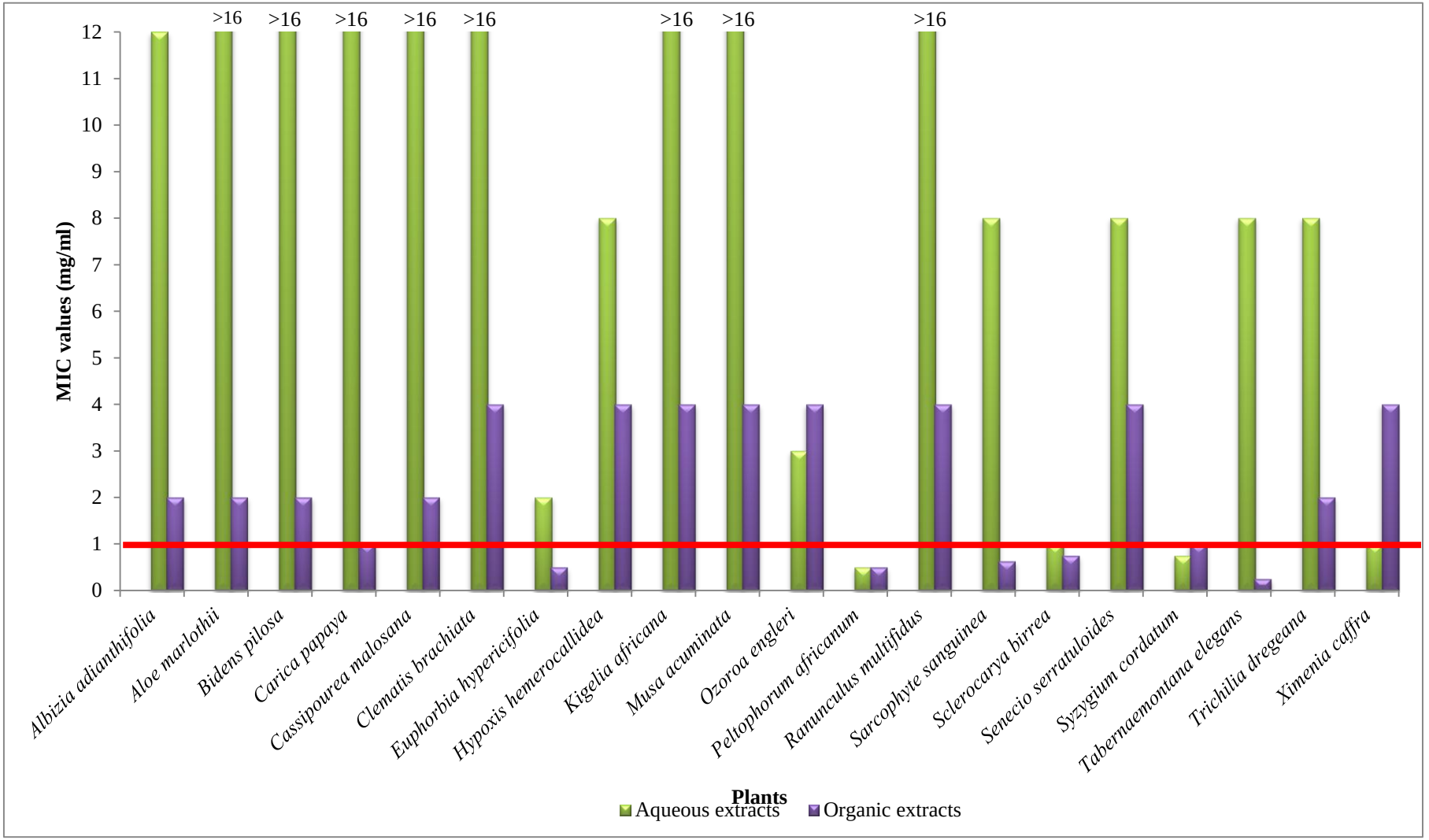


Figure 3.5 Antimicrobial activity for the individual plants (aqueous and organic extracts) against *G. vaginalis* (ATCC 14018).

3.2.2.6 Plants tested against *N. gonorrhoeae*

The aqueous plant extracts of *H. hemerocallidea* (corm), *P. africanum* (root) and *S. birrea* (bark) exhibited noteworthy antimicrobial activity with MIC values of 0.50 mg/ml against *N. gonorrhoeae* (Figure 3.6). Further noteworthy antimicrobial activity was obtained from the aqueous extracts of *S. sanguinea* (bark), *S. cordatum* (bark) and *X. caffra* (leaf) with MIC values of 0.50 mg/ml. Six of the plants organic extracts' namely, *B. pilosa* (leaf), *C. papaya* (leaf), *C. malosana* (leaf), *P. africanum* (root), *S. birrea* (bark) and *S. cordatum* (bark) exhibited noteworthy antimicrobial activity with MIC values of 0.25 mg/ml. Both aqueous and organic extracts of *P. africanum*, *S. sanguinea*, *S. birrea* and *S. cordatum* exhibited noteworthy activity against *N. gonorrhoeae* (Figure 3.6).

3.3 Discussion

3.3.1 Cytotoxic analysis

In order to ascertain the safety of the plants, toxicological *in vitro* screening against human kidney epithelial cell line is the first step in doing so especially since only half of the plants have been previously investigated for potential toxicity (Table 1.2).

In a previous *in vitro* study by Fouche et al., (2008), the roots and leaves of *K. africana* (6.25-15.0 µg/ml) were found to possess anti-cancer activity against breast, renal and melanoma human cell lines. The dichloromethane extract (leaves and root) inhibited the renal cell line producing 42.9% and 14.9% cell death of, respectively (Fouche et al., 2008). Whilst the organic extract of the leaves tested in the current study produced 16.0% cell death. *K. africana* has been shown to possess toxic properties, where the plants' bark (250 µg/ml) was found to be toxic against white blood cells (Fennell et al., 2004; Nyarko et al., 2005; Eldeen and Van Staden, 2007; Shai et al., 2008). The fruit (500 mg/kg) decreased the white blood cell number in albino rats (Fennell et al., 2004; Nyarko et al., 2005). The unripe fruit (100 µg/ml) of *K. africana* was also found to be toxic by Palmer and Pitman (1972), however, the flower of *K. africana* was shown not to be genotoxic using the AMES test (Elgorashi et al., 2003).

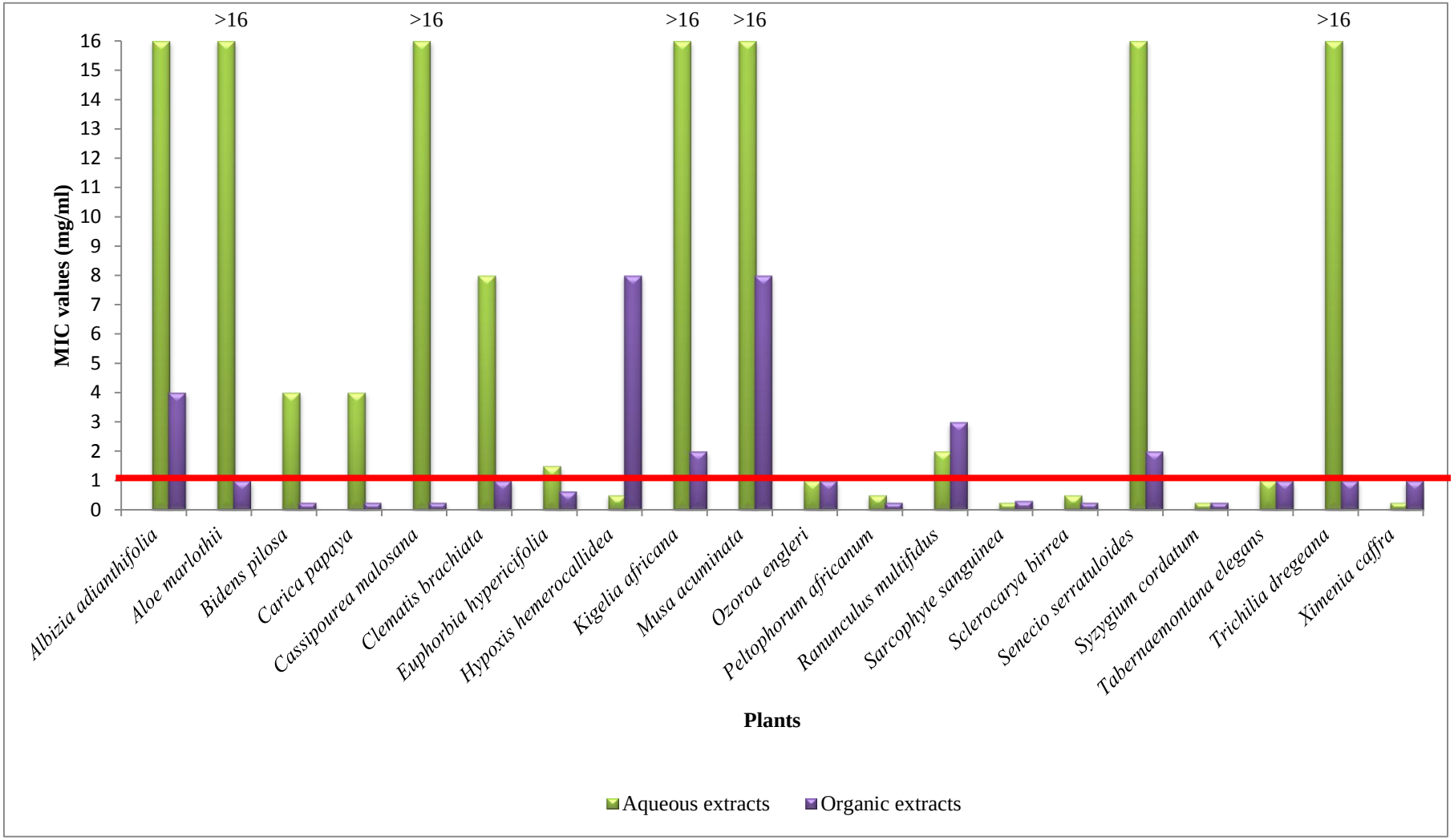


Figure 3.6 Antimicrobial activity for the individual plants (aqueous and organic extracts) against *N. gonorrhoeae* (ATCC 19424).

K. africana (10 and 100 µg/ml stem/bark) was found to be non-toxic when assessed using the anti-proliferation assay against colon adenocarcinoma, cervical and skin carcinoma cell lines (Kamuhabwa et al., 2000). This is to be expected as various cell lines have different sensitivity patterns to drugs and compounds and as such to ascertain if a plant extract has pure toxicity properties it should be tested against a wide range of cell lines to determine the toxicity profile.

Toxicity studies have been reported against some of the plants investigated in this study (Table 1.2), however, according to this study no toxicity was indicated with any plant besides *K. africana* (Table 3.1). There are numerous possible reasons for this outcome. In previous studies the plants (100 µg/l) were tested against different cell lines for example liver and oesophageal cell lines and phototoxicity was tested using *B. pilosa* (Towers et al., 1977; Mirvish, 1979; Yuan et al., 2008), *A. adianthifolia* (100 µg/ml) was tested against the Jukta cell line (Haddad et al., 2004), *S. birrea* and *T. elegans* (20 mg/ml) were tested against human fibroblasts (Loughlin et al., 2008; Luo et al., 2011), as well as *X. caffra* (10-100 µg/ml) against colon adenocarcinoma, skin carcinoma and cervical carcinoma (Kamuhabwa et al., 2000). A further possible reason could be due to different plant parts tested, as is the case with *A. adianthifolia* (100 µg/ml) whereby the root was investigated in a previous study and in this study the bark was investigated (Prozesky et al., 2001; Haddad et al., 2004). In the mutagenic study investigating *S. serratuloides* (100 µg/ml) the AMES assay was used, which is not comparable to that obtained for the cellular viability assay used in the current study, but together do contribute a better understanding of the safety profile of this plant species (Elgorashi et al., 2003). Since pregnant mothers are also treated for STIs at a plasma concentration of between 10-20 mg/ml, *C. papaya* (leaf), *B. pilosa* (leaf) and *M. acuminata* (root/leaf) were found to induce uterine contractions and therefore caution must be taken when these plants are administered to pregnant women infected with STIs (Cherian, 2000; Sarma and Mahanta 2000; Adebiyi et al., 2003; Kamatenesi-Mugisha, 2004; Kamatenesi-Mughisha and Oryem-Origa, 2007; Nikolajsen et al., 2011).

The concentration of the plant being tested should also be taken into consideration. It is important to note that this was only one toxicity assay whereby toxicity could be determined

with the human kidney epithelial cells, it is important to continue tests on other cell lines as well as using other *in vitro* toxicological assays and *in vivo* studies.

3.3.2 Antimicrobial analysis

There have been antimicrobial studies performed on the twenty plants selected in this study (Table 1.2). However, with the exception of *S. cordatum*, studied by Van Vuuren and Naidoo (2010), none of the other nineteen plants have been tested against all six of the selected sexually transmitted pathogens. From the 20 plants investigated in this study, 17 were found to be associated with the treatment of gonorrhoeae (Van Puyvelde et al., 1983; Abbiw, 1990; Mabogo, 1990), syphilis (Chhabra et al., 1991; Hutchings et al., 1996) and unspecified venereal diseases (Arnold and Gulumiam, 1984; Hutchings et al., 1996; Prozesky et al., 2001). The most common pathogens investigated are *Candida albicans* (Motsei et al., 2003; Buwa and Van Staden; 2006; Hamza et al., 2006; Kambizi and Afolayan, 2008; Kuete et al., 2010; Mulaudzi et al., 2011; Ncube et al., 2012), *Neisseria gonorrhoeae* (Silva et al., 2002; Kambizi and Afolayan, 2008; Shokeen et al., 2009; Chomnawang et al., 2009; Mulaudzi et al., 2011) and a few publications report on activities against *Trichomonas vaginalis* (Calzada et al., 2007; Arthan et al., 2008; Moo-Puc et al., 2008; Tiwari et al., 2008; De Villiers et al, 2010). In northern Maputaland, however, no scientific validation has been done for the use of their herbal remedies to treat STIs.

U. urealyticum was the most susceptible pathogen to the selected plants with 13 aqueous and 11 organic plant extracts exhibiting noteworthy antimicrobial activity. The plants *R. multifidus* (aqueous extract) and *P. africanum* (organic extract) obtained the lowest MIC values in this study against *U. urealyticum*. This indicates that *P. africanum* and *R. multifidus* would be the most suitable plants for the treatment of urethritis.

N. gonorrhoeae was the next most susceptible pathogen whereby six aqueous and eight organic plant extracts possessed noteworthy antimicrobial activity. Gonorrhoea is a frequently treated STI in southern Africa, particularly in Maputaland with current strains of the organism demonstrating drug resistance against antibiotics penicillin and tetracycline (Mbwana et al., 1999).

P. africanum (roots) was previously tested by Steenkamp et al., (2007) whereby the results correlated to that found in this study, where the organic extracts (roots and bark) with efficacies of 0.50 mg/ml were observed against *C. albicans*. The aqueous extract (bark) in the current study did possess moderate antimicrobial activity (MIC = 4.00 mg/ml). In a previous ethnobotanical study by Maroyi (2011), the leaves, bark or roots of *P. africanum* were mentioned by the Nhema people to treat syphilis. The people of Maputaland also use *P. africanum* to treat syphilis and urethritis. *P. africanum* has also further demonstrated inhibition of HIV-1 (Bessong et al., 2005) as well as other bactericidal properties (Samie et al., 2005). *P. africanum* (organic extract) further achieved the best antimicrobial activity (lowest MIC value) when compared to all organic extracts against *U. urealyticum*. *R. multifidus* (aqueous extract) demonstrated the most promising antimicrobial activity specific to *U. urealyticum*, therefore this plant may demonstrate exciting future challenges for future antiinfective studies for the treatment of urethritis.

The results in this study with regard to *S. cordatum* (aqueous and organic) against the range of pathogens correlated with a previous study by Van Vuuren and Naidoo (2010), whereby noteworthy activity was indicated against the same pathogens with similar MIC values. A lower MIC value was found, however, with the aqueous extracts of *S. cordatum* against *C. albicans* (0.50 mg/ml) indicating noteworthy antimicrobial activity. A study done by Steenkamp et al., (2007) indicated a similar MIC value of 2.50 mg/ml indicative of moderately good antimicrobial activity. The *S. cordatum* organic extract from this study and the *S. cordatum* methanol extract examined by Steenkamp et al., (2007) were coherent. *S. cordatum* demonstrated antimicrobial activity against four out of the six STI pathogens, thus displaying a broad-spectrum of antimicrobial activity against STIs.

C. papaya possesses a broad-spectrum of activity for the organic plant extracts. Interestingly, the therapeutic efficacy of *C. papaya* does not only extend to the STI efficacy but has demonstrated wound healing properties (Gurung and Ŝkalko-Basnet, 2009). The wound healing property was associated with the latex of the plant where the hydroxyproline content increased collagen turnover and also possessed wound contraction ability (Gurung and Ŝkalko-Basnet, 2009). This could indeed be seen as a great combination for not only treating STIs, but also healing the

external and internal sores and wounds left behind after the infection is healed. Leaves of *C. papaya* have been proven to possess anti-inflammatory and analgesic properties when compared to aspirin (Amazu, 2010; Akah et al., 2002). These beneficial properties may aid in the therapy and healing of STIs. Sexually transmitted infections are directly related to the fertility of male and female reproductive system, therefore relevant to this study was a previous investigation conducted by Lohiya et al., (1999) whereby the seeds of *C. papaya* were found to be effective male contraceptives. According to Lohiya et al., (1999) *C. papaya* kills the male sperm, while not affecting the male libido. *C. papaya* can thus contribute to a reduction in pregnancies due to the dead sperm and with the recurrent STI rate this could potentially reduce the number of babies born with STIs. *C. papaya* (leaf aqueous extract) has been found to treat various infectious diseases (Table 1.2), and to possess an immunomodulatory effect that could stimulate the immune system, thereby aiding in the fight against infection and accelerate wound healing (Mahmood et al., 2005; Otsuki et al., 2010). The leaves of *C. papaya* contain quaternary alkaloids that are responsible for the curative properties of the plant to gynaecological ailments (Oganɬ, 1970). The quaternary alkaloids could have been released in small quantities and thus ineffective individually or their density does not enable them to penetrate the pathogens membrane

S. birrea was found to have a broad-spectrum of antimicrobial activity against the STI pathogens. The aqueous plant extract being effective against *U. urealyticum* and *N. gonorrhoeae*, whereas the organic plant extracts were effective against *U. urealyticum*, *G. vaginalis* and *N. gonorrhoeae*. *S. birrea* has been well investigated (Table 1.2) and the bark has been proven to possess antibacterial activity against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Enterococcus faecalis* (MIC range 0.37 - 2.43 mg/ml) (Eloff, 2001). This was further supported by McGaw et al., (2000), whereby the organic and aqueous bark extracts were found to be effective against *S. aureus* and *Bacillus subtilis*.

T. elegans (ethanolic root) was previously tested and exhibited antibacterial activity against *B. subtilis* and *S. aureus* with MIC values of 64.0 µg/ml and 32.0 µg/ml, respectively (Pallant et al., 2012). The alkaloid extract of *T. elegans* has also been found to possess anti-mycobacterial

activity against *Mycobacterium smegmatis* and *M. tuberculosis* with MIC values of 32.0 µg/ml against both species (Pallant et al., 2012). The aqueous extract of *T. elegans* (bark) has been found in the current study to possess antifungal activity against *C. albicans*. This finding can be substantiated with that by Steenkamp et al., (2007), whereby the aqueous extract of *T. elegans* possessed antimicrobial activity ranging 0.41 - 3.30 mg/ml.

X. caffra (leaf) has been shown to possess antigonococcal, antifungal and antibacterial activity (Mulaudzi et al., 2011). The current study was in agreement with *X. caffra* (leaf) possessing noteworthy antigonococcal activity (Figure 3.6), however, the petroleum ether extract (50 mg/ml) tested by Mulaudzi et al., (2011), demonstrated 73.0% inhibition. The extracts tested in the current study possessed a noteworthy MIC value of 0.25 mg/ml for the aqueous extract and moderately good activity for the organic extract (Figure 3.6). Contradicting results were seen with the antifungal activity results, whereby the aqueous extract in the current study did not possess antifungal activity (MIC value >16.0 mg/ml), whereas Mulaudzi et al., (2011) reported noteworthy antifungal activity (MIC value 0.78 mg/ml). A possible reasoning for the deviation could be that the plants were collected in different provinces as well as possible seasonal variation. *X. caffra* was also mentioned in an ethnobotanical survey conducted with the Nhema people in Zimbabwe, whereby the root/leaves are used to treat venereal infections (Maroyi, 2011). This mirrors the use by the people of Maputaland.

3.3.3 Traditional usage

No previous STI related studies were found for three plants, namely *C. malosana*, *E. hypericifolia* and *S. sanguinea* (Table 1.2). These plants did, however, prove to possess satisfactory antimicrobial activity especially with regards to the organic extracts in this current study. Both the aqueous and organic extracts of *C. malosana* inhibited the pathogen, *U. urealyticum* (Figure 3.2) while the organic extract further inhibited *N. gonorrhoeae* (Figure 3.6). The organic extracts of *E. hypericifolia* exhibited noteworthy activity against pathogens *O. ureolytica* (Figure 3.3), *G. vaginalis* (Figure 3.5) and *N. gonorrhoeae* (Figure 3.6). The aqueous and organic extract of *S. sanguinea* demonstrated noteworthy activity against *N. gonorrhoeae* furthermore the organic extract inhibited the growth of *G. vaginalis* (Figure 3.5 and Figure 3.6).

Plants most often mentioned by the lay people included *R. multifidus*, *S. serratuloides* and *H. hemerocallidea* (De Wet et al., 2012). However, these plants did not always produce the most outstanding antimicrobial activity. The aqueous plant extract of *R. multifidus* did however exhibit the lowest MIC value of all tested plants against the causative organism of urethritis, *U. urealyticum*.

The studies on *N. gonorrhoeae* are particularly interesting as gonorrhoea is frequently seen within the South African setting and is referred to as 'dropsy' in rural villages. In particular the Maputaland study area had more complaints of gonorrhoea than any other STI investigated (De Wet et al., 2012). Most of the plants (organic extracts) studied demonstrated good antimicrobial activity against *N. gonorrhoeae* (Figure 3.6).

Numerous studies have shown organic extracts to be more effective than aqueous extracts (Rabe and Van Staden, 1997; Kambizi and Afolayan, 2001; Ojewole, 2003; Van de Venter et al., 2008; Van Vuuren and Naidoo, 2010). There have been some studies whereby the aqueous extracts were more active than the organic extracts (Lohiya et al., 1999; McGaw et al., 2000), however, this is not a common finding. In this study, the noteworthy antimicrobial efficacies between organic and aqueous extracts was minimal (3% difference) and for many plants the aqueous extracts were found to be just as efficacious as the organic extracts.

Highlighting a few specific examples whereby the aqueous extract performed antimicrobially better than the organic extract, includes *O. engleri* when tested against *C. albicans*, *R. multifidus* against *O. ureolytica* and *H. hemerocallidea* against *N. gonorrhoeae*. *H. hemerocallidea* was one of the most mentioned plants by the lay people of Maputaland and from the results it can be observed that against four out of the six pathogens, the aqueous extract was more efficacious than the organic extract (Figures 3.1, 3.2, 3.3, 3.6). This is an interesting result as it proves the primary method of preparation (aqueous extracts), for this plant, by the lay people is indeed effective. This data justifies that aqueous extracts can be of great benefit under certain circumstances and should therefore never be underestimated.

The only plant which demonstrated toxicity was *K. africana* with the remaining 19 plants being non-toxic against the human kidney epithelial cells. Plants with outstanding antimicrobial activity against one pathogen such as *P. africanum* and *R. multifidus* should be highlighted. Emphasis should also be focused on plants demonstrating noteworthy antimicrobial activity against a minimum of three STI pathogens and furthermore were found to be non-toxic against the human kidney epithelial cells. The plants adhering to such a profile and therefore demonstrating promising results were *C. papaya*, *E. hypericifolia*, *P. africanum*, *S. birrea*, *S. cordatum, T. elegans* and *X. caffra*.

B. pilosa, *C. papaya*, and *M. acuminata* all possess antimicrobial activity against one or more STI pathogen. It should be noted that all three plants have in previous studies been found to increase uterine contractions or have been used as an abortificient agent. Caution must be taken when treating a pregnant woman especially in the first and last trimester for a STI.

3.4 Conclusions

- All of the plants tested in this study are considered non-toxic when tested against human kidney epithelial cells with the exception of *K. africana* (leaf), for both the aqueous and organic plant extracts which was the only plant to inhibit human kidney epithelial cells.
- The best antimicrobial activity was obtained by *R. multifidus* (MIC = 0.02 mg/ml) and *P. africanum* (MIC = 0.04 mg/ml) for the aqueous and organic plant extracts respectively. *R. multifidus* and *P. africanum* were found to be non-toxic against the human kidney epithelial cell line and therefore demonstrates promise as a therapy for the treatment of STIs urethritis especially caused by *U. urealyticum*.
- The STIs most treated by the lay people in Maputaland were urethritis and gonorrhoea and notably the individual plant therapies were most susceptible to *U. urealyticum* and *N. gonorrhoeae*.
- Aqueous extracts are the favoured method of preparation by the lay people and the aqueous plants such as *R. multifidus*, *H. hemerocallidea* and *O. engleri* possessed greater

antimicrobial efficacy compared to the organic extracts. This bares testament to the fact that the aqueous extracts are in some cases superior.

- Not all of the plants mentioned by the lay people; demonstrated outstanding antimicrobial activity when examined alone and this reinforces the need to evaluate their combined effects, as in many cases this is the preferred use traditionally.

Chapter 4

Two plant combinations used to treat STI infections in northern Maputaland

4.1 Introduction

Overall, the ethnopharmacological surveys on medicinal plant STI treatments in Maputaland identified 13 plant combinations of which several were found to be two plant combinations (Table 2.2). The two plant combinations were mostly used to treat gonorrhoea and internal/external sores caused by STI infections.

The aim of this chapter was to investigate the antimicrobial effectiveness of the two plants used in combination. Furthermore, investigation was undertaken to determine whether the plants interacted synergistically and at what ratios the plant remedies could be combined for the best efficacy. It is important to establish if the plant remedies not only possess antimicrobial effectiveness, but also if the plants antagonise each other's pharmacological effect (Berenbaum, 1989; Brooks and Carroll, 2007).

Toxicity studies were also conducted for the combinations, against human kidney epithelial cells to establish if the combinations are safe to use. The concept of herbal remedies being safe with fewer side effects is largely circumstantial and it is important to further determine toxicity of the plants, especially if the combinations are utilized frequently or over long periods (Reid et al., 2006). The plant remedies would be considered less important if the remedies possessed antimicrobial activity, but found to be toxic.

4.2 Results

4.2.1 Toxicity cellular viability assay

The toxicity assay was conducted to determine the safety of plants when used in combination against the human kidney epithelial cell line.

Potential toxicity was detected with only one aqueous plant combination that being *S. birrea* (bark) and *S. cordatum* (bark) (Table 4.1). There was a decrease in cell viability by 25.0% when compared to the untreated cell free control. No toxicity was detected upon testing the plants individually (Table 3.1), However, it was interesting that upon combining the plants in a 1:1 ratio, a vast decrease in cellular viability was noted (Table 4.1). The plant combination was therefore combined in nine various ratios to establish which plant potentiated the toxicity more (Figure 4.1).

Table 4.1 Toxic properties of the combined plants where the values represent percentage (%) cell viability ± standard deviation for at least triplicate experiments.

Plant combinations	**Aqueous extract**	**Organic extract**
A. adianthifolia and *T. dregeana*	98.3± 0.56	97.2± 0.60
A. marlothii and *S. serratuloides*	104.2± 0.46	105.0± 0.52
A. marlothii and *H. hemerocallidea*	110.2± 0.67	105.4± 0.58
H. hemerocallidea and *K. africana*	105.2± 0.55	106.5± 0.68
S. birrea* and *S. cordatum	**75.0± 0.46**	**79.0± 0.51**
X. caffra and *T. elegans*	105.4± 0.68	110.0± 0.70
C. malosana and *H. hemerocallidea*	108.2± 0.57	99.3± 0.65

Untreated control: 100% cell viability Positive control Quinine sulphate IC_{50} = 141.34 ± 22.08µg/ml

It can be noted that for those ratios where *S. cordatum* predominates (3:7, 4:6) there is an increase in cell death of the human kidney epithelial cells (Figure 4.1). However, at the highest ratios of *S. cordatum* (2:8 and 1:9) no toxicity is detected (Figure 4.1). It can be hypothesised that *S. cordatum* combined with *S. birrea* does indicate a certain level of toxicity, however, it should ideally not be used where the combination is in equal ratios or ratios of 6:4, 4:6, 3:7 (*S. birrea*: *S. cordatum*) (Figure 4.1). Since toxicity was determined for this combination a further in

depth analysis into the antimicrobial efficacy was undertaken in Section 2.2.2.5 in order to assess whether or not the combination should be utilized at different ratios.

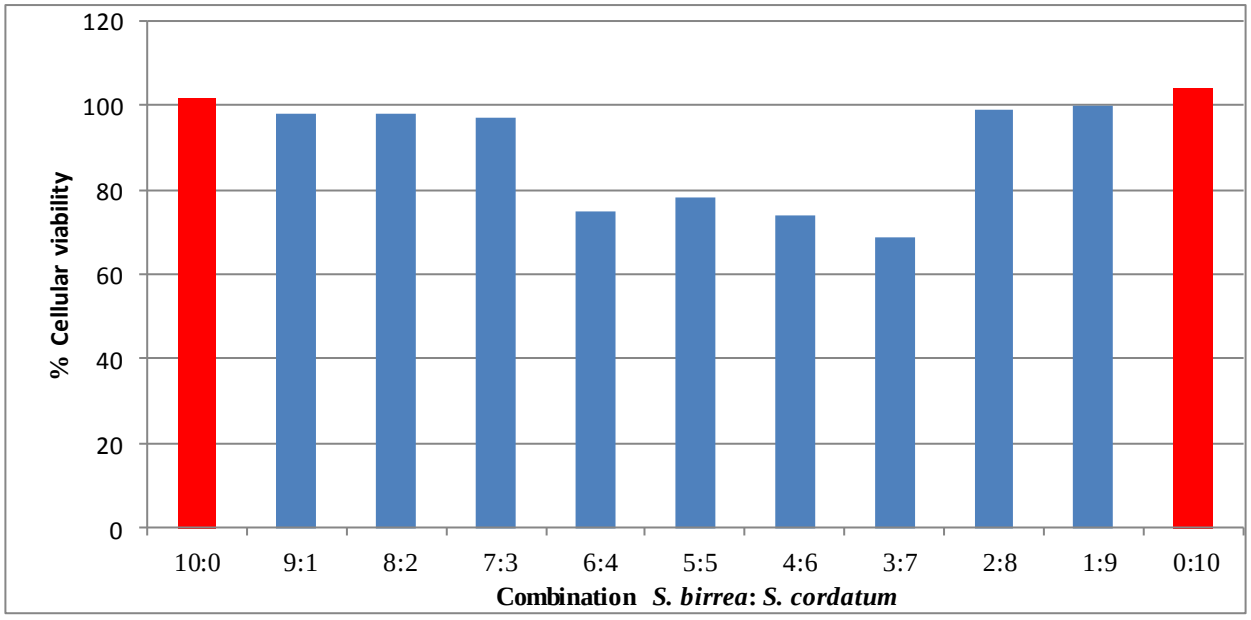


Figure 4.1 Cellular viability percentage cell viability of *S. birrea* combined with *S. cordatum* as determined by the MTT cellular viability assay in nine ratios (red bars are representative of *S. birrea* and *S. cordatum* individually tested).

A. adianthifolia (bark) and *T. dregeana* (bark) on the other hand did not demonstrate any toxic potential against the human kidney epithelial cell line. Thereafter, nine various ratios of the combination were tested against the cell line and a maximum of 3.00% cell death occurred (Figure 4.2). The lack of toxicity was important to determine and becomes more relevant upon a detailed analysis of the antimicrobial efficacy of the combination (Section 2.2.2.1).

4.2.2 Antimicrobial analysis

The MIC assay was conducted to establish antimicrobial efficacy against the six STI associated pathogens using the methods as described in Section 2.5.1. Combinations exhibiting interesting antimicrobial efficacy with synergistic interactions were then further investigated at nine ratio combinations. These were then plotted onto an isobologram for interpretation and discussion. To

avoid repetition the control values determined in all assays used for the toxicity and antimicrobial studies are found in Table 3.1.

4.2.2.1 *Albizia adianthifolia* (bark) with *Trichilia dregeana* (bark)

The 1:1 combination of *A. adianthifolia* with *T. dregeana* against six STI pathogens is given in Tables 4.2 and 4.3. The most interesting antimicrobial activity found for the aqueous combination was against *O. ureolytica*. The ΣFIC value of 0.15 was the lowest ΣFIC value obtained overall when analysing the aqueous plant combination. Thus the combination presents the strongest synergistic interactions against *O. ureolytica* (Table 4.2). This in turn led to further investigation into various ratio combinations of the two plants (Figure 4.2).

Table 4.2 MIC and ΣFIC results when the aqueous plant extracts of *A. adianthifolia* and *T. dregeana* were tested individualy and in 1:1 combination.

Micro-organism	MIC (mg/ml)			ΣFIC	
	A. adianthifolia	***T. dregeana***	***A. adianthifolia* with *T. dregeana***	***A. adianthifolia* with *T. dregeana***	**Interpretation**
C. albicans ATCC 10321	>16.0	>16.0	>16.0	N/D[1]	Indifferent[2]
U. urealyticum Clinical strain	0.25	0.25	1.00	4.00	Indifferent
O. ureolytica ATCC 43534	8.00	3.00	0.75	**0.15**	**Synergistic**
G. vaginalis ATCC 14018	12.0	8.00	8.00	0.84	Additive
T. vaginalis Clinical strain	>16.0	>16.0	16.0	N/D[1]	Indifferent[2]
N. gonorrhoeae ATCC 19424	16.0	1.00	16.0	N/D[1]	Indifferent[2]

N/D[1] No ΣFIC value could be calculated as no MIC end point was established for the either plants investigated individually. [2] Tentative interpretation according to MIC data. Shaded area: results previously discussed in Chapter 3 but included for reference purposes.

The isobologram for the aqueous combination of *A. adianthifolia* and *T. dregeana* highlighted the fact that irrespective of the ratio combination of the two plants, an overall synergistic interaction was observed (Figure 4.2).

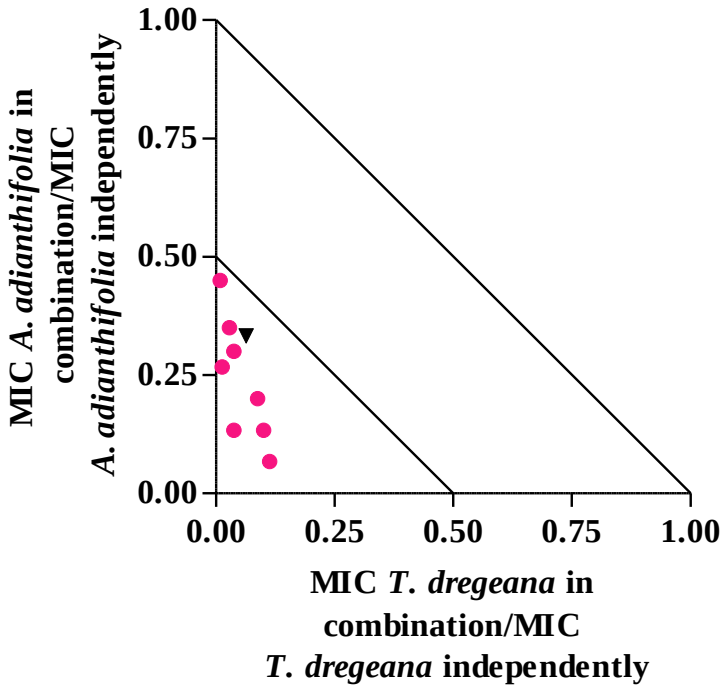


Figure 4.2 Isobologram representation for the relationship between the combination of *A. adianthifolia* and *T. dregeana* against *O. ureolytica*. Where: ● = synergism, ▼ = 1:1 combination.

The organic combination showed an antagonistic interaction against *U. urealyticum* (Table 4.3). The plants when tested singularly possessed noteworthy antimicrobial activity, however, upon combining the plants, the plant to plant interactions were not favourable (Table 4.3). No synergistic interactions were seen with the organic plant extracts.

Table 4.3 MIC and ΣFIC results when the organic plant extracts of *A. adianthifolia* and *T. dregeana* were tested individually and in 1:1 combinations.

Micro-organism	MIC (mg/ml)			ΣFIC	
	A. adianthifolia	***T. dregeana***	***A. adianthifolia* with *T. dregeana***	***A. adianthifolia* with *T. dregeana***	**Interpretation**
C. albicans ATCC 10321	4.00	6.00	12.0	2.50	Indifferent
U. urealyticum Clinical strain	0.25	0.50	3.00	**10.0**	**Antagonistic**
O. ureolytica ATCC 43534	16.0	0.75	3.00	0.94	Additive
G. vaginalis ATCC 14018	2.00	4.00	4.00	1.50	Indifferent
T. vaginalis Clinical strain	2.00	2.00	6.00	3.00	Indifferent
N. gonorrhoeae ATCC 19424	4.00	1.00	1.50	0.57	Additive

N/D[1] No ΣFIC value could be calculated as no MIC end point was established for the either plants investigated individually. [2] Tentative interpretation according to MIC data. Shaded area: results previously discussed in Chapter 3 but included for reference purposes.

4.2.2.2 *Aloe marlothii* (leaf) with *Senecio serratuloides* (leaf)

The aqueous extract for the combination of *A. marlothii* and *S. serratuloides*, demonstrated antagonistic interactions when tested against *C. albicans* (Table 4.4). This may have contributed to the poor antimicrobial activity (MIC = >16.0 mg/ml) noted. The organic extract of the combination showed unfavourable antagonistic interactions against *U. urealyticum* (Table 4.4). This could indicate the individual plants are a better treatment option to treat *U. urealyticum*. The organic extract for the combination did, however, indicate synergistic interactions when tested against *O. ureolytica*. This plant combination had the lowest ΣFIC value (0.31) and warranted further analysis into various ratio mixtures to gain a better understanding into the plants interactions with one another (Figure 4.3).

Table 4.4 MIC and ΣFIC results when the aqueous and organic plant extracts of *A. marlothii* and *S. serratuloides* were tested individually and in 1:1 combinations.

Micro-organism	MIC (mg/ml)			ΣFIC	
	A. marlothii	***S. serratuloides***	***A. marlothii* with *S. serratuloides***	***A. marlothii* with *S. serratuloides***	**Interpretation**
Aqueous extracts					
C. albicans ATCC 10321	16.0	2.00	>16.0	**N/D[1]**	**Antagonistic[2]**
U. urealyticum Clinical strain	0.75	0.50	0.75	1.50	Indifferent
O. ureolytica ATCC 43534	0.38	4.00	0.75	1.10	Indifferent
G. vaginalis ATCC 14018	>16.0	8.00	>16.0	N/D[1]	Indifferent[2]
T. vaginalis Clinical strain	>16.0	>16.0	>16.0	N/D[1]	Indifferent[2]
N. gonorrhoeae ATCC 19424	>16.0	16.0	16.0	N/D[1]	Indifferent[2]
Organic extracts					
C. albicans ATCC 10321	4.00	8.00	8.00	1.50	Indifferent
U. urealyticum Clinical strain	1.00	0.63	2.00	**4.20**	**Antagonistic**
O. ureolytica ATCC 43534	2.00	4.00	0.50	**0.31**	**Synergistic**
G. vaginalis ATCC 14018	2.00	4.00	4.00	1.50	Indifferent
T. vaginalis Clinical strain	6.00	2.00	4.00	1.34	Indifferent
N. gonorrhoeae ATCC 19424	1.00	2.00	1.00	0.75	Additive

N/D[1] No ΣFIC value could be calculated as no MIC end point was established for the either plants investigated individually. [2] Tentative interpretation according to MIC data. Shaded area: results previously discussed in Chapter 3 but included for reference purposes.

The most favorable ratios for the combination of *A. marlothii* : *S. serratuloides* where found to be 1:9, 3:7, 5:5, 6:4, 7:3, where all are plotted within the synergistic quadrant (Figure 4.3). No correlation or trend could be established whereby one plant contributes more towards synergistic interactions than the other, as the most favourable ratios are randomly distributed. There was one combination that did, however, extend into the antagonistic region, that being where a higher ratio of *S. serratuloides* was used (2:8). This could be explained due to the higher MIC value (4.00 mg/ml) found when *S. serratuloides* was tested individually.

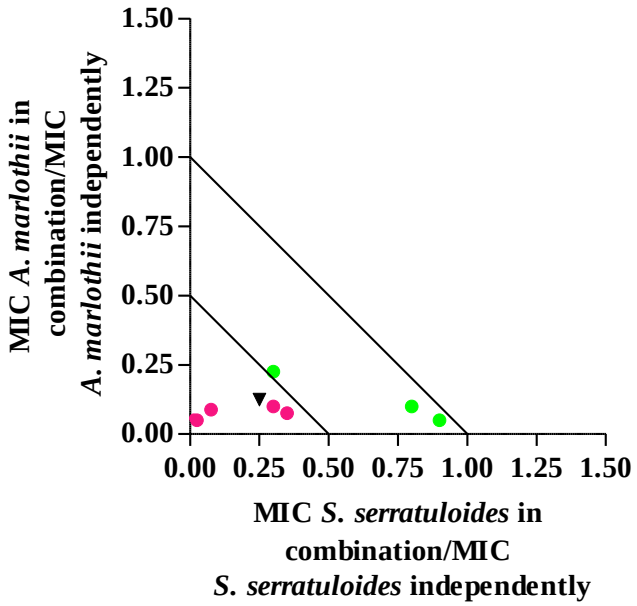


Figure 4.3 Isobologram representation for the relationship between the combination of *A. marlothii* and *S. serratuloides* against *O. ureolytica*. Where: ● = synergism, ● = additive, ▼ = 1:1 combination.

4.2.2.3 *Aloe marlothii* (leaf) with *Hypoxis hemerocallidea* (corm)

The aqueous extract of the combination exhibited antagonistic interactions with no synergistic interactions determined against three (*C. albicans*, *U. urealyticum* and *N. gonorrhoeae*) pathogens (Table 4.5). The organic extract of the combination displayed interactions ranging from indifference to additive, however, no synergism or antagonism was detected.

Table 4.5 MIC and ΣFIC results when the aqueous and organic plant extracts of *A. marlothii* and *H. hemerocallidea* were tested individually and in 1:1 combinations.

Micro-organism	MIC (mg/ml)			ΣFIC	
	A. marlothii	***H. hemerocallidea***	***A. marlothii* with *H. hemerocallidea***	***A. marlothii* with *H. hemerocallidea***	**Interpretation**
Aqueous extracts					
C. albicans ATCC 10321	4.00	1.00	>16.0	**N/D[1]**	**Antagonistic[2]**
U. urealyticum Clinical strain	1.00	0.25	2.00	**5.34**	**Antagonistic[2]**
O. ureolytica ATCC 43534	2.00	2.00	1.00	1.17	Indifferent
G. vaginalis ATCC 14018	2.00	8.00	>16.0	N/D[1]	Indifferent
T. vaginalis Clinical strain	6.00	8.00	>16.0	N/D[1]	Indifferent
N. gonorrhoeae ATCC 19424	1.00	0.50	>16.0	**N/D[1]**	**Antagonistic[2]**
Organic extracts					
C. albicans ATCC 10321	4.00	4.00	>16.0	N/D[1]	Indifferent[2]
U. urealyticum Clinical strain	1.00	3.00	1.50	1.00	Additive
O. ureolytica ATCC 43534	2.00	4.00	2.00	0.75	Additive
G. vaginalis ATCC 14018	2.00	4.00	4.00	1.50	Indifferent
T. vaginalis Clinical strain	6.00	2.00	8.00	2.67	Indifferent
N. gonorrhoeae ATCC 19424	1.00	8.00	1.00	0.57	Additive

N/D[1] No ΣFIC value could be calculated as no MIC end point was established for the either plant or plants in combination. [2] Tentative interpretation according to MIC data. Shaded area: results previously discussed in Chapter 3 but included for reference purposes.

4.2.2.4 *Hypoxis hemerocallidea* (corm) with *Kigelia africana* (leaf)

The aqueous plant extract for the combination of *H. hemerocallidea* and *K. africana* presented with antagonistic interactions against *C. albicans* and *U. urealyticum* (Table 4.6). The high degree of antagonism between the plants against *U. urealyticum* could be clearly observed with the significant increase in MIC value (therefore a decrease in antimicrobial efficacy) upon combination as opposed to the noteworthy antimicrobial activity exhibited by the plants when tested singularly (Table 4.6).

The combination when tested against *O. ureolytica* did, however, possess synergistic interactions (Table 4.6). The synergistic interactions between the plants positively decreased the overall MIC value (therefore indicating better antimicrobial efficacy) when comparing the oblique MIC values obtained with the plants individual activities.

Table 4.6 MIC and ΣFIC results when the aqueous plant extracts of *K. africana* and *H. hemerocallidea* were tested individually and in 1:1 combinations.

Micro-organism	**MIC (mg/ml)**			**ΣFIC**	
	K. africana	***H. hemerocallidea***	***K. africana* with *H. hemerocallidea***	***K. africana* with *H. hemerocallidea***	**Interpretation**
C. albicans ATCC 10321	16.0	1.00	>16.0	**N/D[1]**	**Antagonistic[2]**
U. urealyticum Clinical strain	0.25	0.25	8.00	**32.0**	**Antagonistic**
O. ureolytica ATCC 43534	8.00	2.00	1.00	**0.31**	**Synergistic**
G. vaginalis ATCC 14018	>16.0	8.00	8.00	N/D[1]	Additive[2]
T. vaginalis Clinical strain	>16.0	8.00	8.00	N/D[1]	Additive[2]
N. gonorrhoeae ATCC 19424	>16.0	0.50	2.00	N/D[1]	Indifferent[2]

N/D[1] No ΣFIC value could be calculated as no MIC end point was established for the either plant or plants in combination. [2] Tentative interpretation according to MIC data. Shaded area: results previously discussed in Chapter 3 but included for reference purposes.

From the isobologram there were four ratios which fell within the synergistic quadrant of the isobologram (Figure 4.4). The ratios were (*K. africana* : *H. hemerocallidea*) 1:9, 2:8, 5:5 and 9:1. There was no direct correlation as to what makes the combination synergistic as the ratios are scattered. The ratios that were plotted within the indifferent (blue points) quadrant, however, seem to lean more into the ratios whereby *K. africana* is in the majority (Figure 4.4).

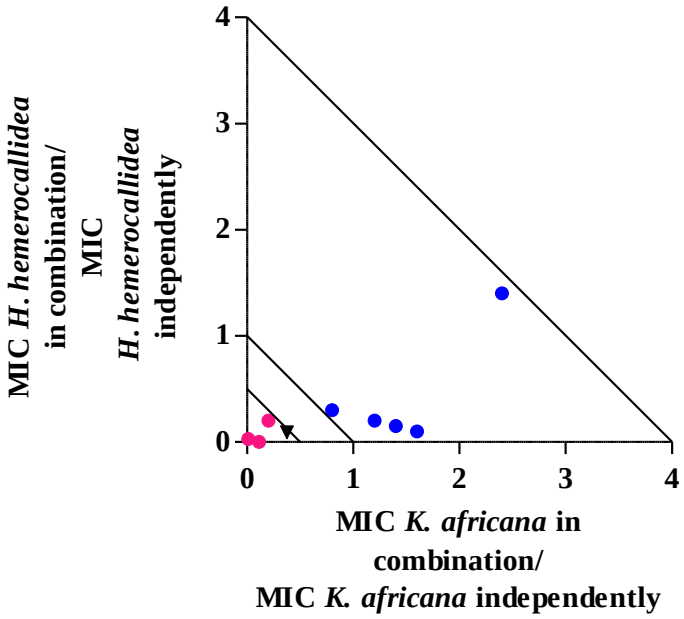


Figure 4.4 Isobologram representation for the relationship between the combination of *K. africana* and *H. hemerocallidea* against *O. ureolytica*. Where: ● = synergism, ● = indifference, ▼ = 1:1 combination.

The individual plant studies in Table 3.1 discovered *K. africana* (aqueous extract) to be the only toxic plant. It is possible that the toxic effect of the plant when combined in a greater portion of the ratio could be influencing the interaction with *H. hemerocallidea*. The organic extract of the combination uncovered interactions ranging from additive to indifference, with no synergistic or antagonistic interactions detected (Table 4.7).

Table 4.7 MIC and ΣFIC results when the organic plant extracts of *K. africana* and *H. hemerocallidea* were tested individually and in 1:1 combinations.

Micro-organism	MIC (mg/ml)			ΣFIC	
	K. africana	***H. hemerocallidea***	***K. africana* with *H. hemerocallidea***	***K. africana* with *H. hemerocallidea***	**Interpretation**
C. albicans ATCC 10321	4.00	4.00	8.00	2.00	Indifferent
U. urealyticum Clinical strain	0.50	3.00	0.75	0.88	Additive
O. ureolytica ATCC 43534	1.00	4.00	1.00	0.63	Additive
G. vaginalis ATCC 14018	4.00	4.00	4.00	1.00	Additive
T. vaginalis Clinical strain	1.50	2.00	6.00	3.50	Indifferent
N. gonorrhoeae ATCC 19424	2.00	8.00	4.00	1.25	Indifferent

Shaded area: results previously discussed in Chapter 3 but included for reference purposes.

4.2.2.5 *Sclerocarya birrea* (root) with *Syzygium cordatum* (root)

S. birrea and *S. cordatum* (aqueous plant combination) exhibited synergistic (ΣFIC = 0.42) interactions when tested against *O. ureolytica* (Table 4.8). The synergistic nature of the plant interactions supportively enhanced the antimicrobial efficacy against *O. ureolytica* when compared to the plants independent antimicrobial activity (Table 4.8). The synergistic results of this combination lead to the investigation of various ratios for further analysis.

Table 4.8 MIC and ΣFIC results when the aqueous and organic plant extracts of *S. birrea* and *S. cordatum* were tested individually and in 1:1 combinations.

Micro-organism	MIC (mg/ml)			ΣFIC	
	S. birrea	***S. cordatum***	***S. birrea* with *S. cordatum***	***S. birrea* with *S. cordatum***	**Interpretation**
Aqueous extracts					
C. albicans ATCC 10321	>16.0	0.50	2.00	N/D[1]	Indifferent[2]
U. urealyticum Clinical strain	0.25	0.25	0.75	3.00	Indifferent
O. ureolytica ATCC 43534	2.00	3.00	1.00	**0.42**	**Synergistic**
G. vaginalis ATCC 14018	1.00	0.75	1.00	0.75	Additive
T. vaginalis Clinical strain	>16.0	8.00	>16.0	N/D[1]	Indifferent[2]
N. gonorrhoeae ATCC 19424	0.50	0.25	0.25	1.50	Indifferent
Organic extracts					
C. albicans ATCC 10321	>16.0	0.50	2.00	N/D	Indifferent
U. urealyticum Clinical strain	0.25	1.00	2.00	**5.00**	**Antagonistic**
O. ureolytica ATCC 43534	1.00	2.00	>16.0	**N/D**	**Antagonistic**
G. vaginalis ATCC 14018	0.75	1.00	1.00	1.17	Indifferent
T. vaginalis Clinical strain	2.00	1.00	2.00	1.50	Indifferent
N. gonorrhoeae ATCC 19424	0.25	0.25	0.25	1.00	Additive

N/D[1] No ΣFIC value could be calculated as no MIC end point was established for the either plant or plants in combination. [2] Tentative interpretation according to MIC data. Shaded area: results previously discussed in Chapter 3 but included for reference purposes.

The isobologram (Figure 4.5) indicates six ratios namely (*S. birrea* : *S. cordatum*) 2:8, 5:5, 6:4, 7:3, 8:2 and 9:1 plotted within the synergistic quadrant. From the ratios, it can be deduced that the synergistic ratios mainly consisted of *S. birrea* being the plant in majority. Therefore, *S. birrea* could possibly be the plant contributing towards synergy.

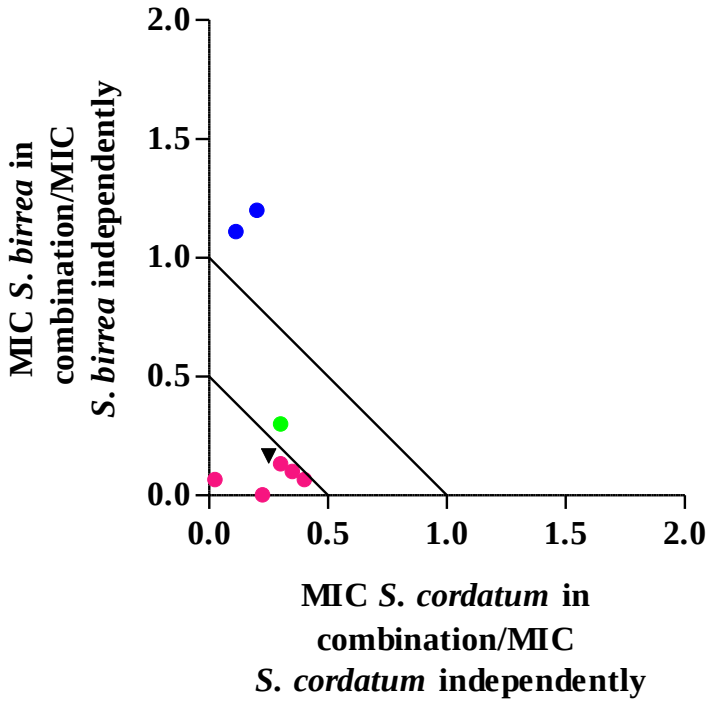


Figure 4.5 Isobologram representation for the relationship between the combination of *S. birrea* and *S. cordatum* against *O. ureolytica*. Where: ● = synergism, ● = additive, ● = indifference, ▼ = 1:1 combination.

On the other hand, contradicting antagonistic interactions were found with the organic extracts of the combination against *O. ureolytica* (Table 4.8). Antagonistic interactions were also further noted when tested against *U. urealyticum*, despite *S. birrea* maintaining noteworthy activity when tested singularly (Table 4.8).

4.2.2.6 *Ximenia caffra* (root) with *Tabernaemontana elegans* (bark)

The aqueous plant extract for the combination of *X. caffra* and *T. elegans* predominantly exhibited antagonistic interactions against *C. albicans*, *U. urealyticum*, *G. vaginalis* and *N. gonorrhoeae* (Table 4.9). This combination demonstrated the most antagonistic interactions when compared to the other two plant combinations. No synergistic interactions were detected. This was disappointing especially when the individual plants possessed noteworthy antimicrobial activity against *U. urealyticum*.

Table 4.9 MIC and ΣFIC results when the aqueous plant extracts of *X. caffra* and *T. elegans* were tested individually and in 1:1 combinations.

Micro-organism	MIC (mg/ml)			ΣFIC	
	X. caffra	***T. elegans***	***X. caffra* with *T. elegans***	***X. caffra* with *T. elegans***	**Interpretation**
C. albicans ATCC 10321	>16.0	0.38	8.00	**N/D[1]**	**Antagonistic[2]**
U. urealyticum Clinical strain	0.25	0.25	4.00	**16.0**	**Antagonistic**
O. ureolytica ATCC 43534	2.00	8.00	2.00	0.63	Additive
G. vaginalis ATCC 14018	1.00	8.00	8.00	**4.50**	**Antagonistic**
T. vaginalis Clinical strain	8.00	>16.0	>16.0	N/D[1]	Indifferent[2]
N. gonorrhoeae ATCC 19424	0.25	1.00	2.00	**5.00**	**Antagonistic**

N/D[1] No ΣFIC value could be calculated as no MIC end point was established for the either plant or plants in combination. [2] Tentative interpretation according to MIC data. Shaded area: results previously discussed in Chapter 3 but included for reference purposes.

The organic plant extracts of the combination exhibited antagonistic interactions against *O. ureolytica* and *G. vaginalis*. There were no synergistic interactions between the plants against any of the selected pathogens (Table 4.10).

Table 4.10 MIC and ΣFIC results when the organic plant extracts of *X. caffra* and *T. elegans* were tested individually and in 1:1 combinations.

Micro-organism	**MIC (mg/ml)**			**ΣFIC**	
	X. caffra	***T. elegans***	***X. caffra* with *T. elegans***	***X. caffra* with *T. elegans***	**Interpretation**
C. albicans ATCC 10321	4.00	0.50	8.00	3.00	Indifferent
U. urealyticum Clinical strain	0.50	1.00	0.63	0.84	Additive
O. ureolytica ATCC 43534	0.75	2.00	6.00	**4.75**	**Antagonistic**
G. vaginalis ATCC 14018	4.00	1.00	12.0	**25.5**	**Antagonistic**
T. vaginalis Clinical strain	2.00	1.00	3.00	2.25	Indifferent
N. gonorrhoeae ATCC 19424	1.00	0.25	3.00	3.00	Indifferent

N/D[1] No ΣFIC value could be calculated as no MIC end point was established for the either plant or plants in combination. [2] Tentative interpretation according to MIC data. Shaded area: results previously discussed in Chapter 3 but included for reference purposes.

4.2.2.7 *Cassipourea malosana* (leaf) with *Hypoxis hemerocallidea* (corm)

The combination of *C. malosana* and *H. hemerocallidea* (aqueous combination) demonstrated antagonistic interactions against three pathogens (*C. albicans*, *U. urealyticum* and *N. gonorrhoeae*). No synergistic interactions were determined (Table 4.11). For the organic plant extracts of *C. malosana* and *H. hemerocallidea* the interactions ranged from indifferent to additivity. With no synergistic or antagonistic interactions were present (Table 4.11).

Table 4.11 MIC and ΣFIC results when the aqueous and organic plant extracts of *C. malosana* and *H. hemerocallidea* were tested individually and in 1:1 combinations.

Micro-organism	MIC (mg/ml)			ΣFIC	
	C. malosana	***H. hemerocallidea***	***C. malosana* with *H. hemerocallidea***	***C. malosana* with *H. hemerocallidea***	**Interpretation**
Aqueous extracts					
C. albicans ATCC 10321	>16.0	1.00	16.0	**N/D[1]**	**Antagonistic[2]**
U. urealyticum Clinical strain	0.25	0.25	8.00	**32.0**	**Antagonistic**
O. ureolytica ATCC 43534	16.0	2.00	4.00	1.13	Indifferent
G. vaginalis ATCC 14018	>16.0	8.00	>16.0	N/D[1]	Indifferent[2]
T. vaginalis Clinical strain	>16.0	8.00	8.00	N/D[1]	Additive[2]
N. gonorrhoeae ATCC 19424	>16.0	0.50	>16.0	**N/D[1]**	**Antagonistic[2]**
Organic extracts					
C. albicans ATCC 10321	4.00	4.00	4.00	1.00	Additive
U. urealyticum Clinical strain	0.75	3.00	1.00	0.84	Additive
O. ureolytica ATCC 43534	3.00	4.00	2.00	0.83	Additive
G. vaginalis ATCC 14018	2.00	4.00	4.00	1.50	Indifferent
T. vaginalis Clinical strain	8.00	2.00	2.00	0.63	Additive
N. gonorrhoeae ATCC 19424	0.25	8.00	1.00	2.00	Indifferent

N/D[1] No ΣFIC value could be calculated as no MIC end point was established for the either plant or plants in combination. [2] Tentative interpretation according to MIC data. Shaded area: results previously discussed in Chapter 3 but included for reference purposes.

4.3 Discussion

Despite toxicity studies found on the plants in Table 3.1 no toxicity studies could be found for any of the combinations mentioned by the people of Maputaland for the treatment of STIs. The lack of toxicology studies for these combinations stresses the importance of further toxicological studies of plant combinations.

There have been other antimicrobial studies done on the twenty plants selected in this study, however, none of them have been used in these particular combinations for the treatment of STIs.

Previously, there have been studies conducted whereby plant combinations have been found to demonstrate synergistic interactions (Boik, 2001; Kamatou et al., 2006; Okusa et al., 2007). Only one previous study could be found in which two plants (*Viola odorata* and *Ruta graveolens*, aqueous extracts) were investigated against *T. vaginalis*. The combination was found to interact synergistically (Al-Heali and Rahemo, 2006). Other studies that have focused on plants from this study in combination, but for the treatment of various other ailments amongst others, diabetes, liver damage, diarrhoea and malaria (Gathirwa et al., 2008; Gbolade, 2009; Sibandze et al., 2010; Mukazayire et al., 2011; York et al., 2011).

4.3.1 Overview of toxicity and antimicrobial efficacy of 1:1 combinations

The antimicrobial properties of *A. adianthifolia* and *T. dregeana* are reported here for the first time as a combination for the effects against STI pathogens. The aqueous leaf extracts of *T. dregeana* have been reported to display antimicrobial activity which correlates with this study's findings (Hutchings et al., 1996). Liminoides have been isolated from *T. dregeana* seeds, which contributed to the antimicrobial effects (Bruneton, 1995). Cycloart-2,3-ene-3,25-diol, which is responsible for the anti-inflammatory effects of *T. dregeana* has been isolated from the leaves (Bruneton, 1995; Eldeen et al., 2007). *A. adianthifolia* when tested individually was ineffective against *O. ureolytica* therefore *T. dregeana* seems to possess a dominating effect. In the ratios where *T. dregeana* is used in minimum quantity, it is possible *T. dregeana* exhibits a stronger effect and synergistically interacts with *A. adianthifolia*, thereby aiding effectiveness against *O.*

ureolytica. The combination of *A. adianthifolia* and *T. dregeana* (aqueous extract) validated all nine various combined ratios to be non-toxic against the human kidney epithelial cells in the MTT assay. The combination is non-toxic against the human kidney epithelial cells and effective against *O. ureolytica*. This is beneficial as the people of Maputaland may continue using water as a solvent to submerge the plants when preparing the remedies.

Upon analysis of the combination of *S. birrea* with *S. cordatum*, it was apparent that the combination on the whole provides good broad-spectrum antimicrobial activity, for both the aqueous and organic extracts (Table 4.8). Broad-spectrum activity was found when the plants were tested individually as well as in combination (Figures 3.1-3.6). Contrasting interactions against the same pathogen was noted, synergistic activity was detected with the aqueous extract whereas antagonistic activity (Table 4.8) was noted with the organic extract against *O. ureolytica* (Table 4.8). The drastic difference in plant interactions could be attributed to specific chemical compounds that are extracted when different solvents are used. Despite synergistic interactions detected against *O. ureolytica* with the aqueous extract toxicity was also detected when combined in the ratios of 6:4, 5:5, 4:6 and 3:7 for *S. birrea*: *S. cordatum*.

Considering noteworthy and moderately good antimicrobial activity *S. birrea* and *S. cordatum* displayed activity for both organic and aqueous extracts against four out of the six pathogens with a MIC range of 0.25 - 2.00 mg/ml. Compounds, terpinen-4-ol and pyrrolidine have been isolated from the aqueous extract of the stem-bark of *S. birrea* (Njume et al., 2011). It cannot be assumed that the terpinen-4-ol and pyrrolidine are present in the aqueous extract as different solvents release different compounds, therefore the aqueous plant extract effectiveness could be a result of other chemical constituents. The compound terpinen-4-ol in a cytotoxicity study previously investigated by Loughlin et al., (2008) was tested against human fibroblast cells and indicated safety at the same concentration used in the current study. Individually *S. birrea* indicated safety against the human kidney epithelial cells using the MTT assay. A study conducted by Ojewole (2003) indicated the methanolic and aqueous extracts of *S. birrea* individually were safe which is in agreement with this study. However, it has been proposed that some secondary metabolites and bio-active chemical compounds such as gallotannins, tannic,

mallic, gallic and citric acids, proanthocyanins and procyanidins, catechins, flavonoids, terpenoids, sesquiterpenoids, coumarins and sterols could be toxic to mammals (Ojewole, 2003). When *S. birrea* was tested against hepatocytes *in vitro*, negligible toxicity was observed and it was proposed that chronic use of the plant over a long period of time may be of concern (Van de Venter et al., 2008). This should not be the issue when using the plants to treat STIs as the plants are administered for short periods of time when required (not continuously), i.e. once/twice daily for up to five days, such that there will not be an accumulation of potentially toxic compounds (Van Heerden and Schwikkard, 2002). In addition, the methanolic and aqueous bark extracts of *S. birrea* had a high LD_{50} values in mice (median LD_{50} values 1215±38 mg/kg), suggesting no acute toxicity (Ojewole, 2003). *S. birrea* has been combined previously with other plants (Gathirwa et al., 2008). When the combinations were tested for anti-malarial activity, synergistic activity was observed with *Boscia saliciafolia* and *S. birrea*, *Boscia saliciafolia*, *Turraea robusta* and *S. birrea* which exhibited high parasite inhibition (>90%) and therefore demonstrated the advantage of combining *S. birrea* (Gathirwa et al., 2008).

S. cordatum, *Breonadia salicina* and *Ozoroa sphaerocarpa* were investigated in combination for anti-diarrhoeal properties (Sibandze et al., 2010). *S. cordatum* with *O. sphearocarpa*, *S. cordatum* with *B. salicina* and the triple combination (*S. cordatum*, *B. salicina* and *O. sphaerocarpa*) were all found to be synergistic (Sibandze et al., 2010). Interestingly, however, when *S. cordatum* and *O. spearocarpa* were combined, the combination was toxic with the MTT assay (human kidney epithelial cells) (Sibandze et al., 2010). This study shows that when including *S. cordatum* in any plant combinations one must be very careful to analyze the toxicity versus therapeutic effect of the combination.

S. cordatum (bark) and *S. birrea* (bark) do possess some of the suggested potentially toxic metabolites that were previously suggested by Ojewole (2003), those being gallic acid and ellagic acid (Candy et al., 1968). These potentially toxic compounds could be enhanced when used in combination and over a long duration and therefore individual therapies could be deemed the preferential treatment option. *S. birrea* and *S. cordatum* interact synergistically, while

possessing noteworthy antimicrobial activity. The combination is potentially safe against the human kidney epithelial cells at the ratios 8:2, 9:1 and 2:8 for *S. birrea*: *S. cordatum*.

For the combination of *A. marlothii* and *S. serratuloides* the antimicrobial effect is considered noteworthy (MIC = 0.50 mg/ml). This indicates that when the plants are combined they exceed their individual antimicrobial potential providing a more effective combination. Aloesin and barbaloin could be the related isolated compounds from *A. marlothii* (leaf exudate) that are responsible for the antimicrobial activity (Van Der Bank et al., 1995; Okamura et al., 1996). Synergistic activity for the organic extract against *O. ureolytica* was apparent across the various ratio mixtures; therefore implying that both plant extracts are contributing to the effective therapy. In an ethnobotanical study conducted by York et al., (2011) with the people of Maputaland, *A. marlothii* as well as *S. serratuloides* was found to be combined with other plants to treat respiratory infections, but the combination has not been investigated against any STI pathogens.

When *H. hemerocallidea* and *K. africana* were combined, the ΣFIC demonstrated synergistic activity (Table 4.6). From the isobologram analysis of the varied ratios the synergistic activity was directly related to the concentration of *H. hemerocallidea* used (Figure 4.5). *H. hemerocallidea* has been combined in a previous study with two other plants *Tulbaghia violacea* and *Merwilla plumbea* (Ncube et al., 2012). The plants in the latter study exhibited better antimicrobial activity, as well as synergy when combined (Ncube et al., 2012). A similar result was found in this study whereby better antimicrobial activity and synergy was noted with the combination (Figure 4.4).

H. hemerocallidea also demonstrates a dominant non-toxic effect when individually tested against the human kidney cell line, whilst *K. africana* (organic extract) was known to be more toxic (Table 3.2). Upon combining the plants, no toxicity was found. Therefore, *H. hemerocallidea* could be counteracting the toxic effects produced by *K. africana* and at the same time potentiating the antimicrobial effects. This is the ideal situation for a combination to eliminate the toxic effects and to be effective.

Some advantages of dual combinations is increasing antimicrobial efficacy (*A. adianthifolia* with *T. dregeana*) and sometimes finding a solution of decreasing toxic effects (*H. hemerocallidea* with *K. africana*). A further advantage is treatment of the patient holistically whereby one plant is effective at inhibiting the pathogen and the other effective in treating a symptom (*A. marlothii* with *S. serratuloides*). The disadvantage will be if the plants interact strongly enough to increase the toxicity in which case individual plant therapy or alternative combinations would be recommended (*S. birrea* with *S. cordatum*).

4.3.2 Traditional usage

Traditionally the two plant combinations were mentioned on average only once by specific households. The only combination that was mentioned on more than one occasion was *S. birrea* and *S. cordatum* for the treatment of gonorrhoea. The combination was discovered to be more favourable when treating urethritis caused by *U. urealyticum* and *O. ureolytica*. The lay people were accurate in the use of this combination for the treatment of gonorrhoea. Despite indifferent interactions, the combination did in fact exhibit noteworthy antimicrobial activity against *N. gonorrhoeae* for both the aqueous and organic plant extracts.

Almost half of the combinations were said to be used to treat gonorrhoea namely *X. caffra* with *T. elegans*, *S. birrea* with *S. cordatum* and *C. malosana* with *H. hemerocallidea*. Surprisingly *S. birrea* with *S. cordatum* was the only combination with noteworthy antimicrobial activity against *N. gonorrhoeae*. Furthermore the aqueous extracts of the remaining two combinations, *X. caffra* with *T. elegans* and *C. malosana* with *H. hemerocallidea* interacted antagonistically, thereby not supporting their use in the treatment of gonorrhoea.

All of the synergistic interactions seen with the combinations were observed with the aqueous plant extracts with only one exception, that of *A. marlothii* and *S. serratuloides*. This is a promising result for the lay people as the traditional solvent used is water.

4.4 Conclusions

- One combination (*S. birrea* combined with *S. cordatum)* of the seven two plant combinations studied possessed toxicity against the human kidney epithelial cells. Furthermore, *K. africana* found to be toxic against the human kidney epithelial cells when tested individually, had less of a toxic effect when combined with *H. hemerocallidea*.

- *S. birrea* and *S. cordatum* (aqueous extract) exhibited antimicrobial activity with synergistic interactions against *O. ureolytica*; however, some toxicity was observed with the MTT assay. The synergistic and 'safe' ratios of the combination were (*S. birrea*: *S. cordatum*) 9:1; 8:2; 7:3; 6:4; 5:5 and 2:8.

- The aqueous extract of the combination *A. adianthifolia* with *T. dregeana* demonstrated the lowest ΣFIC value (0.31) i.e. strongest synergistic interactions, with all nine ratios (9:1; 8:2; 7:3; 6:4; 5:5; 4:6; 3:7; 2:8; 1:9) plotted within the synergistic quadrant.

- Traditionally the lay people use water as a solvent and the aqueous extracts of the 1:1 combinations exhibited some promising activity. *S. birrea* and *S. cordatum* was the most frequently used combination by the lay people and displayed inhibitory effects against pathogens associated with urethritis.

Chapter 5

Poly-herbal combinations (three or more plants combined)

5.1 Introduction

Poly-herbal therapies possessing antifungal antibacterial, antipyretic, analgesic and anti-inflammatory activity are often used to broaden the spectrum of activity thereby increasing the therapeutic effect of the treatment (Kwon et al., 2008). According to Dahlberg and Trygger (2009), lay people do not have the knowledge to combine remedies for more than two plants. A number of combinations consisting of several species was considered stronger and should be administered by someone with more experience, such as by an *inyanga* (traditional healer) (Dahlberg and Trygger, 2009). According to the respondents in this study (lay people of Maputaland) multiple plant combinations are frequently used to treat STIs and therefore these previous presumptions do not necessarily hold true for all geographical areas in South Africa. Six poly-herbal combinations (Table 2.2) are commonly used by the lay people to treat STIs. None of the poly-herbal plant combinations have previously been scientifically validated for their efficacy against STI pathogens nor, has the toxicity of these combinations been evaluated.

The aim of this chapter was thus, to investigate the antimicrobial effectiveness of the poly-herbal plant combinations against the STI pathogens, to determine if the plants interact synergistically, and if the plant combinations exhibit toxicity against the human kidney epithelial cell line.

5.2 Results

5.2.1 Toxicity cellular viability assay

Toxicity studies were conducted with all six of the poly-herbal combinations for both the organic and aqueous plant extracts. The results indicated no toxicity with either the aqueous or the organic plant extracts for all six combinations when tested against the human kidney epithelial cells (Table 5.1). The only plant combination of *B. pilosa*, *R. multifidus*, *S. sanguinea* and *C. brachiata* (organic extract) exhibited a slight stimulatory effect on cell viability (17%) (Table 5.1).

Table 5.1 Toxic properties of poly-herbal combinations where the values represent percentage (%) cell viability ± standard deviation for the poly-herbal combinations of at least triplicate experiments.

Plant species in combination	**% Cell viability**	
	Aqueous extract	**Organic extract**
C. papaya *S. serratuloides* *H. hemerocallidea*	100.3 ± 0.57	106.5 ± 0.61
M. acuminata *S. serratuloides* *H. hemerocallidea*	100.4 ± 0.45	98.2 ± 0.47
B. pilosa *R. multifidus* *S. sanguinea* *C. brachiata*	100.2 ± 0.58	117.1 ± 0.67
E. hypericifolia *S. serratuloides* *H. hemerocallidea* *O. engleri*	112.5 ± 0.63	110.3 ± 0.48
P. africanum *S. serratuloides* *H. hemerocallidea* *K. africana*	99.4 ± 0.55	100.2 ± 0.74
H. hemerocallidea *S. serratuloides* *A. marlothii* *S. birrea* *S. cordatum*	102.4 ± 0.66	105.2 ± 0.73

Untreated control: 100% cell viability Positive control: Quinine IC_{50} = 141.34 ± 22.08 µg/ml

5.2.2 Antimicrobial analysis

5.2.2.1 *Carica papaya* (leaf), *Senecio serratuloides* (leaf) and *Hypoxis hemerocallidea* (corm)

The plants in multiple combinations are presented in Table 5.2. A breakdown of 1:1 interactions for each of the plants within the mixture is provided in Table 5.3. The combination *C. papaya*, *S. serratuloides* and *H. hemerocallidea* aqueous extract demonstrated moderately good antimicrobial activity against pathogens *O. ureolytica*, *G. vaginalis* and *N. gonorrhoeae* (Table 5.2). The lowest MIC value this combination achieved was with the aqueous plant extracts of 1.00 mg/ml.

The aqueous plant extracts were found to interact synergistically (Table 5.2) against *G. vaginalis* whereby moderately good antimicrobial activity was indicated. However, the plants *C. papaya*, *S. serratuloides* and *H. hemerocallidea* individually possessed poor antimicrobial activity against *G. vaginalis* (MIC values ranging 8.00 - >16.0 mg/ml). The rationale of the combination possessing moderately good antimicrobial activity and interacting synergistically is a positive result for the treatment of bacterial vaginosis. The combination predominantly interacted antagonistically and indifferently when combined, however, with the 1:1 plant combinations for the aqueous extracts, additive interactions were predominantly demonstrated (Table 5.3). Antagonistic interactions were demonstrated with the combination against *C. albicans* and *U. urealyticum*. Upon analysis of the plants 1:1 combinations for *C. albicans*, antagonistic effects were dominated whenever *S. serratuloides* was present (Table 5.3).

The organic extracts displayed antagonistic interactions against *C. albicans* and *N. gonorrhoeae* (Table 5.2). *U. urealyticum* on the other hand, demonstrated synergistic interactions for the organic extracts with noteworthy antimicrobial activity (ΣFIC = 0.32, MIC value = 0.25 mg/ml). However, the 1:1 combinations, of these plants (Table 5.3), displayed indifferent and additive interactions with no synergism present. The 1:1 combination of *S. serratuloides* with *H. hemerocallidea*, was found to interact synergistically. The combinations of aqueous extracts

interacted synergistically against *G. vaginalis* and *O. ureolytica* whereas the organic extract exhibited synergistic interactions against *C. albicans* and *G. vaginalis* (Table 5.3).

Table 5.2 MIC and ΣFIC results when the aqueous plant extracts of *C. papaya*, *S. serratuloides* and *H. hemerocallidea* were tested individually and in combination (1:1:1).

Micro-organism	MIC (mg/ml)				ΣFIC	
	C. papaya	***S. serratuloides***	***H. hemerocallidea***	**Triple combination**	**Triple combination**	**Interpretation**
Aqueous extract						
C. albicans ATCC 10321	>16.0	2.00	1.00	>16.0	**N/D[1]**	**Antagonistic[2]**
U. urealyticum Clinical strain	0.38	0.50	0.25	>16.0	**N/D[1]**	**Antagonistic[2]**
O. ureolytica ATCC 43534	0.50	4.00	2.00	1.00	0.92	Additive
G. vaginalis ATCC 14018	>16.0	8.00	8.00	2.00	**N/D[1]**	**Synergistic[2]**
T. vaginalis Clinical strain	>16.0	>16.0	8.00	>16.0	N/D[1]	Indifferent[2]
N. gonorrhoeae ATCC 19424	4.00	16.0	0.50	2.00	1.54	Indifferent
Organic extracts						
C. albicans ATCC 10321	1.00	8.00	4.00	>16.0	**7.33**	**Antagonistic**
U. urealyticum Clinical strain	0.50	0.63	3.00	0.25	**0.32**	**Synergistic**
O. ureolytica ATCC 43534	0.50	4.00	4.00	0.75	0.63	Additive
G. vaginalis ATCC 14018	1.00	4.00	4.00	4.00	2.00	Indifferent
T. vaginalis Clinical strain	2.00	2.00	2.00	2.00	1.00	Additive
N. gonorrhoeae ATCC 19424	0.25	2.00	8.00	2.00	**3.08**	**Antagonistic**

N/D[1] No ΣFIC value could be calculated as no MIC end point was established for the either plant or plants in combination. [2] Tentative interpretation according to MIC data. Shaded area: results previously discussed in Chapter 3 but included for reference purposes.

Table 5.3 The ΣFIC inter-relationship (1:1) interpretations between *C. papaya*, *S. serratuloides* and *H. hemerocallidea* when used in combination.

STI pathogens	*C. papaya* + *S. serratuloides*		*C. papaya* + *H. hemerocallidea*		*S. serratuloides* + *H. hemerocallidea*	
Aqueous extracts						
C. albicans	N/D	ANT	N/D	INDIF	4.50	ANT
U. urealyticum	N/D	ANT	0.84	ADD	0.78	ADD
O. ureolytica	0.57	ADD	0.63	ADD	0.19	SYN
G. vaginalis	N/D	ADD	N/D	ADD	0.06	SYN
T. vaginalis	N/D	SYN	N/D	ADD	N/D	ADD
N. gonorrhoeae	1.25	INDIF	4.50	ANT	2.07	INDIF
Organic extracts						
C. albicans	0.57	ADD	0.63	ADD	0.01	SYN
U. urealyticum	1.30	INDIF	0.88	ADD	3.54	INDIF
O. ureolytica	0.35	SYN	0.57	ADD	1.00	ADD
G. vaginalis	1.25	INDIF	1.25	INDIF	0.50	SYN
T. vaginalis	1.50	INDIF	1.00	ADD	1.00	ADD
N .gonorrhoeae	1.41	INDIF	1.29	INDIF	8.50	ANT

ANT = antagonism, INDIF = indifferent, ADD = additive, SYN = synergism, N/D = no end point.

5.2.2.2 *Musa acuminata* (root), *Senecio serratuloides* (leaf) and *Hypoxis hemerocallidea* (leaf)

The 1:1:1 combination of *M. acuminata*, *S. serratuloides* and *H. hemerocallidea* aqueous extract displayed synergistic interactions against *O. ureolytica* (ΣFIC = 0.36), despite the moderate antimicrobial activity achieved by the plants individually (Table 5.4). Furthermore, the aqueous

extract of the combination exhibited synergistic interactions against the pathogen *G. vaginalis* (Table 5.6). Most interesting and promising is each 1:1 plant combinations within the poly-herbal combination tested demonstrated synergistic interactions against *O. ureolytica* and *G. vaginalis* (Table 5.4). The overall synergistic interactions within the combination and the moderately good antimicrobial activity, is indicative of a promising rationale towards the usage of the poly-herbal combination for the treatment of urethritis caused by *O. ureolytica* and bacterial vaginosis caused by *G. vaginalis*. On the contrary unfavourable antagonistic interactions were indicated when the combination was studied against *C. albicans, U. urealyticum* and *N. gonorrhoeae* (Table 5.4).

Table 5.4 The MIC and ΣFIC results when the aqueous plant extracts of *M. acuminata* and *S. serratuloides* and *H. hemerocallidea* were tested individually and in combination (1:1:1).

Micro-organism	**MIC (mg/ml)**				**ΣFIC**	
	M. acuminata	***S. serratuloides***	***H. hemerocallidea***	**Triple combination**	**Triple combination**	**Interpretation**
Aqueous extract						
C. albicans ATCC 10321	>16.0	2.00	1.00	>16.0	**N/D[1]**	**Antagonistic[2]**
U. urealyticum Clinical strain	>16.0	0.50	0.25	8.00	**N/D[1]**	**Antagonistic[2]**
O. ureolytica ATCC 43534	3.00	4.00	2.00	1.00	**0.36**	**Synergistic**
G. vaginalis ATCC 14018	>16.0	8.00	8.00	4.00	**N/D[1]**	**Synergistic[2]**
T. vaginalis Clinical strain	>16.0	>16.0	8.00	8.00	N/D[1]	Additive[2]
N. gonorrhoeae ATCC 19424	>16.0	16.0	0.50	>16.0	**N/D[1]**	**Antagonistic[2]**

N/D[1] No ΣFIC value could be calculated as no MIC end point was established for the either plant or plants in combination. [2] Tentative interpretation according to MIC data. Shaded area: results previously discussed in Chapter 3 but included for reference purposes.

For the organic extract of the poly-herbal combination *M. acuminata, S. serratuloides* and *H. hemerocallidea*, the calculated ΣFIC = 0.33 interpreted synergistic interactions against *O.*

ureolytica. The 1:1 combination interactions revealed indifferent and additive interactions with no synergistic interactions present (Table 5.6). The moderately good antimicrobial activity and synergistic interactions allows for the organic extract of the combination to be an effective treatment against the STI pathogen *O. ureolytica* (Table 5.5).

Table 5.5 The MIC and ΣFIC results when the organic plant extracts of *M. acuminata*, *S. serratuloides* and *H. hemerocallidea* were tested individually and in combination (1:1:1).

Micro-organism	MIC (mg/ml)				ΣFIC	
	M. acuminata	***S. serratuloides***	***H. hemerocallidea***	**Triple combination**	**Triple combination**	**Interpretation**
Aqueous extract						
C. albicans ATCC 10321	8.00	8.00	4.00	4.00	0.67	Additive
U. urealyticum Clinical strain	0.75	0.63	3.00	2.00	3.25	Indifferent
O. ureolytica ATCC 43534	2.00	4.00	4.00	1.00	**0.33**	**Synergistic**
G. vaginalis ATCC 14018	4.00	4.00	4.00	3.00	0.75	Additive
T. vaginalis Clinical strain	12.0	2.00	2.00	6.00	2.17	Indifferent
N. gonorrhoeae ATCC 19424	8.00	2.00	8.00	2.00	0.50	Additive

N/D[1] No ΣFIC value could be calculated as no MIC end point was established for the either plant or plants in combination. [2] Tentative interpretation according to MIC data. Shaded area: results previously discussed in Chapter 3 but included for reference purposes.

The 1:1 plant combinations for the poly-herbal combination indicate a number of synergistic interactions with 16.6% of the interactions also demonstrating some antagonism (Table 5.6). *S. serratuloides* and *H. hemerocallidea*, in combination for both the organic and aqueous extracts demonstrated synergistic interactions against *O. ureolytica* (aqueous extract), *C. albicans* (organic extract) and *G. vaginalis* (aqueous and organic extracts) (as observed in Chapter 4). The organic extract of *M. acuminata* with *S. serratuloides* demonstrated synergistic interactions against *C. albicans*, *O. ureolytica*, *G. vaginalis* and *N. gonorrhoeae*.

Table 5.6 The ΣFIC inter-relationship (1:1) interpretations between *M. acuminata*, *S. serratuloides* and *H. hemerocallidea* when used in combination.

STI pathogens	***M. acuminata* + *S. serratuloides***		***M. acuminata* + *H. hemerocallidea***		***S. serratuloides* + *H. hemerocallidea***	
Aqueous extracts						
C. albicans	N/D	INDIF	N/D	INDIF	4.50	ANT
U. urealyticum	N/D	ANT	N/D	INDIF	0.78	ADD
O. ureolytica	0.08	SYN	0.11	SYN	0.19	SYN
G. vaginalis	N/D	SYN	N/D	SYN	0.06	SYN
T. vaginalis	N/D	INDIF	N/D	ADD	N/D	ADD
N. gonorrhoeae	N/D	ADD	N/D	ANT	2.07	INDIF
Organic extracts						
C. albicans	0.13	SYN	0.75	ADD	0.01	SYN
U. urealyticum	4.54	ANT	0.20	SYN	3.54	INDIF
O. ureolytica	0.08	SYN	0.10	SYN	1.00	ADD
G. vaginalis	0.50	SYN	1.00	ADD	0.50	SYN
T. vaginalis	0.58	ADD	0.88	ADD	1.00	ADD
N .gonorrhoeae	0.20	SYN	0.08	SYN	1.00	ADD

ANT = antagonism, INDIF = indifferent, ADD = additive, SYN = synergism, N/D = No end point.

5.2.2.3 *Bidens pilosa* (leaf), *Ranunculus multifidus* (leaf), *Sarcophyte sanguinea* (stem) and *Clematis brachiata* (leaf)

The aqueous plant extracts for the combination of *B. pilosa*, *R. multifidus*, *S. sanguinea* and *C. brachiata* demonstrated poor antimicrobial activity (MIC value = 6.00 mg/ml) against *G. vaginalis*, however, synergistic interactions were noted (Table 5.7). The 1:1 combinations established synergistic and additive interactions against *G. vaginalis* between all the plant species with one exception that being *B. pilosa* with *S. sanguinea* (Table 5.8). Antagonism was further detected when the combination was studied against pathogens *U. urealyticum* and *T. vaginalis* (Table 5.7). The 1:1 plant combinations for the pathogen *U. urealyticum* demonstrated predominantly antagonistic interactions explaining that each plant in the combination plays a role in the observed antagonistic interactions.

The organic plant extract for the poly-herbal combination *B. pilosa*, *R. multifidus*, *S. sanguinea* and *C. brachiata* demonstrated moderately good antimicrobial activity against *U. urealyticum*, *O. ureolytica*, *G. vaginalis*, *T. vaginalis* and *N. gonorrhoeae* (Table 5.7). For the combination, synergistic interactions were demonstrated against the pathogen *O. ureolytica* (Table 5.8). The 1:1 combination studies for this pathogen further revealed interesting results whereby every 1:1 plant within the combination interacted synergistically (Table 5.8).

Antagonistic interactions were observed when the poly-herbal combination was studied against *C. albicans*. Unexpectedly, the 1:1 plant combinations revealed contradictory interactions with a predominating synergistic interaction. Furthermore, no antagonism was detected with these results for this pathogen (Table 5.8). It was interesting to note that the combination of *R. multifidus* and *S. sanguinea* (aqueous and organic extracts) interacted synergistically for three STI associated pathogens. The aqueous plant extract indicated synergism against *C. albicans*, *O. ureolytica* and *G. vaginalis*; similarly the organic plant extracts interacted synergistically against *C. albicans*, *O. ureolytica* and *U. urealyticum* (Table 5.7). These two plants seemed to predominantly contribute towards the synergy of the whole plant mixture.

Table 5.7 The MIC and ΣFIC results when the aqueous and organic plant extracts of *B. pilosa*, *R. multifidus*, *S. sanguinea* and *C.brachiata* and were tested individually and in combination (1:1:1:1).

Micro-organism	MIC (mg/ml)					ΣFIC	
	B. pilosa	***R. multifidus***	***S. sanguinea***	***C. brachiata***	**Combination**	**Combination**	**Interpretation**
N/D= not determined	**Aqueous Extracts**						
C. albicans ATCC 10321	2.00	12.0	>16.0	>16.0	4.00	N/D[1]	Additive[2]
U. urealyticum Clinical strain	0.25	0.02	3.00	4.00	2.00	**28.5**	**Antagonistic**
O. ureolytica ATCC 43534	1.00	3.00	8.00	1.50	2.00	1.06	Indifferent
G. vaginalis ATCC 14018	16.0	16.0	8.00	>16.0	6.00	**N/D[1]**	**Synergistic[2]**
T. vaginalis Clinical strain	2.00	>16.0	>16.0	16.0	>16.0	**N/D[1]**	**Antagonistic[2]**
N. gonorrhoeae ATCC 19424	4.00	2.00	0.25	8.00	1.00	1.22	Indifferent
Organic Extracts							
C. albicans ATCC 10321	4.00	16.0	>16.0	1.00	16.0	**N/D[1]**	**Antagonistic[2]**
U. urealyticum Clinical strain	0.50	0.25	1.00	1.00	0.63	1.26	Indifferent
O. ureolytica ATCC 43534	1.00	>16.0	2.00	2.00	0.75	**N/D[1]**	**Synergistic[2]**
G. vaginalis ATCC 14018	2.00	4.00	0.63	4.00	2.00	1.29	Additive
T. vaginalis Clinical strain	1.00	2.00	1.00	2.00	4.00	3.00	Indifferent
N. gonorrhoeae ATCC 19424	0.25	2.00	0.31	1.00	0.50	1.78	Indifferent

N/D[1] No ΣFIC value could be calculated as no MIC end point was established for the either plant or plants in combination . [2]Tentative interpretation.

Table 5.8 The ΣFIC inter-relationship (1:1) interpretations when *B. pilosa*, *R. multifidus*, *S. sanguinea* and *C. brachiata* when used in combination.

Plant combinations		**STI pathogens ΣFIC**											
Aqueous extracts		*C. albicans*		*U. urealyticum*		*O. ureolytica*		*G. vaginalis*		*T. vaginalis*		*N. gonorrhoeae*	
B. pilosa	*R. multifidus*	N/D	ANT	N/D	ANT	1.34	INDIF	0.13	SYN	N/D	ANT	0.38	SYN
B. pilosa	*S. sanguinea*	N/D	ADD	N/D	ANT	2.25	INDIF	4.15	ANT	N/D	INDIF	0.67	ADD
B. pilosa	*C. brachiata*	N/D	ANT	N/D	ANT	1.67	INDIF	N/D	SYN	2.25	INDIF	0.24	SYN
R. multifidus	*S. sanguinea*	N/D	SYN	N/D	ANT	0.46	SYN	0.28	SYN	N/D	ADD	0.70	ADD
R. multifidus	*C. brachiata*	N/D	INDIF	N/D	ANT	1.00	ADD	N/D	SYN	N/D	ADD	0.39	SYN
S. sanguinea	*C. brachiata*	N/D	SYN	N/D	ADD	0.20	SYN	N/D	ADD	N/D	ADD	0.65	ADD
Organic extracts													
B. pilosa	*R. multifidus*	0.08	SYN	1.50	INDIF	N/D	SYN	0.75	ADD	3.00	INDIF	1.41	INDIF
B. pilosa	*S. sanguinea*	N/D	SYN	N/D	INDIF	0.06	SYN	2.19	INDIF	4.00	ANT	2.25	INDIF
B. pilosa	*C. brachiata*	0.16	SYN	0.56	ADD	0.12	SYN	0.75	ADD	3.00	INDIF	1.57	INDIF
R. multifidus	*S. sanguinea*	N/D	SYN	N/D	SYN	N/D	SYN	8.38	ANT	3.00	INDIF	1.16	INDIF
R. multifidus	*C. brachiata*	1.07	INDIF	1.13	INDIF	N/D	SYN	1.00	ADD	1.50	INDIF	0.45	SYN
S. sanguinea	*C. brachiata*	N/D	ADD	0.50	SYN	0.16	SYN	1.40	INDIF	1.50	INDIF	1.32	INDIF

ANT = antagonism, INDIF = indifference, ADD = additive, SYN = synergism, N/D = no end point.

5.2.2.4 *Euphorbia hypericifolia* (leaf), *Senecio serratuloides* (leaf), *Hypoxis hemerocallidea* (leaf) and *Ozoroa engleri* (leaf)

The aqueous plant extract for the combination of *E. hypericifolia, S. serratuloides*, *H. hemerocallidea* and *O. engleri* displayed moderately good antimicrobial activity against *U. urealyticum*, *O. ureolytica*, *G. vaginalis* and *N. gonorrhoeae* (Table 5.9). Synergistic interactions were found with the poly-herbal sample against *G. vaginalis* (ΣFIC value = 0.27), however, only one 1:1 plant combination (*S. serratuloides* with *H. hemerocallidea*) indicated a synergistic interaction when examining this combination of plants against *G. vaginalis*. The lowest MIC and therefore, best antimicrobial activity was seen against *U. urealyticum* with an MIC value of 0.50 mg/ml. Furthermore, synergistic interactions (ΣFIC value = 0.32) were displayed for the poly-herbal combination (Table 5.9). When examining the 1:1 plant combinations against this pathogen, the majority of the observed interactions were additive and synergistic interactions occurred (Table 5.10). Unfavourable antagonistic interactions were obtained against *C. albicans* for the poly-herbal combination with predominantly antagonistic interactions found with the 1:1 plant combinations (Table 5.10). This could have possibly contributed to the poor antimicrobial activity (MIC value = >16.0 mg/ml) observed with the poly-herbal combination against *C. albicans* (Table 5.9).

For the organic extracts of the poly-herbal combination, synergistic interactions (ΣFIC values of 0.19) were observed for *U. urealyticum* (Table 5.9). When taking an in-depth analysis of the 1:1 plant combinations, synergistic interactions were displayed against *U. urealyticum* whereby *E. hypericifolia* seemed to be the common plant for all synergistic interactions (Table 5.10). Antagonistic interactions were obtained with the poly-herbal combination against *C. albicans* which was probably the factor attributing to the poor antimicrobial activity (MIC value = >16.0 mg/ml) of the combination. Unexpectedly, the in-depth analysis of the 1:1 plant combinations resulted in the increased observation of synergistic interactions the complete converse of the overall interactive result. This could result from two plants interacting synergistically whereas when multiple plants are combined there could be an increase of competition toward the binding site on the microbe therefore providing an antagonistic interaction between all the plants.

Table 5.9 The MIC and ΣFIC results when the aqueous and organic plant extracts of *E. hypericifolia*, *S. serratuloides*, *H. hemerocallidea* and *O. engleri* and were tested individually and in combination (1:1:1:1).

Micro-organism	MIC (mg/ml)					ΣFIC	
	E. hypericifolia	***S. serratuloides***	***H. hemerocallidea***	***O. engleri***	**Combination**	**Combination**	**Interpretation**
N/D= not determined	**Aqueous Extracts**						
C. albicans ATCC 10321	2.00	2.00	1.00	0.75	>16.0	**N/D[1]**	**Antagonistic[2]**
U. urealyticum Clinical strain	3.00	0.50	0.25	>16.0	0.50	**N/D[1]**	**Synergistic[2]**
O. ureolytica ATCC 43534	2.00	4.00	2.00	>16.0	2.00	N/D[1]	Additive[2]
G. vaginalis ATCC 14018	2.00	8.00	8.00	3.00	1.00	**0.27**	**Synergistic**
T. vaginalis Clinical strain	>16.0	>16.0	8.00	>16.0	>16.0	N/D[1]	Indifferent[2]
N. gonorrhoeae ATCC 19424	1.50	16.0	0.50	1.00	2.00	1.87	Indifferent
Organic Extracts							
C. albicans ATCC 10321	1.00	8.00	4.00	16.0	>16.0	**N/D[1]**	**Antagonistic[2]**
U. urealyticum Clinical strain	1.50	0.63	3.00	2.00	0.25	**0.19**	**Synergistic**
O. ureolytica ATCC 43534	0.50	4.00	4.00	0.50	0.63	0.71	Additive
G. vaginalis ATCC 14018	0.50	4.00	4.00	4.00	4.00	2.75	Indifferent
T. vaginalis Clinical strain	1.50	2.00	2.00	1.00	2.00	1.33	Indifferent
N. gonorrhoeae ATCC 19424	0.63	2.00	8.00	1.00	2.00	3.50	Indifferent

N/D[1] No ΣFIC value could be calculated as no MIC end point was established for the either plant or plants in combination . [2] Tentative interpretation

Table 5.10 The ΣFIC inter-relationship (1:1) interpretations when *E. hypericifolia*, *S. serratuloides*, *H. hemerocallidea* and *O. engleri* when used in combination.

Plant combinations		**STI pathogens ΣFIC**											
Aqueous extracts		*C. albicans*		*U. urealyticum*		*O. ureolytica*		*G. vaginalis*		*T. vaginalis*		*N. gonorrhoeae*	
E. hypericifolia	*S. serratuloides*	2.00	INDIF	0.88	ADD	0.19	SYN	1.25	INDIF	N/D	ADD	0.73	ADD
E. hypericifolia	*H. hemerocallidea*	12.0	ANT	0.19	SYN	1.00	ADD	1.25	INDIF	N/D	ADD	2.67	INDIF
E. hypericifolia	*O. engleri*	5.50	ANT	N/D	SYN	N/D	INDIF	2.50	INDIF	N/D	ADD	0.84	ADD
S. serratuloides	*H. hemerocallidea*	4.50	ANT	0.78	ADD	0.19	SYN	0.06	SYN	N/D	ADD	2.07	INDIF
S. serratuloides	*O. engleri*	3.67	INDIF	N/D	ADD	N/D	SYN	0.92	ADD	N/D	SYN	1.07	INDIF
H. hemerocallidea	*O. engleri*	4.67	ANT	N/D	INDIF	N/D	SYN	0.92	ADD	N/D	ADD	0.38	SYN
Organic extracts													
E. hypericifolia	*S. serratuloides*	0.28	SYN	0.29	SYN	1.69	INDIF	2.25	INDIF	1.17	INDIF	0.66	ADD
E. hypericifolia	*H. hemerocallidea*	0.63	ADD	0.19	SYN	4.50	ANT	0.57	ADD	2.34	INDIF	0.54	ADD
E. hypericifolia	*O. engleri*	0.14	SYN	0.42	SYN	4.00	ANT	1.13	INDIF	1.67	INDIF	0.82	ADD
S. serratuloides	*H. hemerocallidea*	0.01	SYN	3.54	INDIF	1.00	ADD	0.50	SYN	1.00	ADD	8.50	ANT
S. serratuloides	*O. engleri*	0.03	SYN	1.05	INDIF	4.50	ANT	0.50	SYN	2.25	INDIF	0.47	ADD
H. hemerocallidea	*O. engleri*	0.32	SYN	0.67	ADD	4.50	ANT	0.25	SYN	1.50	INDIF	0.35	SYN

ANT = antagonism, INDIF = indifference, ADD = additive, SYN = synergism, N/D = no end point.

5.2.2.5 *Peltophorum africanum* (root), *Senecio serratuloides* (leaf), *Hypoxis hemerocallidea* (leaf) and *Kigelia africana* (leaf)

When analyzing the MIC and ΣFIC data where the aqueous extracts of *P. africanum*, *S. serratuloides*, *H. hemerocallidea* and *K. africana* were combined most interactions consisted of indifference. Antagonism was noted for the pathogen, *U. urealyticum* (MIC value = 3.00 mg/ml; ΣFIC = 7.88) and *G. vaginalis* (MIC value = 8.00 mg/ml). The antimicrobial efficacy against these pathogens demonstrated poorer activity in combination. The combination tested against *O. ureolytica* demonstrated synergistic interactions (ΣFIC value = 0.47; MIC value = 2.00 mg/ml) and the 1:1 plant combinations corresponded with the overall result depicting predominantly synergistic interactions, with one exception namely, the combination between *P. africanum* and *H. hemerocallidea* which interacted additively (ΣFIC = 0.57) (Table 5.11 and 5.12).

The MIC and ΣFIC data for the organic extracts displayed similar results to the aqueous extracts, with indifferent interactions being mainly observed. Unfavourable antagonistic interactions were noted against *U. urealyticum* (ΣFIC value = 5.49) despite the fact that noteworthy antimicrobial activity (MIC value = 0.75 mg/ml) was indicated. Even though the combination resulted in additive interactions with *C. albicans* the analysis of the 1:1 plant combinations indicated mostly synergistic interactions (Table 5.12).

This four plant combination demonstrated the most antagonistic interactions against the STI pathogens tested (Table 5.11).

5.2.2.6 *Aloe marlothii* (leaf), *Hypoxis hemerocallidea* (corm), *Senecio serratuloides* (leaf), *Sclerocarya birrea* (root) and *Syzygium cordatum* (root)

The aqueous plant extracts of *A. marlothii*, *H. hemerocallidea*, *S. serratuloides*, *S. birrea* and *S. cordatum* combined obtained dominating indifferent interactions with the exception against *U. urealyticum* in which antagonistic interactions (MIC value = 8.00 mg/ml; ΣFIC value = 24.5) was observed (Table 5.13).

Table 5.11 The MIC and ΣFIC results when the aqueous and organic plant extracts of *P. africanum*, *S. serratuloides*, *H. hemerocallidea* and *K. africana* and were tested individually and in combination (1:1:1:1).

Micro-organism	MIC (mg/ml)					ΣFIC	
	P. africanum	***S. serratuloides***	***H. hemerocallidea***	***K. africana***	**Combination**	**Combination**	**Interpretation**
N/D= not determined	**Aqueous Extracts**						
C. albicans ATCC 10321	4.00	2.00	1.00	16.0	4.00	1.81	Indifferent
U. urealyticum Clinical strain	2.00	0.50	0.25	0.25	3.00	**7.88**	**Antagonistic**
O. ureolytica ATCC 43534	16.0	4.00	2.00	8.00	2.00	**0.47**	**Synergistic**
G. vaginalis ATCC 14018	0.50	8.00	8.00	>16.0	8.00	**N/D[1]**	**Antagonistic[2]**
T. vaginalis Clinical strain	>16.0	>16.0	8.00	>16.0	>16.0	N/D[1]	Indifferent[2]
N. gonorrhoeae ATCC 19424	0.50	16.0	0.50	>16.0	2.00	N/D[1]	Indifferent[2]
Organic Extracts							
C. albicans ATCC 10321	>16.0	8.00	4.00	4.00	4.00	N/D[1]	Additive[2]
U. urealyticum Clinical strain	0.04	0.63	3.00	0.50	0.75	**5.49**	**Antagonistic**
O. ureolytica ATCC 43534	1.00	4.00	4.00	1.00	2.00	1.25	Indifferent
G. vaginalis ATCC 14018	0.50	4.00	4.00	4.00	1.50	1.03	Indifferent
T. vaginalis Clinical strain	2.00	2.00	2.00	1.50	2.00	1.08	Indifferent
N. gonorrhoeae ATCC 19424	0.25	2.00	8.00	2.00	1.00	1.28	Indifferent

N/D[1] No ΣFIC value could be calculated as no MIC end point was established for the either plant or plants in combination . [2] Tentative interpretation .

Table 5.12 The ΣFIC inter-relationship (1:1) interpretations when *P. africanum*, *S. serratuloides*, *H. hemerocallidea* and *K. africana* used in combination.

Plant combinations		**STI pathogens ΣFIC**											
Aqueous extracts		*C. albicans*		*U. urealyticum*		*O. ureolytica*		*G. vaginalis*		*T. vaginalis*		*N. gonorrhoeae*	
P. africanum	*S. serratuloides*	1.50	INDIF	12.7	ANT	0.16	SYN	2.13	INDIF	N/D	ANT	1.29	INDIF
P. africanum	*H. hemerocallidea*	3.75	INDIF	N/D	ANT	0.57	ADD	6.38	ANT	N/D	INDIF	1.13	INDIF
P. africanum	*K. africana*	0.32	SYN	N/D	ANT	0.09	SYN	N/D	ANT	N/D	ADD	N/D	INDIF
S. serratuloides	*H. hemerocallidea*	4.50	ANT	0.78	ADD	0.19	SYN	0.06	SYN	N/D	ADD	2.07	INDIF
S. serratuloides	*K. africana*	N/D	ANT	N/D	ANT	0.06	SYN	N/D	ADD	N/D	ADD	N/D	SYN
H. hemerocallidea	*K. africana*	N/D	ANT	6.00	ANT	0.20	SYN	N/D	ADD	N/D	ADD	N/D	ANT
Organic extracts													
P. africanum	*S. serratuloides*	N/D	SYN	6.81	ANT	N/D	ANT	0.85	ADD	1.00	ADD	1.41	INDIF
P. africanum	*H. hemerocallidea*	N/D	SYN	13.0	ANT	N/D	ANT	0.85	ADD	1.00	INDIF	1.30	INDIF
P. africanum	*K. africana*	N/D	SYN	6.90	ANT	1.00	ADD	1.13	INDIF	2.34	INDIF	1.41	INDIF
S. serratuloides	*H. hemerocallidea*	0.09	SYN	3.54	INDIF	1.00	ADD	0.50	SYN	1.00	ADD	8.50	ANT
S. serratuloides	*K. africana*	0.75	ADD	1.30	INDIF	0.20	SYN	1.00	ADD	3.50	ANT	0.32	SYN
H. hemerocallidea	*K. africana*	2.00	INDIF	0.88	ADD	0.63	ADD	0.75	ADD	3.50	INDIF	1.25	INDIF

ANT = antagonism, INDIF = indifference, ADD = additive, SYN = synergism, N/D = no end point.

Table 5.13 The MIC and ΣFIC results when the aqueous and organic plant extracts of *A. marlothii*, *S. serratuloides*, *H. hemerocallidea* and *S. birrea* and *S. cordatum* were tested individually and in combination (1:1:1:1).

Micro-organism	MIC (mg/ml)						ΣFIC	
	A. marlothii	***H. hemerocallidea***	***S. serratuloides***	***S. birrea***	***S. cordatum***	**Combination**	**Combination**	**Interpretation**
N/D= not determined	**Aqueous Extracts**							
C. albicans ATCC 10321	16.0	1.00	2.00	>16.0	0.50	4.00	N/D[1]	Indifferent[2]
U. urealyticum Clinical strain	0.75	0.25	0.50	0.25	0.25	8.00	**24.5**	**Antagonistic**
O. ureolytica ATCC 43534	0.38	2.00	4.00	2.00	3.00	2.00	1.69	Indifferent
G. vaginalis ATCC 14018	>16.0	8.00	8.00	1.00	0.75	4.00	N/D[1]	Indifferent[2]
T. vaginalis Clinical strain	>16.0	8.00	>16.0	>16.0	8.00	>16.0	N/D[1]	Indifferent[2]
N. gonorrhoeae ATCC 19424	>16.0	0.50	16.0	0.50	0.25	1.00	N/D[1]	Indifferent[2]
Organic Extracts								
C. albicans ATCC 10321	4.00	4.00	8.00	>16.0	0.50	4.00	N/D[1]	Indifferent[2]
U. urealyticum Clinical strain	1.00	3.00	0.63	0.25	1.00	1.00	1.58	Indifferent
O. ureolytica ATCC 43534	2.00	4.00	4.00	1.00	2.00	0.75	0.68	Additive
G. vaginalis ATCC 14018	2.00	4.00	4.00	0.75	1.00	1.50	1.00	Additive
T. vaginalis Clinical strain	6.00	2.00	2.00	2.00	1.00	3.00	1.60	Indifferent
N. gonorrhoeae ATCC 19424	1.00	8.00	2.00	0.25	0.25	0.75	1.44	Indifferent

N/D[1] No ΣFIC value could be calculated as no MIC end point was established for the either plant or plants in combination . [2] Tentative interpretation.

Table 5.14 The ΣFIC inter-relationship (1:1) interpretations when *A. marlothii*, *S. serratuloides*, *H. hemerocallidea*, *S. birrea* and *S. cordatum* aqueous extract were used in combination.

Plant combinations		**STI pathogens ΣFIC**											
Aqueous extracts		*C. albicans*		*U. urealyticum*		*O. ureolytica*		*G. vaginalis*		*T. vaginalis*		*N. gonorrhoeae*	
A. marlothii	*S. serratuloides*	N/D	ANT	0.67	ADD	2.92	INDIF	N/D	ADD	N/D	ADD	N/D	SYN
A. marlothii	*H. hemerocallidea*	8.50	ANT	5.34	ANT	1.59	INDIF	N/D	SYN	N/D	INDIF	N/D	INDIF
A. marlothii	*S. birrea*	N/D	SYN	21.3	ANT	0.40	SYN	N/D	INDIF	N/D	ADD	N/D	ADD
A. marlothii	*S. cordatum*	4.13	ANT	0.67	ADD	0.75	ADD	N/D	INDIF	N/D	INDIF	N/D	INDIF
H. hemerocallidea	*S. serratuloides*	4.50	ANT	2.50	INDIF	0.19	SYN	0.06	SYN	N/D	ADD	2.07	INDIF
H. hemerocallidea	*S. birrea*	N/D	ANT	28.0	ANT	0.01	SYN	0.85	ADD	N/D	ADD	1.25	INDIF
H. hemerocallidea	*S. cordatum*	6.00	ANT	1.00	ADD	0.21	SYN	1.10	INDIF	1.00	ADD	1.25	INDIF
S. serratuloides	*S. birrea*	N/D	ADD	0.67	ADD	0.38	SYN	2.25	INDIF	N/D	ADD	N/D	INDIF
S. serratuloides	*S. cordatum*	1.88	INDIF	0.84	ADD	0.08	SYN	2.19	INDIF	N/D	ADD	0.65	ADD
S. birrea	*S. cordatum*	N/D	INDIF	N/D	ANT	0.11	SYN	0.58	ADD	N/D	ANT	0.64	ADD

ANT = antagonism, INDIF = indifference, ADD = additive, SYN = synergism, N/D = no end point.

Table 5.15 The ΣFIC inter-relationship (1:1) interpretations when *A. marlothii*, *S. serratuloides*, *H. hemerocallidea*, *S. birrea* and *S. cordatum* organic extract were used in combination.

Plant combinations		**STI pathogens ΣFIC**											
Organic extracts		*C. albicans*		*U. urealyticum*		*O. ureolytica*		*G. vaginalis*		*T. vaginalis*		*N. gonorrhoeae*	
A. marlothii	*S. serratuloides*	0.38	SYN	1.05	INDIF	0.12	SYN	0.75	ADD	0.67	ADD	6.00	ANT
A. marlothii	*H. hemerocallidea*	0.75	ADD	0.25	SYN	3.00	INDIFF	0.75	ADD	0.67	ADD	0.35	SYN
A. marlothii	*S. birrea*	N/D	SYN	0.94	ADD	0.15	SYN	0.46	SYN	1.75	INDIF	10.0	ANT
A. marlothii	*S. cordatum*	1.13	INDIF	0.50	SYN	1.50	INDIF	0.75	ADD	1.17	INDIF	3.13	INDIF
H. hemerocallidea	*S. serratuloides*	0.01	SYN	3.54	INDIF	1.00	ADD	0.50	SYN	1.00	ADD	8.50	ANT
H. hemerocallidea	*S. birrea*	N/D	SYN	0.54	ADD	2.50	INDIF	0.79	ADD	1.50	INDIF	1.30	INDIF
H. hemerocallidea	*S. cordatum*	1.13	INDIF	0.17	SYN	1.50	INDIF	0.94	ADD	2.25	INDIF	2.58	INDIF
S. serratuloides	*S. birrea*	N/D	SYN	1.80	INDIF	0.16	SYN	0.79	ADD	1.00	ADD	1.41	INDIF
S. serratuloides	*S. cordatum*	1.07	INDIF	1.05	INDIF	0.75	ADD	0.63	ADD	3.00	INDIF	1.41	INDIF
S. birrea	*S. cordatum*	N/D	ADD	5.00	ANT	N/D	ANT	0.29	SYN	1.50	INDIF	2.50	INDIF

ANT = antagonism, INDIF = indifference, ADD = additive, SYN = synergism, N/D = no end point.

Even though the aqueous extract demonstrated indifferent interactions against *O. ureolytica* it was interesting to note that the 1:1 plant combinations consisted of an array of synergistic interactions for this pathogen (Table 5.14). With the interactions, *A. marlothii*, however, had a lesser effect (Table 5.14).

The organic extract for the combination presented predominating indifferent interactions. The only two exceptions were the additive interactions when tested against *O. ureolytica* and *G. vaginalis*. Noteworthy antimicrobial activity (MIC value = 0.75 mg/ml) was detected for pathogens *O. ureolytica* and *N. gonorrhoeae* (Table 5.13). The 1:1 combinations for *O. ureolytica* did indicate three synergistic interactions (Table 5.15). The only 1:1 combination demonstrating synergy for *N. gonorrhoeae* was found with the combination of *A. marlothii* and *H. hemerocallidea* (ΣFIC value = 0.35). The poly-herbal combination (organic extract) against *C. albicans* exhibited indifferent interactions yet when the 1:1 combinations were evaluated; a predominantly synergistic interaction was observed (Table 5.15).

Overall, no synergistic interactions were displayed by the poly-herbal combination against any of the selected six pathogens for both the aqueous and organic plant extracts.

5.3 Discussion

5.3.1 Cytotoxic analysis

No previous toxicity studies could be found on any of the poly-herbal combinations therefore the results of this study will be this first documented scientific knowledge on the plant combinations. Athough there was a promise of safety with the lack of toxic effect against the human kidney epithelial cell line; caution must be taken when using the poly-herbal combination whereby antagonism has been established. The plant *K. africana* was found to show some toxic potential against the human kidney epithelial cells when singularly tested (Table 3.1), but when combined with *H. hemerocallidea* (Table 4.1) with no toxicity observed, as well as being non-toxic when used in a poly-herbal combination (Table 5.1). Since *K. africana* is known to be toxic it is not impossible that the lay people have seen the adverse effects when used singularly and the people combine the plants in order to reduce toxic effects or simply dilute out the causative compound.

Due to the counteracted toxicity the aqueous plant extract used in a two plant combination or in the poly-herbal combination is an effective treatment against *O. ureolytica*.

5.3.2 Antimicrobial analysis

The only previous study to take note of poly-herbal combinations, as a treatment for STIs, was the ethnobotanical review by De Wet et al., (2012). From a global perspective, there have been some studies on combinations for the treatment of STIs. In 1999, an array of 19 compounds from 18 different plant extracts were investigated to establish which compounds could be used in combination to formulate a topical microbicide to provide protection against *Herpes simplex* virus type 2 (Bourne et al., 1999).

In India, two poly-herbal preparations demonstrated promising efficacy, namely Praneem and Basant (Talwar et al., 2000; Bhengraj et al., 2008). The poly-herbal formula Praneem is used intravaginally and prepared from a synergistic combination of *Azadirachtae indica* extract, *Sapindus mukerossi* and *Mentha citrate* oil (Talwar et al., 2000). Praneem has been reported to inhibit *N. gonorrhoeae, C. albicans, Candida krusei, Candida tropicalis* as well as *Escherichia coli* (Bagga et al., 2006). Praneem further prevents vaginal transmission of *H. simplex* virus 2 and *Chlamydia trachomatis*. After a seven day human trial there was evidence of regression of vaginal discharge associated with a *G. vaginalis*, *C. albicans* and *T. vaginalis* infections. No toxicity or side effects were evident (Bagga et al., 2006). The poly-herbal formula BASANT, which is applied topically is prepared from plants *Aloe vera, Curcumin longa, Emblica officinalis* and *Sapindus mukerossi*. The plants interact synergistically and were found to inhibit *N. gonorrhoeae, C. albicans* and *C. trachomatis* at a concentration of 30 µg/ml. Furthermore, no toxicity was detected using the MTT cytotoxicity assay against adenocarcinoma cells (HeLa 229) (Bhengraj et al., 2008). Furthermore the poly-herbal formulations Praneem and BASANT were found to possess virucidal activity against the HIV-1 cell line (Talwar et al., 2000; Bhengraj et al., 2008).

In this study the most susceptible pathogen for both the aqueous and organic extracts was *U. urealyticum* which was in agreement with the antimicrobial results with the individual plants

(Figure 3.2). When analyzing the antimicrobial efficacy with the FIC, the most synergistic interactions were found to be against the STI pathogens, *U. urealyticum* and *O. ureolytica*. Furthermore, the majority of these poly-herbal combinations exhibited noteworthy antimicrobial activity against urethritis associated pathogens. Four of the aqueous plant extracts for the poly-herbal combinations interacted synergistically when tested against *G. vaginalis*, however, this was not beneficial as the combinations did not demonstrate noteworthy antimicrobial efficacy.

The majority of the poly-herbal combinations that demonstrated synergistic interactions against specific pathogens indicated at least one synergistic interaction when tested in 1:1 combinations. There were a few exceptions where the 1:1 combinations displayed no synergistic interactions (e.g. the organic extract of *C. papaya*, *S. serratuloides* and *H. hemerocallidea* against *U. urealyticum*). In such cases combining plants within the mixture seems to be more advantageous from an antimicrobial perspective.

The converse was also true whereby antagonistic interactions were displayed in the overall poly-herbal combination, however, for some cases predominance of synergistic and additive interactions were demonstrated with the analysis of the 1:1 combinations (e.g. the organic extract of *E. hypericifolia*, *S. serratuloides*, *H. hemerocallidea* and *O. engleri* against *C. albicans*). A trend was detected with the organic extracts of the poly-herbal combinations against the pathogen *C. albicans*. Whereas antagonistic interactions dominated the overall poly-herbal combination many of the 1:1 combinations displayed a synergistic interaction. This was extremely fascinating as this shows that investigating the 1:1 combinations is a valuable tool to try to understand and interpret plant dynamics.

Although the poly-herbal combinations did have some antimicrobial activity, it seemed that the more plants within the combination the less chance there was for synergistic interactions to occur. Perhaps consideration should be given to combining multiple plants as this may not always yield the most efficacious antimicrobial option. Alternatively, with poly-herbal combinations it is possible that not every plant is meant to have an antimicrobial effect. In fact, some plants may be used for relieving other symptoms such as pain and inflammation (Table 1.2).

Interestingly, all six poly-herbal combinations (both aqueous and organic extracts) were antimicrobially effective against STI pathogen *N. gonorrhoeae*, however, to much dismay the combinations depicted indifferent interactions. The one exception, *M. acuminata*, *S. serratuloides* and *H. hemerocallidea* whereby the aqueous extract demonstrated additive, indifferent, antagonistic interactions and the organic extracts exhibited synergistic and additive interactions (Table 5.6).

H. hemerocallidea was mentioned in five out of the six poly-herbal combinations, yet the plant when investigated individually did not demonstrate outstanding antimicrobial activity (Figures 3.1-3.6). It is possible that *H. hemerocallidea* could act as a catalyst or transporter, facilitating the absorption of plants to which it is combined with, therefore resulting in an increased bioavailability of the antimicrobially effective plants as was observed in a previous study for nevirapine absorption (Brown et al., 2008). *H. hemerocallidea* could further be attributing variable amounts of antibacterial activity against pathogens through chemical components flavonoids and gallotannins which it possesses (Brown et al., 2008, Drewes et al., 2008).

5.3.3 Traditional usage

The aqueous plant extracts of the poly-herbal combinations possessed almost double the synergistic interactions when compared to the organic extracts. This result was promising for the lay people, as the primary method of preparation is the use of aqueous extracts. In the circumstances whereby the plants exhibit more synergistic activity in the 1:1 combinations and antagonistic interactions as a poly-herbal therapy it is more beneficial to use the plants as 1:1 combinations. The plants could be working in synergy if there is less competition towards a binding site therefore better as 1:1 combinations. Alternatively with multiple plants the holistic approach could be demonstrated whereby only one or two plants are inhibiting the STI pathogen and the remaining plants are there for symptomatic treatment. Caution needs to be adhered to with the administration of the poly-herbal combinations demonstrating antagonism. Antagonistic interactions could be inadvisable for traditional healing practices as the plants are counteracting one another. The antagonistic interactions between the plants decreases the overall antimicrobial effect of the combination and therefore would not benefit the people of Maputaland.

H. hemerocallidea and *S. serratuloides* were used in 66.7% of the poly-herbal combinations. Interestingly these two plants were the most mentioned plants by the lay people, however, singularly their activity was not outstanding. Upon analyzing the results of *H. hemerocallidea* with *S. serratuloides*, the antimicrobial efficacy broadened and including this combination into the poly-herbal combination seemed to be advantageous.

The majority of the plants used in combination were said to be used to treat gonorrhoea, unspecified venereal infections and external/internal sores (Table 1.2). The results indicated that antimicrobially the poly-herbal combinations are of benefit against *N. gonorrhoeae*, however, the plants interact indifferently in combination. The 1:1 combinations highlighted the most effective plant interactions but the remaining plants could be included to treat the external/internal sores. Furthermore, with the exception of *M. acuminata*, *S. serratuloides* and *H. hemerocallidea* all poly-herbal combinations exhibited noteworthy antimicrobial activity against at least one STI pathogen and validated the use of the poly-herbal combinations to treat STIs.

Beneficial combinations with no toxicity against the human kidney epithelial cells that interacted synergistically with noteworthy antimicrobial activity, were few. These combinations, *C. papaya*, *S. serratuloides* and *H. hemerocallidea* (organic extract) and *E. hypericifolia*, *S. serratuloides*, *H. hemerocallidea* and *O. engleri* (aqueous and organic extracts) were most effective against *U. urealyticum* (Table 5.2 and 5.9). Whereas the organic extract of the combination *B. pilosa*, *R. multifidus*, *S. sanguinea* and *C. brachiata* were most effective against *O. ureolytica* (Table 5.7). These are the poly-herbal combinations that will be best suited for the treatment of urethritis.

5.4 Conclusions

- The poly-herbal therapies demonstrated no toxicity against the human kidney epithelial cell line and antimicrobially effective were *C. papaya, S. serratuloides* with *H. hemerocallidea* (organic extract) and *E. hypericifolia*, *S. serratuloides*, *H. hemerocallidea* with *O. engleri* (aqueous and organic extracts) against *U. urealyticum* as well as *B. pilosa*, *R. multifidus*, *S. sanguinea* and *C. brachiata* against *O. ureolytica*.

- Plants used in combinations of ≥ 3 do not necessarily interact synergistically and therefore do not always produce a better antimicrobial result.

- The 1:1 combinations of plants within multiple combinations do not always correspond with the overall poly-herbal combination interaction.

- The poly-herbal combinations were most effective for the treatment of urethritis and to a lesser extent to treat gonorrhoea and external/internal sores. The combination of *C. papaya*, *S. serratuloides* with *H. hemerocallidea* was most effective against urethritis (*U. urealyticum*) with synergistic interactions (ΣFIC = 0.32) and noteworthy antimicrobial activity (MIC = 0.25 mg/ml).

Chapter 6

Design of Experiments (DoE) analysis

6.1 Introduction

Since the lay people use the plant combination in equal ratios (one handful of each plant) the MODDE 9.1® design software could offer an alternative ratio to combine the plants in order to optimize the antimicrobial activity. Umetrics design of experiments (DoE) is a carefully prepared set of representative experiments in which all relevant factors (plants) are varied simultaneously. The software allows for a better understanding of the effect the plants have on the overall MIC value and therefore on antimicrobial activity. The graphs constructed in the software MODDE 9.1® will establish if the MIC value obtained for the selected triple combination can be improved. The most important benefit of this software is the precise result output acquired from fewer experiments (Eriksson et al., 2008).

The three plant combinations were considered to be used on the software as the two plant combinations used isobolograms to establish ratios and the poly-herbal combination consisted of too many plants for the software design. The two plant combinations considered for the analysis of the software were *C. papaya, S. serratuloides* with *H. hemerocallidea*, and *M. acuminata*, *S. serratuloides* with *H. hemerocallidea*. The combination of *C. papaya, S. serratuloides* and *H. hemerocallidea* was the combination selected for optimization as this triple combination was the one mostly used and mentioned by the lay people of Maputaland. Thus the objective of this chapter was to investigate this triple combination in more detail, whereby the concentration of each plant within the mixture can be varied for optimum results.

6.2 Results

This study used MODDE 9.1® software to predict the lowest MIC value for the combination of *C. papaya, S. serratuloides* with *H. hemerocallidea*. This is a step by step process whereby the methods and analysis provided in Chapter 2, Section 2.6. For the sake of brevity only one example (*T. vaginalis*, organic extract) is provided with all graphs. Thereafter the results from

the program are provided in Table 6.2 and Table 6.3 and select graphs in Appendix B (B1.1-B4.2). Step one was to enter the factors (plants) into the program with a target MIC value. The target value in this case is an MIC value of below 0.50 mg/ml indicating noteworthy antimicrobial activity. The program then provided a list of ratios Table 6.1 (shaded area) the plants needed to be combined in and tested in the laboratory using the MIC microplate methodology to obtain a set of MIC values (Table 6.1). The experiments were performed in triplicate.

Table 6.1 Experimental combinations of various ratios for the three plant combination.

Experiment No	Experiment name	*C. papaya*	*S. serratuloides*	*H. hemerocallidea*	MIC of experiment
1	N1	1.00	0.00	0.00	2.00
2	N2	0.00	1.00	0.00	2.00
3	N3	0.00	0.00	1.00	2.00
4	N4	0.67	0.17	0.17	2.00
5	N5	0.17	0.67	0.17	1.00
6	N6	0.17	0.17	0.67	1.00
7	N7	0.00	0.50	0.50	2.00
8	N8	0.50	0.00	0.50	2.00
9	N9	0.50	0.50	0.00	4.00
10	N10	0.33	0.33	0.33	2.00
11	N11	0.33	0.33	0.33	2.00
12	N12	0.33	0.33	0.33	2.00
13	N13	1.00	0.00	0.00	2.00
14	N14	0.00	1.00	0.00	2.00
15	N15	0.00	0.00	1.00	2.00
16	N16	0.67	0.17	0.17	1.00

*ratios 17-24 were excluded for the sake of brevity.

Once all antimicrobial experiments were undertaken, the MIC values were entered into the software. The experiments were all run in triplicates in order to obtain accurate MIC values (Table 6.1). The software then created a replicate plot.

6.2.1 The replicate plot

From Figure 6.1 it can be seen that all experiments were evenly distributed. The three centre points indicated on the X axis point 10, 11, 12 (point A) indicates the replicate points deviated marginally and this indicates a promising prediction of the MIC value.

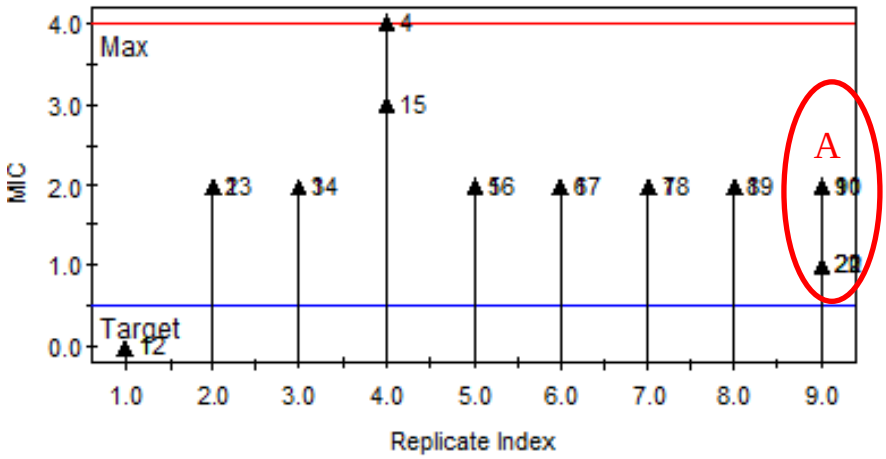


Figure 6.1 Plot of replications for MIC experiments for the combination of *C. papaya*, *S. serratuloides* and *H. hemerocallidea* against *T. vaginalis*. Whereby point A represents the centre points.

Since the laboratory tested experiments produced accurate results, the prediction of the MIC values from the model should be accurate and therefore the best antimicrobial activity can be established. The second step in the design of experiment software was to interpret the histogram.

6.2.2 The histogram of skewness

The histogram for the combination *C. papaya, S. serratuloides* and *H. hemerocallidea* indicated an ideal 'bell shaped' response (Figure 6.2). The response variable (MIC values) is approximately normally distributed. The normally distributed responses provide for better model estimates and statistics. The better the model estimates and statics are the more reliable the prediction of the MIC values by the model will be. This then leads to the analysis of the summary of fit for the model.

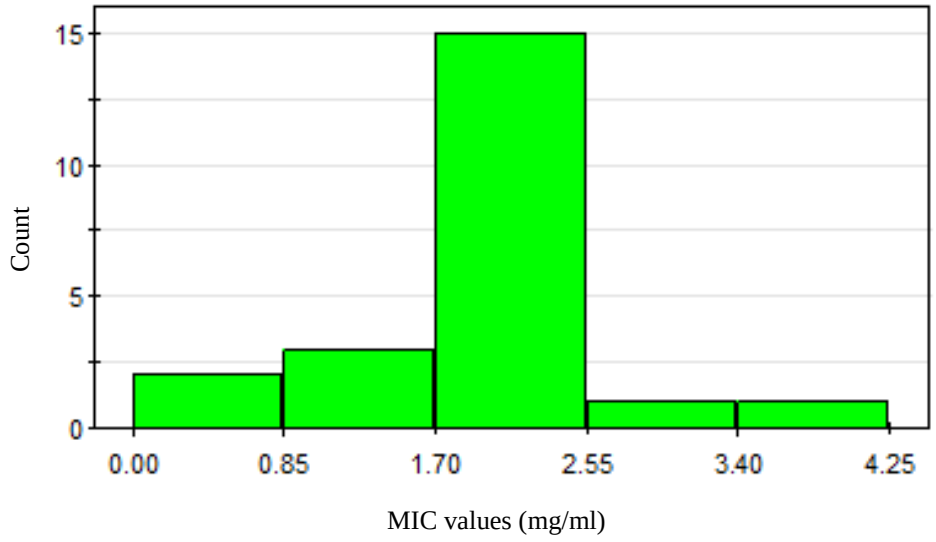


Figure 6.2 Histogram of skewness for MIC experiments for the combination of *C. papaya*, *S. serratuloides* and *H. hemerocallidea* against *T. vaginalis*.

6.2.3 The summary of fit

The R_2 value is 0.81 (81.0%) indicating that the model design is significant. The Q_2 value of 0.73 (73.0%) is also significantly high. The Q_2 value indicates this study is significant and the model fits the study design adequately and therefore will effectively predict the response (lowest MIC values). The model validity is dependent on the pure error (environmental factors not taken into consideration such as temperature and time) and in this result, the replicates are well distributed.

The reproducibility of the model is indicated at approximately 0.80 (80.0%) which is significant and indicates a decreased variation of replicates as observed in Figure 6.2.

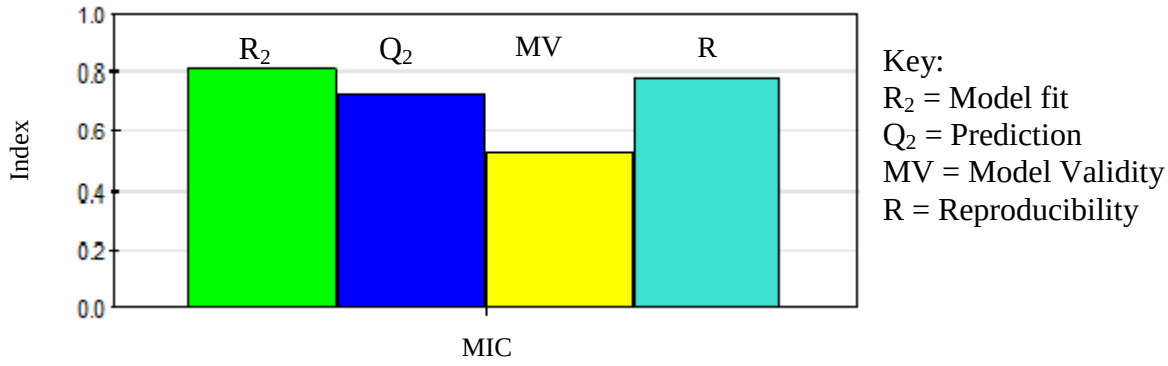


Figure 6.3 Summary of fit of experiments for the combination of *C. papaya*, *S. serratuloides* and *H. hemerocallidea* against *T. vaginalis*.

The summary of fit plot has established that the program will be able to successfully predict and reproduce the lowest MIC values, possible for the combination. This study is a perfect fit for the optimization of the MIC values therefore it is possible to obtain the best antimicrobial activity from the combination. The next step of the software was the analysis of the co-efficient plot. The coefficient's being the three plants *C. papaya*, *S. serratuloides* and *H. hemerocallidea*.

6.2.4 The coefficient plot

It can be seen from the coefficient plot *C. papaya* with *H. hemerocallidea* and *S. serratuloides* with *H. hemerocallidea* produce better antimicrobial effect compared to when the plants are individually tested. *H. hemerocallidea* is thus classified as a non-significant (less important) term and could be eliminated as a coefficient hence the exclusion in Figure 6.4. The reason *H. hemerocallidea* was not removed was due to the overall influence the term will exhibit on the Q_2 value, as removing it would decrease the Q_2 value significantly from 0.73 (73.0%) to 0.29 (29.0%). The *H. hemerocallidea* may exhibit lower antimicrobial activity individually, however, when combined it is a significant (good) factor. *H. hemerocallidea* becomes important as two significant interactions are seen whereby the activity between *C. papaya* with *H. hemerocallidea*

and *S. serratuloides* with *H. hemerocallidea* extend into the negative region proving that antimicrobial activity is increasing upon the interactions of the plants. Figure 6.4 proves that despite plants being less effective singularly, when combined it can add benefit by contributing towards more effective antimicrobial activity.

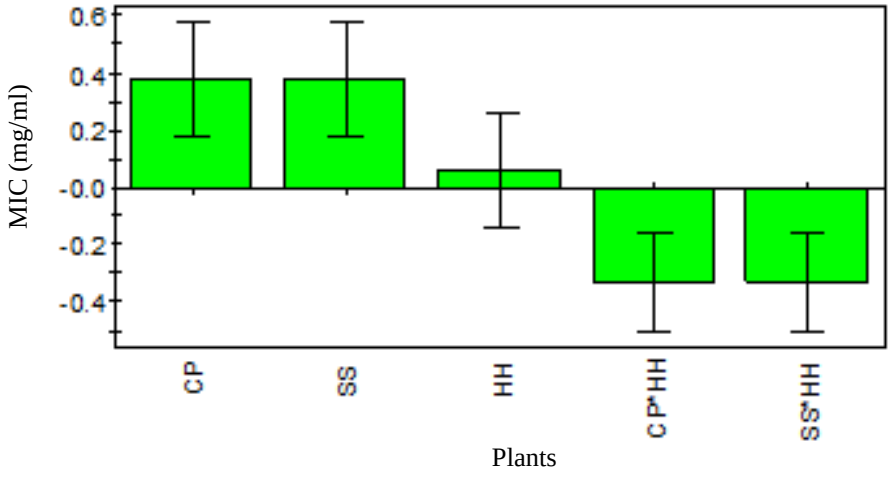


Figure 6.4 Coefficients plot of MIC experiments for the combination of *C. papaya*, *S. serratuloides* and *H. hemerocallidea* against *T. vaginalis* where CP = *C. papaya*, SS = *S. serratuloides* and HH = *H. hemerocallidea*.

Once the factors (plants) are established to be useful within a combination, the next step is to indicate if the experiments in Table 6.1 were influenced by any outside factors (outliers). This was undertaken by examining the residual plot.

6.2.5 The residual plot

The residual plot is representative of the outside factors that may be captured as 'noise'. The residual should be evenly distributed in order to be acceptable. The residual plot was scattered with a slight curvature. This is not ideal, however, the residual is directly related to the outliers ('noise') and all environmental factors (temperature, time, geographical location etc) could not be taken into consideration for this factorial design. According to the full factorial design, the residual scattering will be accepted (Eriksson et al., 2008). The graph also further depicts that the

model may have some difficulty predicting the results, but the results will still be reliable and reproduceable as established in Figure 6.5.

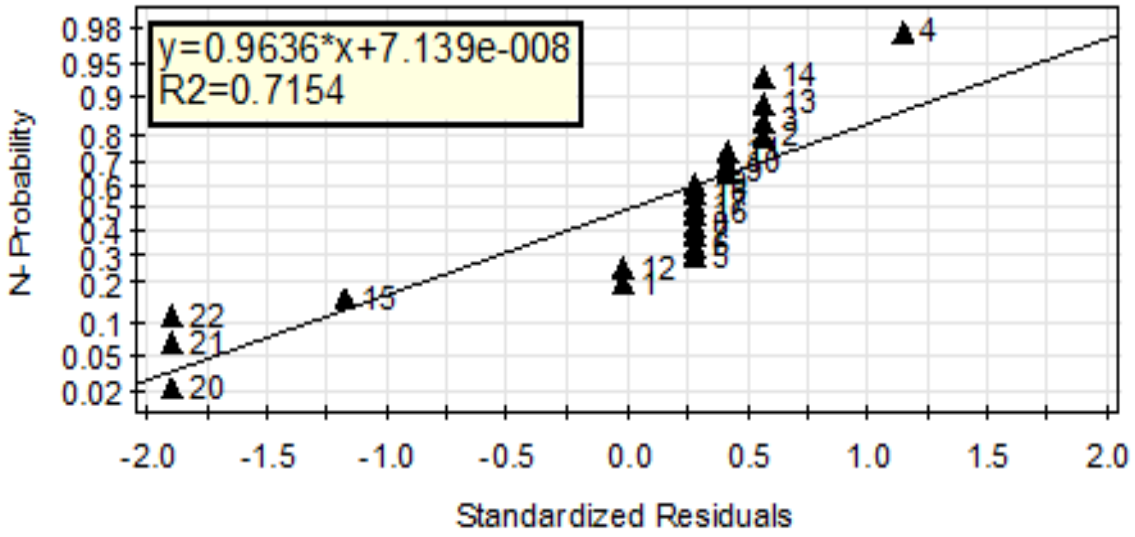


Figure 6.5 Residual plot for MIC experiments for the combination of *C. papaya, S. serratuloides* and *H. hemerocallidea* against *T. vaginalis*.

The MIC values determined by the software will therefore still be valid and the next step is to see a correlation between the observed vs the predicted MIC values.

6.2.6 The observed versus predicted

The observed versus (vs) predicted plot is a relationship between the measured and calculated MIC values. Therefore the plot indicates the correlation of data provided initially in Table 6.1 to that of the calculated MIC values by the software. This plot is a crucial indication as to whether the DoE and study is a good model fit (Figure 6.6). The closer the plotted points are towards the diagonal line the better predictablilty of the software for the lowest MIC value will be.

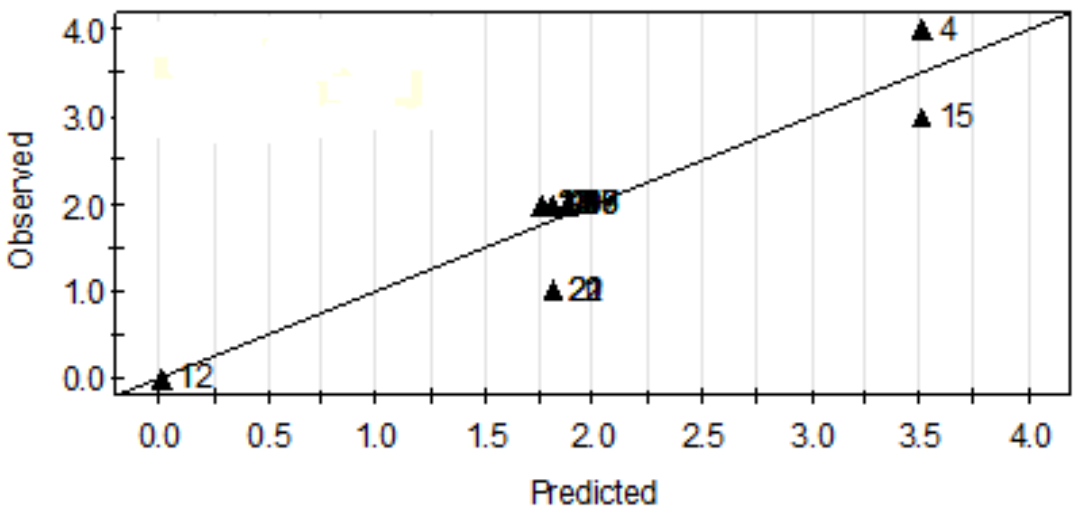


Figure 6.6 Observed versus predicted plot for MIC experiments for the combination of *C. papaya, S. serratuloides* and *H. hemerocallidea* against *T. vaginalis*.

The experimental plot points fall on the straight line indicating a precise correlation between the observed and predicted results for the response. This indicates the MIC values predicted in the varied ratios by the software should be very reliable and accurate. There are a few points that fall either below or above the line, however, these points are closely situated along the line and are therefore accepted (Eriksson et al, 2008). This indicates the observed vs the predicted results are directly correlated and this is a good factorial design model.

6.2.7 The contour plot

The contour plot is subjective to the study approach. The approach taken with this study is to find the minimum value and therefore the green shaded area indicated on the contour plot highlights the lower MIC values (Figure 6.7). *C. papaya* was the plant kept at a constant value of 0.50 when optimization of results were undertaken. *H. hemerocallidea* would have been excluded as explained in Section 6.2.4 therefore *C. papaya* and *S. serratuloides* demonstrated equal antimicrobial activity (Figure 6.4). The software then selected *C. papaya* to be constant.

The plant that is kept constant is usually the plant that possesses the most dominant antimicrobial activity. The plant ratios are then calculated in order to reproduce the optimized MIC value. The values provided for all the plants to be combined are represented as ratios.

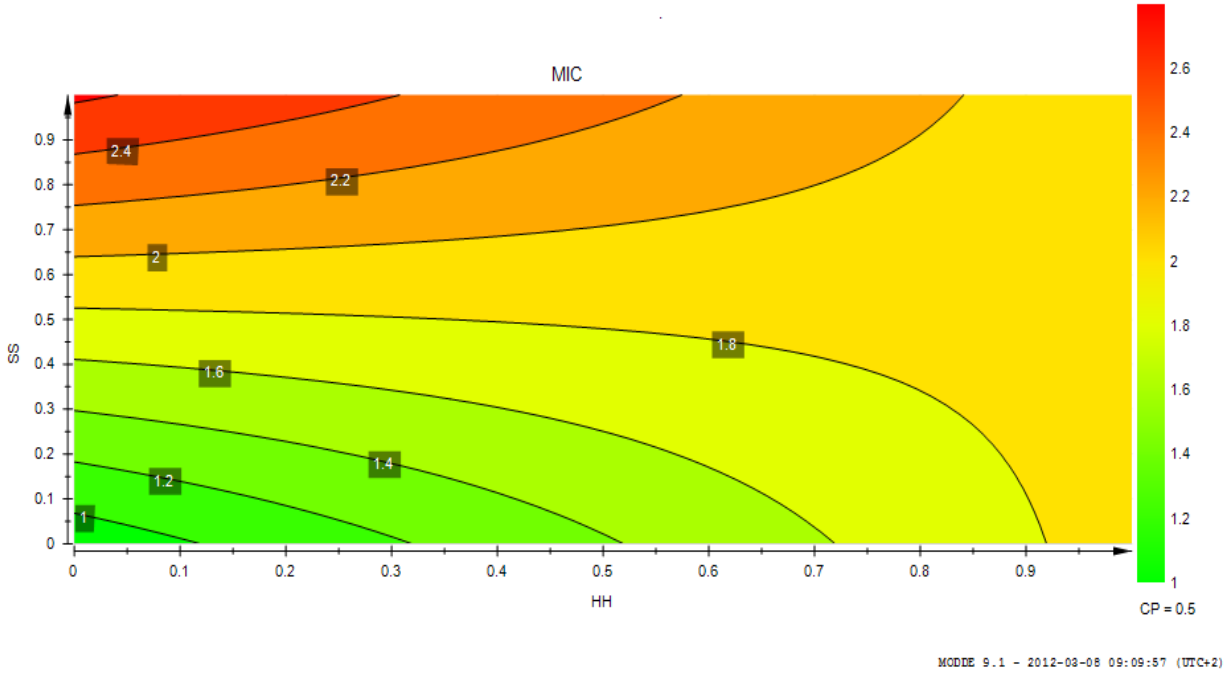


Figure 6.7 Contour plot for the optimization of MIC values for the combination of *C. papaya*, *S. serratuloides* and *H. hemerocallidea* against *T. vaginalis*. Where: CP = *C. papaya*, SS = *S. serratuloides* and HH = *H. hemerocallidea*.

The green region of the contour plot revealed that at a constant *C. papaya* ratio of 0.50 adding *S. serratuloides* 0.0346 and *H. hemerocallidea* 0.00357 would lead to a minimum MIC value of 0.97 (Figure 6.7). When the plants were tested to mimic the traditional preparation (1:1:1), the combination exhibited an MIC value of 2.00 mg/ml (Table 5.2). The software was able to decrease the MIC value by more than half, thereby doubling the antimicrobial efficacy of the combination against *T. vaginalis*.

6.3 Summary of Results

The summary of the results (all graphs) of the combinations against all the test pathogens in this study were analyzed by the full factorial design (Table 6.2). A limitation of the software is that results are only attainable for the combinations against pathogens where an end point MIC value is obtained, therefore results > 16 mg/ml, was not acceptable and excluded for optimization.

MODDE 9.1® was a successful model fit for the prediction of MIC values for the combination *C. papaya*, *S. serratuloides* and *H. hemerocallidea*. The combination of *C. papaya*, *S. serratuloides* and *H. hemerocallidea* across all related pathogens in this study (Table 6.2) produced a histogram that was normally to almost congruently distributed, whether transformation was required or not, the end result was positive. The experimental replications were all simultaneous and quite accurate. The explained variation (Q_2 values) and the predicted variation (R_2 values) were above the accepted levels which lead to a high reproducibility of the software. The only result that deviated was the combination tested against *O. ureolytica* (aqueous extract) for which the Q_2 and R_2 levels were slightly lower than expected (Table 6.2). There was some deviation from the residual plot whereby the outside factors may have caused some interference with the running of the software; however, the influences were not crucial enough to interfere with the final outcome obtaining the lowest MIC value (mg/ml). The observed vs predictability graph correlated well with minor point deviations, therefore the model fit was still appropriate. The plot lines for the residual plot as well as the observed vs predictability graphs, were fairly close to the diagonal line just as the graphs in the pharmaceutical study by Bodea and Leucuta (1997) which indicated a good model fit.

From the coefficients plot, the identification of the most dominant plant could be identified. *C. papaya* was the plant selected by the full factorial design to be the constant factor for 86.0% (six of seven) plant samples. When *C. papaya* was kept constant (Table 6.3), it was found that *C. papaya* when tested individually possessed the lowest MIC value when compared to *S. serratuloides* and *H. hemerocallidea* (Appendix B).

Table 6.2 MODDE 9.1® software analysis for combination *C. papaya*, *S. serratuloides* and *H. hemerocallidea* against selected STI pathogens.

Organism	Extract	Replicate plot	Histogram	Summary of fit				Coefficient plot-Dominant plant	Distribution of Residual plot	Distribution of Observed vs predicted
				Q_2	R_2	Model validity	Reproducibility			
N. gonorrhoeae	Organic	Ideal	Bell shaped	0.96	0.99	Absent	1.00	*C. papaya*	Ideal	Ideal
N. gonorrhoeae	Aqueous	Ideal	Almost congruent	0.76	0.85	Negative	> 0.90	*H. hemerocallidea*	Slight deviation	Ideal
O. ureolytica	Organic	Ideal	Bell shaped	0.71	0.87	Absent	1.00	*C. papaya*	Ideal	Slight deviation
O. ureolytica	Aqueous	Ideal	Almost congruent	0.54	0.70	Negative	>0.90	*C. papaya*	Ideal	Ideal
G. vaginalis	Organic	Ideal	Almost congruent	0.61	0.86	Negative	>0.90	*C. papaya*	Slight deviation	Slight deviation
U. urealyticum	Organic	Ideal	Bell shaped	0.72	0.80	Negative	1.00	*C. papaya*	Ideal	Ideal

One aqueous experimental sample tested against *N. gonorrhoeae* required *H. hemerocallidea* to be the constant factor as when tested individually it possessed the lowest MIC value when compared to *C. papaya* and *S. serratuloides*. The general trend was the plant with the lowest individual MIC value was the plant most dominant in antimicrobial activity and would therefore be used at a greater ratio to optimize the overall combined antimicrobial efficacy. This trend was, however, challenged by the organic experimental sample when tested against *T. vaginalis*, whereby all the independent MIC values were found to be equivalent to one another (MIC value = 2.00 mg/ml). In that instance, *C. papaya* was selected as the constant factor which did however upon optimization improved the antimicrobial efficacy of the combination.

6.4 Optimized results versus laboratory tested results

The target MIC value was set for 0.50 mg/ml which would indicate noteworthy antimicrobial activity. MODDE 9.1® managed to successfully achieve this target with 71.4% of the tested samples reaching MIC values below 0.50 mg/ml. The two exceptions was with the organic experimental samples when tested against *G. vaginalis* and *T. vaginalis* which was expected for *G. vaginalis* as the equal ratio combination possessed the highest MIC value compared to the other samples (MIC value 4.00 mg/ml). In this case, the MIC value for predicted ratio combinations were considerable, however, ideal noteworthy activity was not achieved.

A correlation between the predicted MIC values by the MODDE 9.1® software and the laboratory tested MIC values demonstrated very similar efficacies. The only two exceptions were against the two lowest predicted MIC values, 0.08 mg/ml and 0.19 mg/ml, against *N. gonorrhoeae* and *U. urealyticum* respectively (Table 6.3).

When comparing the MODDE 9.1® predicted MIC values to that of the plants combined in equal ratios previously tested, there was a decrease in MIC value for all experiments, with the highest being noted by a 25 fold decrease with the organic extract against *N. gonorrhoeae* (MIC value 2.00 to 0.08 mg/ml) (Table 6.3). Unfortunately upon testing the ratios in the laboratory, only a 4 fold decrease was noted against *N. gonorrhoeae* (MIC value 2.00 to 0.50 mg/ml) (Table 6.3).

Table 6.3 Comparison of MIC values calculated with MODDE 9.1 ® software and laboratory tested MIC values.

Organism	**Extract**	**MIC values (mg/ml) for plants combined in equal ratios (1:1:1)**	**Optimized MODDE 9.1® ratio**			**MODDE 9.1® predicted MIC value (mg/ml)**	**MODDE 9.1® ratios tested in laboratory MIC values (mg/ml)**
			C. papaya	***S. serratuloides***	***H. hemerocallidea***		
N. gonorrhoeae	Organic	2.00	0.50	0.023	0.010	0.08	0.50
N. gonorrhoeae	Aqueous	2.00	0.035	0.060	0.50	**0.25**	**0.25**
O. ureolytica	Organic	0.75	0.50	0.016	0.0013	**0.41**	**0.50**
O. ureolytica	Aqueous	1.00	0.50	0.053	0.0044	**0.26**	**0.50**
G. vaginalis	Organic	4.00	0.50	0.100	0.0044	**1.73**	**2.00**
U. urealyticum	Organic	0.25	0.50	0.034	0.0074	0.19	1.00
T. vaginalis	Organic	2.00	0.50	0.035	0.0036	**0.97**	**2.00**

The shaded area depicts the results obtained previously when the plants were combined in equal ratios.
The bold figures are the results determined in the laboratory that were similar to the results determined by MODDE 9.1®.

6.5 Discussion

MODDE 9.1® software has been previously selected to be used in pharmacy related fields for the analysis and optimization of the relevant scientific data (Persson and Ăstroöm, 1997; Brunnkvist et al., 2004; Elfstrand et al., 2007; Bigan et al., 2008; Tosi et al., 2009; Snorradóttir et al., 2011). The software has been used previously for the optimization of the extraction of plant extracts; however, the determination of optimization of MIC values is still fairly recent concept.

Traditionally the combination *C. papaya*, *S. serratuloides* with *H. hemerocallidea* was mentioned by three different homesteads which was more than the combination of *M. acuminata*, *S. serratuloides* with *H. hemerocallidea*. *C. papaya* has proven to be antimicrobially effective against the STI pathogens. Furthermore, published literature has indicated *C. papaya* to have numerous other pharmacological properties that may aid with the STI symptoms. *S. serratuloides* and *H. hemerocallidea* on the other hand are plants mostly mentioned by the lay people for the treatment of STIs. The lay people combined these plants in equal ratios which exhibited satisfactory antimicrobial results. MODDE 9.1® has successfully predicted an improvement of results and provided ratios that could be used which would improve the overall antimicrobial efficacy of the combination. It would be beneficial to use this combination in the ratios provided by MODDE 9.1® for the aqueous extract against *N. gonorrhoeae* and the organic extract against *G. vaginalis* (Table 6.3).

Overall the plant combination of *C. papaya*, *S. serratuloides* and *H. hemerocallidea* is traditionally used as a 1:1:1 combination by the lay people and proved to be satisfactory. The software MODDE 9.1® was beneficial for three of the experiments. However, overall when the traditional 1:1:1 combination was compared to the improved ratios, there was a demonstrated minimal improvement for four of the experiments.

Even though the software MODDE 9.1® was a valuable tool in studying optimization of triple combinations, a number of limitations need to be considered when using this software. A limitation to the model was identified when all three plants must be included in a triple combination even if a plant is considered insignificant according to the co-efficient plot. The

MIC values were first analyzed against all six pathogens and if the individual MIC values achieved >16.0 mg/ml, the plant combination had to be excluded from analysis as no end point was established. With plant extracts there could be numerous background 'noise' that is not considered, amongst others extraction time, extraction temperature, geographical difference of samples (Sun et al., 2009). The model cannot give a clear indication as to whether or not there are underlining chemical interactions that could interfere with the predicting of results, and these may be falsely indicated as a bad model fit. It would be recommended that all plants have an MIC value with an end point.

Whereas the MODDE 9.1® software has been used pharmaceutically, the use of this software on plant extract combinations is still fairly new. The MODDE 9.1® software should be used to gain insight into ratios that could optimize results. The software is not as simplistic as just optimizing results but also leads to the observation of all plants interactions, identifies the dominant plant within the combination, as well as gaining insight into the accuracy of results of the study. The software has proven to be an effective tool in the optimization of the MIC values with a minimum number of experiments.

6.6 Conclusions

- The full factorial design was a good model fit for the aqueous extract combination of *C. papaya, S. serratuloides* and *H. hemerocallidea* which achieved an optimal ratio mixture of ratios 0.035 *C. papaya,* 0.060, *S. serratuloides* and 0.50 *H. hemerocallidea* against *N. gonorrhoeae,* which was the highest efficacy noted and achieved.

- The MODDE 9.1® predicted MIC values displayed a close correlation compared to the laboratory tested MIC values. This therefore justifies the use of MODDE 9.1® and utilisation of the full factorial design software.

- There was improvement (decrease) in MIC values when the combination was tested against the selected pathogens when comparing the optimized MODDE 9.1® predicted MIC values and the tested equal ratio combined MIC values.

- MODDE 9.1® software does have merit comparatively to the MIC values established by 1:1:1 for the majority of the experiments. Therefore, the lay people might achieve benefit with the 1:1:1 ratio methods of preparation.

Chapter 7

Summary and general conclusions

The lay people of Maputaland have for centuries relied on their surrounding natural habitat as a source of medicine. This study focused on one facet of treatment, that being the antimicrobial treatment of STIs. An intense ethnobotanical survey of the four geographical areas was previously conducted (De Wet et al., 2012), and the results from that led to this investigation which was to scientifically validate the use of these plants against six STI test pathogens. Furthermore, the aim was to test for *in vitro* toxicity against human kidney epithelial cell line.

The twenty plants were tested first for an indication of antimicrobial activity. The single plants were then combined into various plant combinations according to the combinations the lay people use for the treatment of symptoms associated with the STIs. The two plant combinations were further assessed for interactions. One of the multiple plant combinations (*C. papaya*, *S. serratuloides* and *H. hemerocallidea*) was further investigated for optimization of the combined MIC values using a full factorial design software program MODDE 9.1®. The plant combination was selected for optimization based on the frequency of use by the lay people. All plants (individually and combined) were tested for toxicity using the human kidney epithelial cells.

7.1 Individual plants

Upon testing the plants it was found that only *K. africana* possessed some toxic effect against the human kidney epithelial cells for both the aqueous and organic plant extracts (Table 3.1). An array of antimicrobial results was observed with regard to the plants being tested independently. According to a literature search the plants were also found to demonstrate other contributory properties (such as anti-inflammatory, anti-pyretic, would healing) which may assist with symptomatic relief caused by the STI, as discussed extensively in Chapter 3.2.

The aqueous and organic plant extracts exhibit similar noteworthy activity (Figure 7.1). More than half of the aqueous plant extracts demonstrated poor antimicrobial activity (Figure 7.1). The most significant values for the aqueous extract were *R. multifidus* (MIC value 0.02 mg/ml) and

for the organic extract was *P. africanum* (MIC value 0.04 mg/ml) against *U. urealyticum*. The plant with a broadest spectrum of activity was *S. cordatum* which was effective against four STI related pathogens.

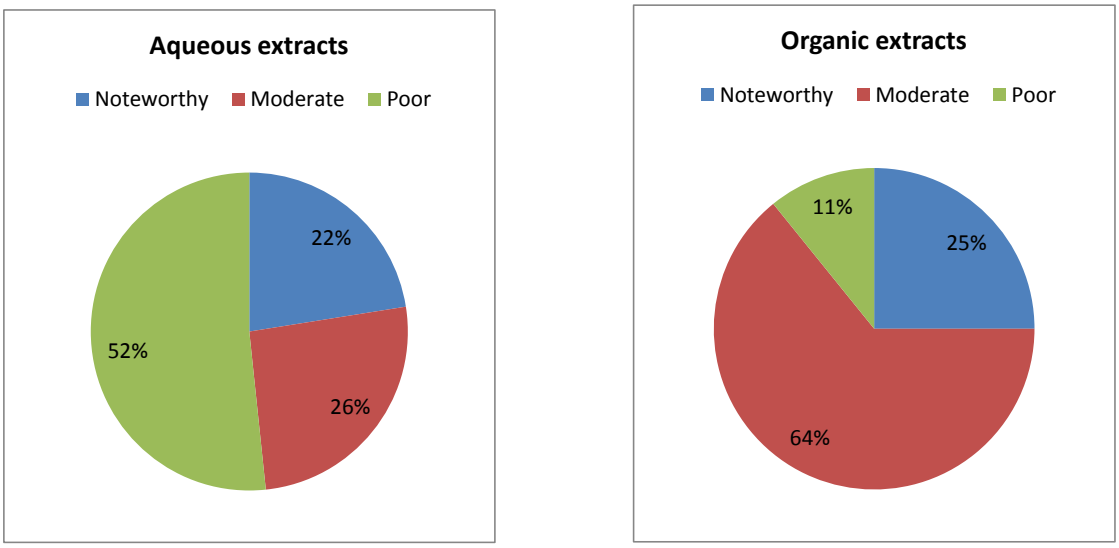


Figure 7.1 Distribution of antimicrobial activity exhibited by the aqueous and organic extracts against the six STI associated pathogens.

The plants that were mostly mentioned by the people of Maputaland (*E. hypericifolia*, *S. serratuloides* and *H. hemerocallidea*) were not the most active in terms of antimicrobial activity. Therefore, it cannot be assumed that plants most commonly used will be the most antimicrobially effective (York et al., 2012). Although *C. papaya*, *M. acuminata* and *B. pilosa* have been found to possess antimicrobial activity in this study and exhibit non-toxic effects against the human kidney epithelial cell line, the plants must be used with caution when administered to pregnant woman as the plants have been reported to induce uterine contractions (Cherian, 2000; Sarma and Mahanta, 2000; Adebiyi et al., 2003; Kamatenesi-Mugisha., 2004; Kamatenesi-Mughisha and Oryem-Origa, 2007; Nikolajsen et al., 2011). The lay people should also exercise caution with plant combinations which contain these plants in the mixture as no

research has been conducted to determine if the combinations exhibit the same uterine contractile properties.

7.2 Two plant combinations

The two plant combination of *A. adianthifolia* with *T. dregeana* (aqueous extract) possessed noteworthy antimicrobial activity against *O. ureolytica* and were found to interact synergistically. On closer examination of the various ratio combinations all nine combinations interacted synergistically (Table 7.1). *A. adianthifolia* with *T. dregeana* was also found to be non-toxic against the human kidney epithelial cells. This two plant combination may prove to be an effective treatment for urethritis caused by *O. ureolytica*.

The two plant combination of *S. birrea* with *S. cordatum* (aqueous extract) resulted in moderately good antimicrobial activity and interacted synergistically against *O. ureolytica* (Table 7.1). Unlike *A. adianthifolia* with *T. dregeana,* the combination was found to be toxic at 1:1 combinations when tested at specific ratios (Table 7.1), thus caution would be advised if *S. birrea* and *S. cordatum* are to be used in combination by the people of Maputaland.

Table 7.1 Most favourable 1:1 plant combinations used for the treatment of STIs.

Plant combination	**Plant preparation**	**Test organism**	**MIC value (mg/ml)**	**ΣFIC**	**ΣFIC interpretation**	**Toxicity**
A. adianthifolia + *T. dregeana*	Aqueous	*O. ureolytica* (ATCC 43534)	0.75	0.15	Synergism	Non-toxic
A. marlothii + *S. serratuloides*	Organic	*O. ureolytica* (ATCC 43534)	0.50	0.31	Synergism	
H. hemerocallidea + *K. africana*	Aqueous	*O. ureolytica* (ATCC 43534)	1.00	0.31	Synergism	
S. birrea + *S. cordatum*	Aqueous	*O. ureolytica* (ATCC 43534)	1.00	0.42	Synergism	Safe ratios: (*S. birrea*: *S. cordatum*) (2:8; 8:2; 9:1)

7.3 Poly-herbal combinations

The lay people of Maputaland use six poly-herbal combinations for the treatment of STIs. The aqueous plant extracts (19%) demonstrated somewhat more synergistic effects than the organic extracts (11%). Despite the encouraging overall result of the aqueous extracts it should also be noted that the aqueous extracts more than doubled the antagonistic activity when the poly-herbal combinations where combined (Figure 7.2). An unexpected trend was observed with the organic extracts of the poly-herbal combinations and *C. albicans*, where no synergistic interactions were observed when multiple plants are combined. However, the 1:1 combinations exhibited a number of synergistic interactions.

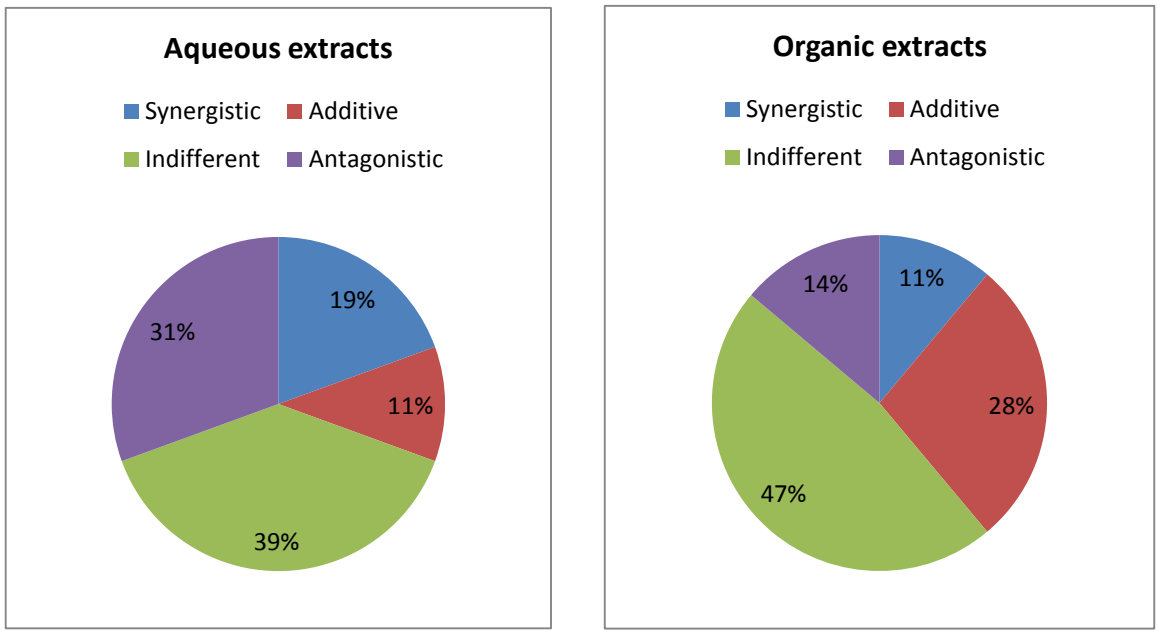


Figure 7.2 The overall distribution of the ΣFIC interpretations for the poly-herbal combinations against the six STI pathogens.

Plant combinations that possessed antagonistic interactions must be administered with caution. Ideal combinations are those with good antimicrobial activity and interact synergistically and in

an additive manner. The combination that displayed overall synergistic/additive interactions and possessed good antimicrobial activity consisted of *E. hypericifolia*, *S. serratuloides*, *H. hemerocallidea* and *O. engleri*. This would be a strongly recommended combination to be administered by the lay people.

Interestingly, all the combinations (aqueous and organic extracts) were antimicrobially effective (MIC range 0.50-2.00 mg/ml), although interact indifferently, against *N. gonorrhoeae*. This is an important scientific validation for the use of plant therapies as gonorrhoea is one of the most treated STI by the people of Maputaland. The next common STI condition treated by the lay people is urethritis whereby the causative organisms are *U. urealyticum* and *O. ureolytica* against which several of the combinations have demonstrated efficacy.

Plants such as *H. hemerocallidea* and *S. serratuloides* were frequently found (five out of the six) in poly-herbal combinations. Individually the two plants possessed moderate antimicrobial activity, however, both plants have been reported to possibly add another contributory property to their therapeutic use (Table 1.2). It was additionally observed that within every plant combination there is at least one plant that could exhibits other beneficial properties to contribute towards the symptoms associated with STIs (Table 1.2). This demonstrates that even though every plant may not be beneficial in inhibiting growth or killing the invading micro-organisms, within the combination other pharmacological effects are targeted. This emphasises the fact that one shouldnot just narrowly focus on the antimicrobial aspects when investigating the ethnobotanical usage of plants for infectious diseases, but also examine other pharmacological effects. Dual effects could substantiate the belief of why so many people continue to trust and use herbal remedies despite the availability of western pharmaceuticals.

When the poly-herbal plant combinations were examined in 1:1 combinations similarities of distribution were found between the aqueous and organic extracts (Figure 7.3).

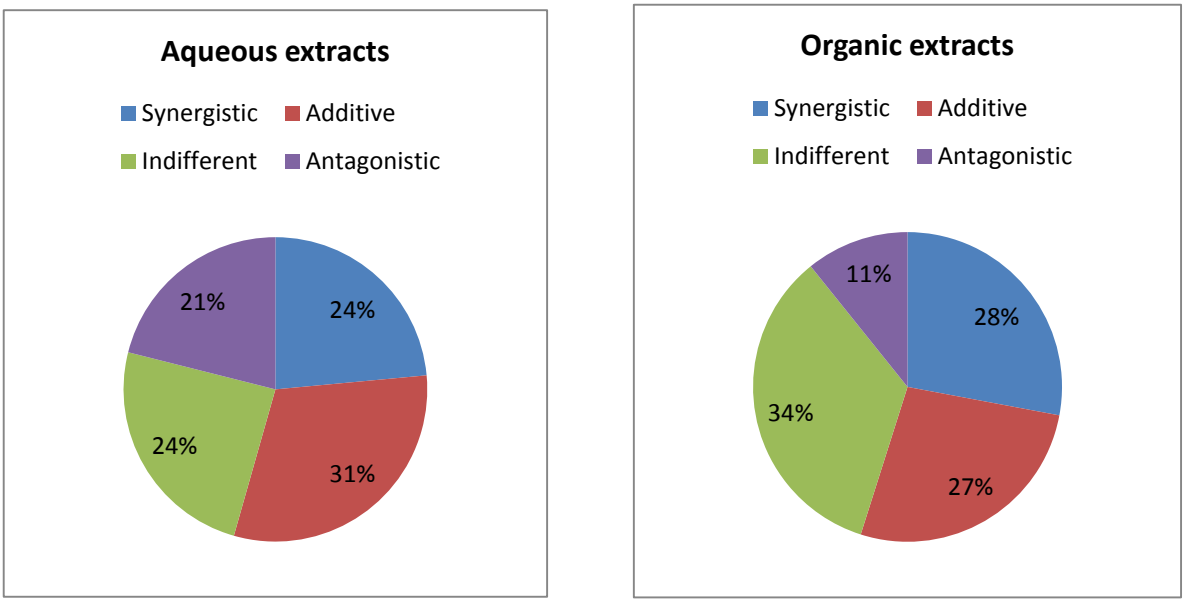


Figure 7.3 The overall distribution of the ΣFIC interpretations for the 1:1 combinations against the six STI pathogens.

7.4 Optimization

The three plant combination of *C. papaya*, *S. serratuloides* and *H. hemerocallidea* exhibited noteworthy to moderately good antimicrobial activity against five of the six STI pathogens (Table 7.2). The idea of optimizing the plant extracts in order to achieve the best MIC value was successful for two experiments therefore the traditional combination used by the lay people (1:1:1) proved to be satisfactory (Table 7.2).

7.5 Future recommendations

In vivo studies should be conducted for all plants (individual as well as combinations) demonstrating efficacy and verifying synergistic interations. Most of the plants when studied individually and in combination were non-toxic when tested aginst the human kidney epithelial cell line. The toxicity of the plants and the combinations should be tested against other cell lines and in other toxicological assays, before being studied *in vivo* in order to establish a full toxicity profile. The KwaZulu-Natal region has the highest number of reported cases of HIV compared to

other provinces in South Africa. Plants such as *H. hemerocallidea* and *P. africanum* are commonly used by the community to treat people infected with HIV (Nzama, 2009). The combinations used in this study should also be tested for their effectiveness against viruses such as HIV and *Herpes simplex*. Additionally the plants could be tested against other sexually transmitted diseases, such as syphilis and chlamydia.

Since the plant combinations are used to holistically treat a patient other pharmacological properties such as anti-inflammatory and anti-pyretic effects could be investigated in order to understand the potential and extent of the use in combination therapy.

Table 7.2 MIC values obtained by MODDE9.1® software and the various provided ratios tested in the laboratory.

Plant combination	**Plant preparation**	**Plant organism**	**MIC value (mg/ml)**	**Optimized MIC value (mg/ml)**	**Laboratory tested MIC value (mg/ml)**
C. papaya + *S. serratuloides* + *H. hemerocallidea*	Organic	*U. urealyticum* (clinical strain)	0.25	0.19	0.50
	Aqueous	*O. ureolytica* (ATCC 43534)	**1.00**	0.26	**0.25**
	Organic	*O. ureolytica* (ATCC 43534)	0.75	0.41	0.50
	Organic	*G. vaginalis* (ATCC 14018)	**4.00**	1.73	**0.50**
	Organic	*T. vaginalis* (clinical strain)	2.00	0.97	2.00
	Aqueous	*N. gonorrhoeae* (ATCC 19424)	2.00	0.25	1.00
	Organic	*N. gonorrhoeae* (ATCC 19424)	2.00	0.08	2.00

Bold figures indicate an improved MIC value of more than one dilution factor.

7.6 Final conclusions

The aqueous extracts for both the individual and plant combinations, surprisingly, faired well in comparison to the organic extracts. This is an extremely reassuring result as this mimics the form in which the decoctions and infusions are prepared traditionally.

The overall outcome of this study has scientifically validated the traditional use of plants used in combination by the lay people of Maputaland as treatment against STIs and has provided some positive and encouraging results in combating STIs. Furthermore, the study also provided some cautionary results that will aid the people in Maputaland when selecting and dispensing future treatments.

References:

Abbiw, D.K. 1990. Useful plants of Ghana. West African uses of wild and cultivated plants. Intermediate Technology Publications, London and Royal Botanic Gardens, Kew.

Abdou, R., Scherlach, K., Dahse, H-M., Sattler, I and Hertweck, C. 2010. Botryorhodines A-D, antifungal and cytotoxic depsidones from *Botryosphaeria rhodina*, an endophyte of the medicinal plant *Bidens pilosa*. Phytochemistry. 71, 110-116.

Adebiyi, A., Adaikan, A.G and Prasad, R.N.V. 2003. Tocolytic and toxic activity of papaya seed extract on isolated rat uterus. Life Sciences. 74, 581-592.

Akah, P.A., Akunyili, D.N and Egwuata, C.N. 2002. Investigations on the analgesic and antipyretic activities of aqueous extract of *Carica papaya* leaves. Nigerian Journal of Neuroscience. 5, 29-34.

Akunyili, D.N., Houghton, P.J and Raman, A. 1991. Antimicrobial activities of the stembark of *Kigelia pinnata*. Journal of Ethnopharmacology. 35, 173-177.

Al-Heali, F.M.G and Rahemo, Z.I.F. 2006. The combined effect of two aqueous extracts on the growth of *Trichomonas vaginalis, in vitro*. Turkish Society for Parasitology. 30, 272-274.

Amazu, L.U., Azikiwe, C.A., Njoka, C.J., Osulala, F.N., Nwosu, P.J.C., Ajugwo, A.O and Enye, J.C. 2010. Anti-inflammatory activity of the methanolic extract of the seeds of *Carica papaya* in experimental animals. Asian Pacific Journal of Tropical Medicine. 2, 884-886.

Aremu, A.O., Ndhlala, A.R., Fawole, O.A., Light, M.E., Finnie, J.F and Van Staden, J. 2010. *In vitro* pharmacological evaluation and phenolic content of 10 South African medicinal plants used as antihelminths. South African Journal of Botany. 76, 558-566.

Arnold, H.J and Gulumian, M. 1984. Pharmacopoeia of traditional medicine in Venda. Journal of Ethnopharmacology. 12, 35-74.

Arthan, D., Sithiprom, S., Thima, K., Limmatvatirat, C., Chavalitshewinkoon-Petmitr, P and Svasti, J. 2008. Inhibitory effects of Thai plants ß-glycosides on *Trichomonas vaginalis*. Parasitology Research. 103, 443-448.

Azarkan, M., Wintjens, R., Looze, Y and Baeyens-Volant, D. 2004. Detection of three wound-induced proteins in papaya latex. Phytochemistry. 65, 525-534.

Bagga, R., Raghuvanshi, P., Gopalan, S., Das, S.K., Baweja, R., Suri, S., Malhotra, D., Khare, S and Talwar, G.P. 2006. A polyherbal vaginal pessary with spermicidal and antimicrobial action: evaluation of its safety. Transactions of the Royal Society of Tropical Medicine and Hygiene. 100, 1164-1167.

Baker, J., Borris, R., Carte, B., Cordell, G., Soejarto, D., Cragg, G., Gupta, M., Iwo, M., Madulid, D and Tyler, V. 1995. Natural product discovery and development: new perspectives on international collaboration. Journal of Natural Products. 58, 1325-1357.

Berenbaum, M.C. 1989. What is synergy? Pharmacological Reviews. 41, 96-97.

Bessong, P.O., Obi, C.L., Andréola, M.L., Rojas, L.B., Pouységu, L., Igumbor, E., Meyer, J.J.M., Quideau, S and Litvak, S. 2005. Evaluation of South African medicinal plants for inhibitory properties against human immunodeficiency virus type 1 reverse transcriptase and intergrase. Journal of Ethnopharmacology. 99, 83-91.

Bhengraj, A.R., Sajad, A.D., Talwar, G.P and Mittal, A. 2008. Potential of a novel polyherbal formulation BASANT for the prevention of *Chlamydial trachomatis* infection. International Journal of Antimicrobial Agents. 32, 84-88.

Bigan, M., Bigot, J., Mutel, B and Coqueret. X. 2008. Grafting of copolymer styrene maleic anhydride on poly (ethyleneterephthalate) film by chemical reaction and by plasma method: Optimization of the grafting reaction using experimental design. Applied Surface Science. 254, 2300-2308.

Bodea, A and Leucuta, S.E. 1997. Optimization of hydrophilic matrix tablets using D-optimal design. International Journal of Pharmaceutics. 153, 247-255.

Bodley-Tickell, A.T., Olowokure, B., Bhaduri, S., White, D.J., Ward, D., Ross, J.D.C., Smith, G., Duggal, H.V and Goold, P. 2008. Trends in sexually transmitted infections (other than HIV) in older people: analysis of data from an enhanced surveillance system. Sexually Transmitted Infections. 84, 312-317.

Boik, J. 2001. Natural Compounds in Cancer Therapy. Oregon Medicinal Press, LCC, USA.

Boily, Y and Van Puyvelede, L. 1986. Screening of medicinal plants of Rwanda (Central Africa) for antimicrobial activity. Journal of Ethnopharmacology. 16, 1-13.

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

Bourne, K.Z., Bourne, N., Reising, S.F and Stanberry, L.R. 1999. Plant products as topical microbicide candidates: assessment of *in vitro* and *in vivo* activity against herpes simplex virus type 2. Antiviral Research. 42, 219-226.

Brãndao, M.G., Krettli, A.U., Soares, L.S., Nery, G.C and Marinuzzi, H.C. 1997. Antimalarial activity of extracts and fractions from *Bidens pilosa* and other *Bidens* species (Asteraceae) correlated with the presence of acetylene and flavonoid compounds. Journal of Ethnopharmacology 57, 131-138.

Brooks, G.F and Carroll, K.C. 2007. Bacteriology. Jawets, Melnick & Adelberg's Medical Microbiology. 24th edition. McGraw-Hill Companies Incoporated. United States of America.

Brown, L., Heyneke, O., Brown, D., Van Wyk, J.P.H and Hamman, J.H. 2008. Impact of traditional medicinal plant extracts on antiretroviral drug absorption. Journal of Ethnopharmacology. 119, 588-592.

Bruneton, J. 1995. Pharmacognosy, Phytochemistry, Medicinal Plant. Intercept, Hampshire, UK.

Brunnkvist, H., Karlberg, B., Gunnarsson, L and Granelli, I. 2004. Experimental design as a tool when evaluating stationary phases for the capillary electrochromatographic separation of basic peptides. Journal of Chromatography. 813, 67-73.

Bussmann, R.W., Malca-Garcìa, G., Glenn, A., Sharon, D., Nilsen, B., Parris, B., Dubose, D., Ruiz, D., Saled, J., Martinez, M., Carillo, L., Walker, K., Kuhlman, A and Townesmith, A. 2011. Toxicity of medicinal plants used in traditional medicine in Northern Peru. Journal of Ethnopharmacology. 137, 121-140.

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

Calzada, F., Yepez-Mulia, L and Tapia-Contretas, A. 2007. Effect of Mexican medicinal plant used to treat trichomoniasis on *Trichomonas vaginalis* trophozoites. Journal of Ethnopharmacology. 113, 248-251.

Camacho-Corona, M.R., Ramìrez-Cabrera, M.A., González-Santiago, O., Garza- González, E., Palacios, I.P and Luna-Herrera, J. 2008. Activity against drug resistant tuberculosis strains of

plants used in Mexican traditional medicine to treat tuberculosis and other respiratory diseases. Phytotherapy Research. 22, 82-85.

Candy, H.A., McGarry, E.J and Pegal, K.H. 1968. Constituents of *Syzygium cordatum*. Phytochemistry. 17, 889-890.

Carmicheal, J., DeGraaf, W.G., Gazdar, A.F., Minna, J.D and Mitchell, J.B. 1987. Evaluation of a tetrazolium-based semiautomated colorimetric assay: assessment of chemo-sensitivity testing. Cancer Research. 47, 936-942.

Cẻline, V., Adriana, P., Eric, D., Joaquina, A-C., Yannick, E., Augusto, L.F., Rosario, R., Dionicia, G., Michel, S., Denis, C and Geneviéve, B. 2009. Medicinal plants from the Yanesha (Peru): Evaluation of the leishmanicidal and antimalarial activity of selected extracts. Journal of Ethnopharmacology. 123, 413-422.

Cherian, T. 2000. Effect of papaya latex extract on gravid and non-gravid rat uterine preparations *in vitro*. Journal of Ethnopharmacology. 70, 205-212.

Chhabra, S.C., Mahunnah, R.L.A and Mshiu, E.N. 1991. Plants used in traditional medicine in eastern Tanzania.V. Angiosperms (Passifloraceae to Sapindaceae). Journal of Ethnopharmacology. 33, 143-157.

Chiang, L.C., Chang, J.S., Chen, C.C., Ng, L.T and Lin, C.C. 2003. Anti-*Herpes simplex* virus activity of *Bidens pilosa* and *Houttuynia cordata*. American Journal of Chinese Medicine. 31, 355-362.

Chinoy, N.J., D'Souza, J.M and Padman, P. 1994. Effects of crude aqueous extract of *Carica papaya* seeds in male albino mice. Reproductive Toxicology. 8, 75-79.

Chomnawang, M.T., Trinapakul, C and Gritsanapan, W. 2009. *In vitro* antigonococcal activity of *Coscinium fenestratum* stem extract. Journal of Ethnopharmacology. 112, 445-449.

Clarkson, C., Maharaj, V.J., Crouch, N.R., Grace, O.M., Pillay, P., Matsabisa, M.G., Bhagwandin, N., Smith, P.J and Folb, P.I. 2004. *In vitro* antiplasmodial activity of medicinal plants native to or naturalised in South Africa. Journal of Ethnopharmacology. 92, 177-191.

Cohen, M.S., Hoffman, I.F., Royce, R.A., Kazembe, P., Dyer, J.B., Daly, C.G., Zimbu, D., Vernazza, P.L., Maida, M., Fiscus, S.A and Eron, J.J. 1997. Reduction of concentration of HIV-1 in semen after treatment of urethritis, implication for prevention of sexually transmission of HIV-1. The Lancet. 349, 1869-1873.

Dahlberg, A.C and Trygger, S.B. 2009. Indigenous medicine and primary healthcare: the importance of lay knowledge and use of medicinal plants in rural South Africa. Human Ecology. 37, 79-94.

Deba, F., Xuan, T.D., Yasuda, M and Tawaka, S. 2008. Chemical composition and antioxidant, antibacterial and antifungal activities of the essential oils from *Bidens pilosa* Linn. Var. *Radiata*. Food Control. 19, 346-352.

Debnath, S., Rahman, S.M.H., Deshmukh, G., Duganath, N., Pranitha, C and Chiranjeevi, A. 2011. Antimicrobial screening of various fruit seed extracts. Journal of Pharmacognosy. 19, 83-86.

Déciga-Campos, M., Rivero-Cruz, I., Arriaga-Alba, M., Castañeda-Corral, G., Angeles-López, G.E., Navarrete, A and Mata, R. 2007. Acute toxicity and mutagenic activity of Mexican plants used in traditional medicine. Journal of Ethnopharmacology. 110, 334-342.

De Mesquita, M.L., De Paula, J.E., Pessoa, C., De Moraes, M.O., Costa-Lotufo, L.V., Grougnet, R., Michel, S., Tillequin, F and Espindola, L.S. 2009. Cytotoxic activity of Brazilian *Cerrado* plants used in traditional medicine against cancer cell lines. Journal of Ethnopharmacology. 123, 439-445.

De Sá Ferreira, I.C.F and Ferrão Vargas, V.M. 1999. Mutagenicity of medicinal plant extracts in *Salmonella* microsome assay. Phytotherapy Research. 13, 397-400.

De Villiers, B.J., Van Vuuren, S.F., Van Zyl, R.L and Van Wyk, B.E. 2010. Antimicrobial and antimalarial activity of *Cussonia* species (Araliaceae). Journal of Ethnopharmacology. 129, 189-196.

De Wet, H., Dludla, P.M and Makhaliphi, S.N.M. 2008. An ethnopharmacological investigation of home grown plants used for treating diarrhea and wound infections in the Mbazwana area, Maputaland. South African Journal of Botany. 74, 365-368.

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

Desta, B. 1993. Ethiopian traditional herbal drugs. Part 2. Antimicrobial activity of 63 medicinal plants, Journal of Ethnopharmacology. 39, 129-139.

Dimo, T., Rakotonirina, S.V., Tan, P.V., Azay, J., Dongo, E., Kamtchouing, P and Cros, G. 2007. Effect of *Sclerocarya birrea* (Anacardiaceae) stem bark methylene chloride/methanol extract on streptozotocin-diabetic rats. Journal of Ethnopharmacology. 110, 434-438.

Donovan, B. 2004. Sexually transmissible infections other than HIV. The Lancet. 363, 545-556.

Drewes, S.E., Elliot, E., Khan, F., Dhlamini, J.T.B and Gcumisa, M.S.S. 2008. *Hypoxis hemerocallidea*-Not merely a cure for benign prostatic hyperplasia. Journal of Ethnopharmacology. 119, 593-598.

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

Eldeen, I.M.S and Van Staden, J. 2007. *In vitro* pharmacological investigation of extracts from some trees used in Sudanese traditional medicine. South African Journal of Botany. 73, 435-440.

Elfstrand, L., Eliasson, A-C., Jönsson, M., Reslow, M., Thelin, B and Wahlgren, M. 2007. Recrystallization of waxy maize starch during manufacturing of starch microspheres for drug delivery: Optimization by experimental design. Carbohydrate Polymers. 68, 568-576.

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

Eloff, J.N. 1998. A sensitive and quick microplate method to determine the minimal inhibitory concentration of plant extracts for bacteria. Planta Medica. 64, 711-713.

Eloff, N. 2001. Antibacterial activity of Marula (*Sclerocarya birrea* (A. rich.) Hochst. subsp. caffra (Sond.) Kokwaro) (Anacardiaceae) bark and leaves. Journal of Ethnopharmacology. 76, 305-308.

Eriksson, L., Johansson, E., Kettaneh-Wold, N., Wikström, C and Wold, S. 2008. Design of Experiments Principles and Applications. Third edition. Published in Sweden.

Fabrey, W., Okemo, P.O and Ansorg, R. 1998. Antibacterial activity of East African medicinal plants. Journal of Ethnopharmacology. 60, 70-84.

Farnsworth, N.R. 1998. The ethnobotanical approach to drug discovery: strengths and limitations, Ethnobotany and the search for new drugs. Ciba Foundation Symposium. 42-59.

Fawole, O.A., Amoo, S.O., Ndhlala, A.R., Light, M.E., Finnie, J.F and Van Staden, J. 2010. Anti-inflammatory, anticholinesterase, antioxidant and phytochemical properties of medicinal plants used for pain-related ailments in South Africa. Journal of Ethnopharmacology. 127, 235-241.

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

Fouche, G., Cragg, G.M., Pillay, P., Kolesnikova, N., Maharaj, V.J and Senabe, J. 2008. *In vitro* anticancer screening of South African plants. Journal of Ethnopharmacology. 119, 455-461.

Fyhrquist, P., Mwasumbi, L., Haeggstrom, C.A., Vuorela, H; Hiltunen, R and Vourela, P. 2002. Ethnobotanical and antimicrobial investigation of some species of *Terminalis* and *Combretum* (Combretaceae) growing in Tanzania. Journal of Ethnopharmacology. 79, 169-177.

Gabrielson, J., Hart, M., Jarelov, A., Kuhn, L., McKenzie, D and Mollby, R. 2002. Evaluation of redox indicators and the use of digital scanners and spectrophotometer for qualification of microbial growth in microplates. Journal of Microbiological Methods. 50, 63-73.

Gaidamashvili, M and Van Staden, J. 2002. Interaction of lectin-like proteins of South African medicinal plants with *Staphylococcus aureus* and *Bacillus subtilis*. Journal of Ethnopharmacology. 80, 131-135.

Gaillard, Y and Paquin, G. 1999. Poisoning of plant material: review of human cases and analytical determination of main toxins by high-performance liquid chromatography-(tandem) mass spectrometry. Journal of Chromatography, Biomedical Sciences and Applications. 733, 181-229.

Gathirwa, J.W., Rukunga, G.M., Njagi, E.N.M., Omar, S.A; Mwitari, P.G., Guantai, A.N., Tolo, F.M., Kimani, C.W., Muthaura, C.N., Kirira, P.G., Ndunda, T.N., Amalemba, G., Mungai, G.M and Ndiege, I.O. 2008. The *in vitro* anti-plasmodial and *in vivo* anti-malarial efficacy of combinations of some medicinal plants used traditionally for the treatment of malaria by the Meru community in Kenya. Journal of Ethnopharmacology. 115, 223-231.

Gbolade, A.A. 2009. Inventory of antidiabetic plants selected in districts of Lagos State, Nigeria. Journal of Ethnopharmacology. 121, 135-139.

Githens, T.S. 1949. Drug Plants of Africa. African Handbooks 8. University of Pennsylvania Press, Philadelphia.

Green, E. 1992. Sexually transmitted disease, ethnomedicine and health policy in Africa. Social Science and Medicine. 35, 121-130.

Green, E.C. 1997. The participation of African traditional healers in AIDS/STD prevention programmes. Tropical Doctor. 27, 56-59.

Green, E., Samie, A., Obi, C.L., Bessong, P.O and Ndip, R.N. 2010. Inhibitory properties of selected South African medicinal plants against *Mycobacterium tuberculosis*. Journal of Ethnopharmacology. 130, 151-157.

Grierson, D.S and Afolyan, A.J. 1999. Antibacterial activity of some indigenous plants used for the treatment of wounds in Eastern Cape, South Africa. Journal of Ethnopharmacology. 66, 103-106.

Gurung, S and Ŝkalko-Basnet, N. 2009. Wound healing properties of *Carica papaya* latex: *In vitro* evaluation in mice burn model. Journal of Ethnopharmacology. 121, 338-341.

Haddad, M., Laurens, V and Lacaille-Dubois, M.A. 2004. Induction of apoptosis in a leukemia cell line by triterpene saponins from *Albizia adianthifolia*. Bioorganic and Medicinal Chemistry. 12, 4725-4734.

Hamza, O.J.M., Van den Bout-van den Beukel, C.J.P., Matee, M.I.N., Moshi, M.J., Mikx, F.H.M., Selemani, H.O., Mbwambo, Z.H., Van der Ven, A.J.A.M and Verweij, P.E. 2006. Antifungal activity of some Tanzanian plants used traditionally for the treatment of fungal infections. Journal of Ethnopharmacology. 108, 124-132.

Hay, P and Ugwumadu, A. 2009. Detecting and treating common sexually transmitted diseases. Acute Gynaecological Vol 2: Infection, uterine and ovary pathology. 23, 647-660.

Hutchings, A. 1989. Observations of plant usage in Xhosa and Zulu medicine. Bothalia. 19, 225-235.

Hutchings, A., Scott, A.H., Lewis, G and Cunningham, A.B. 1996. Zulu Medicinal Plants-An Inventory. University of Natal Press, Pietermaritzburg.

Jäger, A.K., Hutchings, A and Van Staden, J., 1996. Screening of Zulu medicinal plants for prostaglandin-synthesis inhibitors. Journal of Ethnopharmacology. 52, 95-100.

Jäger, A.K. 2005. Is traditional medicine betteroff 25 years later? Journal of Ethnopharmacology. 100, 3-4.

Johnson, L. 2008. The burden of disease attributable to sexually transmitted infections in South Africa in 2000. South African Medicinal Journal. 97, 287-288.

Kamatenesi-Mugisha, M. 2004. Medicinal plants used in reproductive healthcare in western Uganda: documentation, phytochemicaland bioactivity evaluation. Ph.D. Thesis. Department of Botany, Makerere University, Kampala, Uganda.

Kamatenesi-Mugisha, M and Oryem-Origa, H. 2007. Medicinal plants used to induce labour during childbirth in western Uganda. Journal of Ethnopharmacology. 109, 1-9.

Kamatou, G.P.P., Viljoen, A.M., Van Vuuren, S.F and Van Zyl, R.L. 2006. *In vitro* evidence of antimicrobial synergy between *Salvia chamelaeagnea* and *Leonotis leonurus*. South African Journal of Botany. 72, 634-636.

Kambizi, L and Alfolayan, A.J. 2001. An ethnobotanical study of plants used for the treatment of sexually transmitted diseases (njohera) in Guruve District, Zimbabwe. Journal of Ethnopharmacology. 77, 5-9.

Kambizi, L and Afolayan, A.J. 2008. Extracts from *Aloe ferox* and *Withania somnifera* inhibit *Candida albicans* and *Neisseria gonorrhoeae*. African Journal of Biotechnology. 7, 012–015.

Kamuhabwa, A., Nshimo, C and De Witte, P. 2000. Cytotoxicity of some medicinal plant extracts used in Tanzanian traditional medicine. Journal of Ethnopharmacology. 70, 143-149.

Kassie, F., Parzefall, W., Musk, S., Johnson, I., Lamprecht, G., Sontag, G and Knasmueller, S. 1996. Genotoxic effects of crude juices from *Brassica* vegetables and juices and extracts from

phytopharmaceutical preparations and spices of cruciferous plants origin in bacterial and mammalian cells. Chemical Biological Interactions. 102, 1-16.

Katzung, B., Masters, S and Trevor, A. 2011. Basic and Clinical Pharmacology. 12th edition. Published by McGraw-Hill Companies Incorporated.

Kelmanson, J.E., Jäger, A.K and Van Staden, J. 2000. Zulu medicinal plants with antibacterial activity. Journal of Ethnopharmacology. 69, 241-246.

Khan, M.R., Kihara, M and Omoloso, A.D. 2001. Anti-microbial activity of *Bidens pilosa*, *Bischofia javanica*, *Elmerillia papuana* and *Sigesbekia orientalis*. Fitoterapia. 72, 662-665.

Kirira, P.G., Rukunga, G.M., Wanyonyi, A.W., Muregi, F.M., Gathirwa, J.W., Muthaura, C.N., Omar, S.A., Tolo, F., Mungai, G.M and Ndiege, I.O. 2006. Anti-plasmodial activity and toxicity of extracts of plants used in the traditional malaria therapy in Meru and Kilifi Districts of Kenya. Journal of Ethnopharmacology. 106, 403-407.

Koch, A., Tamez, P., Pezzuto, J and Soejarato, D. 2005. Evaluation of plants used for antimalarial treatment by the Maasai of Kenya. Journal of Ethnopharmacology. 101, 95-99.

Kuete, V., Tabopda, T.K., Ngameni, B., Nana, F., Tshikalange, T.E and Ngadjui, B.T. 2010. Antimycobacterial, antibacterial and antifungal activities of *Terminalia superba* (Combretaceae). South African Journal of Botany. 76, 125-131.

Kumar, P and Clark, M. 2005. Clinical Medicine. 6th edition, Published by Elsevier 2005. 117-128.

Kwon, H.A., Kwon, Y-J., Kwon, D-Y and Lee, J.H. 2008. Evaluation of antibacterial effects of a combination of *Coptidis Rhizoma*, *Mume Fructus* and *Schizandrae Fructus* against *Salmonella*. Journal of Food Microbiology. 127, 180-183.

Laga, M., Manoka, A., Kivuvu, M., Malele, B., Tuliza, M., Nzila, N., Goeman, J., Behets, F., Batter, V and Alary, M. 1993. Non-ulcerative sexually transmitted diseases at risk factors for HIV-1 transmission in women: results from cohert study. AIDS. 7, 95-102.

Leone, P., Menu-Bouaoiche, L., Peumans, W.J., Payan, F., Barre, A., Van Damme, E.J.M and Rouge. P., 2006. Resolution of the structure of the allergenic and antifungal banana fruit thaumatin-like protein at 1.7-Å. Biochemie. 88, 45-52.

Liu, Y.B., Peterson, D., Kimura, H and Schubert, D. Mechanism of cellular 3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) reduction. Journal of Neurochemistry. 69, 581-593.

Lohiya, N.K., Pathak, N., Mishra, P.K and Manivannan, B. 1999. Reversible contraception with chloroform extract of *Carica papaya* linn. seeds in male rabbits. Reproductive Toxicology. 13, 59-66.

Loughlin, R., Gilmore, B.F., McCarron, P.A and Tunney, M.M. 2008. Comparison of the cidal activity of tea tree oil and terpinen-4-ol against clinical bacterial skin isolates and human fibroblast cells. Letters in Applied Microbiology. 46, 428-433.

Luo, X., Pires, D., Aìnsa, J.A., Gracia, B., Mulhovo, S., Duarte, A., Anes, E and Ferreira, M.U. 2011. Anti-mycobacterial evaluation and preliminary phytochemical investigation of selected medicinal plants traditionally used in Mozambique. Journal of Ethnopharmacology. 137, 114-120.

Mabogo, D.E.N. 1990. The Ethnobotany of Vhavenda. Published Master of Science Thesis. University of Pretoria.

Mahmood, A.A., Sidik, K and Salmah, I. 2005. Wound healing activity of *Carica papaya* L aqueous leaf extracts in rats. International Journal of Molecular Medicine and Advanced Sciences. 1, 398-401.

Maïkere-Faniyo, R., Van Puyvelde, L., Mutwewingabo, A and Habiyaremye, F.X. 1989. Study of Rwandese medicinal plants used in the treatment of diarrhoea. Journal of Ethnopharmacology. 26, 101-109.

Maregesi, S.M., Pieters, L., Ngassapa, O.D., Apers, S., Vingerhoets, R., Cos, P., Van Berghe, D.A and Vlietinck, A.J. 2008. Screening of some Tanzanian medicinal plants from Bunda district for antibacterial, antifungal and antiviral activities. Journal of Ethnopharmacology. 119, 58-66.

Maroyi, A. 2011. An ethnobotanical survey of medicinal plants used by the people in Nhema communal area, Zimbabwe. Journal of Ethnopharmacology. 136, 347-354.

Mathabe, M.C., Nikolova, R.V., Lall, N and Nyazema, N.Z. 2006. Antibacterial activities of medicinal plants used for the treatment of diarrhea in Limpopo Province, South Africa. Journal of Ethnopharmacology. 105, 286-293.

McGaw, L.J., Jäger., A.K and Van Staden, J. 2000. Antibacterial, antihelminth and anti-amoebic activity in South African medicinal plants. Journal of Ethnopharmacology. 72, 247-263.

Mbwana, J., Mhalu, F., Mwakagile, D., Masesa, J., Moshiro, C and Sandstrom, E. 1999. Susceptibility pattern of *Neisseria gonorrhoeae* to antimicrobial agents in Dar es Salaam. East African Medical Journal. 76, 330-334.

Mirvish, S.S. 1979. Studies on the esophagus. II. Enhancement of [^{3}H] thymidine incorporation in the rat esophagus by *Bidens pilosa* (A plant eaten in South Africa) and by *Croton* oil. Cancer Letters. 6, 159-165.

Mølgaard, P., Nielsen, S.B., Rasmussen, D.E., Drummond, R.B., Makazi, N and Andreassen, J. 2001. Antihelminth screening of Zimbabwean plants traditionally used against schistosomiasis. Journal of Ethnopharmacology. 74, 257-264.

Moo-Puc, R., Robledo, D and Freile-Pelegrin, Y. 2008. Evaluation of selected tropical seaweed s for *in vitro* anti-trichomonal activity. Journal of Ethnopharmacology. 120, 92-97.

Morgan, D.M. 1998. Tetrazolium (MTT) assay for cellular viability and activity. Methodology Molecular Biology. 79, 179-183.

Mosmann, T. 1983. Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxic assays. Journal of Immunological Methods. 65, 55-63.

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

Moyo, M., Finnie, J.F and Van Staden. 2011. Antimicrobial and cyclooxygenase enzyme inhibitory activities of *Sclerocarya birrea* and *Harpephyllum caffrum* (Anacardiaceae) plant extracts. South African Journal of Botany. 77, 592-597.

Mugisha, M.K., Origa, H.O., Odyek, O and Makawiti, D.W. 2008. Medicinal plants used in the treatment of fungal and bacterial infections in and around Queen Elizabeth Biosphere Reserve, western Uganda. African Journal of Ecology. 46, 90-97.

Mukazayire, M-J., Minani, V., Ruffo, C.K., Bizuru, E., Stévigny, C and Duez, P. 2011. Traditional phytotherapy remedies used in Southern Rwanda for the treatment of liver diseases. Journal of Ethnopharmacology. 138,415-431.

Mulaudzi, R.B., Ndhlala, A.R., Kulkarni, M.G., Finnie, J.F and Van Staden, J. 2011. Antimicrobial properties and phenolic contents of medicinal plants used by the Venda people for conditions related to venereal diseases. Journal of Ethnopharmacology. 135, 330-337.

Muli, F.W and Struthers, J.K. 1998. The growth of *Gardnerella vaginalis* and *Lactobacillus acidophilus* in Sorbarod biofilms. The Pathological Society of Great Britain and Ireland. 47, 401-405.

Mundodi, V., Kucknoor, A.S., Chang, T.H and Alderete, J.F. 2006. A novel surface protein of *Trichomonas vaginalis* is regulated independently by low iron and contact with vaginal epithelial cells. BMC Microbiology. 6, 1307-1317.

Muñoz, V and Sauvain, M. 2002. A search for natural bioactive compounds in Bolivia through a multidisciplinary approach. Part 1. Evaluation of the antimalarial activity of plants used by the Chacobo Indians. Journal of Ethnopharmacology, 69, 127-137.

Muthaura, C.N., Rukunga, G.M., Chhabra, S.C., Mungai, G.M and Njagi, E.N.M. 2007. Traditional phytotherapy of some remedies used in treatment of malaria in Meru district of Kenya. South African Journal of Botany. 73, 402-411.

Ncube, B., Finnie, J.F and Van Staden, J. 2012. *In vitro* antimicrobial synergism within plant extract combinations from three South African medicinal bulbs. Journal of Ethnopharmacology. 139, 81-89.

Ndubani, P. 1997. Knowledge about and herbal treatment of sexually transmitted diseases among the Goba of Chiawa, Zambia. Central African Journal of Medicine. 43, 283-287.

Ndubani, P and Höjer, B. 1999. Traditional healers and the treatment of sexually transmitted illnesses in rural Zambia. Journal of Ethnopharmacology. 67, 15-25.

Ngogang, J., Nkongmeneck, B.A., Biyiti Bi Essam, L.F., Oyono, J.L.E., Tsabang, N., Zapfack, L., Mballa, R.N and Tamze, V. 2008. Evaluation of acute and sub-acute toxicity of four medicinal plant extracts used in Cameroon. Toxicology Letters. 180, s185-s186.

Nikolajsen, T., Nielson, F., Rasch, V., Sørensen, P.H., Ismail, F., Kristiansen, U and Jäger, A.K. 2011. Uterine contraction induced by Tanzanian plants used to induce abortion. Journal of Ethnopharmacology. 137, 921-925.

Njume, C., Afolayan, A.J., Green. E and Ndip, R.N. 2011. Volatile compounds in the stem bark of *Sclerocarya birrea* (Anacardiaceae) possess antimicrobial activity against drug-resistant strains of *Helicobactor pylori*. International Journal of Antimicrobial Agents. 38, 319-324.

Nyarko, A.K., Okine, L.K.N., Wedzi, R.K., Addo, P.A and Ofosuhene, M. 2005. Subchronic toxicity studies of the antidiabetic herbal preparation ADD-199 in the rat: absence of organ toxicity and modulation of cytochrome P450. Journal of Ethnopharmacology. 97, 319-325.

Nzama, V. 2009. An Ethnobotanical Investigation of Medicinal Plants used traditionally to treat Sexually Transmitted Infections in Maputaland, KwaZulu Natal. Honors Thesis, University of Zululand.

Ochsendorf, F.R. 2008. Sexually transmitted infections: impact on male fertility. Andrologia. 40, 72-75.

Ogan‡, A.U. 1970. The basic constituents of the leaves of *Carica papaya*. Phytochemistry. 10, 2544-2547.

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

Ojewole, J.A.O. 2006. Antinociceptive, anti-inflammatory and antidiabetic properties of *Hypoxis hemerocallidea* Fisch. & C.A Mey. (Hypoxidaceae) corm [‘African Potato’] aqueous extract in mice and rats. Journal of Ethnopharmacology. 103, 126-134.

Okamura, N., Asai, M., Hine, N and Yagi, A. 1996. High-performance liquid chromatographic determination of phenolic compounds in *Aloe* species. Journal of Chromatography. 746, 225-231.

Okoli, A.S and Iroegbu, C.U. 2004. Evaluation of extracts of *Anthocleista djalonensis*, *Nauclea latifolia* and *Uvaria afzalii* for the activity against bacterial isolates from cases of non-gonococcal urethritis. Journal of Ethnopharmacology. 92, 135-144.

Okusa, P.N., Penge, O., Debleeschouwer, M and Duez, P. 2007. Direct and indirect antimicrobial effects and antioxidant activity of *Cordia gilletii* De Wild (Boraginaceae). Journal of Ethnopharmacology. 112, 476-481.

Osato, J.A., Santiago, L.A., Remo, G.M., Cuadra, M.S and Mori, A. 1993. Antimicrobial and antioxidant activities of unripe papaya. Life Sciences. 53, 1383-1389.

Otsuki, N., Dang, N.H., Kumagai, E., Kondo, A., Iwata, S and Morimoto, C. 2010. Aqueous extract of *Carica papaya* leaves exhibits anti-tumor activity and immunomodulatory effects. Journal of Ethnopharmacology. 127, 760-767.

Owolabi, O.J., Omogbai, E.K.I and Obasyi, O. 2007. Antifungal and antibacterial activities of the ethanolic and aqueous extract of *Kigelia africana* (Bignoniaceae) stem bark. African Journal of Biotechnology. 6, 1677-1680.

Pallant, C.A and Steenkamp, V. 2008. *In vitro* bioactivity of Venda medicinal plants used in the treatment of respiratory conditions. Human and Experimental Toxicology. 27, 859-866.

Pallant, C.A., Cromarty, A.D and Steenkamp, V. 2012. Effect of an alkaloidal fraction of *Tabernaemontana elegans* (Stapf.) on selected micro-organisms. Journal of Ethnopharmacology. 140, 398-404.

Palmer, E and Pitman, N. 1972. Trees of Southern Africa. Volume 3, Balkema, Cape Town.

Pereira, R.L., Ibrahim, A.T., Lucchetti, L., Da Silva, A.J and Goncalves de Moraes, V.L. 1999. Immunosuppressive and anti-inflammatory effects of methanolic extract and the polyacetylene isolated from *Bidens pilosa* L. Immunopharmacology. 43, 31-37.

Persson, K and Ăströom, O. 1997. Fractional factorial design optimization of the separation of pilocarpine and its degradation products by capillary electrophoresis. Journal of Chromatography. 697, 207-215.

Philips, B.J. 1996. Development of cell culture techniques for assessment of the toxicity of plant products. Toxicology in Vitro. 10, 69-76.

Pillay, P., Maharaj, V.J and Smith, P.J. 2008. Investigating South African plants as a source of new antimalarial drugs. Journal of Ethnopharmacology. 119, 438-454.

Pooley, E. 1998. The complete field guide to trees of Natal Zululand and Transkei. Natural Flora Publication Trust. Durban.

Pooley, E. 2005. A Field Guide to wild flowers KwaZulu–Natal and the Eastern Regions. Natal Flora Publications Trust, Durban.

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

Rabe, T and Van Staden, J. 1997. Antibacterial activity of South African plants used for medicinal purposes. Journal of Ethnopharmacology. 56, 81-87.

Reid, K.A., Maes, J., Van Staden, J., De Kimpe, N., Mulholland, D.A and Verschaeve, L. 2006. Evaluation of the mutagenic and antimutagenic effects of South African plants. Journal of Ethnopharmacology. 106, 44-50.

Richardson, M.D and Warnock, D.W. 2003. Fungal infection- Diagnosis and Mangement. Blackwell Scientific Publications, London. 61-73.

Richter, E.R and Vore, L.A. 1989. Antimicrobial activity of banana puree. Food Microbiology. 6, 179-187.

Runyaro, D.K.B., Ngassapa, O.D., Matee, M.I.N., Joseph, C.C and Moshi, M.J. 2006. Medicinal plants used by Tanzanian traditional healers in the management of Candida infections. Journal of Ethnopharmacology. 106, 158–165.

Rybalchenko, N.P., Prykhodko, V.A., Nagorna, S.S., Volynets, N.N., Ostapchuk, A.N., Klochko, V.V., Rybalchenko, T.V and Avdeeva, L.V. 2010. *In vitro* antifungal activity of phenylheptatriyne from *Bidens cernua* L. against yeasts. Fitoterapia. 81, 336-338.

Samie, A., Obi, C.L., Bessong, P.O and Lall, N. 2005. Activity profiles of fourteen selected medicinal plants from rural Venda community in South Africa against fifteen clinical bacterial species. African Journal of Biotechnology. 4, 1443-1451.

Sarma, H.N and Mantha, H.C. 2000. Modulation of morphological changes of endometrial surface epithelium by administration of composite root extract in albino rat. Contraception. 62, 51-54.

Scheltz. Z., Molnar, J and Hohmann, J. 2006. Antimicrobial and antiplasmid activities of essential oils. Fitoterapia. 77, 279-285.

Scherlie, R. 2011. The MTT assay as a tool to evaluate and compare excipient toxicity in vitro on respiratory epitheliail cells. International Journal of Pharmaceutics. 411, 98-105.

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

Shokeen, P., Bala, M and Tandon, V. 2009. Evaluation of the activity of 16 medicinal plants against *Neisseria gonorrhoeae*. International Journal of Antimicrobial Agents. 33, 86-91.

Sibandze, G.F., Van Zyl, R.L and Van Vuuren, S.F. 2010. The anti-diarrhoeal properties of *Breonadia salicina, Syzygium cordatum* and *Ozoroa sphaerocarpa* when used in combination in Swazi traditional medicine. Journal of Ethnopharmacology 132, 506-511.

Silva, O., Ferreira, E., Vaz Pato, M., Caniça, M and Gomes, E.T. 2002. *In vitro* anti-*Neisseria gonorrhoeae* activity of *Terminalia macroptera* leaves. FEMS Microbiology Letters. 217, 271-274.

Snorradóttir, B.S., Pálmat, I.G, Freygardur, T and Már, M. 2011. Experimental design for optimizing drug release from silicone elastomer matrix and investigation of transdermal drug delivery. European Journal of Pharmaceutical Sciences. 42, 559-567.

SAMF (South African Medical Formulary). 2011. 8th edition. Published South African Medical Association. Cape Town.

Sowemimo, A.A., Fakoya, F.A., Awopetu, I., Omobuwajo, O.R and Adesanya, S.A. 2007. Toxicity and mutagenic activity of some selected Nigerian plants. Journal of Ethnopharmacology. 113, 427-432.

Spartz, M., Smith, D.W.E., McDaniel, E.G and Laqueur, G.L. 1967. Role of intestinal microorganisms in determining cycasin toxicity. Proceedings of the Society for Experimental Biology and Medicine. 124, 691-697.

Starley, I.F., Mohammed, P., Schneider, P and Bickler, W. 1999. The treatment of pediatric burns using topical papaya. Burns. 25, 636-639.

Steenkamp, V. Stewart, M.J and Zuckerman, M. 2000. Clinical and analytical aspects of pyrrolizidine poisoning caused by South African traditional medicines. Therapeutic Drug Monitoring. 22, 302-306.

Steenkamp. P.A, Van Heerden. F.R and Van Wyk. B.E. 2002. Accidental fatal poisoning by *Nicotiana glauca*: Identification of anabasine by high performance liquid

chromatography/photodiode array/mass spectrometry. Forensic Science International. 127, 208-217.

Steenkamp, P.A. 2005. Chemical Analysis of Medicinal and Poisonous Plants of Forensic Importance in South Africa. PhD thesis. University of Johannesburg. Page 80.

Steenkamp, V., Gouws, M.C., Gulumian, M., Elgorashi, E.E and Van Staden. 2006. Studies on antibacterial, anti-inflammatory and antioxidant activity of herbal remedies used in the treatment of benign prstatic hyperplasia and prostatitis. Journal of Ethnopharmacology. 103, 71-75.

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

Steward, M.J., Steenkamp, V and Zuckeman. M. 1998. Toxicology of African herbal remedies. Drug Monitoring Therapeutics. 20, 510-516.

Sokmen, A., Jones, B.M and Erturk, M. 1999. The *in vitro* antibacterial activity of Turkish medicinal plants. Journal of Ethnopharmacology. 67, 79-86.

Sun, Y., Li, Y., Li, M., Tong, H., Yang, X and Liu, J. 2009. Optimization of extraction technology of *Anemone raddeana* polysaccharides (ARP) by orthogonal test design and evaluation of its anti-tumor activity. Carbohydrate Polymers. 75, 575-579.

Talwar, G.P., Raghuvanshi, P., Mishra, R., Banerjee, U., Rattan, A., Whaley, K.J., Zeitlin, L., Achilles, S.L., Barre-Sinoussi, F., David, A and Doncel, G.F. 2000. Polyherbal formulations with wide spectrum antimicrobial activity against reproductive tract infections and sexually transmitted pathogens. American Journal of Reproductive Immunology. 43, 144-151.

Thompson, S.A. 2000. South African government genocide and ethnopiracy. The Gaia Research Institute, 12 April 2002.

Tiwari, P., Singh, D and Singh, M.M. 2008. Anti-Trichomonas activity of *Sapindus* saponins, a candidate for development as microcidal contraceptive. Journal of Antimicrobial Chemotherapy. 62, 526-534.

Tona, L., Kambu, K., Mesia, K., Cimanga, K., Apers, S., De Bruyne, T., Pieters, L., Totté, J and Vlietinck, A.J. 1999. Biological screening of traditional preparations from some medicinal plants used as antidiarrhoeal in Kinshasa, Congo. Phytomedicine. 6, 59-66.

Tor-Agbidye, J., Palmer, V.S., Laserev, M.R., Craig, A.M., Blythe, L.L., Sabri, M.I and Spencer, P.S. 1999. Bioactive of cyanide to cyanate in sulphur amino acid deficiency: relevance to neurological disease in humans subsisting on cassava. Toxicological Sciences. 50, 228-235.

Tosi, A., Mazzitelli, S., Capretto, L., Guerrieri, R and Nastruzzi, C. 2009. Optimization of lipospheres production by factorial design and their performances on a dielectrophoretic lab-on-a-chip platform. Colloids and Surfaces A: Physiochemical and Engineering Aspects. 340, 77-85.

Towers, G.H.N., Wat, C.K., Graham, E.A., Bandoni, R.J., Chan, G.F.Q., Mitchell, J.C and Lam, J. 1977. Ultraviolet-mediated antibiotic activity of species of Compositae caused by polyacetylenic compounds. Lloydia. 40, 487-498.

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

Tunney, M.M., Ramage, G., Field, T.R., Moriarty, T.F and Storey, D.G. 2004. Rapid calorimetric assay for antimicrobial susceptibility testing of *Pseudomonas aeruginosa*. Antimicrobial Agents and Chemotherapy. 48, 1879-1881.

Turner, Q., 2001. Medicinal plants of Maputaland. Southern African botanical diversity Network, South Africa.

UNAIDS. 2002. AIDS Epidemic Update. Cited on 17/09/2011. Retrieved from www.onlinedatingmagazine.com/STD/aidshivstats.html

UNAIDS. 2009. AIDS Epidemic Update. Cited on 12/08/2011. Retrieved from www.onlinedatingmagazine.com/STD/aidshivstats.html

Van de Venter, M., Roux, S., Bungu, L.C., Louw, J., Crouch, N.R., Grace, O.M., Maharaj,V., Pillay, P., Sewnarain, P., Bhagwandin, N and Folb, P. 2008. Antidiabetic screening and scoring of 11 plants traditionally used in South Africa. Journal of Ethnopharmacology. 119, 81-86.

Van Der Bank, H., Van Wyk, B-E and Van Der Bank, M. 1995. Genetic variation in two economical important *Aloe* species (Aloeceae). Biochemistry Systematics and Ecology. 23, 251-256.

Van Dyk, S., Griffiths, S., Van Zyl, R.L and Malan, S.F. 2009. The importance of including toxicity assays when screening plant extracts for antimalarial activity. African Journal of Biotechnology. 8, 5595-5601.

Van Heerden, F.A and Schwikkard, S. 2002. Antimalarial activity of plant metabolites. Natural Product Reports. 19, 1-19.

Van Puyvelde, L., Geiser, I., Rwangabo, P.C and Sebikali, B. 1983. Rwandese herbal remedies used against gonorrhoea. Journal of Ethnopharmacology. 8, 279-286.

Van Vuuren, S.F. 2008. Antimicrobial activity of South African medicinal plants. Journal of Ethnopharmacology. 119, 462-472.

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

Van Vuuren, S.F and Naidoo, D. 2010. An antimicrobial investigation of plants used traditionally in southern Africa to treat sexually transmitted infections. Journal of Ethnopharmacology. 130, 552-558.

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

Van Wyk, B.E., Van Heerdenn, F.R and Van Oudtshoorn, B. 2002. Poisonous plants of South Africa. Briza Publications, Arcadia, Pretoria. Page 108.

Van Wyk, B.-E., Van Ouddshoorn, B and Gericke, N. 2009. Medicinal Plants of South Africa. Briza, South Africa.

Van Zyl, R.L and Viljoen, A.M. 2002. *In vitro* activity of *Aloe* extracts against *Plasmodium falciparum* . South African Journal of Botany. 68, 106-110.

Vermani, K and Garg, S. 2002. Herbal medicines for sexually transmitted diseases and AIDS. Journal of Ethnopharmacology. 80, 49-66.

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

Wainwright, J., Schonland, M.M and Candy, H.A. 1977. Toxicity of *Callilepsis laureola*. South African Medical Journal. 52, 313-315.

Wang, H., Cheng, H., Wang, F., Wei, D and Wang, X. 2010. An improvement 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) reduction assay for evaluating the viability of *Escherichia coli* cells. Journal of Microbological Methods. 82, 330-333.

Watt, J.M and Breyer-Brandwijk, M.G. 1962. The Medicinal and Poisonous plants of Southern and Eastern Africa. 2nd edition, Livingstone, London.

WHO. 2001. Prevalence and incidence of sexually transmitted infections, overview and estimates. World Health Organisation. Cited on 16/02/2010. Retrieved from: http://www.who/int/topics/sexuallytransmittedinfections/en/

WHO, 2008. Traditional medicine: Key facts. World Health Organision. Cited on 29/09/2011. Retrieved from: http://www.who.int/mediacentre/factsheets/fs134/en/

WHO., 2011. Sexually transmitted infections. WHO Factsheet No 110. Cited on 21/07/2012. Retrieved from: http://www.who.int/mediacentre/factsheets/fs110/en/index.html#

York, T., De Wet, H and Van Vuuren, S.F. 2011. Plants used for treating respiratory infections in rural Maputaland, KwaZulu-Natal, South Africa. Journal of Ethnopharmacology. 135, 696-710.

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

Yuan, L., Chen, F., Ling, L., Dou, P., Bo, H., Zhong, M and Xia, L. 2008. Protective effects of total flavonoids of *Bidens pilosa* L. (TFB) on animal liver injury and liver fibrosis. Journal of Ethnopharmacology. 116, 539-546.

APPENDIX A - Ethics Waiver

University of the Witwatersrand, Johannesburg

Human Research Ethics Committee (Medical)
(formerly Committee for Research on Human Subjects (Medical))

Secretariat: Research Office, Room SH10005, 10th floor, Senate House • Telephone: +27 11 717-1234 • Fax: +27 11 339-5708
Private Bag 3, Wits 2050, South Africa

Ref: W-CJ-110202-5 07/02/2011

TO WHOM IT MAY CONCERN:

Waiver: This certifies that the following research does not require clearance from the Human Research Ethics Committee (Medical).

Investigator: **Dr R L van Zyl & Miss D Naidoo (student no 0603932M).**

Project title: **Safety and efficacy of traditional medicinal plant combinations for the treatment of sexually transmitted infections in Maputuland, South Africa..**

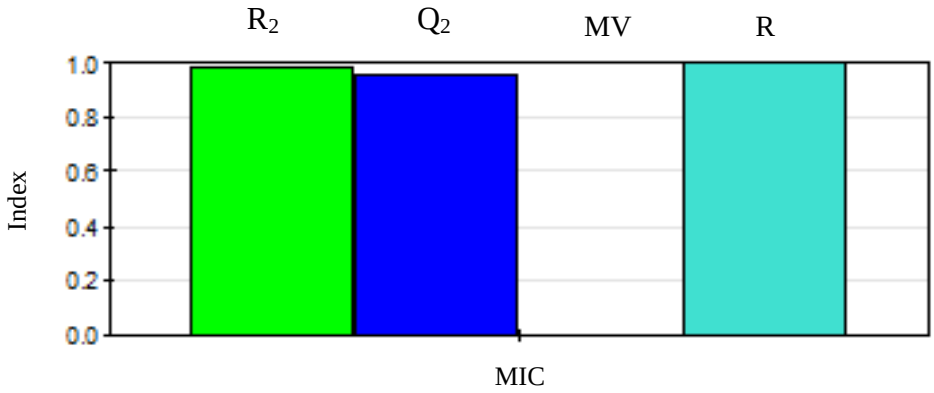
Reason: This is a laboratory study using a commercial cell line - Graham and one or more of the following assays tetrazolium (MTT), sulforhodamine B (SRB), [^{3}H]- thymidine incorporation. No humans are involved.

UNIVERSITY OF THE WITWATERSRAND
PROF PE CLEATON - JONES
HREC (MEDICAL)
2011 -02- 07
JOHANNESBURG

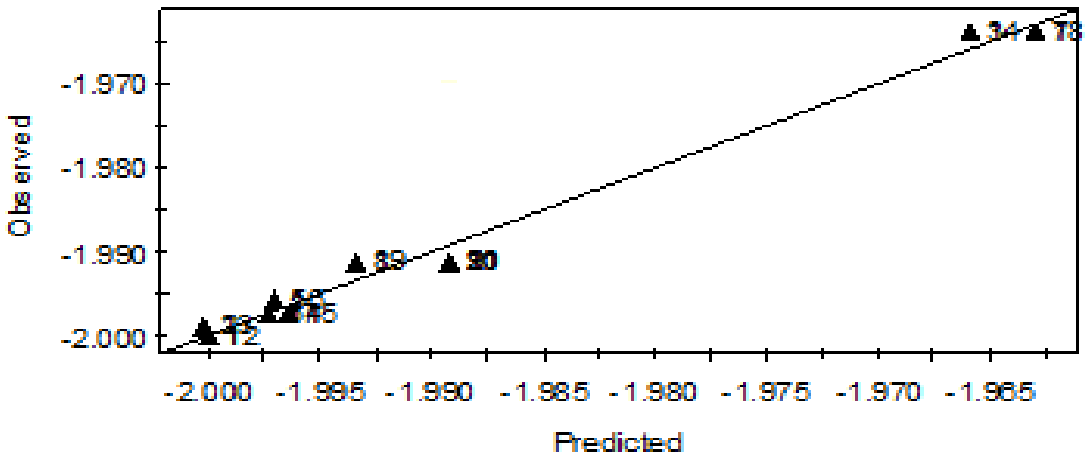
Professor Peter Cleaton-Jones
Chair: Human Research Ethics Committee (Medical)

copy: Anisa Keshav, Research Office, Senate House, Wits

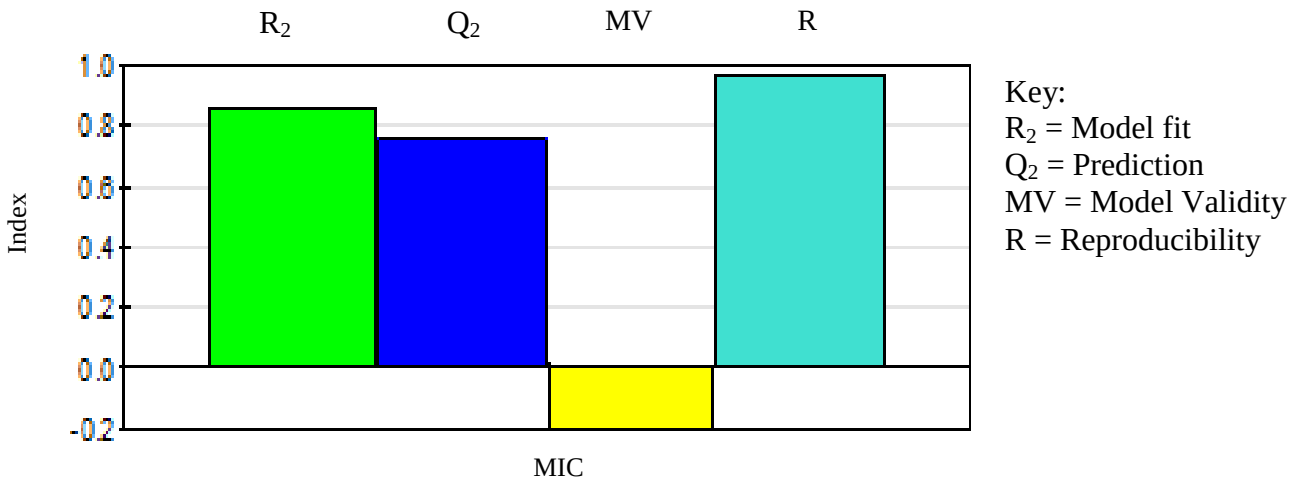
APPENDIX B - Graphs for the optimization of *C. papaya*, *S. serratuloides* and *H. hemerocallidea*



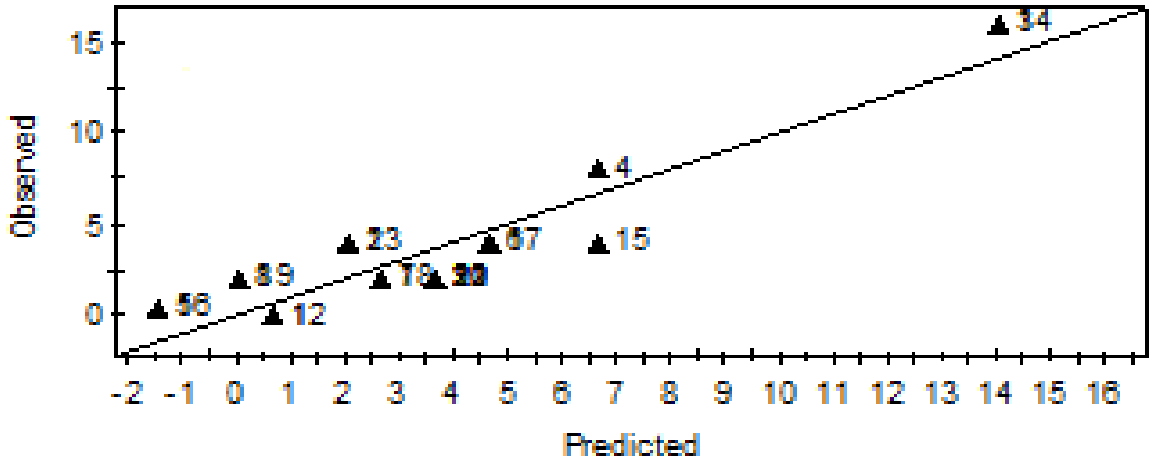
B1.1 Summary of fit of experiments for the combination of *C. papaya*, *S. serratuloides* and *H. hemerocallidea* organic extract against *N. gonorrhoeae.*



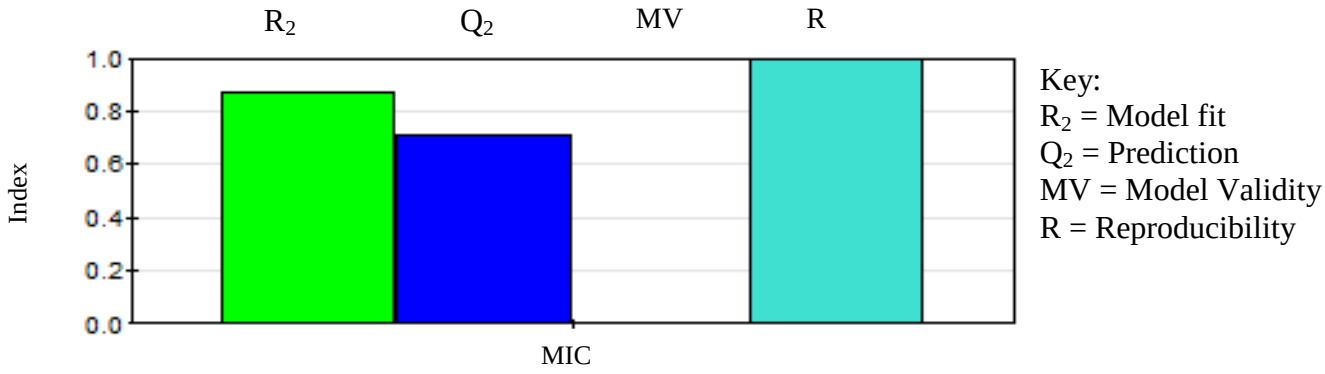
B1.2 Observed vs predicted plot for the combination of *C. papaya*, *S. serratuloides* and *H. hemerocallidea* organic extract against *N. gonorrhoeae*.



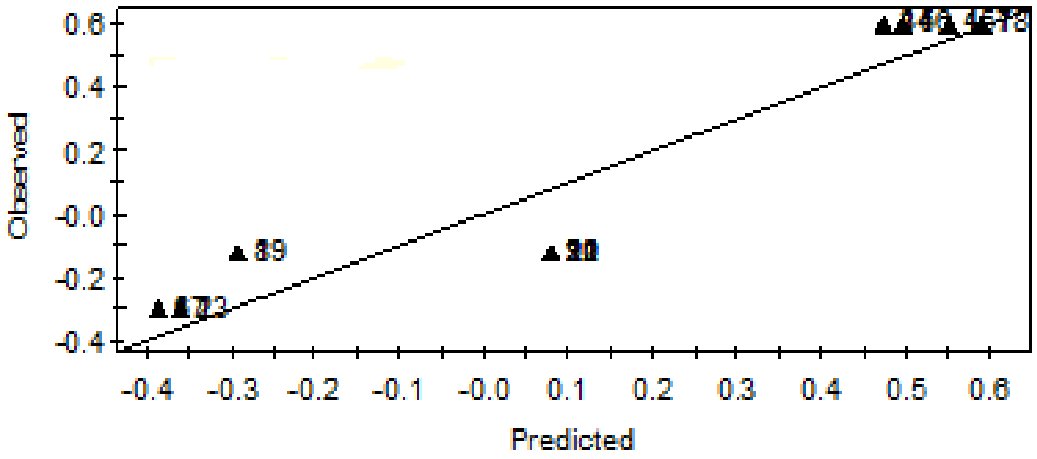
B1.3 Summary of fit of experiments for the combination of *C. papaya*, *S. serratuloides* and *H. hemerocallidea* aqueous extract against *N. gonorrhoeae.*



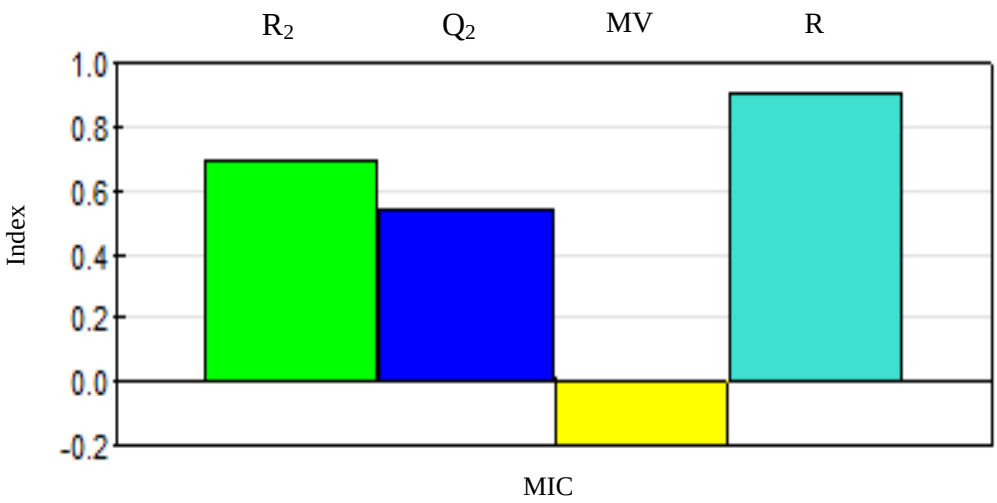
B1.4 Observed vs predicted plot for the combination of *C. papaya*, *S. serratuloides* and *H. hemerocallidea* organic extract against *N. gonorrhoeae.*



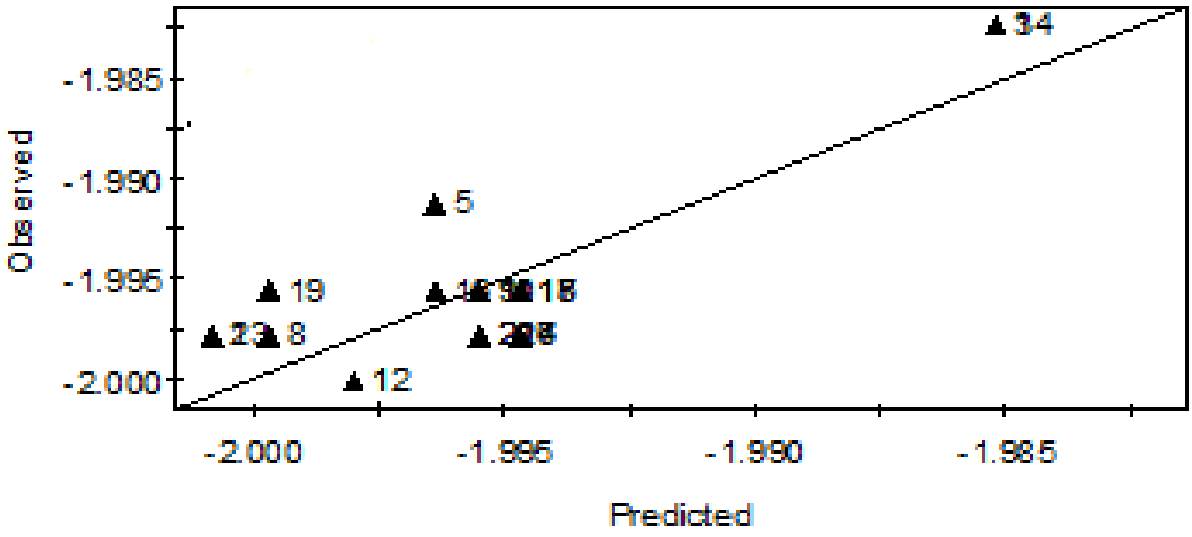
B2.1 Summary of fit of experiments for the combination of *C. papaya*, *S. serratuloides* and *H. hemerocallidea* organic extract against *O. ureolytica.*



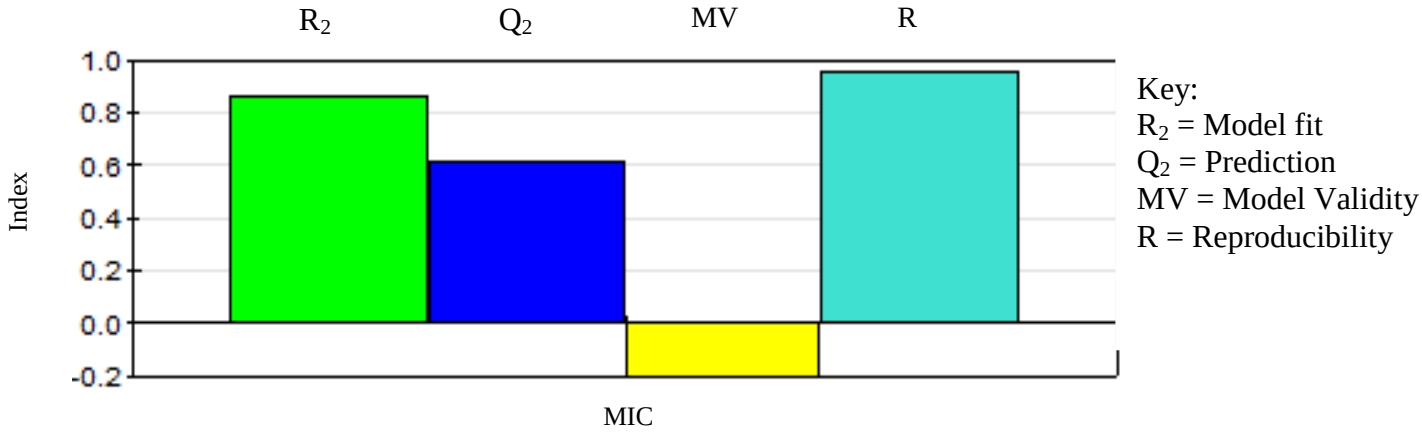
B2.2 Observed vs predicted plot for the combination of *C. papaya*, *S. serratuloides* and *H. hemerocallidea* organic extract against *O. ureolytica.*



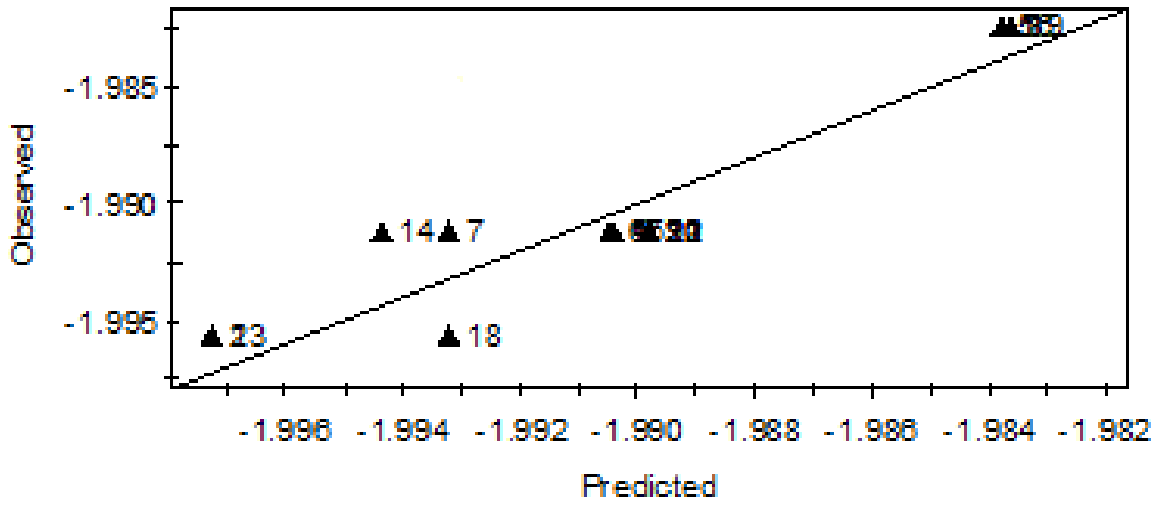
B.2.3 Summary of fit of experiments for the combination of *C. papaya*, *S. serratuloides* and *H. hemerocallidea* aqueous extract against *O. ureolytica.*



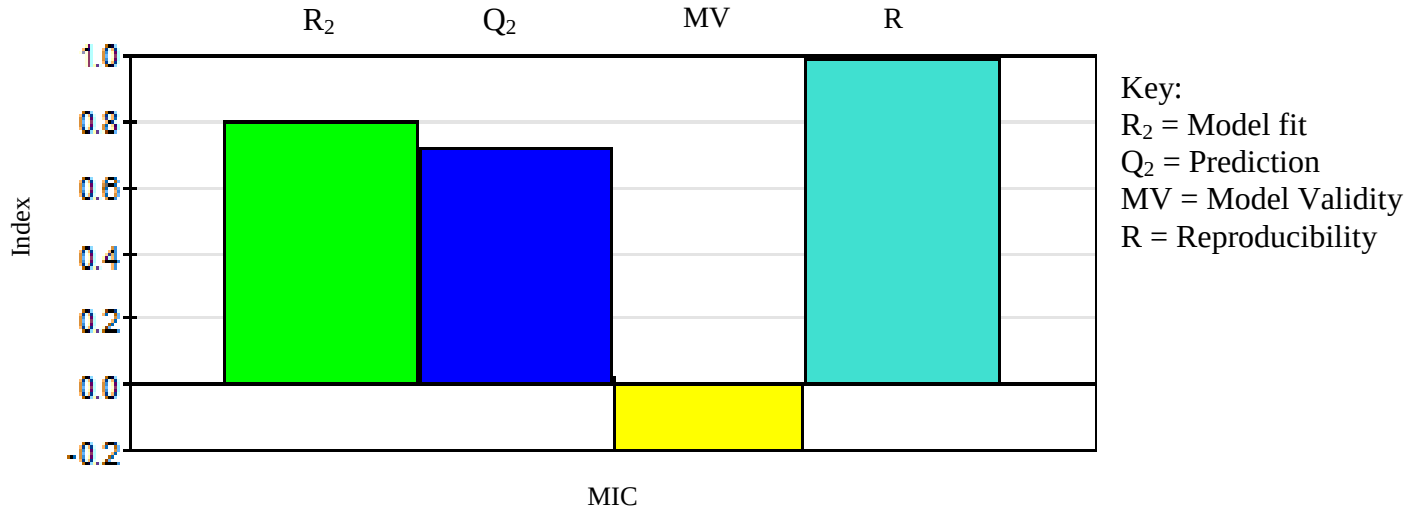
B.2.4 Observed vs predicted plot for the combination of *C. papaya*, *S. serratuloides* and *H. hemerocallidea* organic extract against *O. ureolytica.*



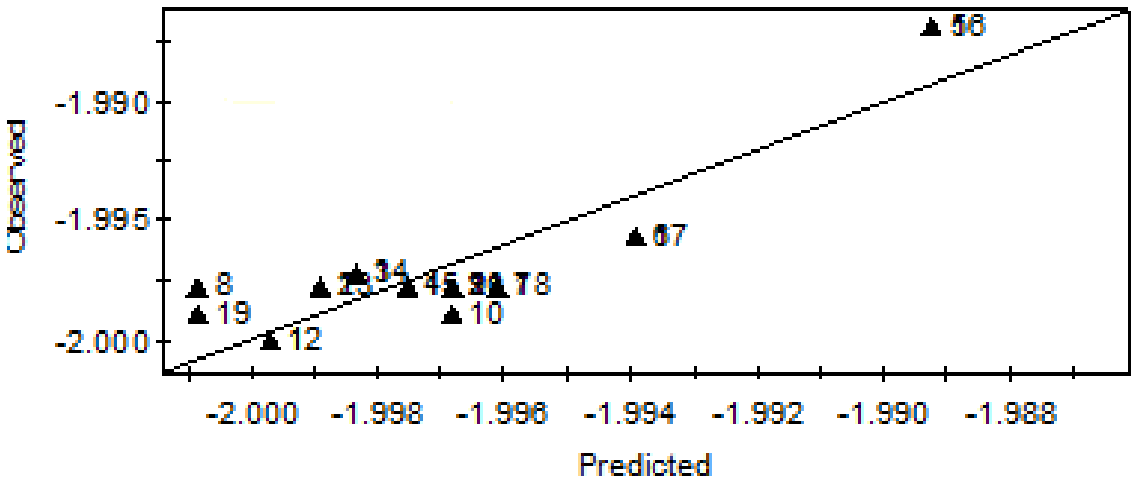
B.3.1 Summary of fit of experiments for the combination of *C. papaya*, *S. serratuloides* and *H. hemerocallidea* organic extract against *G. vaginalis.*



B.3.2 Observed vs predicted plot for the combination of *C. papaya*, *S. serratuloides* and *H. hemerocallidea* organic extract against *G. vaginalis.*



B4.1 Summary of fit of experiments for the combination of *C. papaya*, *S. serratuloides* and *H. hemerocallidea* organic extract against *U. urealyticum.*



B4.2 Observed vs predicted plot for the combination of *C. papaya*, *S. serratuloides* and *H. hemerocallidea* organic extract against *U. urealyticum.*

APPENDIX C – Abstracts of presentations/conference presentations/posters

An antimicrobial investigation of plants used traditionally in southern Africa to treat sexually transmitted infections

Naidoo D., van Vuuren SF.

Department of Pharmacy and Pharmacology, Faculty of Health Sciences, University of Witwatersrand, 7 York Road, Parktown 2193, South Africa

Aims: Eighteen plants were assessed for antimicrobial activity against pathogens associated with prevalent urogenital/sexually transmitted infections. Plant selection was based on information obtained from the ethnobotanical literature.

Methods: Dried plant material was submerged in a 1:1 mixture of methanol and dichloromethane for 24 h. Aqueous extracts were prepared by submerging dried plant material in sterile distilled water for 24 h followed by lyophilisation. Essential oils were distilled from the two aromatic plant species (*Tarchonanthus camphoratus* and *Croton gratissimus*). Antimicrobial activity was assessed using the micro-well minimum inhibitory concentration (MIC) assay with specific modifications to facilitate fastidious growth of pathogens.

Results: *Trichomonas vaginalis*, *Oligella ureolytica*, *Neisseria gonorrhoeae* and *Candida albicans* as well as 0.70 mg/ml against *Gardnerella vaginalis*. The most noteworthy activity for the essential oils was observed for *T. camphoratus* (0.80 mg/ml) against *Oligella ureolytica*. The highest noteworthy activity noted against *Ureaplasma urealyticum* was for *Psidium guajava* (CH_2Cl_2:MeOH plant extract) at 0.80 mg/ml. Psidium guajava (solvent extract) did however prove to possess further antimicrobial activity when tested against *Trichomonas vaginalis* (1.0 mg/ml*), Gardnerella vaginalis* (1.0 mg/ml) and *Candida albicans* (0.80 mg/ml).

Conclusion: *T. camphoratus* which has been cited only twice for unspecified STI-related diseases has shown significant activity towards the pathogens tested. These newly discovered sensitivities are encouraging and should be investigated further to identify the possible compound or combination of compounds responsible for activity. Antimicrobial activity was

observed for a number of the plant samples against at least one or more pathogen, thus validating the ethnobotanical use as an anti-infective to treat sexually transmitted diseases.

Antimicrobial efficacy of traditional medicinal plant combinations for the treatment of sexually transmitted infections in northern Maputaland, South Africa

D. (Deshnee) Naidoo[1], S.F van Vuuren[1] and H. de Wet[2]

1. Department of Pharmacy and Pharmacology. University of Witwatersrand, Johannesburg

2. Department of Botany , Faculty of Agriculture and Science. University of Zululand. Zululand

The validation of medicinal plants to treat sexually transmitted infections (STI) is of utmost importance considering that diseases of the urogenital tract are one of the main reasons for consultation with traditional healers in southern Africa.

Thirteen plant combinations were assessed for antimicrobial activity against pathogens selected on the basis of their prevalent urogenital/sexually transmitted infection rates. Plant selection was based on ethnobotanical information obtained from lay people in a rural community of northern Maputaland.

Methanol and dichloromethane (CH_2Cl_2:MeOH) extracts as well as aqueous extracts were prepared for individual plants. Antimicrobial activity was assessed using the micro-well minimum inhibitory concentration (MIC) assay with specific alterations to facilitate fastidious growth of pathogens. Fractional inhibitory concentrations were calculated using MIC values with the combined plants.

When the plants were tested individually 28.3% of the aqueous extract and 45% of the solvent extracts demonstrated noteworthy against the test STI pathogens. When 1:1 combinations as well as multiple combinations were studied the aqueous extracts resulted in, 55.9% antagonism, 44% indifferent, 0.04% additive and 0.013% synergism and the solvent extracts resulted in, 53% antagonism, 47% indifference and 0.03% additive. Only one combination (*Albizia adianthifolia* and *Trichilia dregeana*, aqueous extract) demonstrated synergistic activity (FIC value 0.29).

Analysis of multiple combinations (more than one plant) indicate that when interactive studies were undertaken in-depth on each plant combination a more favorable (synergistic and additive) interaction was noted.

Although individual plants showed antimicrobial efficacy against the STI pathogens tested, in combination (traditional use) the majority were antagonistic. Thus plants used traditionally for the treatment of STI's in combination may not necessarily be the best option.

Antimicrobial and toxicological analysis of independent plants and plants in combination used to treat sexually transmitted infections in northern Maputaland

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1. Department of Pharmacy and Pharmacology. University of Witwatersrand, Johannesburg

2. Department of Botany , Faculty of Agriculture and Science. University of Zululand. Zululand

The validation of medicinal plants to treat sexually transmitted infections (STI) is of utmost importance considering that diseases of the urogenital tract are one of the main reasons for consultation with traditional healers in southern Africa. A scientific investigation was conducted into the antimicrobial activity of plants independently as well as the plants combined in 13 combinations.

Methanol and dichloromethane (CH_2Cl_2:MeOH) extracts as well as aqueous extracts were prepared for individual plants and the 13 combinations. Antimicrobial activity was assessed using the MIC micro-dilution assay with specific alterations to facilitate fastidious growth of pathogens.

There were a total of 20 individual plants tested against six STI pathogens which resulted in 22.5% of the aqueous extracts and 25% of the CH_2Cl_2: MeOH extracts exhibiting noteworthy antimicrobial activity. The most susceptible pathogen was *U. urealyticum* (clinical strain) with 65% of the independent plants possessing antimicrobial activity in aqueous and CH_2Cl_2: MeOH extracts. *Ranunculus multifidus* (aqueous extract) demonstrated the most noteworthy activity against *U. urealyticum* (MIC=0.019 mg/ml). The antimicrobial activity of the combined plants was analyzed using the ΣFIC index. Of the 13 combinations studied, several synergistic combinations were evident, the most prominent being the combination of *Albizia adianthifolia* and *Trichilia dregeana* (aqueous extract) a ΣFIC of 0.15. *Syzygium cordatum* and *Sclerocarya birrea* was also a combination of interest as when combined, antagonistic (ΣFIC=5) as well as

synergistic (∑FIC=0.42) interactions were evident. Toxicological profiling was performed using the 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay with kidney epithelial cells (Graham cells). From the 20 plants studied the only plant found to possess some toxicity by producing 20% cell death was *Kigelia africana* (aqueous extract). When *S. birrea* (aqueous extract) and *S. cordatum* (aqueous extract) were independently studied they possessed no toxicity, however, when combined the toxicity levels increased leading to an approximately 20% cell death. Combined ratios of the combination were further investigated to find the safest option using isobolograms as well as toxicity studies. It was found that ratios (6:4, 5:5, 4:6, 3:7) *S.birrea*: *S. cordatum* were toxic by resulting in cell death.

This study has concluded that the scientific investigation was warranted and that plants used in combinations do possess antimicrobial activity however toxicological profiling is of utmost importance for the safety of the patient and if not combined in its correct ratio can lead to potential bodily harm.

Are plants used traditionally for sexually transmitted infection treatment in northern Maputaland effective and safe?

Deshnee Naidoo.[1], Professor Sandy Van Vuuren[1], Professor Robyn Van Zyl[1], Dr Helene de Wet [2]

[1]***Department of Pharmacy and Pharmacology, Faculty of Health Sciences. University of the Witwatersrand, 7 York Road, Parktown, 2193***

[2]***Department of Botany. University of Zululand, Private Bag X101, Kwa-Dlangewa 3886, South Africa***

Purpose:

Traditional healing is widely practiced in South Africa, however, there has been very little documented research undertaken on the use of medicinal plants in Maputaland, in particular the use of combined plants for the treatment of sexually transmitted infections (STI's). An investigation was conducted on the antimicrobial activity of plants independently as well as in combination.

Methods:

Antimicrobial study: Methanol and dichloromethane (CH_2Cl_2: MeOH) as well as aqueous extracts were prepared for 20 individual plants and 13 combinations which were then tested against six STI pathogens. Antimicrobial activity was assessed using the MIC micro-dilution assay with specific alterations to facilitate fastidious growth of pathogens. The antimicrobial activity of the combined plants was analyzed using the ΣFIC index in order to determine if the combination was antagonistic, indifferent, additive or synergistic.

Toxicity study: Toxicological profiling was performed using the 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay with kidney epithelial cells (Graham cells).

Results:

From the 20 individual plants tested against six STI pathogens 22.5% and 25% (aqueous and CH_2Cl_2: MeOH extracts respectively) exhibited noteworthy antimicrobial activity. The most susceptible pathogen was *U. urealyticum* (clinical strain) with 65% of the independent plants possessing antimicrobial activity. *Ranunculus multifidus* (aqueous extract) demonstrated the

most noteworthy activity against *U. urealyticum* (MIC = 0.019 mg/ml). Of the 13 combinations studied, several synergistic combinations were evident, the most prominent being the combination of *Albizia adianthifolia* and *Trichilia dregeana* (aqueous extract) with a ΣFIC of 0.15. Regarding the toxicity plant profiling the only independent plant found to possess some toxicity by producing approximately 20% cell death was *Kigelia africana* (aqueous and solvent extract). When *Sclerocarya birrea* (aqueous extract) and *Syzygium cordatum* (aqueous extract) were independently studied they possessed no toxicity, however, when combined the toxicity levels increased leading to an approximately 20% cell death. Furthermore selected ratios (6:4, 5:5, 4:6, 3:7) of the two plants were toxic resulting in cell death.

Conclusion:

This study not only confirmed the antimicrobial activity of many of the plants independently and in combination for the treatment of STI in northern Maputaland, but also demonstrated to some extent the safety.

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An antimicrobial investigation of plants used traditionally in southern Africa to treat sexually transmitted infections

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ABSTRACT

Aim of the study: Eighteen plants were assessed for antimicrobial activity against pathogens associated with prevalent urogenital/sexually transmitted infections. Plant selection was based on information obtained from the ethnobotanical literature.

Materials and methods: Dried plant material was submerged in a 1:1 mixture of methanol and dichloromethane for 24 h. Aqueous extracts were prepared by submerging dried plant material in sterile distilled water for 24 h followed by lyophilization. Essential oils were distilled from the two aromatic plant species (*Tarchonanthus camphoratus* and *Croton gratissimus*). Antimicrobial activity was assessed using the micro-well minimum inhibitory concentration (MIC) assay with specific modifications to facilitate fastidious growth of pathogens.

Results: *Tarchonanthus camphoratus* (solvent extract) showed the most significant broad spectrum activity with MIC values ranging between 0.50 and 0.70 mg/ml against five of the six pathogens tested. Other noteworthy activity was found for *Hypericum aethiopicum* (root) at 0.3 mg/ml (*Neisseria gonorrhoeae*). *Polygala fruticosa* and the solvent root extract of *Hypericum aethiopicum* showed highest sensitivities towards *Gardnerella vaginalis* at 0.2 mg/ml. Efficacy of the solvent extracts against the pathogen *Oligella ureolytica* demonstrated antimicrobial activity (MIC values $\leq$1.0 mg/ml) for seven plant species. The highest activity noted against *Ureaplasma urealyticum* was for *Psidium guajava* (solvent extract) at 0.8 mg/ml. In general the aqueous extracts displayed mostly poor anti-STI activity. The most noteworthy susceptibility for the aqueous extracts was noted with the plant extract *Syzygium cordatum* (MIC value 0.1 mg/ml against *Candida albicans*). The most noteworthy activity for the essential oils was observed for *Tarchonanthus camphoratus* (0.8 mg/ml) against *Oligella ureolytica*.

Conclusions: Antimicrobial activity was observed for a number of the plant samples against at least one or more pathogen, thus validating the ethnobotanical use as an anti-infective to treat sexually transmitted diseases.

An Antimicrobial Investigation Of Plants Used Traditionally In Southern Africa To Treat Sexually Transmitted Infections.

NATIONAL HEALTH LABORATORY SERVICE

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Introduction

The rate of sexually transmitted infections (STIs) in Southern Africa is among one of the highest in world. Many patients fail to seek therapy from medical health authorities and the majority of patients infected with STIs in South Africa initially seek help from traditional healers. Furthermore, STIs are one of the most prominent reasons for patients consulting traditional healers. Although antimicrobial research has been done on a number of plants, few studies have focused on pathogens associated with STIs using indigenous South African plants. In this study, a selection of plant species which are traditionally used for the treatment of STIs were identified using several literature sources. Relevant plant species were tested against six of the most commonly encountered micro-organisms associated with infections of the urogenital tract.

Objectives

- To review the recorded literature and identify plants used in traditional healing for the treatment of sexually transmitted diseases
- To investigate the antimicrobial efficacy of a selection of these plants against pathogens associated with STIs

Table 1: Literature review of plants used to treat infections of the urogenital tract

Scientific name and family	Zulu name	Part of plant used	STI related use	Preparation	Reference
Aloe ferox Mill. Asphodelaceae	umhlaba	leaf	syphilis, gonorrhoea, *Candida albicans*	leaf juice	Watt and Breyer-Brandwijk, 1962 Hutchings et al., 1996 Kambizi and Afolayan 2008
Bowiea volubilis Harv. ex Hook. f. Hyacinthaceae	igibisila, iguleni, ugibisisila	bulb	STI related bladder pains, dropsy	decoction, poultice	Van Wyk et al., 1997 Felhaber, 1997 Bowa and Van Staden, 2016
Carpobrotus edulis (L.) L. Bol. Mesembryanthemaceae	ikhambi-lamabulawo, umgongozi	leaf	Herpes simplex virus, *Candida albicans*	strained juice from the pounded leaf	Van Wyk et al., 1997 Neuwinger, 2000 Thring and Weitz, 2006
Cassia occidentalis (L.) Link Fabaceae	isinyembane, umnwanda-nyoka	leaf/seeds/root	gonorrhoea, STIs syphilitic sores, swollen testicles	decoctions	Watt and Breyer-Brandwijk, 1962 Cáceres et al., 1995 Neuwinger, 2000
Catharanthus roseus G. Don pocynaceae	*ikhetinini*	unspecified parts/roots	venereal diseases	decoctions	Hutchings et al., 1996
Cissus quadrangulis L. Vitaceae	isinwazi	leaf	gonorrhoea	decoction	Neuwinger, 2000
Croton gratissimus Burch. Euphorbiaceae	thubeshane-elikhulu, ilabela, intumbanhlosi,	leaf	sores associated with STIs	steam baths	Hutchings et al., 1996 Van Wyk et al., 1997
Rauclea natalensis. Rubiaceae	ichitamuzi, idungamuzi, inkunzana, umnyama	leaf/bark	syphilis, venereal diseases	leaf gel is applied to skin directly	Watt and Breyer-Brandwijk, 1962 Hutchings et al., 1996
Hypericum aethiopicum Thunb. Clusiaceae	isimoyisana, isimonyo, isimoyisane, isiyamdwane,	unspecified parts	pain in the loins or abdominal complaints	decoctions	Hutchings et al., 1996
Polygala fruticosa P. J. Bergius Polygalaceae	ithethe	root	gonorrhoea	whole plant in infusions and snuffs	Hutchings et al., 1996 Van Wyk et al., 1997
Psidium guajava L. Myrtaceae	ugwava	leaf/root	gonorrhoea, non specified STIs	infusions	Gutiérrez et al., 2008 Kamatenesi-Mugisha et al., 2008
Sansevieria aethiopica Thunb. Asparagaceae	isikholokotho, isikwendle , isiphokoloko	root/ leaf	unspecified venereal diseases	unspecified	Hutchings et al., 1996
Scabiosa columbaria L. Dipsacaceae	ibhuka, igwaluza	root	venereal sores	ointment made from the charred root	Watt and Breyer-Brandwijk, 1962 Hutchings et al., 1996
Strelitzia reginae Ait. Strelitziaceae	isagudu, isigude	leaf/ inflorescence	enemas for inflamed glands assoc with STIs	strained decoctions	Hutchings et al., 1996
Syzygium cordatum Hochst. Myrtaceae	umdoni	leaf	have purgative effects, for stomach ailments	infusions	Hutchings et al., 1996
Tarchonanthus camphoratus L. Asteraceae	igqeba-elimhlophe, isiduli-sehlathi	unspecified parts/ leaf	sexually transmitted diseases	leaf infusions smoked, inhaled, and chewed, topically applied	Watt and Breyer-Brandwijk, 1962 Hutchings et al., 1996
Terminalia sericea Burch. ex DC. Combretaceae	amangwe, umkhonono	roots	Syphilis, gonorrhea, *Candida albicans*	decoction of the roots	Watt and Breyer-Brandwijk, 1962 Hutchings et al., 1996 Tshikalange et al., 2008
Typha capensis Typhaceae	ibhuma, ibuma	rhizomes/root	STI's, problems relating to genitalia	decoction	Hutchings et al., 1996