Synergistic Effects of Plant Extracts and Penicillins on *Staphylococcus aureus* and *Enterococcus faecalis*

By

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Thesis submitted in fulfilment for the requirements of the Degree of Doctor of Medical Laboratory sciences in the Faculty of Health Sciences at the Durban University of Technology.

I Nhlanhla Wiseman Nsele do hereby declare that these investigations represent my original work, while registered for the Doctor: Medical Laboratory Science degree in the Department of Biomedical and Clinical Technology at the Durban University of Technology, and have not been submitted in any form for any Diploma or Degree to another University. When use was made of the work of others it has been duly acknowledged in the text.

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ABSTRACT

Introduction

Medicinal plants have been used for centuries as remedies for human diseases because they contain components of therapeutic value. Recently, the acceptance of traditional medicine as an alternative form of health care and the development of microbial resistance to the available antibiotics has led scientists to investigate the antimicrobial activity of medicinal plants.

Aim

The aim of this study is to evaluate the interaction between water and ethanolic extracts of *Psidium guajava* (ugwava) and *Sutherlandia frutescens* (unwele) alone and then synergy testing of these extracts with known penicillins using both disc diffusion and microdilution method on *Staphylococcus aureus*(*S. aureus)* and *Enterococcus faecalis*(*E. faecalis).*

Methodology

The plants used in this study *Sutherlandia frutenscens (S. fruitescens)* and *Psidium guajava (P. guajava)* were harvested from the Silverglen Nature Reserve (Chatsworth) early in the morning (8 am). The leaves of *S. frutescens* and *P. guajava* were used to prepare the extracts. All plant extracts were prepared according to modified method of the German Homeopathic Pharmacopoea. Two solvents, water and ethanol were used for extraction.

The extracts were then assessed for their antibacterial activity against methicillin-resistant *Staphylococcus aureus* (MRSA), methicillin-sensitive *Staphylococcus aureus (*MSSA) and *Enterococcus faecalis.* The effect of the plant extracts on these bacteria was determined by the disk diffusion test, which was used as the screening test. Positive results were further subjected to the minimum inhibitory concentration and minimum bactericidal concentration assays using the agar dilution method. Dilutions that showed no growth on non-selective plates were taken as minimum inhibitory concentration and minimum bactericidal concentrations. Bacterial sensitivity testing was carried out in accordance with modified Kirby-Bauer Antimicrobial Sensitivity Test.

The synergy testing was conducted by combining the extracted plants with penicillins. The combinations were then tested against MRSA, MSSA and *E. faecalis* and the results were compared with both the individual plant and the penicillins.

Results

Only the water-based extracts of plants were able to inhibit MRSA, MSSA and *E. faecalis*. None of the test organisms were inhibited by the ethanol extracts of all plants used in this study. In the screening test, the zones of inhibition for water-based extracts against MRSA, MSSA and *E. faecalis* ranged from 17 mm to 35 mm. The minimum inhibitory concentration ranged from 0 % to 100 % inhibition depending on the dilution of the extract. In the combination studies, the zones of inhibition for water-based extracts against MRSA, MSSA and *E. faecalis* ranged from 18 mm to 50 mm. The minimum inhibitory concentration ranged from 0 % to 100 % inhibition depending on the dilution of the extract.

Conclusion

The results obtained in this study prove that *S. frutescens* and *P. guajava* extracts contain antibacterial substances. The water-based extracts of all plants in this study inhibited the growth of MRSA, MSSA and *E. faecalis*. The combination studies produced zones that were greater than the individual penicillins indicating that synergistic effects do exist. Ethanol-based plant extracts did not inhibit the growth of any bacteria in this study. The results obtained in this study might be considered sufficient for further studies aimed at isolating and identifying the active compounds. The herbs should be tested *in vivo* by means of clinical trials and they should also be tested for their toxicity to cells. Different parts of the plants should also be tested for antibacterial activity to a wide range of bacteria.

DEDICATION

This work is dedicated with love and gratitude to my family for their support and for believing in me. I would also like to dedicate this work to the Department of Biomedical Science at Mangosuthu University of Technology.

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CHAPTER 1: INTRODUCTION

1.1. Introduction

Bacterial infections are a common aetiology for mortality globally. Resistance of microorganisms is becoming a worldwide burden (Liu *et al.,* 2016). The efficiency of a number of current antimicrobial agents is being endangered by the appearance of a large number of pathogenic microorganisms that are resistant to many drugs (Oliphant & Eroschenko, 2015). A number of bacterial infections have in the past been cured with plant extracts all the way through the ages (Marceau, 2015). Plant extracts or pure compounds present an enormous amount of chances for the discovery of novel therapeutic medicines and drugs. This is due to the accessibility of biochemical variety of the therapeutic plants. There needs to be an urgent and continuous discovery of novel compounds or metabolites that are antibacterial, with various chemical constructions and innovative mechanisms of action for new and recurring bacterial infections (Al Momani *et al*., 2013).

It is therefore, for this reason that scientists are more and more diverting their devotion to herbal medicine when looking for fresh means to develop better antimicrobial agents to deal with bacterial infections (Mahomoodally & Gurib-Fakim, 2013). There is an ever growing inefficiency of treatments as well as the growing resistance to contemporary drugs displayed by microorganisms that are pathogenic. Therefore this necessitates the screening for the possible antimicrobial action of certain pharmaceutical herbs and plant (Rath & Padhy, 2013).

Bacterial Infections are the leading cause of deaths, contributing to about 50 percent of all deaths in most countries worldwide (Weber *et al*., 2016). Their mortality rates are escalating in third world countries like South Africa. These increases are due to the rise in Human Immunodeficiency Virus (HIV) as well as the resultatnt Acquired Immune Deficiency Syndrome (AIDS) infections. Nosocomial and community acquired infections are additional factors contributing to the increase in the resistance of drugs (Mantadakis *et al.,* 2015). Alarming escalations are seen between the ages of 15 to 45 years. This acceleration is attributed to the proposition that young people engage in increased high - risk sexual activity, thus explaining the dramatic intensification of the risk of getting HIV infections.

An approximated 3000 to 4000 plant species are utilised for therapeutic or medicinal purposes throughout South Africa, with an estimated 27 million of South African population using them for their renowned medicinal properties (Ryno, 2009). Of this 27 million, three million South Africans use herbal prescriptions as their main source of treatment of their diseases. There are 250 000 to 500 000 known plant species, however, a scarce number of these plants have been investigated to discover their potential pharmacological capabilities or qualities. Thus metabolites and compounds that could be of invaluable therapeutic benefit are yet to be discovered in the majority of the plants (Joshi *et al.,* 2013).

The massive utilisation of antimicrobial agents in the curing of infectious diseases contributes to the development of bacterial strains that are resistant. A major challenge in nosocomial infections is the methicillin–resistant strain of *Staphylococcus aureus.* These infections are difficult to treat because methicillin-

resistant *Staphylococcus aureus* strains are resistant to most clinically available penicillin antibiotics. These strains are mostly treated by a glycopeptide type of a drug known as vancomycin. However, vancomycin – resistant *S. aureus* (VRSA) is emerging. Therefore, it becomes necessary to find new ways that are effective for the treatment of infections caused by drug-resistant bacteria such as MRSA.

The efficacy of antibacterial agents against pathogens can be improved by combiningthem with plant extracts. Some South African plants exhibit significant potency against human bacteria but plant extracts as antimicrobial agents are rarely used as systematic antibiotics currently (Ghaleb & Mohammad, 2008). This may be due to their low level of activity, especially against Gram negative bacteria (Ghaleb &Mohammad, 2008). Plants used in the present study are *Psidium guajava (P. guajava)* and *Sutherlandia frutescens (S. frutescens).* This is because both these plants have been shown to have antibacterial activity in different studies. The ethanol extract of *P. guajava* was shown to be antibacterial active against *S. aureus* and *Bacillus cereus* (Biswas *et al.,* 2013). The hexane extract of *S. frutescens* was shown to be antibacterial active against *S. aureus, Escherichia coli (E. coli)* and *Enterococcus feacalis (E. faecalis)* (Katerere & Eloff, 2005).

Scientists established that, on top of the production of antibacterial composites, medicinal plants yield multi-drug resistant (MDR) inhibitors. These inhibitors improve the action of antimicrobial compounds (Aiyegoro & Okoh, 2009). The action of supposed plant antibacterial agents against Gram positive and Gram negative organisms were improved by synthetic MDR inhibitors of related efflux proteins (Aiyegoro & Okoh, 2009). These discoveries provided a foundation that medicinal

plants may be used as foundations of ordinary MDR inhibitors which are able to modify the performance of antimicrobials against resilient strains of microorganisms. The assessment of medicinal plant extracts for their synergistic activity with antimicrobials can deliver ways for the identification of MDR inhibitors.

Samples of *S. frutescens* and *P. guajava* were acquired from Silverglen Nature Reserve (Chartswoth). The plants were collected in the early morning (8 am) when the activity of the cells is high. For both *S. frutescens* and *P. guajava*, leaves were utilised. The study was conducted at Mangosuthu University of Technology in the Department of Biomedical Science.

This study was undertaken to evaluate the interaction between water extracts of *Psidium guajava* and *Sutharlanda frutescens* only, followed by synergy testing of these extracts with penicillins which are the Beta-lactam agents. The following penicillins were used in this study: penicillin G, procaine penicillin, benzathine penicillin, and penicillin V. Each penicillin was tested against each bacteria used in the study before synergy with plant extracts was assessed. This study was conducted against two *S. aureus* strains; Methicillin-resistant *S. aureus* and Methicillin-sensitive *S. aureus*. It was also conducted against *E. faecalis.*

Ethanolic and water extracts from each plant was tested six times with each bacterium. For each replication, the zone of inhibition produced and minimum inhibitory concentrations (MIC) were recorded. Each bacterium response was coded: growth or no growth. The statistician was consulted for the determining the number of replicates.

In South Africa the lack of health care centres drive a large number of people to traditional healers. The South African government developed Act 22 of 2007 as an attempt of recognising and regulate the use of traditional medicine (Boyane, 2015). The rate of infections and the development of new resistant strains of bacteria are also very high and therefore, any available means to deal with these infections should be used. Scientific investigation of the synergistic effects of plant extracts and penicillins on *S. aureus* and *E. faecalis* was undertaken in order to identify possible future sources of antibacterial drugs. These evaluations will assist in better understanding and in the use of traditional medicine. It will also help in the identification of possible future sources of antibacterial drugs. Once the results are available the traditional healers will be trained to extract the plants using the method in this study.

The aim of this study is to evaluate the interaction between ethanolic extracts of *P. guajava* (ugwava) and *S. frutescens* alone and then synergy testing of these extracts with known penicillins using both disc diffusion and microdilution method on *S. aureus* and *E. faecalis.* Essential oils from leaf extracts of *P. guajava* was demonstrated to contain antimicrobial active compounds against *S. aureus* (Biswas, 2013) and the hexane, dichloromethane and ethylacetate extracts of *S. frutescens* have been shown to be antibacterially active against *S. aureus (*Keterere & Eloff, 2005).

The first objective was to determine zones of inhibition produced, MIC and minimum bactericidal concentration (MBC), of *P. guajava* and *S. frutescens* extracts on *S.*

aureus and *E. faecalis.* The second objective was to compare the antibacterial activity of water and ethanolic extracts for *P. guajava and S. frutescens*. The third objective was to determine the synergistic effects of plant extracts (*P. guajava and S. frutescens)* and penicillins on *S. aureus* and *E. faecalis*.

It was hypothesised that there is a synergistic effect of plant extracts (*P. guajava and S. frutescens)* and penicillins on *S. aureus* and *E. faecalis*.

CHAPTER 2: LITERATURE REVIEW

2.1. Introduction

There has been a extensive investigation of secondary plant metabolites in the recent past. These plants were previously with unidentified pharmacological activities to serve as basis of antibacterial agents (Singh & Kumar, 2013). Therefore, it is predicted that secondary plant metabolites with satisfactory antimicrobial efficiency can be utilised for the cure of infectious diseases (Pakekh & Chanda, 2007). Since ancient times, man has used numerous fragments of plants in the therapy and prevention of several illnesses (Munuswamy *et al.,* 2013).

2.2. Problems of antibiotic resistance

The challenge of resistance of bacteria to antibiotics started a long time ago. It spread out to the past and it exposes the attack and counter attack of multifaceted bacterial flora so that ecological niches and survival can be established. Failures of treatments with antimicrobials signify a major clinical problem. This is due to additional classes of agents, having diverse cellular targets that are becoming available. Presence of numerous drug resistances is bringing about huge difficulties in the management options of infectious diseases today. A number of factors such as extended-spectrum agents and developments in remedial procedures such as organ transplantation and cancer chemotherapy drove this state of affairs. The consequence has been an enormous discriminatory pressure in favour of numerous resistant strains. When comparing the 30 years to the 20-year period following the Second World War, there has been a reduction in the establishment of antimicrobials that act on new cellular targets. The resilient organisms causing worry in the midst of

Gram positive organisms currently are glycopeptides intermediate sensitivity *S. aureus* (GISA), methicillin resistant *S. aureus* and *Staphylococci epidermidis*, penicillin-resistant *Streptococcus pneumoniae* and vancomycin-resistant *Enterococcus* (VRE) species (Revira & Boucher, 2011). Difficulties amongst Gram negative organisms include bacteria such as multidrug-resistant *Pseudomonas aeruginosa,* members of the *Enterobacteriaceae* with extended-spectrum Beta-lactamases, *Acinetobacter baumannii* and *Stenotrophomonas maltophilia* to mention a few (Mattner *et al*., 2012).

There are other micro-organisms that are developing resistance to antibiotics. The contributing factor is that drugs are abused by users. Treatment has to be altered to second-or third-line drugs when infectious diseases become resistant to first choice or first line antibiotics and these are almost always costly (Rivera & Boucher, 2011).

In countless countries in developing states, the high cost of such substitution of drugs is difficult to get a hold of, with the outcome that a few ailments can never again be dealt within territories where resistance to first-line drugs is far reaching (Ventola, 2015). The disturbing difficulties facing doctors and drug specialist now, are the need to create elective methodologies notwithstanding the hunt of new antimicrobial mixes (Sibanda & Okoh, 2007). Plants may give an answer for battling the issue of antibiotic resistance.

2.3. Antibiotic resistance mechanisms in pathogenic bacteria

Enzymatic inactivation of the medication (Wright, 2005), adjustment of target locales (Alekshun & Levy, 2007), decreased cell take-up (Hajipour *et al*., 2012) and

exclusion by efflux (Alekshun & Levy, 2007) are the four primary mechanisms that bacteria use to create resistance to antibacterial agents. The reports have demonstrated that chemical alterations could be critical in antimicrobial resistance, nevertheless elimination from the cell of unaltered antibiotic describes the main means in dismissing the antimicrobial access to its objectives and this is thought to enhance resistance even in events where modification is the key mechanism (LaRock & Nizet, 2015).

2.3.1. Alteration of target site

Decreased liking of the antimicrobial agent to its coupling site might be the consequence of chemical modification of target site (LaRock & Nizet, 2015). This mechanism is utilized by various pathogens in beating the impact of antimicrobial agents and is usually facilitated by enzymes. Methylation of the N6 amino group of an adenine residue in 23S rRNA is the instrument that is utilized by *Streptococcus* species to end up resistant for example to macrolides, lincosamide and streptogramin B antibiotic agents. Beta-lactams antibiotics work by binding to and hindering the biosynthetic activity of Penicillin Binding Proteins (PBPs), along these lines blocking cell wall synthesis. Due to target site adjustment this may not happen along these lines rendering the antibiotic in-effective.

2.3.2. Enzymatic inactivation

The approach utilized by various pathogenic microbes in getting away from the impact of antimicrobial agents is the making of hydrolytic enzymes and group transferases (Wright, 2005). This occurs at a genetic level. Genes that code for antibiotic degrading enzymes are generally carried on plasmids and other hereditary

components. The resistance to Beta-lactam anti-microbials by both Gram negative and Gram positive microorganisms have for quite some time been ascribed to Beta - lactamases (Chishimba *et al*., 2016). These enzymes present critical antibacterial resistance to their bacterial hosts by hydrolysis of the amide bond of the four membered-lactam ring (Wilke *et al*., 2005; Drawz & Bonomo, 2010). Resistance to aminoglycosides in Gram negative microorganisms is frequently mediated by a variety of enzymes that change the antibiotic molecule by acetylation, adenylation or phosphorylation (Ramirez &Tolmasky, 2010).

2.4. Role of ethnopharmacology in the treatment of bacteria

Traditional medicine is used world-wide and has a quickly growing economic significance. In third world countries, herbal medicine is frequently the most reachable and inexpensive cure available. In Africa, a large percentage of people use herbal treatments as the primary health care system (Benzie & Wachtel-Galor; 2011).

National governments and health researchers are giving careful consideration on natural solution. As of late Peru's national program in complementary medicine and the Pan American health organisation analyzed alternative medicine in health care centres and hospitals inside the Peruvian social security system (Benzie & Wachtel-Galor, 2011; Mahomoodally, 2013).

For a huge number of years, plants have been utilized as a part of natural prescription (Abu– Rabia, 2005; Idu *et al*.,2009; Hosseinzadeh *et al*., 2015).The data about medicinal plants has been accumulated over the span of numerous times in

view of different therapeutic framework. There has been a developing consideration in the investigation of therapeutic plants and their conventional use in different parts of the world during the past few eras (Murthy, 2015). There are generous monetary advantages in the utilization of medicinal plants for the administrations of various infections (Azaizeh, 2003).

Most of the rural communities are obligated to consult traditional healers for their common everyday diseases because of inaccessibility to modern health facilities, poverty and poor communication means. These populations constitute the poorest connection in the trade of medicinal plants. A gigantic measure of data on the most proficient method to utilize the plants against various ailments might be expected to have accumulated in regions where the utilization of plants is of extraordinary essentialness (Xavier *et al*, 2015). In the first world countries, 25 % of the therapeutic medications are established on plants and their by-products (Tabasum & Khare, 2015)

Individuals from the World Health Organization specialists, who met in Congo, Brazzaville in 1976, needed to portray customary African medicine as the amount of practices, measures, ingredients and techniques of various types whether material or not, which has allowed the African to guard against illnesses to ease his/her torment (WHO, 2010). Conventional therapeutic information in plants and their practice by local societies are important for community health care and medication improvement in the present and future (Saha *et al*., 2014).

A variety of scientific scholars have assessed and confirmed the anti-typhoidal activities of phytochemicals (herbs and plants) and the future prospects are promising. Aliero & Wara (2009), assessed the effectiveness of *Leptadenia hastate* (*L. hastate*) extracts against fungi and bacteria. Water extracts significantly inhibited the growth of *Salmonella paratyphi*, *Escherichia coli (E. coli)* and *Pseudomonas aeruginosa*. These outcomes have afforded scientific evidence for the much commended ethno-pharmacological efficiency of aqueous extracts of *L. hastate* in the treatment of bacterial ailments and are suggestive of the likely potential of the plant methanol extract as a promising source of antifungal agent.

Evans *et al*., (2002), assessed the capacity of *Euphobia hirta; Citrus aurantifolia, Cassia occidentalis*, and *Cassia eucalyptu* that is guaranteed by the Nupes tribe of Nigeria to be powerful in the eradication of typhoid fever. Antimicrobial resistance and the possibilities of medicinal plants in the administration of *Salmonellosis* investigation affirmed that, only *Cassia eucalyptus* had inhibited *Salmonella typhi* growth. They established the effectiveness of the plant, and that the contained active natural compounds are useful for the treatment of typhoid fever.

2.5. Plants as sources of new antimicrobials and resistance modifying agents

Plants have traditionaly given a source of hope for novel drug compounds, as plant natural blends have made substantial contributions to human wellbeing and health (Iwu *et al*., 1999; Selvamohan *et al*., 2012; Das *et al*., 2014). Owing to their popularity and general therapeutic use for various infectious diseases, investigations for plants with antimicrobial activity are now recurrent (Shibata *et al*., 2005; Betoni *et al*., 2006; Bhalodia & Shukla, 2011; Tabassum *et al*, 2013).

Plants consist of a variety of bioactive metabolites, such as terpenoids, tannins, flavonoids, and alkaloids. These have been found, *in vitro,* to possess antimicrobial activity (Lewis & Ausubel, 2006). Literature has shown a number of compounds from a variety of medicinal plants. Regardless of this abundant literature on the antimicrobial properties of plant extracts, none of the plant inferred chemicals have effectively been investigated for clinical use as antibacterial agents (Dahiya & Purkayastha, 2012).

The diverse chemicals formed by herbs and plants, is grasped to secure plants against pathogenic microbes. A wide range of these phytochemicals often classified as antibacterial, produce MIC above 1,000 µg/ml, which from a clinical perspective are of no relevance (Tegos *et al*., 2002; Sibanda & Okoh, 2007; Aliero & Ibrahim, 2012). Furthermore, they proposed that a dominant part of plant mixes with negligible *in vitro* antibacterial action are not really antimicrobial, rather they possess an indirect role in the plant defence against bacterial infections.

Many researchers have confirmed that generally plant derived compounds have minimal efficiency in comparison to commercially available anti-fungal and antibacterial antibiotics. However, the very same compounds have been proven to display a significantly increased activity against Gram positive bacteria compared to Gram negative species (Aliero & Ibrahim, 2012).

Moreover this evidence resulted in Tegos *et al.,* (2002), theorising that herbs and plants yield compounds assumed to be effective antimicrobials when they gain entry into the barrier double membrane of Gram negative bacteria (Sibanda & Okoh,2007; O'Bryan *et al.,*2015).

2.6. Major groups of antimicrobial compounds from plants

2.6.1. Phenolic compounds

Phenolic compounds encompass an extensive variety of plant constituents which have an aromatic ring that bears a hydroxyl substitute (Harbone, 1984; Nsele, 2012; Nwanna *et al*., 2013). They are located within the cell vacuole, and dissolve in water since they exist, bound to sugar. Harbone (1984), further reported that among the natural phenolic compounds, flavonoids form the major cluster, but simple monocyclic phenols, (phenol propanoids and phenolic quinines) exist in sizeable quantities (Jain *et al*., 2013; Działo *et al*., 2016; Sahelian, 2016).

In several occurrences, these substances safeguard components of plants against predation by microbes, insects and herbivores (Marjorie, 1999; War *et al*., 2012; Fürstenberg-Hägg *et al.,* 2013). Terpenoids provide plants their smells; while others (tannins and quinines) give plant colouring. Aromatic compounds give plant flavour (Nsele, 2012; Redondo *et al*., 2014).

2.6.2. Simple phenols and phenolic acids

Phenols, occasionally refered to as phenolics, are a class of synthetic mixes comprising of a hydroxyl useful gathering (- OH) joined to a fragrant hydrocarbon gathering. The easiest of the class is phenol ($C6H_5OH$). A few phenols are germicidal and are utilized as a part of formulating disinfectants (Nsele, 2012; Prasad, 2015; Sahelian, 2016). The parent compound phenol is utilised for decontamination as well as chemical synthesis (Ball *et al*., 2016). Propolis is an emerging natural remedy that has maintained popularity for a long period (Deswele *et al.,* 2016). The propolis, flavonoids, phenolic acids and their esters are known to be pharmacologically useful particles (Huang *et al*., 2014).

Together these segments have numerous effects on bacteria, fungi and viruses. In addition, propolis possesses anti-inflammatory and immunomodulatory activities. Moreover, propolis has of late been discovered to lower cholesterol and blood pressure levels. Be that as it may, information of clinical investigations to learn and substantiate these cases is inadequate. Coffee consists of bound phenolic acids (caffeic acid, ferulic acid, and P-coumaric acid) (Huang *et al*., 2014). Spices have phenolics that contribute to food flavour, taste and medicinal properties. The phenolic acids contained in spices are tannic, gallic, caffeic, cinnamic, chlorogenic, ferulic and vanillic acids. Black mustard and clove has a high amount of tannic and gallic acids. Cumin has a high concentration of caffeic, chlorogenic as well as ferulic acids. Lastly onion seeds have vanillic and cinnamic acids (de Oliveira *et al*., 2014).

Another phenolic compound is salicylic acid which is an instigator compound for aspirin (Montenegro *et al*., 2009). This compound is routinely utilised for pain alleviation in headaches as salicylate. Headache medicine is orally taken and is

quickly ingested at the acidic pH of the stomach. Slower absorption is seen with other formulations due to the rate limiting step of tablet disintegration, this latter factor being maximal in alkaline pH (Yahya & AL-Dabbagh, 2012). The rate of aspirin absorption is dependent on both the formulation and the rate of gastric emptying. The half-life of aspirin ranges from five to sixteen minutes and absorption follows first-order kinetics. Aspirin is hydrolysed in the liver and some in the stomach by nonspecific esterases to salicylic acid, so just 68 percent of the measurements achieve the systemic flow as headache medicine (Nordt *et al*., 2011; Sahelian, 2016).

Albumin is the protein that binds and carries aspirin and salicylic acid in serum and both are disseminated to the joints, central nervous system, and saliva (Sahelian, 2016). Aspirin in serum has an approximated half-life of twenty minutes. The serum aspirin concentration will decline as the salicylic acid concentration increases in the circulation (Holford, 2012). Salicylic acid is excreted through the renal system, and the elimination rate is subject to the urinary pH, whether there are organic acids or not, and the glomerular filtration output (Nsele, 2012).

2.6.3. Benefit of *phenols*

Phenols are compounds generally spread all through the plant kingdom. They are important for the propagation and development of plants, and are created as a reaction for shielding injured plants against pathogens. The latest awareness in phenolic acids arises from their protective role, through consumption of vegetables and fruits, against a wide range of oxidative damage diseases (cancers, stroke and coronary heart disease) (Aleksic & Knezevic, 2014; Sahelian, 2016; Xu *et al*., 2016).

The bioavailability and absorption of phenolics in humans are controversial. There is minimal amount of data available which highlights the necessity for all-encompassing investigations of the behaviour of phenolics in the digestive system and subsequent metabolism and systemic uptake (D'Archivio *et al.,* 2010; Sahelian, 2016). Phenolic plant compounds are not only assorted in physical arrangement, additionally they are secondary metabolites with an aromatic ring that is hydroxylated (Nelson and Raul, 2017). Nevertheless the functional role of phenolic acids in plants is not well understood but they may be polymerised into larger molecules (Działo *et al.,* 2016; Sahelian, 2016). Moreover, phenolic acids occur in food plants as esters or glycosides conjugated with other natural compounds such as flavonoids*,* alcohols, hydroxyl fatty acids, sterols, and glucosides (Kabera *et al*., 2014; Sahelian, 2016).

Hydroxylated phenols such as catechol, pyrogallol, and plants such as thyme and tarragon have a phenolic acid known as caffeic acid which has generally shown to be antimicrobial (Marjorie, 1999; Arif *et al*., 2011, Sahelian, 2016). Some authors have found that more highly oxidized phenols are inhibitory (Sahelian, 2016). The phenol microbial toxicity actions includes enzyme inhibition owing to the oxidized compounds, possibly through reaction with sulfhydryl groups or through more non-specific interactions with the proteins (Naza *et al*., 2006; Nasrin *et al.,* 2012). Phenolic compounds possessing a C_3 side chain which is often cited as antimicrobial. This C_3 side chain is at a lower level of oxidation (Kumar & Pandey, 2013).

2.7. Terpenoids and essential Oils

These highly enriched molecules from isoprene structures are oils which are secondary metabolites that are responsible for plant fragrance termed terpenes (Marjorie, 1999; Aleksic & Knezevic, 2014). They are hydrocarbons made by plants (Aleksic & Knezevic, 2014). If they bring an element, such as oxygen, they are termed terpenoids; routine examples are Camphor (monoterpes), farnesol and Artemisin (sesquiterpenoids). A routine example is methanol.

Just like fatty acids they are made from acetate units (Marjorie, 1999; Upadhyay *et al*., 2014). But the distinction from fatty acids is that compounds are cyclized and broad branched. These compounds are known to posess antibacterial properties. (Aleksic & Knezevic, 2014). The speculated terpenes mode of action is membrane disruption (Marjorie, 1999; Jasmine *et al*., 2007). Nutritional researchers discovered that *Listeria monocytogenes* may be controlled by the terpenoids contained in the essential oils of plants (Jasmine *et al*., 2007; Pandey *et al*., 2014).

2.7.1. Therapeutic benefits of terpenoids and essential Oils

The consumption of aromatic herbs and other dietary supplements can supply the body with essential oils. There are a number of specific dietary sources of essential oils, such as orange and citrus peel, caraway, dill; cherry, spearmint, black pepper and lemongrass. Human exposure to essential oils through the diet or environment is widespread. Essential oils may be absorbed from food or as pure products and they are able to cross the blood brain barrier easily. This property is due to the lipophilic character of volatile compounds and their small size. The action of essential oils occurs by entering the human body via three possible ways including

direct absorption through inhalation, ingestion or diffusion through the skin tissue (Aleksic & Knezevic, 2014).

2.7.1.1 Absorption through the skin

Since essential oil compounds are fat soluble, they are able to permeate the membranes of the skin before being captured by the micro-circulation and drained into the systemic circulation, which reaches all target organs (Adorjan & Buchbauer, 2010).

2.7.1.2 Inhalation

Essential oils also enter the body by inhalation. Because they are volatile, they can be inhaled easily through the respiratory tract and lungs, which then distribute them into the bloodstream (Moss *et al*., 2003). The respiratory tract is the most rapid way of entry followed by the dermal pathway.

2.7.1.3 Ingestion

Ingested essential oil compounds and/or their metabolites may be absorbed and delivered to the rest of the body by the bloodstream and then distributed to all parts of the body. Once essential oil molecules are in body, they interact with physiological functions by three distinct modes of action:

- Biochemical (pharmacological): Interacting in the bloodstream and interacting chemically with hormones and certain enzymes.

- Physiological: By acting on specific physiological function. For example, the essential oil of fennel contains a form of oestrogen-like compounds that may be effective for female problems such as lactation and menstruation.
- Psychological: by inhalation, the olfactory area of the brain (limbic system) undergoes an action triggered by the essential oil molecules and then, chemical and neurotransmitter messengers provide changes in the mental and emotional behaviour of the person (Johnson, 2011; Shibamoto *et al.,* 2010).

2.8. Flavones, flavonoids and flavonols

These are phenolic structures bound to a single carbonyl group. When a 3-hydroxyl group is added, it then forms a flavonol. They are hydroxylated phenolic compounds that occur as a C_6-C_3 unit that is linked to an aromatic ring (Marjorie, 1999). These plant synthesized compounds combat microbial infection (Dixon & Lamb, 1983). Ample *in vitro* evidence reveals them to possess an antimicrobial activity for a variety of microbes. This action is most likely attributed to their capability to form complexes with the cell walls of bacteria, and disrupting microbial cell membranes as described for quinines (Marjorie, 1999).

Catechins, a flavonoid compound is contained in oolong green tea (Marjorie, 1999; Chang *et al*., 2016). They are the reason tea is known to possess antimicrobial activity (Marjorie, 1999; Jin *et al*., 2006). These phytochemicals have been discovered *in vitro* to suppress the growth of the causative organism for cholera (*Vibrio cholera*) (Borris, 1996) as well as *Streptococcus mutans* (Batista *et al.,* 1994; Jin *et al*., 2006). Plants consist of a considerable variety of *flavonol* glycosides.

Above 200 varied glycosides of quercetin, with quercetin 3 rutinose (rutin) being the most common (Harborne, 1984; Kelly, 2011). Flavones occur as glycosides yet the scope of diverse glycosides is less than in case of flavonols. Various examinations found that the derivatives of flavone are inhibitory to respiratory syncytial infection (RSV) (Barnard *et al*., 1993; Chang *et al*., 2016; Kaul *et al*., 1985; Ghanadian *et al*., 2012).

2.8.1. Quercetin

Quercetin is the most prevalent flavone that serves as the pillar for other flavonoids, and is the most dynamic of the flavonoids (Ikan, 1991; Kelly, 2011). A number of curative medicinal plants have substantial quercetin content (Chang *et al*., 2016). Quercetin instigates the wheat germination by conducting itself like auxins. The possible function of this colouring matter in insect-pollinated flowers and edible fruits is to make these organs more conspicuous in order to aid seed dispersion by animals (Ikan, 1991; Chang *et al*., 2016). Small amounts of quercetin that function as heart stimulant are used to help support frail capillary blood vessels (Ikan, 1991; Chang *et al*., 2016).

2.8.2. Catechin

Flavonoids may join to yield other monomeric flavonoids such as catechin. These are yellow in colour and are able to formulate compounds which are bonded and organized in various configurations with very diverse physical characteristics (Nsele, 2012). Dimers are formed when two of the catechins are bonded, a trimer for 3 proceeding up to oligomers and polymers termed proanthocyanidis, or condensed

tannins. Oligomeric proantho cyanidins are oligomeric flavonoids which are routinely synthesised by the bark of pineapple trees (Wijesekera, 1991; Nirengi *et al*., 2016).

Catechin easily binds to proteins, making them inaccessible to microorganisms. This compound decreases the serum cholesterol levels. It further prevents the circulation of the unhealthy LDL cholesterol which is derived from oxidation. LDL is unhealthy because it accumulates in blood vessels hindering their constriction (Nirengi *et al*., 2016). Moreover the benefit of catechin has been reported by scholars in management and treatmentof immune-suppressive substances and agents (Wijesekera, 1991; Chacko *et al*., 2010). It is antibacterial, antiviral, an antioxidant and prevents influenza (Nirengi *et al*., 2016).

2.8.3. Benefits of flavones, flavonoids and flavonols

Fruit and vegetables (F&V) intake is a major source of flavonoid consumption. Fruits and vegetables (F&V's) intake has beneficial health effects including reduced risk to different types of cancers (Krupp *et al*., 2016). Catechin is not only an antioxidant but effectively suppresses the flourishing of bacterial and virial species, for instance it prevents influenza (Nirengi *et al*., 2016). Added to these, convincing experimental evidence indicates that the growth hormone insulin like growth factor axis itself can be influenced by dietary flavonoids; this is important because this growth hormone insulin like axis has been linked to insulin metabolism and an increased risk of malignancy (Krupp *et al*., 2016). Moreover quecertin can be used to fortify fragile capillary blood vessels (Ikan, 1991; Chang *et al*., 2016).

2.9. Tannins

Tannins have the ability to combine with protein, yielding stable water insoluble co-polymers (Harbone, 1984; Jain *et al*., 2013). They are plant derived, which through their capacity to cross-connect with protein are fit for changing crude animal skin into leather. Plant tissues high in tannin content confer an astringent taste, which goes about as a deterring hindrance to most feeders (Jain *et al*., 2013).). These compounds are isolated into two, hydrolysable and consolidated tannins. Hydrolysable tannins depend on gallic acid, ordinarily as different esters with D-glucose while the consolidated tannins are gotten from flavonoid monomers (Marjorie, 1999; Samy & Gopalakrishnakone, 2010). Tannins consist mainly of gallic acid residues which are linked to glucose by means of glycosidic bonds (Negri & Tabach, 2013).

Tannins may be derived from polymerisation of quinone units. Endless human physiological utilities have been assigned to tannins, for example, have intervened tumour action, incitement of phagocytic cells, and an extensive variety of anti-infective activities. Tannins may be designed by polymerisation of quinone units. Countless human physiological activities have been assigned to tannins, such as host mediated tumour (Haslam, 1996; Pandey & Kumar, 2013). Enzymes inactivation, microbial adhesions and cell envelope transport proteins, and ability to form complexes with polysaccharides have been speculated to be the mode of action of tannins. Moreover there is scientific proof in support of the ability of tannins to inactivate micro-organisms. Condensed tannins have been found to bind cell wall of bacteria, counteracting development and protease action (Jones *et al*., 1994; Mohanraj, 2014).

2.9.1. Benefits of tannins

The tannins are phytochemicals prevalent in fruits (grapes, blackberries and cranberries), certain natural products and herbal preparations, tea, coffee, cocoa used to make chocolates as well as red wine. The majority of the tannins are useful for prevention against tooth decay and cavities, loose bowels, and some even offer preventative means for heart illnesses and malignancy. These compounds will inhibit bacterial growth and prevent plague build-up in the buccal cavity of humans, they forestall tooth rot yet they recolour and stain teeth (Nsele, 2012). This effectiveness is attributed to the astringency property. It is responsible for the sour taste usually felt while eating improperly ripened fruits. When bound to salivary proteins they create a rough, sensation of the mouth which resembles sandpaper, these are believed to protect the plants against predations, hence they act like pesticides (Nsele, 2012).

Tannins also bind some minerals, including iron-particularly the iron in plant foods. (Nsele, 2012). In groups at risk of iron deficiency the advice should be to drink tea between meals and to wait a minimum of an hour after eating before drinking tea. The addition of milk or lemon to tea helps to reduce the effect of tannins. The tannins bind to anything that is added to it

The term tannin frequently confounds individuals, as there are singular tannins which vary significantly. The vast majority of them are polyphenols, while, some others are antioxidants. Antioxidants shields from heart illnesses and they avoid cancer by averting cell harm. Since they are notable for their astringent property, they have been utilized as a base for a few home grown medicines. Some clinical specialists suggest the utilization of some tannin-containing herbs keep away certain ordinary illnesses (Nsele, 2012; Sahelian, 2016)

2.10. Quinones

Quinones contain aromatic rings (Marjorie, 1999; Lu *et al.*, 2013). Being universal in nature they are highly reactive. Although they are extensively spread and display pronounced physical dissimilarity, they make generally little contribution to colour in higher plants (Harbone, 1984; Lu *et al.*, 2013). They are found in bark, roots, or in leaves where their shades are covered by other colours (Nsele, 2012; Lu *et al.*, 2013). These phytochemicals, being shaded in colour, are responsible for browning response in cut or harmed vegetables as well as fruits. They are likewise linked in the melanin biosynthesis pathway of the human skin (Nsele 2012; Lu *et al.*, 2013). Quinones offer a wellspring of unfaltering free radicals and are recognized to form complexes that are irreversible with nucleophilic amino acids in proteins (Stern *et al.,* 1996; Lu *et al*, 2013).

Quinones frequently prompt inactivation of protein and loss of capacity. It is for this reason, that the potential scope of quinone antimicrobial impact is incredible. The possible targets could be that quinones focus in the microbial cell, surface-uncovered bonds, and layer bound chemicals (Marjorie, 1999; Lu *et al.*, 2013). Quinones may likewise decrease substrates inaccessible to the microorganisms (Rietjens *et al*., 2016).

2.10.1. Benefits of quinones

A number of these phytochemicals like genistein have clearly defined health applications such as cancer therapy, however others are poisonous. For instance, genistein prevents breast cancer; on the contrary, extended contact to chemically

similar compounds or oestrogens is linked with high occurrence of breast cancer. The catechol oestrogen quinones utilises the redox cycling or depurination to modify the cellular DNA (Rietjens *et al*., 2016).

2.11. Types of herbal extracts

There are different types of plant and herb extracts that can be formulated using diverse methods of extraction of the product. Extraction methods are classified into water-based, alcohol based vinegar, glycerine and fat based. The frequently utilised extraction solvents are water and alcohol.

2.11.1. Alcohol based tinctures

Tinctures are conventionally made by employing the herb in an organic solvent and allowing it to soak for weeks. An alternative and quicker method is for the solvent to be infiltrated through the herb (Werbach & Murray, 2002; Ahmad *et al*., 2013). For marketable preparations, an alcohol solvent is the most valuable, frequently ethyl alcohol (ethanol). In the manufacture of an extract, depending on the pharmacopoeia and monograph followed, the raw herb is immersed in an alcohol-water mixture for 2-4 weeks. The fluid is then strained to isolated tincture from the herb (Ahmad *et al*., 2013). A water-alcohol mixture safeguards the extraction of both the water soluble and alcohol soluble ingredients. Alcohol–based extracts are frequently stronger than infusions as alcohol can extract ingredients that are water–insoluble (Rotblatt & Zimet, 2002; Ahmad *et al*., 2013). Alcohol is also an appropriate effective natural preservative. Because a tincture is effortlessly embraced by the body, it is a very effective method to administer herbal compound. Tinctures are resolute and economical (Rotblatt & Zimet, 2002) and they have a longer shelf life.

2.11.2. Water based extractions

There are massive amounts of writing and logical examinations at present existing on herbal tinctures under general. Nonetheless, writing and logical examinations on water-based extractions are as of now insufficient (Ahmad *et al*., 2013). That is the reason water-based extractions are an exceptionally under-used asset.

Water-based extractions are set up as infusions or decoctions. For sensitive plant parts, for example, leaves, flowers, delicate stems and fruits, infusions are utilized. The herbal material is situated in a suitable vessel and boiling water is administered over. The mixture is soaked for 5 to 10 minutes. Decoctions are for the most part more concentrated than infusions, and the technique is significant for fibrous plant material, for example, bark, roots, and stems (Sultana *et al*., 2009). The herbal material is situated in a container, enclosed in cold water and conveyed to boil inorder to make decoctins. It is covered and permitted to simmer for 5 to 10 minutes (Rotblatt & Ziment, 2002; Sultana *et al*., 2009).

For the making of an infusion, the plant material and water is allowed to remain at room temperature overnight before being strained off. This is significant in occasions where there are various volatile oils that might be lost if heat is utilized. In both these cases, the water executes as a solvent to extract those constituents that are soluble in water. It might be appropriately applied to extrcat tannins and glycosides, yet is not sensible for the extraction of resins, volatile and non-volatile oils or alkaloids (Thakur *et al.,* 2011).

Water-based extracts are the safest kind of extracts, since toxic alkaloids are generally not soluble in water. Since they have a short timeframe of realistic usability because of bacterial contamination, they should be stored at a low temperature and thrown out following a couple of days. They are also not easy to standardise and are frequently bitter tasting except when flavour additives are added (Rotblatt &Zimet, 2002; Mapfunde *et al*., 2016). Circumstances exist where water-based extractions are more required than conventional alcohol tinctures. One such condition is where alcohol consumption is forbidden by religion. The use of alcohol-containing medicines is also objectionable for use in children and pregnant women as it is not known what the safe level of alcohol consumption is throughout pregnancy (ADF, 1998). Alcohol use throughout pregnancy may lead to foetal alcohol syndrome.

Water-based extractions have procedural advantages over alcohol based tinctures regarding antibacterial investigations. Research at Pretoria University by the Faculty of Medicine Research Committee expressed that if an extract is to be analyzed for anti-microbial properties, the extract ought not to hinder the bioassay techniques (Nsele 2012). Alcohol itself has antibacterial properties; consequently, antimicrobial investigations by methods for alcohol based tinctures can be incompetent in finding the antibacterial properties of the definate plant substance. The alcohol control should always be incorporated when conducting such examinations (Singh, 2004b).

2.11.3. Other Methods of Herbal Extractions

2.11.3.1. Vinegar

Vinegar is also refered to as acetrata. Vinegar is a sensibly decent solvent; however, the timeframe of realistic usability is just around three months (Singh, 2004b). As a result of the unfriendly taste, the medicine is generally blended with honey. It might be important while managing herbs to a young child with suppressed liver function since vinegar is exceptionally delicate on the body (Singh, 2004b).

2.11.3.2. Glycerine

Glycerine is also termed glycerata or glycerol. Glycerine is a colourless, unscented and gelatinous fluid with solvent capabilities somewhere between alcohol and water. A glycerata is frequently used to preserve fresh expressed plant juices and to make syrup (Singh, 2004b). It has a sweet taste and the shelf life is 6 to 12 months.

2.11.3.3. Fat extractions

Constituents that are fat or alcohol dissolvable e.g. gums, resins, volatile oils, waxes and alkaloids, might be extracted utilizing fat as a solvent. The two techniques utilized are as per the following:

1. Enfleurage: Fresh plant material (normally flowers) is positioned over a layer of fat with a low boiling point (e.g. cocoa butter) and left for three days at room temperature. A mild organic solvent is then utilized to extract the plant component from fat (Singh, 2004b).

2. Absorption:

This is set up similarly to effleurage, however the fat is warmed to around 35 ^{o}C and kept up at that level for various hours to a couple of days. The warm oil separates the plant material and draws out the fat-soluble constituents.

2.12. Penicillins

Penicillins are a group of antibiotics that are derived from *Penicillium* fungi. They include the following drugs viz. penicillin G, procaine penicillin, benzathine penicillin, and penicillin V. Penicillin antibiotics are usually effective Beta-lactama antibiotics that prevent the synthesis of peptidoglycan cross-links in the bacterial cell wall. Penicillins are routinely used, despite numerous microbes which have developed resistance. These Beta-lactam antibiotics are ordinarily utilised for management of bacterial infections usually caused by Gram positive organisms. However, the hydrolysis of the antibiotic by a Beta- lactamase enzyme has been the primary reason for resistance (Shaikh *et al*., 2015).

Penicillins are usually used to refer to benzylpenicillin (penicillin G), procaine benzylpenicillin (procaine penicillin), benzathine benzylpenicillin (benzathinepenicillin), and phenoxymethylpenicillin (penicillin V). Although the antibacterial activity of procaine penicillin and benzathine penicillin is similar, benzylpenicillin remains active for longer periods of time. On the contrary Phenoxymethylpenicillin has very minimal utility against Gram negative bacteria in comparison to benzylpenicillin (Garrod, 1960; Bhattacharya, 2010). Phenoxymethylpenicillin may be administered orally but Benzylpenicillin, procaine

penicillin and benzathine penicillin are all administered intramuscularly (Ventola, 2015).

2.12.1. Penicillin G

Penicillin G is routinely used for the treatment and management of syphilis, meningitis, endocarditis, pneumonia, lung abscesses and septicaemia in children via intravenous or intramuscular means (Rossi, 2013; Stamm, 2016). A parenterally injection of penicillin G is purposely given to avoid the stomach because it is unstable in the exceedingly acidic environment of stomach. Thus, greater tissue concentrations of penicillin G can be attained than is likely with phenoxymethylpenicillin. These higher concentrations translate to increased antibacterial activity (Ogawara, 2015).

This antibiotic is effective against Gram positive organisms. A few Gram negative organisms (*Neisseria gonorrhoeae* and *Neisseria meningitidis)* are also susceptible (Ogawara, 2015). Adverse effects include hypersensitivity reactions such as urticaria, fever, joint pains, rashes, angioedema, anaphylaxis, serum sickness-like reaction. When overdosed the characteristic features noted are related to the central nervous system (CNS) toxicity with convulsions seen especially in severe renal impairment. Furthermore, diarrhoea including colitis can occur. Studies report interstitial nephritis, haemolytic anaemia, leucopeania thrombocytopeania and coagulation disorders (Ogawara, 2015).

The serum levels of this antibiotic can be monitored by the process of therapeutic drug monitoring using traditional microbiological assay or chromatographic techniques (Roberts *et al*., 2012). This is particularly vital in avoiding the above

mentioned adverse effects in chronically ill patients receiving high doses; predominantly. They are more relevant to patients with renal failure. These patients have a tendency to accumulate the drug due to reduced urinary excretion rate (Ogawara, 2015).

2.12.2. Procaine penicillin

This antibiotic is made up benzylpenicillin and procaine (a local anaesthetic agent) utilised for various bacterial infections. Post intramuscular administration it is gradually taken up into the system and hydrolysed to benzylpenicillin, thus it is used where prolonged low concentrations of benzylpenicillin are required (Stamm, 2016).

The goal of this combination is to alleviate pain and discomfort associated with a large intramuscular injection of penicillin. Procain penicillin is used for diseases such as syphilis, respiratory tract infections, *Streptococcus* throat infections, cellulitis, erysipelas and anthrax. Respiratory tract infections where compliance with oral treatment is unlikely, alongside pen V and erythromycin, it is used to treat *Streptococcus* throat infection (Stamm, 2016), given as one intramuscular injection. At high doses procaine penicillin can cause CNS abnormalities e.g. seizures (Moulton & Koychev, 2015).

2.12.3. Benzathine Penicillin

Benzathine benzylpenicillin is commercially known as Bicillin, it is also an antibiotic useful for the treatment of various bacterial infections (Riedner *et al*., 2005). These range from prevention of rheumatic fever and early or latent syphilis. It is absorbed into the circulation gradually, after intramuscular injection, and hydrolysed to benzylpenicillin *in vivo* (Riedner *et al.,* 2005). It is most preferred when prolonged low

concentrations of benzylpenicillin are required and appropriate; allowing prolonged antibiotic action over 2–4 weeks after a single intramuscular dose (Riedner *et al*., 2005). Benzathine benzylpenicillin may cause seizures and CNS abnormalities when overdosed.

2.12.4. Penicillin V

Penicillin V is an orally active antibiotic, although it has a range of antimicrobial activity against Gram positive bacteria, it has shown to be less effective than benzylpenicillin (penicillin G) against Gram negative bacteria (Garrod, 1960; Dhotre *et al*., 2016). However, it is more acid-stable, which allows for the oral administration. It produces a bactericidal action against penicillin-sensitive bacteria during the stage of active multiplication. It acts by inhibiting the biosynthesis of cell-wall peptidoglycan. Be that as it may, this antibiotic isn't effective against Beta-lactamase-producing bacteria, which include MRSA (Stapleton & Taylor, 2002; Dhotre *et al*., 2016).

Phenoxymethylpenicillin is usually used only for the treatment of mild to moderate infections but not for severe or deep-seated infections because its absorption cannot be predictable. Besides treatment or prevention of infection with *Streptococcus pyogenes* which is commonly sensitive to penicillin, treatment should be guided by sensitivity testing and by clinical response. Patients treated initially with parenteral benzylpenicillin may continue oral treatment with phenoxymethylpenicillin once a satisfactory clinical response has been obtained (Dhotre *et al.,* 2016; Rose *et al*., 2016). For prophylaxis against rheumatic fever, it can be given orally two times a day as a substitute to injections of benzathine penicillin given every two weeks (Sprenger *et al*., 2016).

Specific uses for phenoxymethylpenicillin include infections caused by *Streptococcus pyogenes,* for example tonsillitis, pharyngitis and skin infections, anthraxlyme disease (early stage in pregnant women or young children), rheumatic fever (primary and secondary prophylaxis), *Streptococcal* skin infections, spleen disorders (pneumococcal infection prophylaxis), initial treatment for dental abscesses, moderate-to-severe gingivitis (with metronidazole), avulsion injuries of teeth (as an alternative to tetracycline) and blood infection prophylaxis in children with sickle cell disease. It is occasionally utilized in the treatment of odontogenic infections (Dhotre *et al*., 2016; Rose *et al*., 2016).

Phenoxymethylpenicillin is typically well tolerated, however, may periodically cause transient nausea, vomiting, epigastric pain, diarrhoea, constipation, acidic smell to urine and dark hairy tongue. A past excessive hypersensitivity reaction to any penicillin is a contraindication (Dhotre *et al*., 2016; Rose *et al*., 2016).

Common unfriendly drug reactions related with the utilization of the penicillins incorporate diarrhoea, hypersensitivity, nausea, rash, neurotoxicity, urticaria, and superinfection (counting candidiasis). Rare unfriendly effects incorporate fever, vomiting, erythema, dermatitis, angioedema and seizures (Dhotre *et al*., 2016; Rose *et al*., 2016).

Bacteria continually rebuild their peptidoglycan cell wall, at the same time building and separating bits of the cell wall as they grow and devide. Beta-lactam antimicrobials inhibit the arrangement of peptidoglycan links in the bacterial cell, yet have no direct impact on cell wall degradation. The Beta-lactam moiety of penicillin

ties to the compound DD-transpeptidase that connects the peptidoglycan molecules in microorganisms. The enzyme that hydrolyze the peptidoglycan cross-links keep on functioning, which weakens the cell wall of the bacterium (in other words, the antimicrobial agent cause's cytolysis or demise due to osmotic pressure (Scheffers & Pinho, 2005; Dhotre *et al*., 2016; Rose *et al*., 2016).

Also, the development of peptidoglycan precursors triggers the initiation of bacterial cell wall hydrolases and autolysins, which additionally digest the microorganisms existing peptidoglycan (Dhotre *et al*., 2016; Rose *et al*., 2016). This irregularity between cell wall generation and degradation is responsible for the fast cell-killing activity of this class of medications, even without cell division. What's more, the moderately little size of the penicillin atom enables it to enter profoundly into the cell wall, influencing its whole depth. This is as opposed to the other significant class of antimicrobials that hinder cell wall synthesis (Rose *et al*., 2016).

2.13. Sutherlandia frutescens

Sutherlandia frutescens and its closely related plant *Sutherlandia microphylla* is a plant that belongs to the third largest family of flowering plants, the Legume family of Fabaceae. These two species have proven a challenge to differentiate, thus some botanists consider them merely different forms of a single, large and variable taxon. *Sutherlandia frutescens* is a lax spreading shrub and approximately 1.2 metres in height (Gonyela, 2016). *S. frutescens* is widespread in the drier areas of the South Western and Northern Cape Provinces. The plant is also found in Botswana, Zimbabwe and Namibia (Gonyela, 2016). It consists of leaves are compound pinnate with leaflets oblong to linear-elliptic, slightly too densely hairy, and they appear

silvery in appearance. *S. frutescens* flowers between July and December; the flowers are bright red; fruits are inflated leathery pods, bearing a persistent upturned style and the seeds are black and flattened (Gonyela, 2016).

The hexane, dichloromethane and ethylacetate concentrates of *S. frutescens* have been shown to be antibacterial active against *S. aureus* (Keterere & Eloff, 2005; Masoko *et al*., 2016). *S. frutescens* was usually utilized all through its natural distribution to treat the indications of flu during the 1918 flu pandemic in Southern Africa. It is as still used to treat flu right up until the present time. *S. frutescens* is for the most part viewed as the most valuable of the medicinal plants in Southern Africa, and has in this manner been utilized by all societies including the San, Khoi, Sotho and Nguni-talking individuals (Masoko *et al*., 2016).

Moreover, *S. frutescens* appreciates a long history as a much esteemed segment of African traditional prescription and a portion of the vernacular names utilized by nearby occupants in southern Africa mirror its significance. In Setswana it is called "*petola"* which signifies 'it changes', suggesting that the plant changes the course of numerous diseases towards a great result. The North Sesotho vernacular name "*lerumolamadi"* signifies 'the spear for the blood' demonstrating that *S. frutescens* is an intense blood purifier and universally handy tonic (Oluwaseyi *et al*., 2014; Gonyela, 2016; Masoko *et al*., 2016).

The indigenous and contemporary uses of *S. frutescens* include: enhancing well-being, immune support, TB and AIDS, treatment for cancer (Gelderblom *et al*., 2016)

(hence its common English name is ‘cancer bush’ and Afrikaans name is ‘kankerbos’) (Masoko *et al*., 2016).

The analgesic, anti-inflammatory and antidiabetic effects of water extracts of *S. frutescens* have been reported (Oluwaseyi *et al.,* 2014). Using an animal model, (Kundu *et al., 2005)* showed that *Sutherlandia* could inhibit phorbol ester-induced COX-2(Cyclo-oxygenase 2) expression.

The hexane extract was shown to be the most active against *S. aureus*, *E. faecalis* and *E. coli* with MIC values of 0.31, 1.25 and 2.50 mg/ml, respectively (Katerere *et al.,* 2005). Ethanolic extracts of commercial preparations of *S. frutescens* have been reported to inhibit proliferation of malignant cells. *S. frutescens* also inhibits enzymes involved in the human immunodeficiency virus life cycle (Lei *et al*., 2016).

Sutherlandia promotes glucose uptake either by increasing insulin sensitivity at a cellular level or by substituting insulin itself, thereby alleviating the demand on Beta cells (Lei *et al*., 2016). This correlated with a study conducted in 2014 in which the plant normalised insulin levels and glucose uptake in peripheral tissues and suppressed intestinal glucose uptake with no weight gain, in treated rats (Lei *et al*., 2016).

Currently *in vivo* clinical trials involving human subjects are being done to assess safety and efficacy of *S. frutescens*. The chemistry of *S. frutescens* is complex and it is most probable that it is the combined effect of several phytochemicals (e.g. triterpenoids, amino acids and sugars), rather than a single key active compound

that accounts for the efficacy of this coveted indigenous ethnomedicinal plant (Oluwaseyi *et al*., 2014).

2.13.1. Chemistry

The chemistry of *Sutherlandia* was contemplated by Prof. Ben-Erik van Wyk & Dr. Carl Albrecht. Four known key compounds adding to the proficiency of this therapeutic plant are the amino acid L-canavanine; pinitol; GABA and asparagine. Furthermore a novel triterpenoid glucoside has been identfied and categorised (Van Wyk *et al*., 2000; Van der Walt *et al*., 2016).

The accessible biological activities of these compouds appear to confirm a portion of the traditional employments of the plant, and additionally bolster the utilization of the plant as a satisfaction tonic in cancer and AIDS patients (Gonyela, 2016).

Microchemical examinations in laboratories demonstrated the presence of tannins however, no alkaloids, cardiac glycosides, saponins or anthraquinone derivatives. It has been expressed that the anti- HIV free amino acids are accounted for as common ingredients of *Sutherlandia frutescens* (Van der Walt *et al*., 2016).

2.13.2. Pharmacology

Scholars utilizing 50 percent ethanol extracts of fresh flowers of *Sutherlandia frutescens* found no antitumor action against CA-Lewis lung, Leuk-L1210 or Sarcoma 180 (strong) tumors in the mouse. Comparable extracts, evaluated for cytotoxicity against CA-9KB cell lines, at a concentration of 20.0 mg/ml, exhibited inactivity (Deutschländer, 2010; Van der Walt *et al.,* 2016).

No *in vitro* antibacterial action against *Pseudomonas aeruginosa*, *Candida albicans* or *Mycobacterium smegmatis* was recognized in the concentrations utilized for disc diffusion assays in laboratories. Some action was archived against *S. aureus* (Mayeku *et al.,* 2013).

2.13.3. L-Canavanine

L-Canavanine is recognized to occur in high levels in specific seeds. What is occasional is that generous levels of this compound are found in *Sutherlandia* leaves. This strong non-protein amino acid is structurally related to L-arginine with reported anti-fungal, antiviral, anticancer and antibacterial activities (Oluwaseyi *et al*., 2014). An average of 2.2 mg of L-canavanine per dry gram of leaf material of *Sutherlandia* was established. L-Canavanine is a powerful L-arginine analogue that has unproved anticancer (Oluwaseyi *et al*., 2014) and antiviral activity, and in addition against influenza virus and retroviruses (Oluwaseyi *et al*., 2014). L-Canavanine is moreover a selective inhibitor of inducible nitric oxide synthase and in this way has likely application in the treatment and administration of septic shock and chronic inflammation (Oluwaseyi *et al*., 2014). The non-protein amino acid canavanine has been identified in the seeds of this species yet not in different organs.

2.13.4. Pinitol

Pinitol, a documented antidiabetic agent (Arif *et al*., 2014), has been identfied from *Sutherlandia* leaves, and quantifiable work is in advance. A United States of America (US) Patent (Ostlund, 1996) suggests that pinitol may have clinical application in

treating the squandering in cancer and AIDS patients (Skerman *et al*., 2011; Arif *et al*., 2014).

2.13.5. GABA

GABA was isolated from dry *Sutherlandia* leaves in levels scarce to 14 mg/g dry weight. This inhibitory neurotransmitter could legitimize the utilization of the plant for stress and nervousness, and for the improvement of the mood and well-being experienced by numerous patients (Oluwaseyi *et al*., 2014).

2.13.6. Novel triterpenoid glucoside

A new triterpenoid glucoside has been identfied and categorized, and is one of the main compounds used in the selection of raw material for promulgation. This compound has encouraging biological activities, but this is still the subject of ongoing scientic investigations.

2.13.7. Safety

In keeping with World Health Organization (WHO) guidelines of the evaluation of herbal medications, *Sutherlandia* is normally regarded as safe on the premise of its long history of safe use in South Africa. Extensive scientific studies have been carried out on the safety, quality, and the efficacy of this medicinal plant, to validate the traditional claims, and elucidate the bioactive components (Oluwaseyi *et al*., 2014).

Sutherlandia is one of the few medicinal plants on the world market that has been formally studied for safety, in this situation in Vervet monkeys. Elite chemotype

Sutherlandia dried leaf powder was tried for safety in 2001 by the Medical Research Council of South Africa. The investigation was part of a Medical Research Council Indigenous Knowledge Systems (IKS) procedure to build up a "clinical stage" to assess the safety and efficacy of promising South African indigenous medicinal plants. No toxicity was apparent in any variable examined by the Medical Research Council (MRC).

No severe adverse effects are known, however, symptoms such as occasional reports of dry mouth, a mild diuretic effect; diarrhoea and constipation have been noted. Slight dizziness has been occasionally noted in very wasted and weak patients (e.g, in an ill adult weighing 35 kg) who take *Sutherlandia* without meals. This is corrected by instructing wasted patients to take the product after meals (Oluwaseyi *et al* 2014).

Although there is a well-established traditional use of taking *Sutherlandia* in pregnancy with no adverse effect, scientific data does not exist to validate with evidence the safety of use of the plant by pregnant women (Oluwaseyi *et al.,* 2014).

2.14. *Psidium guajava*

P. guajava in South Africa is prevalent in the warm subtropical areas of the Northern Province, KwaZulu Natal, and Mpumalanga (Kumari *et al*., 2016). This plant is a small shrub that grows up to no more than four metres in height. When the bark is peeled off it reveals a typically smooth trunk. The plant forms pairs of large leaves opposite each other with prominent veins, mainly on the lower side. It consists of tiny white flowers of approximately 25 mm in width with several stamens that are produced in the early summer, followed by rounded or pear-shaped yellow fruits

(Kumari *et al.*, 2016). Guavas are an important commercial crop because they are edible and delicious, and also contain high vitamin C content.

In South Africa, various regions utilise *P. guajava* leaves as routine remedy for diarrhoea (Watt & Breyer-Brandwijk, 1962; Hutchings, 1996). They are also used for a wide spectrum of ailments, including cough, diabetes, fever, boils, ulcers, and wounds (Van Wyk *et al*., 2000; Abdelmalek *et al*., 2016). The infusion is taken orally as tea or as enema. This liquid mixture is made from leaves that are crushed and boiled in water (Abdelmalek *et al*., 2016).

Numerous active ingredients have been identified namely, tannins, and other phenolic composites, of these compounds, amritoside is of substantial importance (Van Wyk *et al*., 2000; Abdelmalek *et al*., 2016; Esmail *et al.,* 2016). Amritoside is a glycoside of ellagic acid, and ellagic acid is an intestinal astringent and haemostatic agent (Bruneton, 1995; Abdelmalek *et al*., 2016). This characteristic explains the applauded therapeutic value of this specific plant against diarrhoea and dysentery. Furthermore and additional biologically interesting compound in the plant is guiajaverin, this is a glycoside (arabinopyroside) of quercetin (Dictionary of Natural Products, 1996; Sahu *et al.,* 2016).

The leaves also comprise of triterpenoids and important oils. *In vitro* investigation of leaf extracts of *Psidium guajava* has been investigated and confirmed to be antibacterial (Nsele, 2012). The tannins are of great importance due to their capability to form a skin and mucosa protective layer and vasoconstricting effect (Bruneton, 1995; Abdelmalek *et al*., 2016). Quercetin is a flavonoid, in other words, a plant pigment with a molecular structure derived from flavone. This flavonoid

possesses anti-oxidants with anti-HIV, anticarcinogenic, and antibiotic properties (Dictionary of Natural Products, 1996; Abdelmalek *et al*., 2016). Additionally Quercetin has been reported to have a hypoglycaemic effect (Ponglux, 1987; Oluwaseyi *et al.,* 2014). This compound is generally found and extracted from the plant leaves and ellagic acid is mostly found from the bark portion of the plant (Działo *et al*., 2016).

2.15. Antimicrobial synergism in plant products

The synergistic enhancing activity of plants has been discovered in that, regardless of the individual possession or lack of antimicrobial properties, when they are administered concurrently with standard contemporary medications or drugs they enhance the efficiency of that drug (Kamatou *et al*., 2006). The synergistic effect from the association of plant extracts and antibiotic against bacteria may lead to formation of new novel varieties for the treatment and eradication of infectious pathogens and diseases. This effect allows the utilisation of the particular antibiotic when it is no longer effective alone during therapeutic treatment (Nascimento *et al*., 2000; Chanda & Rakholiya, 2011).

The commercial preparation of the antibiotic Augmentin is an example of the application of the synergistic principle. Traditional healers usually utilize plant blended combinations as helpful solution for various ailments (Kamatou *et al*., 2006; Semenya *et al*., 2013). One case from the ethnobotanical literature is the associative administration of different *Salvia* species with *Leonotis leonurus* to treat different diseases (Masika & Afolayan, 2003). Kamatou *et al.,* (2006), affirmed the presence of synergism between Salvia chamelaeagnea and Leonotis leonurus, when these

two plant extracts were joined together and tried against *B. cereus, S. aureus, E. coli* and *K. pneumoniae.* They additionally reported synergism when the tincture of *L. leonurus* and various *Salvia* species were combined together against influenza. Boik (2001), conducted an extensive number of combination studies utilizing various natural substances and the outcomes firmly suggested that when utilized in combination, natural substances can produce synergistic effects. It is thought that *phenolic* compounds, for example, flavonoids may increase the biological action of compounds by synergistic or additional mechanisms (Kumar & Pandey, 2013). Experimental evidence of synergistic activities between plants was additionally shown in a clinical study on the formulation of Chinese herbs used to treat eczema (Aiyegoro *et al*., 2009; Olurishe *et al*., 2016).

2.16. Combinations of bioactive plant products and different classes of antibiotics with specific mechanism of action

Combinations of antibiotics are routinely used in the treatment and management of resistant infections. This method is of advantage because of different mechanisms of action by the antibiotics. The usage of antimicrobial agents exhibiting synergy is one of the well-established indications for combination antimicrobial therapy (Al-Saiym, *et al*., 2015). Evidence suggests that combinations of antimicrobials that show an *in vitro* synergism against bacteria are more likely to produce efficacious therapeutic outcomes. As such, evidence of *in vitro* synergism could be worthwhile in choosing most complimentary combinations of antimicrobials for the practical clinical use in therapy of serious bacterial infections (Hooton *et al*., 1984).

Plants produce intrinsic antimicrobial compounds, as well as multi-drug resistant (MDR) inhibitors which enhance the activity of the antimicrobial compounds (Srivastava *et al*., 2014). Tegos *et al.,* (2002). This showed that the activity of acknowledged plant antimicrobials against Gram negative and Gram positive bacteria was meaningfully enhanced by synthetic MDR inhibitors of associated efflux proteins. These discoveries offered a basis that plants can be favourable prospective sources of natural MDR inhibitors that can modulate the activity and performance of antibiotics against resistant infectious bacteria. The analysis and screening of crude plant extracts for potential synergistic interaction together with antibiotics can offer means for the isolation of MDR inhibitors.

Al-Saiym *et al.,* (2015), carried research on Jordanian herbs and plants and proved that the efficacy of the antibiotics, gentamycin and chloramphenicol against *S. aureus* were seemingly enhanced when used in combination with plant extract materials. Ahmad, & Aqil (2007), also informed that crude extracts of Indian medicinal plants demonstrated a significant synergistic interaction with tetracycline and ciprofloxacin against extended spectrum Beta-lactamase (ESBL)-producing multidrug-resistant enteric micro-organisms (bacteria). Additionally Aiyegoro & Okoh (2009), observed synergistic collaborations between eight antibiotics on *S. aureus* and Brazilian medicinal plants extracts. The use of *Catha edulis* extracts at sub inhibitory levels, has a tendacy to diminish the minimum inhibitory concentration (MIC) values of penicillin G, and tetracycline against oral pathogens *Streptococcus sanguis, Streptococcus oralis* and *Fusobacterium nucleatum.* Large quantities of compounds with an *in vitro* activity of decreasing the MIC's of commercial antibiotics against resistant bacteria have been studied and isolated from herbs or plants.

The Beta-lactam resistance in methicillin-resistant strains of *S. aureus* has been reported to be meritoriously reversed by polyphenols (catechingallate and epicatechin gallate) (Sahelian, 2016). Diterpenes, triterpenes, alkyl gallates, flavones and pyridines have also been reported to have resistance modulating abilities on various antibiotics against resistant strains of *S. aureus* (Marquez *et al*., 2005; Shibata *et al*., 2005).

However, the aforementioned synergy studies were non-specific to antibiotic class or group of organisms. This then suggested that plant crude extracts are a combined blend of compounds that has the potential to enhance diverse antibiotic activity. Plants have been recognised to consist of myriads of antimicrobial metabolites and compounds (Iwu *et al*., 1999) such as flavonoids and polyphenols. Various studies have reported that these naturally occurring compounds; polyphenolic and flavonoids possess the antimicrobial and most importantly resistance modifying capabilities (Cushnie & Lamb, 2005; Sato *et al.,* 2004; Aiyegoro & Okoh, 2009; Pandey & Rizvi, 2009; Sahelian, 2016; Mikulášová *et al.,* 2016).

Some of the compounds including polyphenols have been demonstrated to exercise their antibacterial activities through cell membrane disruption. This disruption of the cell membrane coupled with the action of Beta-lactams on the transpeptidation of the cell membrane could lead to an enhanced antimicrobial effect of the combination (Esimone *et al*., 2006; Sahelian, 2016). It has also been proven that some plant-derived compounds can improve the *in vitro* activities of some peptidomoglycan inhibiting antibiotics by directly attacking the same site, i.e., peptidoglycan in the cell

wall (Aiyegoro & Okoh, 2009). While the above explanations account for the synergy between the extracts and Beta-lactam antibiotics that act on the cell wall, it may not be applicable to cases of the synergy involving other classes of antibiotics with different targets such as chloramphenicol, tetracyclines, ciprofloxacin and erythromycin *(*Aiyegoro *et al*., 2009).

2.16.1. The concept of synergy

Different strategies to overcoming antimicrobial resistance involve combination therapy. Various combinations have demonstrated promising therapeutic outcomes in enhancing antimicrobial effectiveness of existing antimicrobials. This strategy is known as synergy. Synergy is a word which means to work together. It can be defined as a cumulative effect produced by an interaction between two different agents (Bollenbach, 2015), where the cumulative effect is far greater than the effect of the individual agents (Strom *et al.,* 2017).

It has been known that combining drugs when treating patients can often be useful. Therefore, multitherapy has become more popular, as opposed to monotherapy, especially in the treatment of infectious diseases and non-infectious diseases. The combination of known antimicrobials has been found to reduce the development of resistance of micro-organisms towards the antimicrobials (Bollenbach, 2015). This understanding of combination therapy is not only common practice in conventional medicine, but in phytomedicine as well. Medicinal plants have a complex composition and a large diversity of secondary metabolites which increases possibilities for interactions. Plant combinations have been used for centuries, due to the beneficial effects of a combination (Bollenbach, 2015).

Synergy between combinations of known antimicrobials and plant extracts is an idea that has only recently been investigated deeply. Synergy could result in maximum efficacy, minimum toxicity, decreased adverse effects, increased bioavailability, lower dose administration and reduced or delayed antimicrobial resistance (Bollenbach, 2015).

2.16.2. Combination studies of agents with antimicrobial properties

A large amount of methods can be employed to formulate new alternatives with enhanced antimicrobial activity in order to combat drug resistance, such as using multiple antibiotics concurrently, or to combine already available antibiotics with phytochemicals in order to create a potentiating or synergistic effect (Nascimento *et al*., 2000; Sibanda & Okoh, 2007).

2.16.3. Combinations of natural products

Combinations of plant products, to provide a better effect, have been common practice in traditional healing. Essential oils are commonly used in combination and have shown an enhanced effect (Suliman *et al*., 2010). It has been acknowledged that essential oils, when used in combination, have been found to be much higher in inhibitory activity than standard antibiotics (Al-Bayati, 2008). Plant extracts have also been used in combination. A study by Mabona & Van Vuuren (2013) identified various combinations of plant extracts which are used for the treatment of skin ailments, in traditional healing practices in South Africa. These combinations produced enhanced efficacy.

2.16.4. Combinations of natural products with conventional antimicrobial Agents

Compounds in various plants have been found to be synergistic enhancers for conventional antimicrobials, even if the plant compounds do not possess antimicrobial activity themselves (Aiyegoro & Okoh, 2009). Some studies have tested the effects of combining natural products (plant extracts) with conventional antimicrobials (cefuroxime, tetracycline, tobramycin, nystatin, amphotericin B and many more). These have been tested on a number of micro-organisms, including resistant strains of micro-organisms, such as methicillin-resistant *S. aureus* (MRSA) and *Pseudomonas aeruginosa*. In most cases, a synergistic interaction has been identified. Most of these studies have focused on antibiotic combinations with common herbs, such as *Rosmarinus officinalis*, *Origanum vulgare*, *Thymus vulgaris*, *Mentha piperita* and *Melaleuca alternifolia* (Sato *et al*., 2004; Betoni *et al*., 2006). The synergistic effect is represented by a reduced minimum inhibitory concentration (MIC) for the antimicrobial. The reduced MIC signifies a better antimicrobial effect, which could eventually render an ineffective antimicrobial, effective once again. This interaction has resulted in some plant extracts being defined as resistance modifying agents (Sibanda & Okoh, 2007).

Both Adwan *et al.,* & Van Vuuren together with Viljoen (2011), proposed that the potentiating effect of plant extracts on conventional antimicrobials has been neglected and requires further investigation. A study by Van Vuuren & Viljoen (2011) has summarized some combinations of plants with conventional antibiotics and the interactions which were noted. A review by Hemaiswarya *et al*., (2008) also provides

a number of synergistic interactions that have been identified between natural products and antibiotics in the treatment of bacterial infections.

A recent South African study on the combination of the ethanolic extract of *Ziziphus mucronata* with conventional antibiotics (tetracycline, chloramphenicol, amoxicillin and ciprofloxacin) found that more synergistic interactions (54.17 %) occurred between the combinations than those of antagonism (1.39 %) against clinically relevant bacteria (*Bacillus cereus*, *Pseudomonas aeruginosa*, *Enterococcus faecalis* and *Escherichia coli* (Olajuyigbe & Afolayan, 2013). Palaniappan & Holley, (2010), also discovered the synergistic interactions between conventional antibiotics (ampicillin, tetracycline, penicillin, bacitracin, erythromycin and novobiocin) and natural antimicrobials (eugenol, thymol, carvacrol, cinnamaldehyde and allylisothiocyanate) when testing against resistant strains of *Salmonella typhimurium*, *S. aureus*, *E. coli* and *Streptococcus pyogenes,* and it was acknowledged that some plant chemicals have the potential to decrease antimicrobial resistance.

A few combination studies have investigated antimicrobials in combination with isolated phytochemicals, such as phenols, tannins and flavonoids (Sibanda & Okoh, 2007; Hemaiswarya *et al*., 2008; Palaniappan & Holley, 2010; Sahelian, 2016), where again, many synergistic combinations were identified and attributed to the potentiating effect of natural products on conventional antimicrobials (Siebra *et al*., 2016). No antagonistic interactions were observed in this study. Other scientists have investigated the combination of conventional antimicrobial combinations with non-conventional antibiotics, such as tricyclic neuroleptics and antidepressants (Czaplewsk *et al*., 2016).

After conducting a search of published literature, few studies were found that appeared to have investigated interactions between the southern African medicinal plants selected for this study and conventional antimicrobials when used in combination. These plants have been studied for their antimicrobial activity, as well as in many herbal formulations (Suliman *et al*., 2010); however, no evidence is available on their activity when in combination with conventional antimicrobials.

2.17. Prevalence of concurrent use of natural and conventional medicine

In southern Africa, traditional African medicine coexists with conventional medicine, as well as other alternative types of medicine, such as homeopathy, Ayurvedic and Traditional Chinese medicine (Van Wyk & Gericke, 2000; Czaplewsk *et al*., 2016). It has been observed and acknowledged that the 60 % of South Africans consulting traditional healers, very often use modern medical services concurrently (Van Wyk *et al*., 2009). Even if western healthcare is available, traditional medicine still exists side by side with conventional medicine (Czaplewsk *et al*., 2016). Many people in southern Africa use both traditional and conventional medications concurrently (Van Wyk *et al*., 2000), without knowledge of the potential interactions which may exist.

It has also been acknowledged that even in some of the best hospitals in South Africa, traditional medicine is found to be used by patients in conjunction with conventional medicines (Van Wyk *et al*., 2000). The practice of combining traditional remedies with conventional medicine has been found to be practiced not only in southern Africa, but also in most parts of the world. In Israel, it was found that 49.40 % of natural product consumers were also concurrently using conventional drugs

(Czaplewsk *et al*., 2016). A survey performed in the United States of America, indicated that 72 % of patients using herbal remedies were found to be using prescribed drugs and 84 % using over the counter medication in combination. It was also discovered that some patients preferentially combined these two forms of healthcare, with the belief that there would be a synergistic effect (Maizes & Dog, 2010).

The major concern with concurrent use of these two forms of healthcare is the potential for natural product/herb-conventional drug interactions and the clinical consequences of these interactions (Fasinu *et al*., 2012). This provides the reason to study Southern African medicinal plants in combination with conventional medication, in order to identify any interactions which may compromise a patients' therapy.

People believe that traditional medicines are safe for consumption because of the history of their use; but that notion can no longer be valid. It has been found that many phytomedicines that are used in conjunction with over-the-counter or prescription drugs result in many undesirable interactions and effects (Maizes & Dog, 2010).

2.18. Interactions between natural products and conventional drugs

The potential for the interaction between natural products and conventional drugs is worrying and it has become a big concern. Natural products are taken for both the treatment of some diseases and the prevention thereof. Therefore, long-term consumption of natural products usually occurs. This causes an increased frequency

of simultaneous utilisation with prescribed or over-the-counter medicines and the chance for interactions. The concern increases among the old people, where traditional medicines are a popular choice for the treatment of diseases and where conventional prescribed medication is also used. The public is mostly unaware of the possibility for interactions and their adverse effects, which further exacerbates the problem (Newman & Cragg, 2012).

Traditional medicines are often poorly defined and may have different ingredients to that stated on the package, which may further contribute to possible interactions. Many drugs are often used in combination, mostly in the elderly and chronically ill, where interactions commonly occur. This situation can be complicated by the concurrent use of traditional medicine. Often, patients do not disclose the use of natural products to their healthcare providers. A study by Erku & Mekuria (2016), revealed that no less than 40 % of natural product users disclose their use of these medicines to their healthcare providers. The continued practice of using commercial drugs along with 12 traditional medicines has been attributed to the lack of knowledge of interactions and this becomes a major safety concern. The lack of reporting also contributes to the lack of information available on the interactions occurring (Butterweck & Derendorf, 2012; De Lima Toccafondo Vieira & Huang, 2012).

The occurrence of these interactions has been attributed to physicians and their limited knowledge pertaining to herbal medicines and the potential for drug interactions and the impact thereof on the wellbeing of their patients. Another issue is the lack of proactively enquiring natural traditional medicinal use by the healthcare

provider upon their consultation with patients (Fakeye & Onyemadu, 2008). The possibility of interactions between the two forms of healthcare has been identified as a serious healthcare concern in many hospitals throughout the world, with new regulations being implemented to ensure the full disclosure of traditional medicinal use during a consultation, before any conventional medicines are prescribed (Kemper *et al*., 2008). This, however, has not become common practice in South Africa yet.

Herbal products have been found to interact with conventional drugs in many ways. Sometimes the herbal products interact at the site of absorption, and by so doing they affect the rate or extent of absorption of conventional drugs. Herbal products can also interact with protein transporters and compete with conventional drugs for transporters or can interact with the liver enzymes responsible for metabolism of conventional drugs (Tachjian *et al*., 2010). Not only are herbal remedies active on their own, but they are also capable of potentiating or diminishing the therapeutic effects of conventional medication (Yin *et al*., 2013).

The increased use of herbal products throughout the world, has led to an increased number of interactions being identified; where some have been fatal (Ekor, 2013). A few of these interactions have been reviewed by Fasinu *et al*., 2012.

2.19. *Staphylococcus aureus*

S. aureus is found on the human skin and the mucous membranes of around a third of the population. It is known major resistant pathogens. MRSA is commonly acquired in hospitals as nosocomial infections. Bulks of infections caused by this

micro-organism are resistant to penicillin, tetracyclin, methicillin and erythromycin. This has since left vancomycin as the main powerful antibiotic accessible for clinical use. However, in the late 1990s strains with intermediate (4-8 ug/ml) levels of resistance, termed vancomycin intermediate *S. aureus* (VISA) began appearing (Bozdogan *et al.,* 2003; Planet *et al*., 2016). The United States, in 2002, was the country that first documented a strain with complete resistance to vancomycin measuring (>16 ug/ml) (Bozdogan *et al.,* 2003; Gardete & Tomasz, 2014). This emerging strain was termed Vancomycin-resistant *S. aureus* (VRSA). This then called for a formulation of a new class of antibiotics which have an equally comparable effectiveness to vancomycin against MRSA. These antibiotics are available and are commercially known as oxazolidinones (Bozdogan *et al*., 2003; Planet *et al*., 2016).

2.19.1. Classification

S. aureus is a Gram positive coccus about one millimetre in diameter. These are grape-like clustered, but in pathological samples they may be found occurring in singles or pairs (Omer *et al*., 2008). *S. aureus* contains an enzyme coagulase which is able to clot plasma, thus is classified as coagulase positive staphylococcus (Planet *et al*., 2016).

2.19.2. Epidemiology

S. aureus, is available in the skin and nose of a substantial portion of healthy individuals, is an opportunistic pathogen causing routine infections most frequently at locations of depressed or compromised host resistance such as when mucous membranes are injured or damaged skin barrier. *S. aureus* is a common aetiology in

abscesses, boils, skin and wound infections, food poisoning and osteomyelitis. *S. aureus* is susceptible to numerous antibacterial agents; however, there are a few strains that are able to produce the enzyme Beta-lactamase which has the ability to inactivate the action of most Beta-lactam antibiotics. These strains are termed multi-resistant strains of *S. aureus*. Furthermore *S. aureus* is the routine aetiology of both community and socially acquired infections. The most common and prevalent means of transmission (spread) are patient to patient transmission via the hands of the personnel (Planet *et al*., 2016).

2.19.3. Growth characteristics

S. aureus species are classified as facultative anaerobes and have the ability to grow and flourish in any nutrient media. Certain strains can result in beta haemolysis on blood agar plates. This is due to the fact that it does have some *haemolysins*. *S. aureus* fails to grow when cultured on MacConkey agar that has an incorporation of crystal violet, since it is a Gram positive bacterium (Planet *et al*., 2016).

2.19.4. Toxins and enzymes

S. aureus produces a variety of extracellular products viz. enterotoxins, haemolysins, exfoliatin, leukocidin, and enzymes. This type is known as the principal toxin-producing type of *staphylococci*, producing four different types of haemolysins namely; alpha, beta, gamma and delta. The precise character of each kind of these haemolysins is yet to be discovered, however it is alleged that alpha haemolysins possess haemolytic abilities and dermonecrotic activity (Planet *et al*., 2016).

The organism produces leukocidin toxin that has a potential to cause break down of human cells; (white blood cells and macrophages). Whenever produced, these poisons will hinder the patient's immune system. Moreover the leucocytes are a foremost components of the human immune system, thus lymphocyte cell lysis will consequently expose the patient to opportunistic infections (Planet *et al*., 2016). *S. aureus* produces five types of enterotoxins (A to E), it is these toxins that are responsible and in charge of *staphylococcal* food poisoning plus conditions such as toxic shock syndrome that results when *staphylococci* develop and flourish inside a cavity on the human body (Planet *et al*., 2016).

Added to is an exfoliatin toxin that makes layers inside the epidermis of the skin to split, consequently causing shedding of the skin. This is attributed to the fact that it possesses an epidermolytic or exfoliative effect (Ladhani *et al*., 1999).

There are several enzymes yielded by *staphylococci* viz. proteases, lipase, and fibrinolysins. However, *S. aureus* produces the most important enzyme known as coagulase which in addition to cell haemolysis also prevents the bactericidal activity of the normal serum (Planet *et al*., 2016).

2.19.5. *Staphylococcal* infections

S. aureus is the major culprit in the largest amount of infection due to *Staphylococcus.* Invasion is attributed to the production of the enzyme coagulase. The most common type of all *staphylococcal* infections is the local infections. Life threatening invasive infections are rare in healthy individuals, nevertheless may occur in chronically ill or immunocompromised patients.

The known predisposing factors include deficiencies in humoral immunity, leucocyte defects, prior viral infections , injury to normal skin, and alteration of normal flora due to the use of antimicrobial agents to which *S. aureus* is not susceptible. Regrettably this precarious microorganism may gain entry into the blood system stream, and disseminate to various tissue (Planet *et al*., 2016).

2.19.5.1 Methicillin-resistant *Staphylococcus aureus*

Methicillin-resistant *Staphylococcus aureus* is a bacterium that is in charge of a few hard to-treat infections in people. It is additionally called oxacillin-resistant *Staphylococcus aureus* (ORSA) (McDougal et al, 2003). MRSA is any strain of *Staphylococcus aureus* that has created, through the procedure of characteristic choice, resistance to Beta-lactam antimicrobials, which incorporate the penicillins (methicillin, dicloxacillin, nafcillin, oxacillin and cephalosporins). Strains unable to resist these antimicrobial are termed methicillin-sensitive *S. aureus,* or MSSA. The development of such resistance does not make the microorganism to be more harmful than strains of *S. aureus* that have no antibiotic resistance, however, resistance makes MRSA infection harder to treat with standard sorts of antibiotic agents and in this way more dangerous (Planet *et al*., 2016).

MRSA is particularly troublesome in healing centers, detainment facilities, and nursing homes, where patients with open injuries, intrusive gadgets, and debilitated insusceptible frameworks are at more serious danger of nosocomial disease than the general public (Batabyal *et al*., 2012). MRSA started as a nosocomial contamination, however has built up some restricted endemic status and is currently some of the

time group obtained. The terms Heathcare-acquired MRSA (HA-MRSA) and community associated MRSA (CA-MRSA) mirror this refinement (Batabyal *et al*., 2012).

S. aureus ordinarily colonizes the anterior nares. The rest of the respiratory tract, open injuries, intravenous catheters, and the urinary tract are likewise conceivable destinations for infections. Healthy people may carry MRSA asymptomatically for periods extending from half a month to numerous years. Patients with compromised immune systems are at a more serious danger of secondary infection (Batabyal *et al*., 2012).

In many patients, MRSA can be recognized by swabbing the nostrils and identifying the microorganisms found inside the nostrils. Consolidated with additional clean measures for those in contact with contaminated patients, swab screening patients admitted to hospitals can be successful in limiting the spread of MRSA in hospitals (Batabyal *et al*., 2012; Planet *et al*., 2016).

MRSA may advance considerably inside 24–48 hours of starting topical manifestations. Following 72 hours, MRSA can attack human tissues and in the long run end up with noticeably resistant to treatment. The underlying introduction of MRSA is little red bumps that look like pimples or boils. They might be accopanied by fever and sporadically rashes. Inside a couple of days, the bumps wind up noticeably bigger and more agonizing and they in the end open into profound, pus filled boils. Around 75 percent of community associated CA- MRSA infections are confined to skin and soft tissue and they can be dealt with adequately with

vancomycin. Some CA-MRSA strains show improved virulence, spreading more quickly and causing ailment significantly more extreme than traditional HA-MRSA infections. They can affect vital organs and prompt far reaching infection (sepsis), toxic shock syndrome, and necrotizing pneumonia (Planet *et al*., 2016).

Individuals are regularly colonized with CA-MRSA and are totally asymptomatic. The most widely recognized manifestations of CA-MRSA are straightforward skin infections, for example, impetigo, boils, abscesses, folliculitis, and cellulitis (Iroha *et al*., 2012). Rarer, yet more serious, signs can happen, for example, necrotizing fasciitis and pyomyositis, necrotizing pneumonia, infective endocarditis, and bone and joint infections (Raygada & Levine, 2009). CA-MRSA regularly brings about ulcer formation that requires cut and drainage. Prior to the spread of MRSA into the community, abscesses were not viewed as contagious, on the grounds that infection was expected to require violation of skin integrity and the presentation of staphylococci from ordinary skin colonization. Be that as it may, recently emerging CA-MRSA is transmissible (comparative, however with critical contrasts) from HA-MRSA. CA-MRSA is more unusual than other types of MRSA to cause cellulitis (Planet *et al*., 2016).

Diagnostic microbiology laboratories and reference research facilities are imperative in outbreak incidences of MRSA. Ordinarily, the bacterium must be cultured from blood, urine, sputum, or other body-fluid samples, and in adequate amounts to perform confirmatory tests at an opportune time. All things considered, in light of the fact that no snappy and simple strategy exists to diagnose MRSA, starting treatment of the infection is regularly based upon of 'strong suspicion' and strategies by the

treating doctor; these incorporate quantitative polymerase chain reaction (Q-PCR) methodologies, which are utilized in clinical research centers for rapidly recognizing and identifying MRSA strain. Another basic laboratory test is a fast latex agglutination test that detects the PBP2a protein. PBP2a is a variant penicillin-restricting protein that bestows the capacity of *S. aureus* to be resistant to oxacillin (Iroha *et al*., 2012).

Antimicrobial resistance is hereditarily based; resistance is intervened by the procurement of extrachromosomal hereditary components containing resistance qualities (Batabyal *et al*., 2012). Examples are plasmids, transposable hereditary components, and genomic islands, which are exchanged between microorganisms horizontal gene transfer. A defining characteristic of MRSA is its capacity to flourish within the presence of penicillin-like anti-microbials, which typically avoid bacterial development by hindering synthesis of cell wall material. This is because of a resistance gene, mecA, which prevents Beta-lactam anti-microbials from inactivating the enzymes (transpeptidases) basic for cell wall synthesis (Stapleton & Taylor, 2002; Batabyal *et al*., 2012)

2.19.5.2 Methicillin-sensitive *Staphylococcus aureus*

MSSA is a kind of strain of *Staphylococcus* microorganism that reacts well to drugs used to treat *Staphylococcus* infections. Many strains of *Staphylococcus* microorganisms are very normal, and a great many people have *Staphylococcus* bacteria living innocently on their skin or in their noses. *Staphylococcus* bacteria that enter the body through a cut, rub, or rash can cause minor skin infections. The

greater part of these recuperate all alone if the injury is kept perfect and bandaged, however, some of the time antimicrobial agents are required.

MSSA diseases can cause harmful toxic shock syndrome, cellulitis, *Staphylococcus* food poisoning, folliculitis, boils, impetigo, and scalded skin syndrome. Most MSSA infections can be dealt with by washing the skin with an antibacterial chemical, utilizing warm splashes, applying an antimicrobial lotion endorsed by a doctor, and covering the skin with a perfect dressing. Doctors additionally may endorse oral antimicrobial agents to treat MSSA diseases. More serious diseases may require hospitalization.

Most MSSA infections are effortlessly treated with antimicrobial agents or by draining the disease of pus or fluid. Many of these diseases can be averted by washing well and frequently, keeping cuts and scrapes perfect and secured with a wrap, not sharing individual things (like razors, towels, or uniform), and making a point to taking antibiotics as prescribed.

2.19.5.3 Vancomycin-resistant *Staphylococcus aureus*

VRSA is a strains of *S. aureus* that is resistant to the glycopeptide antibiotic vancomycin (Gardete & Tomasz; 2014). Three classes of vancomycin-resistant *S. aureus* have developed that contrast in vancomycin sensitivities: vancomycin-intermediate *S. aureus*, heterogeneous vancomycin- intermediate *S. aureus* (hVISA), and high level VRSA (Gardete & Tomasz; 2014).

Vancomycin-resistant *Staphylococcus aureus* was first isolated in Japan in 1997 and has since been found in hospitals somewhere else in Asia, and in the United Kingdom, France and Brazil. It is likewise called glycopeptide-intermediate *Staphylococcus aureus* (GISA), showing resistance to all glycopeptide antimicrobial agents. These strains exhibit a thickening of the cell wall, which decreases the capacity of vancomycin to diffuse into the division septum of the cell essential for successful vancomycin treatment (Gardete & Tomasz; 2014).

High level vancomycin resistance in *S. aureus* has been once in a while reported (Gould, 2010). *Invitro* and *invivo* experiments publicized in 1992 revealed that vancomycin resistance genes from *Enterococcus faecalis* could be exchanged by gene transfer to *S. aureus,* giving high level vancomycin resistance to *S. aureus* (Gardete & Tomasz; 2014).

2.20. *Enterococcus faecal*

Enterococcus faecalis (*E. faecalis*) is a non-motile, Gram positive, spherical bacterium. It can be observed individually, in pairs, or in short chains, and is regularly found in the large interstine of people. It is a facultative anaerobe with a fermentative breakdown. It can frequently be mistaken for *Streptococcus pneumonia* (*S. pneumonia*) and *Streptococcus viridans* (*S. viridans*), yet *E. faecalis* hydrolyses bile aesculin and it is gamma haemolytic (Mustafa, 2016).

E. faecalis is the third leading source for nosocomial contaminations. The vast majority of these infections happen after surgery of the stomach area or a puncturing injury, however, can likewise be connected to the expanded utilization of internal

valves (IV's) and catheters, which are viewed as compromsing devises. It is additionally accountable for urinary tract infections, bacteraemia, and endocarditis and can be found in wound infections alongside numerous other bacteria (Kristich *et al*., 2014; Mustafa, 2016).

E. faecalis was first isolated as a *Streptococcus* group D bacterium (*Streptococcus faecalis*) as a result of its feature this *Streptococcus* assemble D cell wall carbohydrate. It wasn't until 1984, that it was named an *Enterococcus*. *E. faecalis* is among the most antibiotic resistant microorganism known. It is thought to be a transporter of vancomycin resistance for other genera of microbes. With *E. faecalis* happening much of the time in hospital secondary infections, these multiple drug resistant strains make a scary idea (Kristich *et al*., 2014; Mustafa, 2016).

Treatment of *E*. *faecalis* consists of cell wall-active antibiotics (Ghaleb & Mohammad, 2008). In endeavoring to prevent resistant strains, drug sensitivity testing is unequivocally recommended. New more stronger and more specific antimicrobial agents are being produced (Ghaleb & Mohammad, 2008).

When compared to most *Streptococci*, *Enterococci* are relatively resistant to penicillin and ampicillin. Even when these cell wall–active agents inhibit the growth of enterococci, they regularly don't kill them. Vancomycin is even less bactericidal to *enterococcus*. *Enterococcus faecium* isolates are more resistant to penicillin than *E. faecals* (Kristich *et al*., 2014; Mustafa, 2016).

Enterococci are additionally generally impermeable to aminoglycosides (Hollenbeck & Rice, 2012). In any case, the synchronous utilization of a cell wall–active agent raises the porousness of the cell so that an intracellular bactericidal aminoglycoside concentration can be accomplished without extreme harmfulness. Bactericidal action is justified in clinical conditions of hazardous infection (Murray *et al*., 2016).

Enterococcal isolates are generally examined for sensitivity to ampicillin, penicillin, and vancomycin. The presence of Beta-lactamase gives resistance to penicillin and ampicillin when large quantities of organisms are available (eg, endocarditis vegetation), despite the fact that the organism may test susceptible when utilizing standard laboratory sensitivity testing. To preclude this plausibility for endocarditis or other dangerous *enterococcal* infections, for example, meningitis, specialists recommend that the isolate be screened for Beta-lactamase production (Kristich *et al*., 2014; Mustafa, 2016).

Traditionally, the standard of care for *enterococcal* endocarditis has been a cell wall–active agent combined with an aminoglycoside to produce synergistic, bactericidal action. On the off chance that an aminoglycoside is used, the *enterococcal* isolates ought to be tested for high level resistnce to gentamicin and streptomycin. In the event that the organism is reported as sensitive to high levels of an aminoglycoside, at that point it is assumed that synergism will be accomplished when that aminoglycoside is combined with ampicillin. Strains that are resistant to elevated amounts of gentamicin are resistant to synergism with tobramycin, netilmicin, and amikacin and in addition gentamicin; yet some of these strains need high level

resistance to streptomycin and these will demonstrate synergism with that agent (Kristich *et al*., 2014; Mustafa, 2016).

2.21. Screening methods for natural products with antimicrobial properties

The three most normal techniques utilized to evaluate the antimicrobial properties of natural products are diffusion assay, dilution tests and bioautography tests. This study has utilized the disc diffusion assay which was trailed by dilution assay for minimum inhibitory concentration

2.21.1. Diffusion assays

Diffusion techniques of screening for antimicrobial properties of natural products utilize either a disc or reservoirs for the specimen material (Singh, 2004a; Das *et al*., 2010). This method is established on the rule that the reservoir having an extract is transported into contact with an inoculated medium. The solute will diffuse into the agar. After incubation the diameter of the growth free zone around the resevoir is measured and taken as the antimicrobial activity of that item.

Diffusion assays are proper for the first screening of pure substances, for example, alkaloids, terpenoids and flavonoids (Singh, 2004a; Das *et al*., 2010). These methodologies can't be utilized for samples that are difficult to diffuse in the media, on the grounds that the relationship between diffusion power and antibacterial action has not been established.

Comparison of the zones of inhibition of natural products with those of synthetic antibiotic disc assay is profitable for establishing the susceptibility of the test organism. Correlation of the antimicrobial potency of the natural test materials and the synthetic antimicrobial agent can't be produced from these estimations (Singh, 2004a; Das *et al*., 2010). This is owing to the fact that various different factors, for example, diffusion ability, which can affect the zone size of inhibition can be influential, bringing about vague conclusions. The ideal effectiveness of the disc diffusion technique is gained by utilizing Mueller-Hinton agar and standardised microorganisms American Type Culture Collection (ATCC) (Singh, 2004a; Das *et al.,* 2010).

2.21.2. Dilution tests

Dillution tests require a homogeneous spreading of the specimen. Bacterial propagation is measured by the turbidity of the solution, which is taken as a direct correlation to the measure of microbial growth (Singh, 2004a; Das *et al*., 2010). These examinations can be utilized to produce the minimum inhibitory concentration for the antibacterial specimen. The dilution tests are normally more complex, tedious and costly to perform than the disk diffusion techniques (Balouiri *et al*., 2016).

2.21.3. Bioautographic methods

This method incorporates utilizing paper chromatography or thin layer chromatography to seperate compounds which are then consequently tested utilizing the disc assay technique for antibacterial activity. This technique is not as achievable as the disc diffusion assay and dilution methods for preliminary screening of sample

because of accompanying costs (Singh, 2004a; Das *et al*., 2010; Balouiri *et al*., 2016).

2.22. Choice of extractant

Ethanol is frequently utilized as a part of the generation of plant extracts to permit the extraction of water-insoluble ingredients from the source material and additionally as preservative for the extract (Singh, 2004a). However, ethanol itself has antimicrobial effects. This is the reason an ethanol control was used in this study.

With a specific end goal to balance the variable impact of ethanol altogether, a water-based extract is likewise assessed. Invernizzi (2002) & Pandey and Tripathi (2014), proposed that trials ought to be done utilizing different sorts of extractants to comprehend which is most effective in extracting the active compounds from the plants. They additionally go on about the utilization of acetone, yet this is not a feasible alternative as a therapeutic agent because of its toxic nature (Invernizzi, 2002; Pandey & Tripathi, 2014).

CHAPTER 3: METHODOLOGY

The methods that were used for extraction are reliable and valid as they have been used in the past in similar studies (Naidoo, 2004).

3.1 Study design

This is an investigational study that includes laboratory examination of the synergistic effects of plant extracts and penicillins on *S. aureus* and *E. faecalis.*

3.2 The data

This research involved two types of data: primary and secondary. Primary data were collected through experiments conducted during the advancement of this research study, while the secondary data were gathered through research articles published in journals, books and manuals.

3.3 Criteria governing the admissibility of data

Only data obtained from experimentations conducted by the researcher at the Mangosuthu University of Technology, Department of Biomedical Science Microbiology laboratory were incorporated in the data analysis. It was hypothesized that there is a synergistic effects of plant extracts (*P. guajava* and *S. frutescens)* and penicillins (procain penicillin, benzathin penicillin, penicillin V and penicillin G) on *S. aureus* and *E. faecalis*. It was further hypothesised that both water-based and ethanol extracts would exhibit synergistic effect against *S. aureus* and *E. faecalis.*

3.4 Materials and methods

3.4.1 Sample collection

Samples of *S. frutescens* and *P. guajava* were acquired from Silverglen Nature Reserve. The plants were collected early in the morning (8 am) because the cells are extra active at this time. *S. frutescens* and *P. guajava* leaves were used.

3.4.2 Extraction

3.4.2.1 Preparation of the water-based extraction of *Sutherlandia frutescens* and *Psidium guajava*

S. frutescens was prepared in accordance with an adjusted method HAB 3a of the German Homeopathic Pharmacopoea (Benyunes, 2005). *S. frutescens* (fresh plant part above ground) was collected early in the morning (8 am). Plant material was instantly crushed in an electrical mincer and weighed into a glass jar. Three parts of distilled water were added to one part of minced plant material (1:3) according to calculation 1 (Appendix A). The mixture was shaken for five minutes and then left in a glass jar for 14 days with mixing once a day. Thereafter it was pressed through 100 percent cotton and filtered through a membrane filter (Singh, 2004b). It was then stored in 100 ml glass containers at 2 ºC to 8 ºC until use. Water based extract of *P. guajava* was prepared by utilisation of the same method that was used for water based extract for *S. frutescens*. The diffence was that three parts of distilled water were added to one part of minced plant material (1:3) according to calculation 2 (Appendix A). Distilled water was obtained from the Department of Biomedical Technology at Mangosuthu University of Technology.

3.4.2.2 Preparation of the ethanol tincture of *Sutherlandia frutescens* and *Psidium guajava*

S. frutescens was prepared according to an adjusted method 3a of the German Homeopathic Pharmacopoeia (Benyunes, 2005). *S. frutescens* (fresh plant part above ground) was harvested early in the morning (8 am). Plant material was immediately minced in an electrical mincer and weighed into a glass jar. One part of minced plant material was added to three parts of 96 % ethanol (1:3) according to calculation 3 (Appendix A). The mixture was shaken for five minutes and then left in a glass jar for 10 days at a temperature not exceeding 20 ºC, agitating the mixture once a day. Thereafter it was pressed through 100 percent cotton muslin cloth and filtered through a No 1 Whatman filter paper (Singh, 2004b). It was then stored in 100 ml glass containers at 2 ºC to 8 ºC until use. Ethanol tincture of *P. guajava* was prepared by utilisation of the same method that was used for *S. frutescens*. The difference was that one part of minced plant was added to 3 parts of 96 % ethanol (1:3) according to calculation 4 (Appendix A).

3.4.3 Antibiotic assay (AA) discs

Antibiotic assay discs were used as recommended by Invernizzi (2002). They were purchased from Davies Diagnostics.

3.4.4 Preparation of culture media

Mueller-Hinton agar (Oxoid) was the only agar growth medium that was utilized in this research project for sensitivities. Fresh Mueller-Hinton (MH) was prepared according to the manufacturer's instruction (Oxoid) (Appendix B). Fresh nutrient

broth was the only broth that was used in this project. It was made up according to the manufacturer's instruction (Appendix B). Fresh Nutrient agar was used to maintain the cultures and was made up according to the manufacturer's instructions (Oxoid) (Appendix B).

3.4.5 Microbial cultures

The cultures of MRSA (ATCC 33591), MSSA (ATCC 25923) and *E. faecalis* (ATCC 29212) were maintained on nutrient agar slopes at 4 ^{o}C and subcultured on to blood agar plates for 24 hours before use. These are known American Type Culture Collection strains obtainable from Davies Diagnostics.

3.4.6 Bacterial sensitivity testing (screening)

The method that was used is in accordance with a modification of the Kirby-Bauer Antimicrobial Sensitivity Test Procedure (Cappucino & Sherman, 1992). Inoculum containing 1 x 10^6 colony forming units (CFU) per millilitre (ml) was introduced onto the surface of MH Agar plates. Inoculums were prepared by comparing the bacterial suspension with one MacFaland turbidity standard as described by Thrupp (1980). They were distributed evenly with a sterile swab. A sterile antibiotic assay disc previously soaked in the extract or antibiotic was carefully placed at the centre of the labelled plate of the bacterial suspension.

The disc was soeked in the sovent which was prepared by adding three parts of distilled water to one part of minced plant material. The resultant ratio of the plant to water was 1:3. The ethanol extract was prepared by means of the same technique.

The antibiotics were prepared by dissoving one part of antibiotic to three parts of sterile distilled water. This was prepared immedialy before use. The original concentrations of penicillin were as follows: procain penicillin was 40 mg/ml , benzathine penicillin was 150 mg/ml, penicillin G was 200 mg/ml and lastly penicillin V was 25 mg/ml .

The plates were incubated at 37 ^{o}C and examined for the zone of inhibition after 24 hours, respectively. Disks soaked in sterile distilled water were used as a negative control. The negative control was processed the same way as the tests. The negative control should show no zone of inhibition. A zone of inhibition in the test sample was taken as positive. The zone was measured using a ruler and was reported in mm. Vancomycin was used as the positive control for MRSA and *E. faecalis* and Penicillin G was used as the positive control for MSSA. The ethanol control was included to determine the effect of alcohol on bacteria.

For the synergism effect, 5 ml of each penicillin was mixed with 5 ml of each plant extract. This resulted in mixing equal parts of penicillin and extract. Each penicillin was prepared by mixing it with sterile distilled water in the ratio of 1:3. It was prepared immediately before use.

3.4.7 Determination of MIC by agar plate dilution method

Agar plate dilution test was used to determine the MIC of the antimicrobial agent.

3.4.7.1 Preparation of antimicrobial agents

Individual antibiotics were dissolved in solvents (water) in the ratio 1:3 and then added to molten agar. Dilutions of the antimicrobial agents were prepared in sterile distilled water by way of serial dilutions. The dilutions ranged from 1 in 2 up to 1 in 16. All MIC ranges were according to the National Committee for Clinical Laboratory Standard (NCCLS) guidelines (Kiehlbauch *et al*., 2000; Elisha *et al.,* 2017).

3.4.7.2 Preparations of plates

Two hundred and fifty millilitres MH medium of each flask was autoclaved and allowed to cool at 50 ºC in a water bath. Appropriate volume of intermediate antimicrobial concentration was added to each flask at 10 ml concentration, mixed thoroughly and antibiotic-containing media was poured immediately on the plate. Two fold serial dilutions of the antimicrobial were added with agar medium. The dilution of the penicillin for example in different plates was 1 in 2, 1 in 4, 1 in 8 and 1 in 16. The plant extracts had similar dilutions as the penicillins. At a later stage, dilutions of the combination of penicillins and plant extracts were manufactured in order to determine their synergistic effect.

3.4.7.3 Inoculum preparation for MIC test

Inocula were obtained from an overnight agar culture of the test organism. Inoculum for the MIC test was prepared by taking at least three to five well-isolated colonies of the same morphology from a nutrient agar plate culture. The plate culture was subcultured from a nutrient agar slope. The top of each colony was touched with a sterile loop and the growth was transferred into a tube containing 4 ml to 5 ml of

normal saline. The broth culture was incubated at 37 ºC until it achieved the turbidity of the 0.5 McFarland standards (usually 2 to 6 hours). This resulted in a suspension containing approximately 1 to 2 × 10^8 cfu/ml. The turbidity of the actively growing broth culture was adjusted with sterile broth to obtain turbidity comparable to that of the 0.5 McFarland standards.

3.4.7.4 Turbidity standard for MIC inoculum preparation

To standardize the inoculum density for a susceptibility test, $BaSO_4$ turbidity standard, equivalent to a 0.5 McFarland standard, was used. A 0.5 McFarland standard was prepared as described in NCCLS (NCCLS, 2000a). One percent volume per volume (V/V) solution of sulfuric acid was prepared by adding 1 ml of concentrated sulfuric acid to 99 ml of water and mixing well. A 1.175 % weight per volumr (W/V) solution of barium chloride was prepared by dissolving 2.35 g of dehydrated barium chloride ($BaCl_2.H_2O$) in 200 ml of distilled water. To make the turbidity standard, 0.5 ml of the barium chloride solution was added to 1 % 99.5 ml sulfuric acid solution and mixed well. Small volume of the turbid solution was transferred to screw-capped tubes of the same type as used for preparing the control inoculum and were stored in the dark at room temperature.

3.4.7.5 Inoculation and incubation of the medium

Agar surfaces of the plates containing different concentrations of the antimicrobial agent and the control plate without antimicrobial agent were spot inoculated with a 2 µl suspension using a digital micropipette. Inoculation was done from the plate containing the lowest concentration of the antimicrobial and the control plate was

inoculated last. Inoculated agar plates were allowed to stand until the inoculum spot was completely absorbed and thereafter they were incubated at 37 ºC overnight.

3.4.7.6 Interpretation of MIC results

The MIC represents the concentration of the antimicrobial at which there is complete inhibition of growth. In reading the end points, a barely visible haze of growth or a single colony was disregarded. The results were interpreted according to the recommendation chart of NCCLS (NCCLS, 2000a).

3.5 Data analysis

Water and ethanolic extracts from each plant were tested six times with each bacterium. In total, the experiment was repeated 36 times for each plant. For each replication, the zone of inhibition produced, MIC, and MBC were recorded. The response of each bacterium was also coded: growth or no growth. The number of replicates was determined in consultation with the statistician.

A Fisher's exact test was used to compare the number of replications that responded (grew) among the three bacterium in each extract (ethanol and water) separately. Where a bacterium responded, the response was compared between the two extracts using Fisher's exact test to determine which extract was the more effective. If there was no difference between the extracts, the data was pooled. The analysis was repeated for each plant.

A second analysis compared the zones of inhibitions measured in millimetres using a Kruskal Wallis test because of the small numbers of samples. The comparisons

followed the same analysis plan as the bacterial response. The size of the zone of inhibition between the three bacteria was compared first. Where possible, the zones for each bacterium were compared between the two extracts. If there was no difference the extracts were pooled and the plants compared. For the MIC's the number of colonies were compared between extracts.

3.6 Conclusion

The methods used in this study were able to provide answers to the questions about the synergistic nature of the plants with penicillins. On the screening tests the zones of inhibition were observed and the MICs for each plant against each bacterium were performed. In addition, the synergistic effect of plant extracts and penicillins against each bacterium were performed.

CHAPTER 4: RESULTS

4.1 Introduction

The zones of inhibition for the antibacterial screening tests are displayed in the figures below. The P-values that represent the statistical analysis of data are also displayed below. These figures and statistical values indicate the significant differences between values obtained during experimental work of this study. The tables for the MIC are also displayed. In the data, the ethanol extracts of plants were compared with the ethanol control and the water extracts were compared with the water control. The zones of inhibition produced by water extracts and those produced by ethanol extracts were compared and the comparison zones were recorded in tables and figures. The synergy was analysed by comparing the zones of inhibition for the combination of extracted plants with penicillins against both the individual plant and prepared penicillins.

4.2 Criteria governing the admissibility of data

Only data obtained from the experiments carried out by the researcher at the Mangosuthu University of Technology in the department of Biomedical Sciences were included in the statistical analysis. It was hypothesized that there is a synergistic effects of plant extracts (*P. guajava and S. frutescens)* and penicillins (procain, benzathine, penicillin V and penicillin G) on MRSA, MSSA and *E. faecalis*. It was further hypothesized that both water-based and ethanol extracts will exhibit antibacterial and synergistic effects on MRSA, MSSA *and E. faecalis* when combined with penicillins.

4.3 Effects of *S. frutescens* water-based extract versus water control on MRSA, MSSA and *E. faecalis*

In terms of MRSA snd MSSA, the P value was 0.002 and therefore the alternative hypothesis (H_1) was accepted since P ≤ α. Thus, there was a significant difference in diameter of zones of inhibition between *S. frutescens* water-based extracts and water control on MRSA and MSSA. This is also shown by the zones of inhibition (Figure 4.1) produced in the Kirby-Bauer Antimicrobial Sensitivity Test.

In terms of *E. faecalis,* the P value was 0.003 and therefore the alternative hypothesis (H_1) was accepted since P ≤ α. Thus, there was a significant difference in diameter of zones of inhibition between *S. frutescens* water-based extracts and water control on *E. faecalis.* This was also shown by the zones of inhibition (Figure 4.1) produced in the Kirby-Bauer Antimicrobial Sensitivity Test.

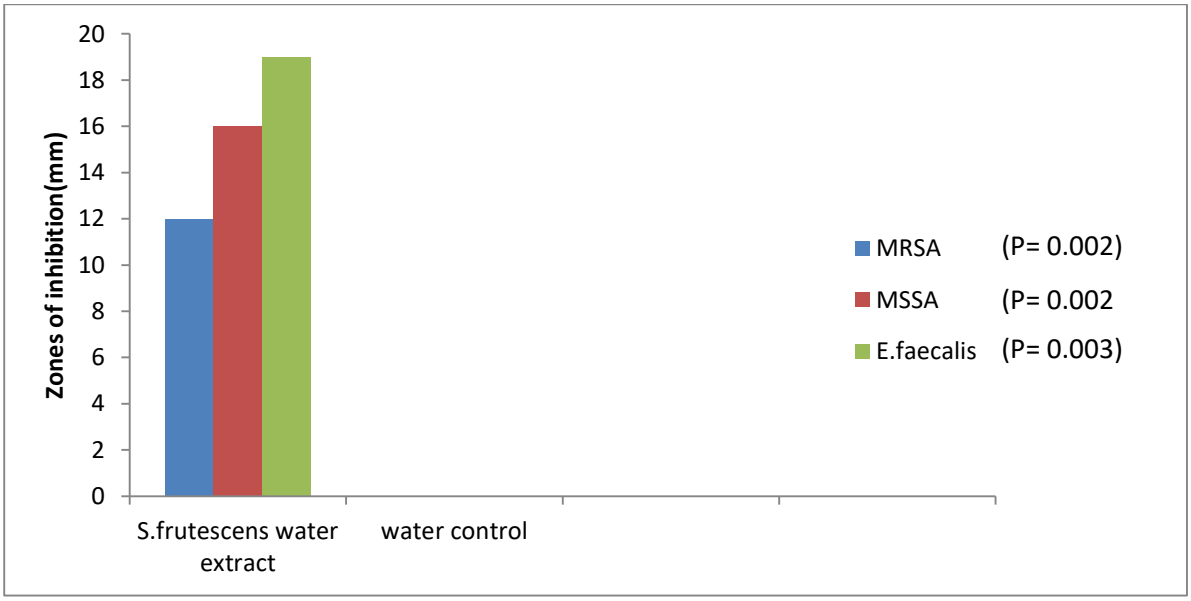


Figure 4.1: Zones of inhibition for *S. frutescens* water-based extract against bacteria

4.4 Effects of *P. guajava* water-based extract versus water control on MRSA, MSSA and *E. faecalis*

In terms of MRSA, the P value was 0.042 and therefore the alternative hypothesis (H_1) was accepted since P ≤ α. Thus, there was a significant difference in diameter of zones of inhibition between *P. guajava* water-based extracts and water control on MRSA. This is also shown by the zones of inhibition (Figure 4.2) produced in the Kirby-Bauer Antimicrobial Sensitivity Test.

In terms of MSSA, the P value was 0.026 and therefore the alternative hypothesis (H_1) was accepted since P ≤ α. Thus, there was a significant difference in diameter of zones of inhibition between *P. guajava* water-based extracts and water control on MSSA. This was also shown by the zones of inhibition (Figure 4. 2) produced in the Kirby-Bauer Antimicrobial Sensitivity Test.

In terms of *E. Faecalis,* the P value was 0.042 and therefore the alternative hypothesis (H_1) was accepted since P ≤ α. Thus, there as a significant difference in diameter of zones of inhibition between *P. guajava* water-based extracts and water control on *E. faecalis.* This was also shown by the zones of inhibition (Figure 4.2) produced in the Kirby-Bauer Antimicrobial Sensitivity Test.

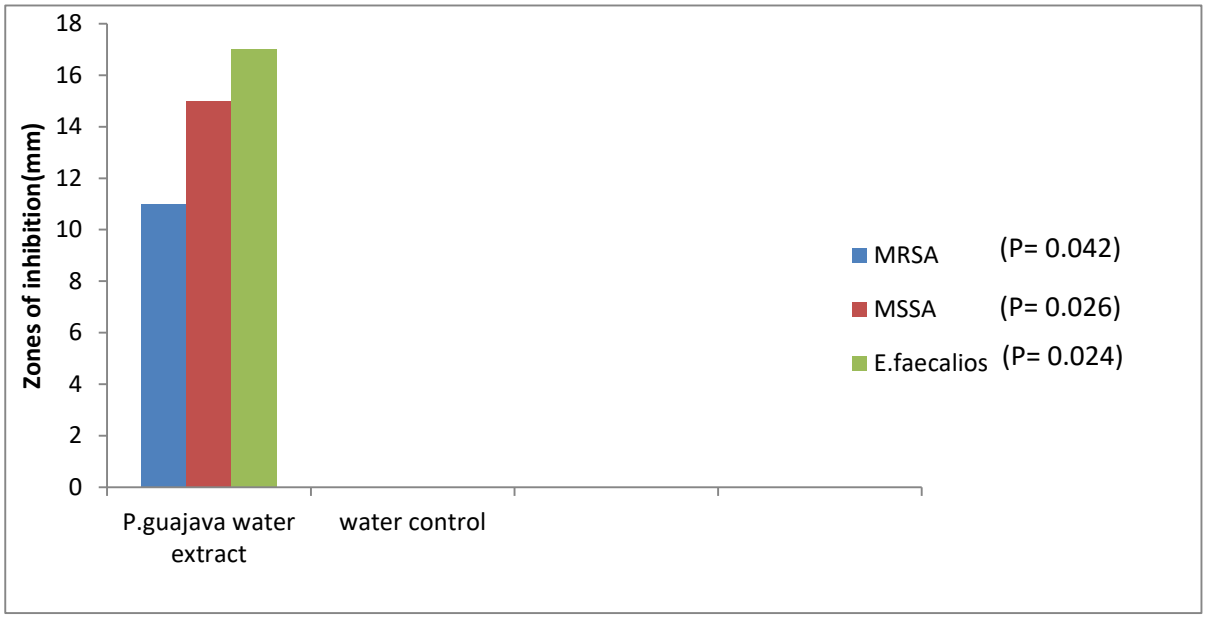


Figure 4.2: Zones of inhibition for *P. guajava* water-based extract against bacteria

4.5 Effects of *S. frutescens* ethanol-based extract versus ethanol control on MRSA, MSSA and *E. faecalis*

The zones of inhibition produced by the ethanol extract of *S. frutescens* against MRSA, MSSA and *E. faecalis* were smaller than those of the ethanol control. Therefore, the ethanol extract of *S. frutescens* was taken as being not effective against the bacteria (Figure 4.3).

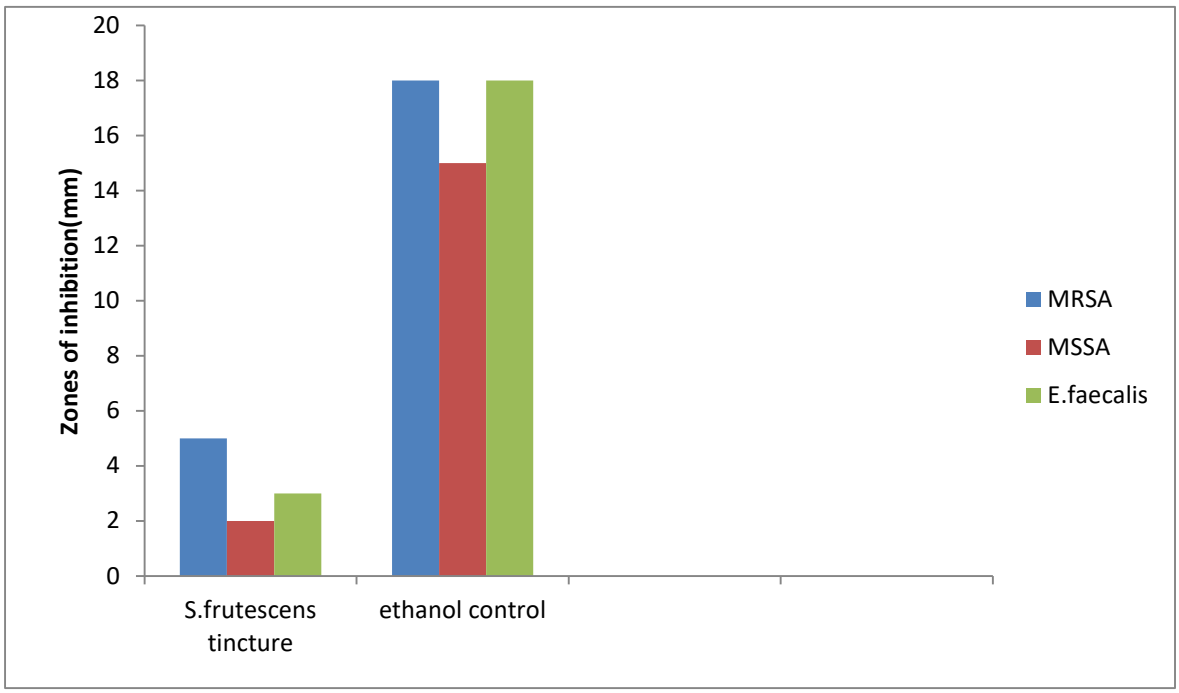


Figure 4.3: Zones of inhibition for *S. frutescens* ethanol-based extract against bacteria

4.6 Effects of *P. guajava* ethanol-based extract versus ethanol control on MRSA, MSSA and *E. faecalis*

The zones of inhibition produced by the ethanol extract of *P. guajava* against MRSA, MSSA and *E. faecalis* were smaller than those of ethanol control. Therefore, the ethanol extract of *P. guajava* was taken as being not effective against the bacteria (Figure 4.4).

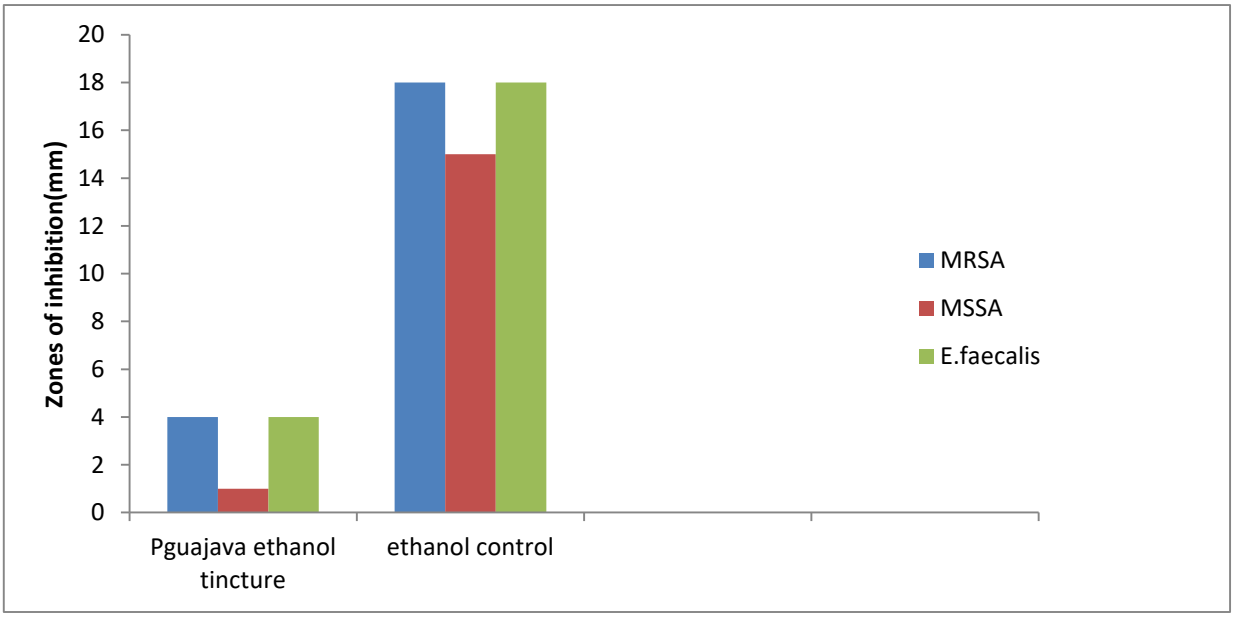


Figure 4.4: Zones of inhibition for *P. guajava* ethanol-based extract against bacteria

4.7 Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

The MICs and MBCs were performed on the extracts of *P. guajava* and *S. frutescens* that gave zones of inhibition against *S. aureus* and *E. faecalis.* If the zones of inhibition of the extract were smaller than those of the control, the MICs were not conducted. The MICs were conducted using the agar dilution technique. The growth or no growth of bacteria indicated the MIC and MBC, respectively. The results of the MICs and MBCs for the extracts were always compared with the water control which always showed 100 % growth.

4.7.1 MIC and MBC results for *S. frutescens* water-based extract against bacteria (reported as percentage growth)

The MIC and MBC for a 1 in 2 dilution to 1 in 16 dilution of *S. frutescens* water-based extract against MRSA was 100 % growth (Table 4.1).

The MIC and MBC for a 1 in 2 dilution of *S. frutescens* water extract against MSSA was 0 % growth, for a 1 in 4 dilution *S. frutescens* water-based extract against MSSA was 66.7 % growth, and for a 1 in 8 dilution to 1 in 16 dilution of *S. frutescens* water-based extract against MSSA was 100 % growth.

The MIC and MBC for a 1 in 2 dilution of *S. frutescens* water-based extract against *E. faecalis* was 0 % growth and for a 1 in 4 dilution to 1 in 16 dilution of *S. frutescens* water-based extract against *E. faecalis* was 100 % growth (Table 4.1).

Table 4.1: Analysis of MIC and MBC results for *S. frutescens* water-based extract against bacteria

Dilution	MRSA (% growth)	MSSA (% growth)	*E. faecalis* (% growth)
1 in 2	100 %	0 %	0 %
1 in 4	100 %	66.7 %	100 %
1 in 8	100 %	100 %	100 %
1 in 16	100 %	100 %	100 %

4.7.2 MIC and MBC results for *P. guajava* water-based extract against bacteria (reported as percentage growth)

The MIC and MBC for a 1 in 2 dilution of *P. guajava* water-based extract against MRSA was 0 % growth and for a 1 in 4 dilution to 1 in 16 dilution of *P. guajava* water-based extract against MRSA was 100 % growth.

The MIC and MBC for a 1 in 2 dilution to 1 in 4 dilution of *P. guajava* water-based extract against MSSA was 0 % growth and for a 1 in 8 dilution to 1 in 16 dilution of *P. guajava* water-based extract against MSSA was 100 % growth.

The MIC and MBC for a 1 in 2 dilution of *P. guajava* water-based extract against *E. faecalis* was 16.7 % growth and for a 1 in 4 dilution to 1 in 16 dilution of *P. guajava* water-based extract against *E. faecalis* was 100 % growth (Table 4.2).

Table 4.2: Analysis of the MIC and MBC results for *P. guajava* water-based extract against bacteria

Dilution	MRSA (% growth)	MSSA (% growth)	*E. faecalis* (% growth)
1 in 2	0 %	0 %	16.7 %
1 in 4	100 %	0 %	100 %
1 in 8	100 %	100 %	100 %
1 in 16	100 %	100 %	100 %

4.8 The synergistic effects of plant extracts and penicillins on *S. aureus* and *E. faecalis* (reported as percentage growth)

4.8.1 Effects of a combination of *S. frutescens* water-based extract and procain penicillin compared to *S. frutescens* water-based extract alone on MRSA, MSSA and *E. faecalis*

The results of a combination of water-based extract of *S. frutescens* and penicillins are presented in Figure 4.5.

In terms of MRSA, MSSA and *E. faecalis,* the null hypothesis (H_O) was accepted since $P \geq \alpha$. There was no significant difference in diameter of the zones of inhibition between a combination of *S. frutescens* water-based extract and procain penicillin against *S. frutescens* water-based extract on MRSA, MSSA and *E. faecalis*. This was also shown by the zones of inhibition produced in the Kirby-Bauer Antimicrobial Sensitivity Test (Figure 4.5).

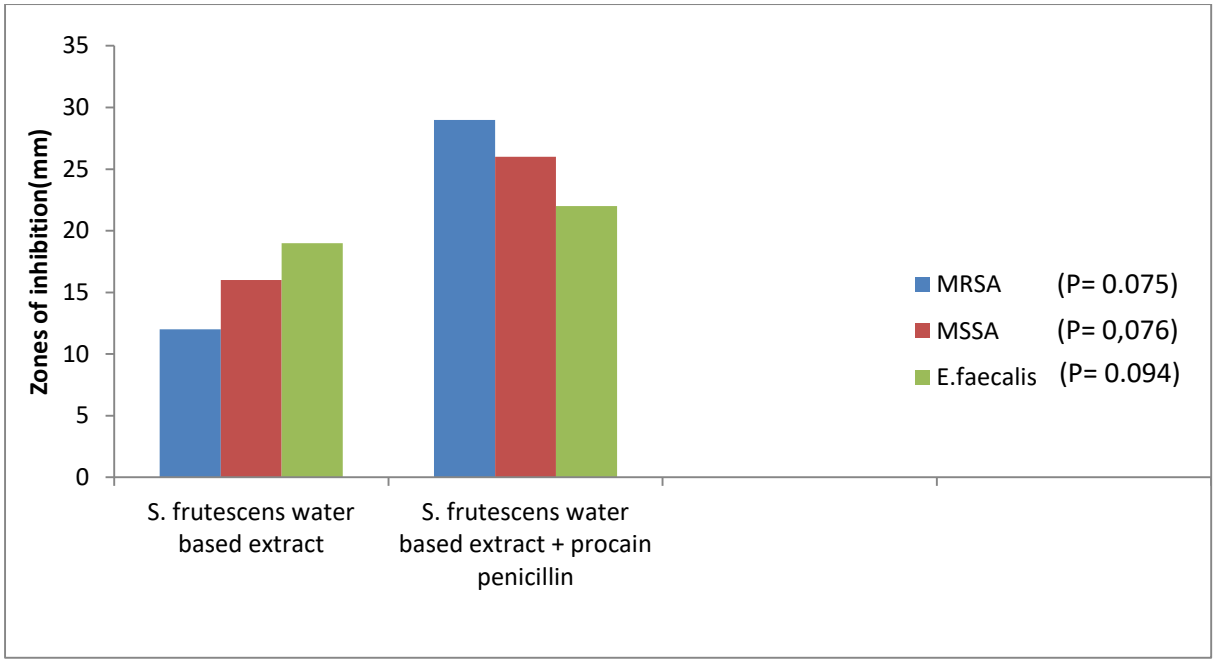


Figure 4.5: Zones of inhibition for the combination of *S. frutescens* water-based extract *S. frutescens* and procain penicillin compared to *S. frutescens* water-based extract alone on bacteria

4.8.2 Effects of a combination of *S. frutescens* water-based extract and procain penicillin compared to procain penicillinon alone on MRSA, MSSA and *E. faecalis*

In terms of MRSA, MSSA and *E. faecalis,* the null hypothesis (H_O) was accepted since $P \geq \alpha$. There was no significant difference in diameter of the zones of inhibition between combinations of *S. frutescens* water-based extract and procain penicillin against procain penicillin on MRSA, MSSA and *E. faecalis*. This was also shown by the zones of inhibition produced in the Kirby-Bauer Antimicrobial Sensitivity Test (Figure 4.6).

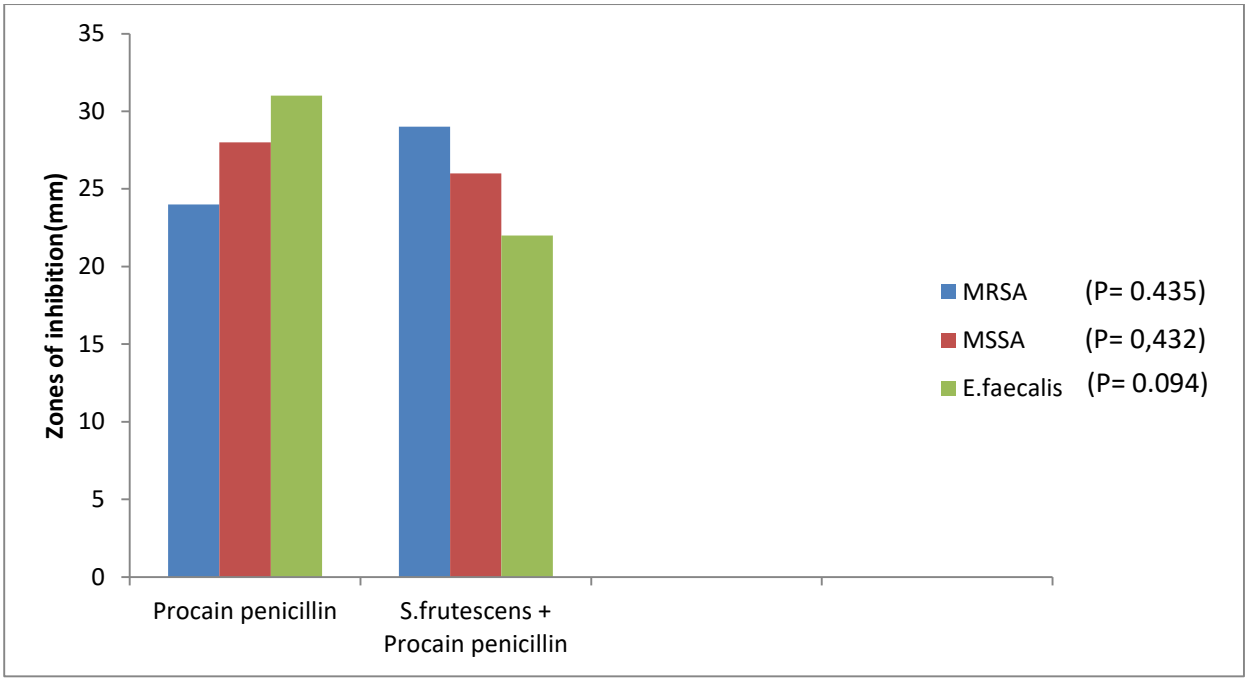


Figure 4.6: Zones of inhibition for a combination of *S. frutescens* water-based extract and procain penicillin compared to procain penicillin alone on bacteria

4.8.3 Effects of a combination of *S. frutescens* water-based extract and benzathine penicillin compared to *S. frutescens* water-based extract alone on MRSA, MSSA and *E. faecalis*

In terms of MRSA, the alternative hypothesis (H_1) was accepted since $P \leq \alpha$. There was a significant difference in diameter of zones of inhibition between a combination of *S. frutescens* water-based extract and benzathine penicillin compared to *S. frutescens* water-based extract alone on MRSA.

In terms of MSSA and *E. faecalis,* the null hypothesis (H_O) was accepted since $P \geq \alpha$. There was no significant difference in diameter of the zones of inhibition between a combination of *S. frutescens* water-based extract and benzathine penicillin compared to *S. frutescens* water-based extract alone on MSSA and *E. faecalis.* This

was also shown by the zones of inhibition produced in the Kirby-Bauer Antimicrobial Sensitivity Test (Figure 4.7).

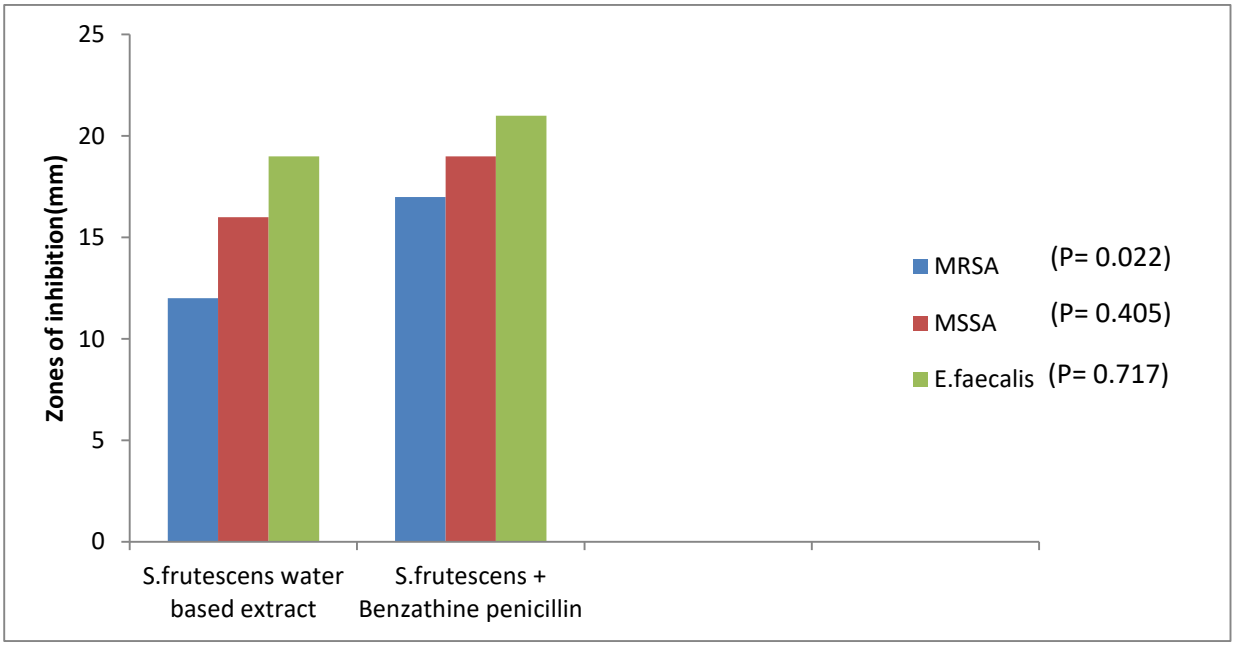


Figure 4.7: Zones of inhibition for a combination of *S. Frutescens* water-based extract and benzathine penicillin against *S. frutescens* water-based extract alone on bacteria

4.8.4 Effects of a combination of *S. frutescens* water-based extract and benzathine penicillin compared to benzathine penicillin alone on MRSA, MSSA and *E. faecalis*

In terms of MRSA and MSSA, the null hypothesis (H_O) was accepted since $P \geq \alpha$. There was no significant difference in diameter of the zones of inhibition between combinations of *S. frutescens* water-based extract and benzathine penicillin conmpared to benzathine penicillin alone on MRSA and MSSA.

In terms of *E. faecalis,* the alternative hypothesis (H_1) was accepted since $P \leq \alpha$. There was a significant difference in diameter of zones of inhibition between

combinations of *S. frutescens* water-based extract and benzathine penicillin compared to benzathine penicillin alone on *E. faecalis* which indicates that there is an antagonistic effect when this combination is used. This was also shown by the zones of inhibition produced in the Kirby-Bauer Antimicrobial Sensitivity Test (Figure 4.8).

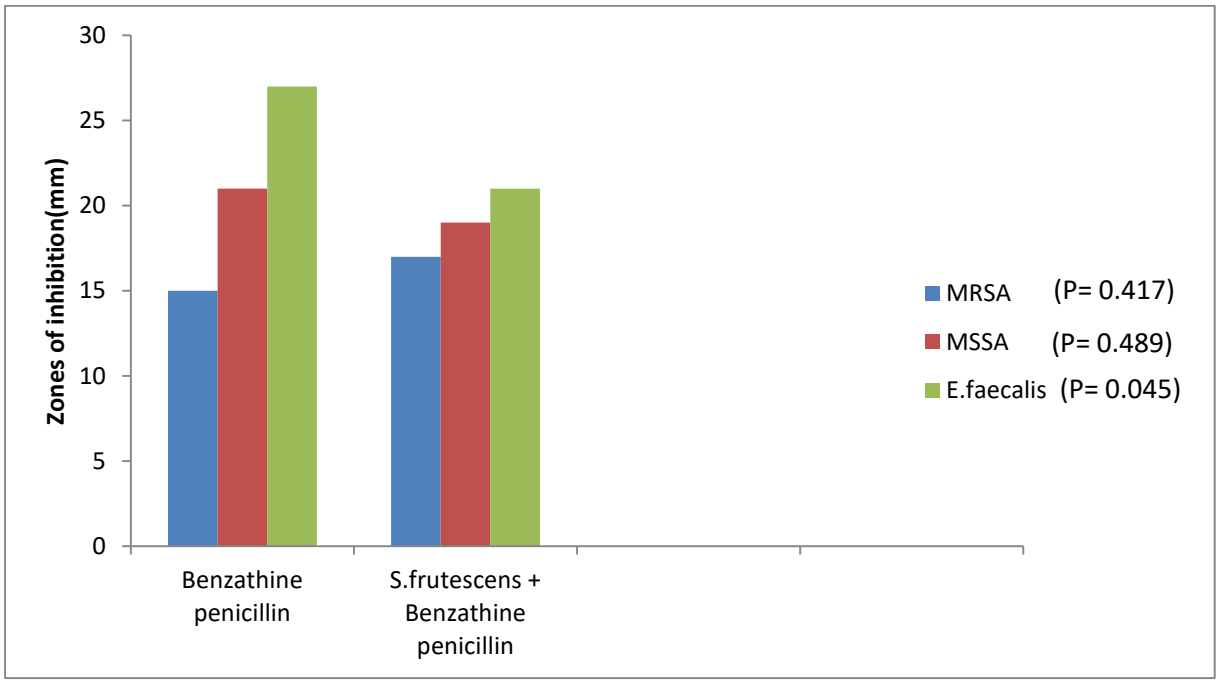


Figure 4.8: Zones of inhibition for a combination of *S. frutescens* water-based extract and benzathine penicillin compared to benzathine penicillin alone on bacteria

4.8.5 Effects of a combination of *S. frutescens* water-based extract and penicillin V compared to *S. frutescens* water-based extract alone on MRSA, MSSA and *E. faecalis*

In terms of MRSA, MSSA and *E. faecalis,* the null hypothesis (H_O) was accepted since $P \geq \alpha$. There was no significant difference in diameter of the zones of inhibition between a combination of *S. frutescens* water-based extract and penicillin V compared to *S. frutescens* water-based extract alone on MRSA, MSSA and *E.*

faecalis. This was also shown by the zones of inhibition produced in the Kirby-Bauer Antimicrobial Sensitivity Test (Figure 4.9).

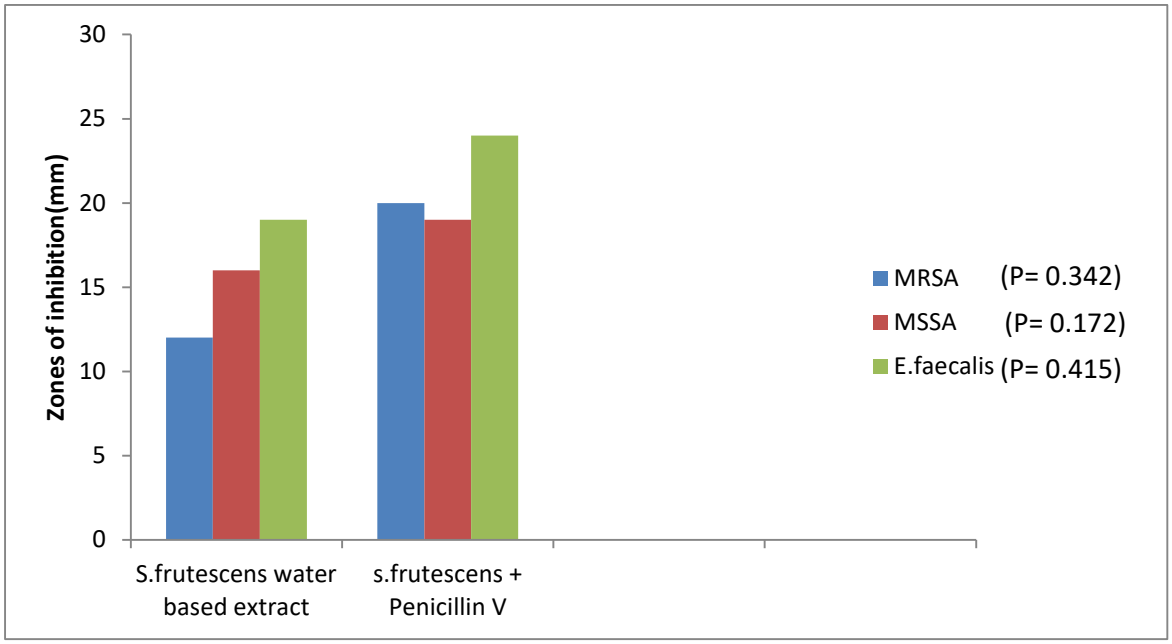


Figure 4.9: Zones of inhibition for a combination of *S. frutescens* water-based extract and penicillin V compared to *S. frutescens* water-based extract alone on bacteria

4.8.6 Effects of a combination of *S. frutescens* water-based extract and penicillin V compared to penicillin V alone on MRSA, MSSA and *E. faecalis*

The null hypothesis (H_O) was accepted since $P \geq \alpha$. Thus, there was no significant difference in diameter of the zones of inhibition between combinations of *S. frutescens* water-based extract and penicillin V compared to penicillin V alone on MRSA, MSSA and *E. faecalis*. This was also shown by the zones of inhibition produced in the Kirby-Bauer Antimicrobial Sensitivity Test (Figure 4.10).

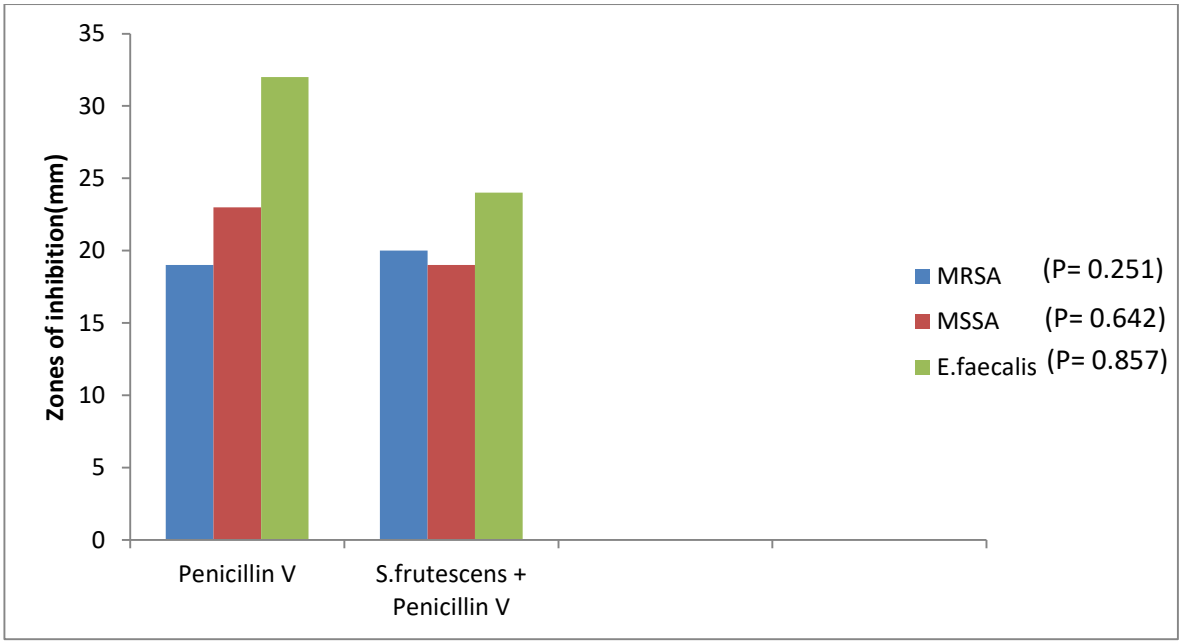


Figure 4.10: Zones of inhibition for a combination of *S. frutescens* water-based extract and penicillin V compared to penicillin V alone on bacteria

4.8.7 Effects of a combination of *S. frutescens* water-based extract and penicillin G compared to *S. frutescens* water-based extract alone on MRSA, MSSA and *E. faecalis*

In terms of MRSA, MSSA and *E. faecalis,* the alternative hypothesis (H_1) was accepted since $P \leq \alpha$. There was a significant difference in diameter of zones of inhibition between a combination of *S. frutescens* water-based extract and penicillin G compared to *S. frutescens* water-based extract alone on MRSA, MSSA and *E. faecalis*. This was also shown by the zones of inhibition produced in the Kirby-Bauer Antimicrobial Sensitivity Test (Figure 4.11).

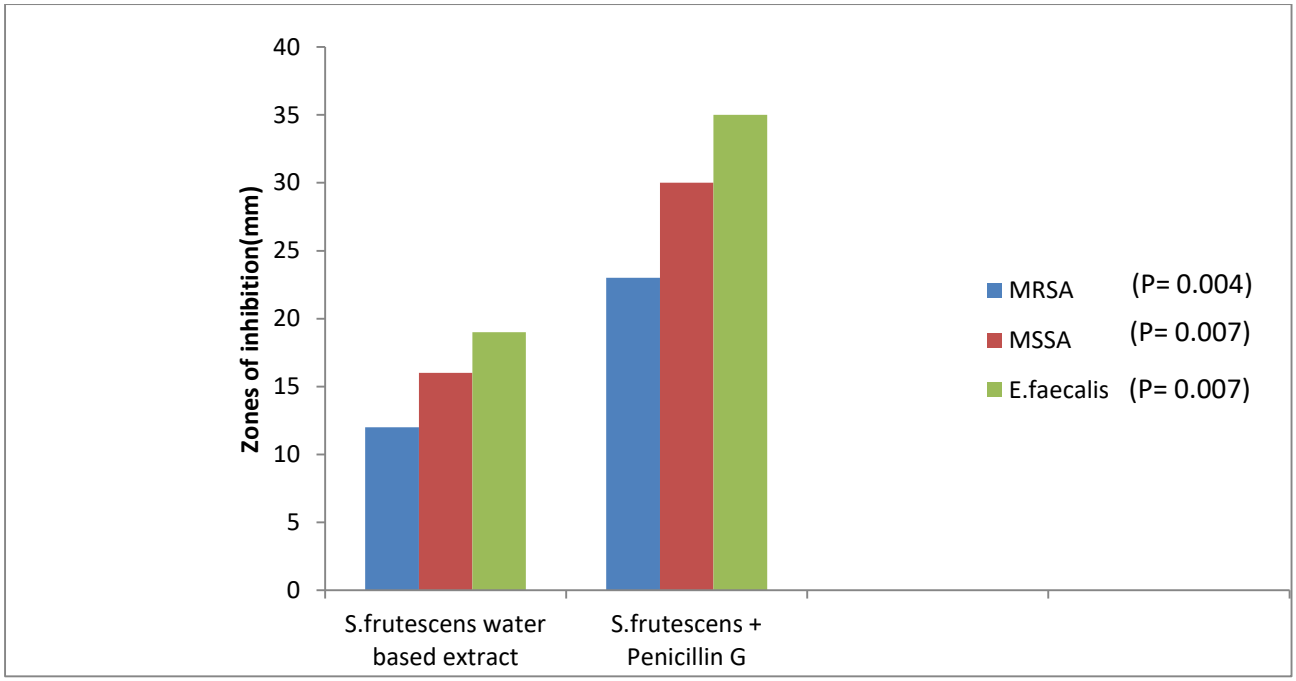


Figure 4.11: Zones of inhibition for a combination of *S. frutescens* water-based extract and penicillin G compared to *S. frutescens* water-based extracton alone on bacteria

4.8.8 Effects of a combination of *S. frutescens* water-based extract and penicillin G compared to penicillin G alone on MRSA, MSSA and *E. faecalis*

In terms of MRSA and MSSA, the alternative hypothesis (H_1) was accepted since P ≤ α. There was a significant difference in diameter of zones of inhibition between a combination of *S. frutescens* water-based extract and penicillin G compared to penicillin G alone on MRSA and MSSA although this difference indicates that there was an antagonistic effect when this combination was used. This was also shown by the zones of inhibition produced in the Kirby-Bauer Antimicrobial Sensitivity Test (Figure 4.12).

In terms of *E. faecalis,* the alternative hypothesis (H_1) was accepted since P ≤ α. There was a significant difference in diameter of zones of inhibition between

combinations of *S. frutescens* water-based extract and penicillin G against penicillin G on *E. faecalis*. This is also shown by the zones of inhibition produced in the Kirby-Bauer Antimicrobial Sensitivity Test (Figure 4.12).

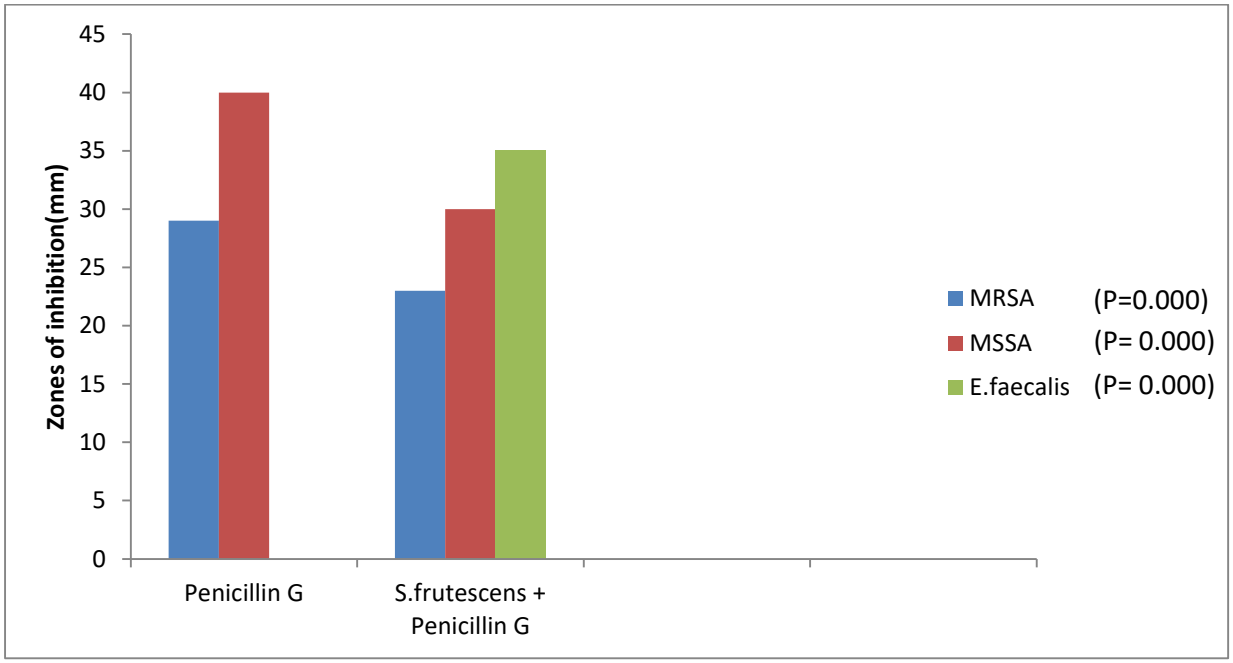


Figure 4.12*:* Zones of inhibition for a combination of *S. frutescens* water-based extract and penicillin G compared to penicillin G alone on bacteria

4.8.9 Effects of a combination of *P. guajava* water-based extract and procain penicillin compared to *P. guajava* water-based extract alone on MRSA, MSSA and *E. faecalis*

In terms of MRSA, MSSA and *E. faecalis,* the alternative hypothesis (H_1) was accepted since $P \leq \alpha$. There was a significant difference in diameter of zones of inhibition between a combination of *P. guajava* water-based extract and procain penicillin compared to *P. guajava* water-based extract alone on MRSA, MSSA and *E. faecalis.* This was also shown by the zones of inhibition produced in the Kirby-Bauer Antimicrobial Sensitivity Test (Figure 4.13).

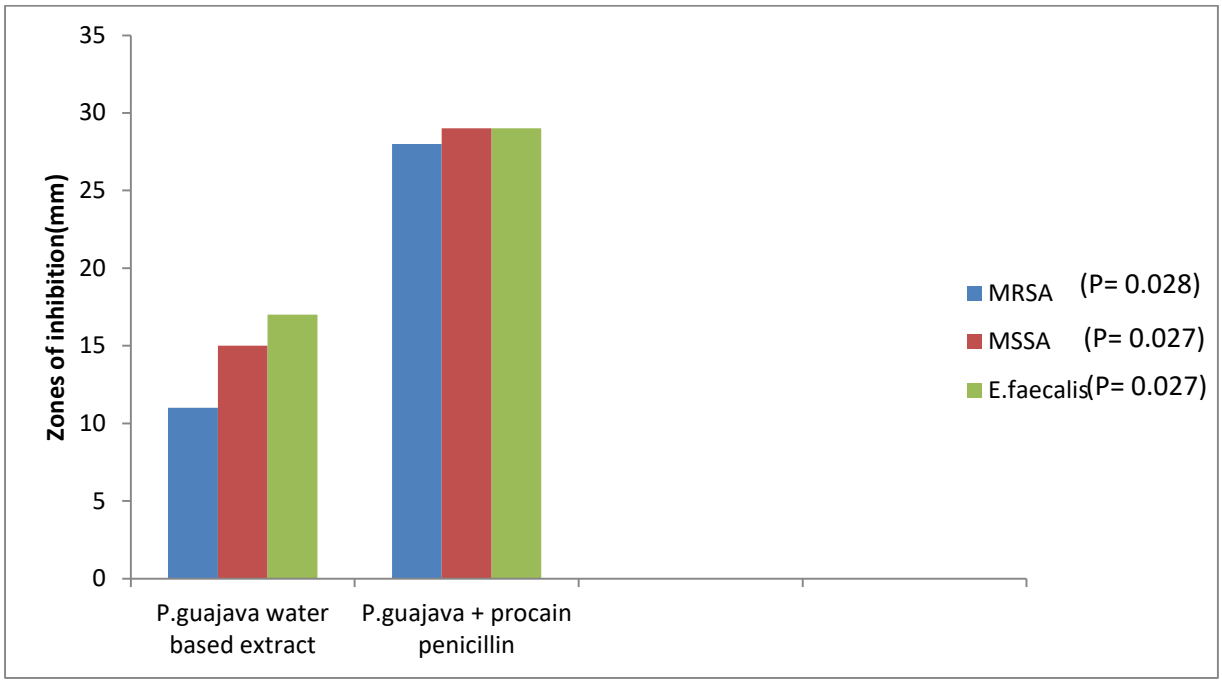


Figure 4.13*: Zones of inhibition for the combination of P. guajava* water-based extract and procain penicillin compared to *P. guajava* water-based extract alone on bacteria

4.8.10 Effects of a combination of *P. guajava* water-based extract and procain penicillin compare to procain penicillin alone on MRSA, MSSA and *E. faecalis*

In terms of MRSA, the alternative hypothesis (H_1) was accepted since $P \leq \alpha$. There was a significant difference in diameter of zones of inhibition between a combination of *P. guajava* water-based extract and procain penicillin compared to procain penicillin alone on MRSA.

In terms of MSSA and *E. faecalis,* the null hypothesis (H_O) was accepted since $P \geq \alpha$. There was no significant difference in diameter of the zones of inhibition between a combination of *P. guajava* water-based extract and procain penicillin compared to

procain penicillin alone on MSSA and *E. faecalis.* This was also shown by the zones of inhibition produced in the Kirby-Bauer Antimicrobial Sensitivity Test (Figure 4.14).

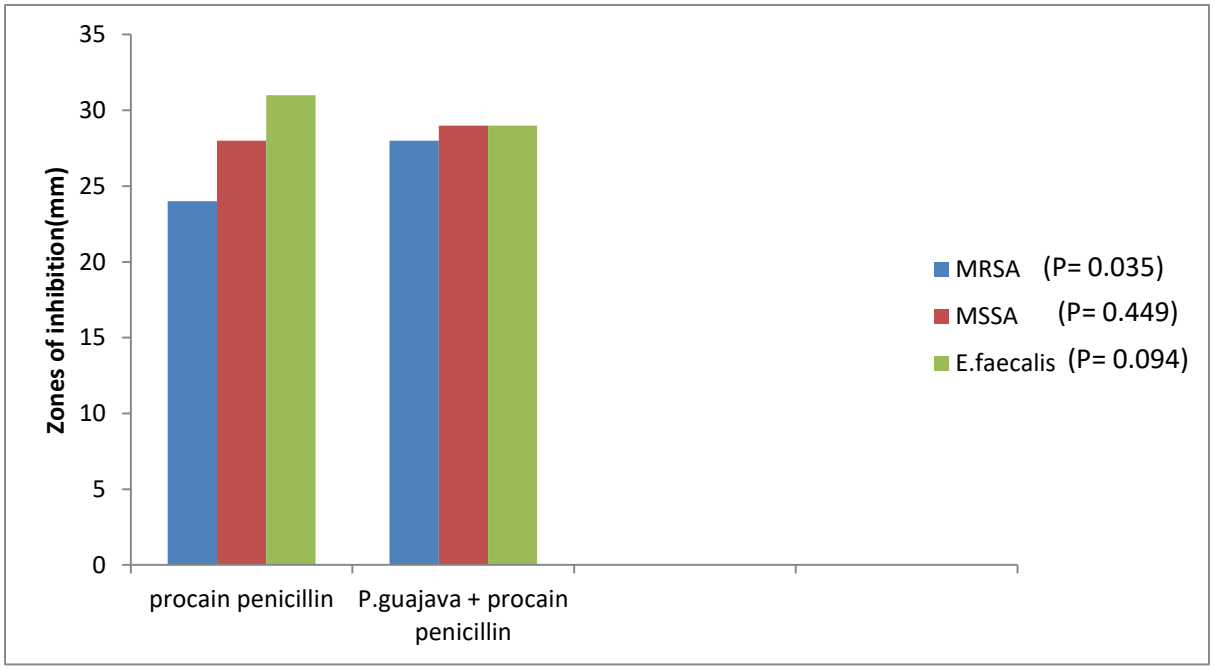


Figure 4.14: Zones of inhibition for a combination of *P. guajava* water-based extract and procain penicillin compared to procain penicillin alone on bacteria

4.8.11 Effects of a combination of *P. guajava* water-based extract and benzathine penicillin compared to *P. guajava* water-based extract alone on MRSA, MSSA and *E. faecalis*

In terms of MRSA, MSSA and *E. faecalis,* the alternative hypothesis (H_1) was accepted since $P \leq \alpha$. There was a significant difference in diameter of zones of inhibition between a combination of *P. guajava* water-based extract and benzathine penicillin compared to *P. guajava* water-based extract alone on MRSA, MSSA and *E. faecalis*. This was also shown by the zones of inhibition produced in the Kirby-Bauer Antimicrobial Sensitivity Test (Figure 4.15).

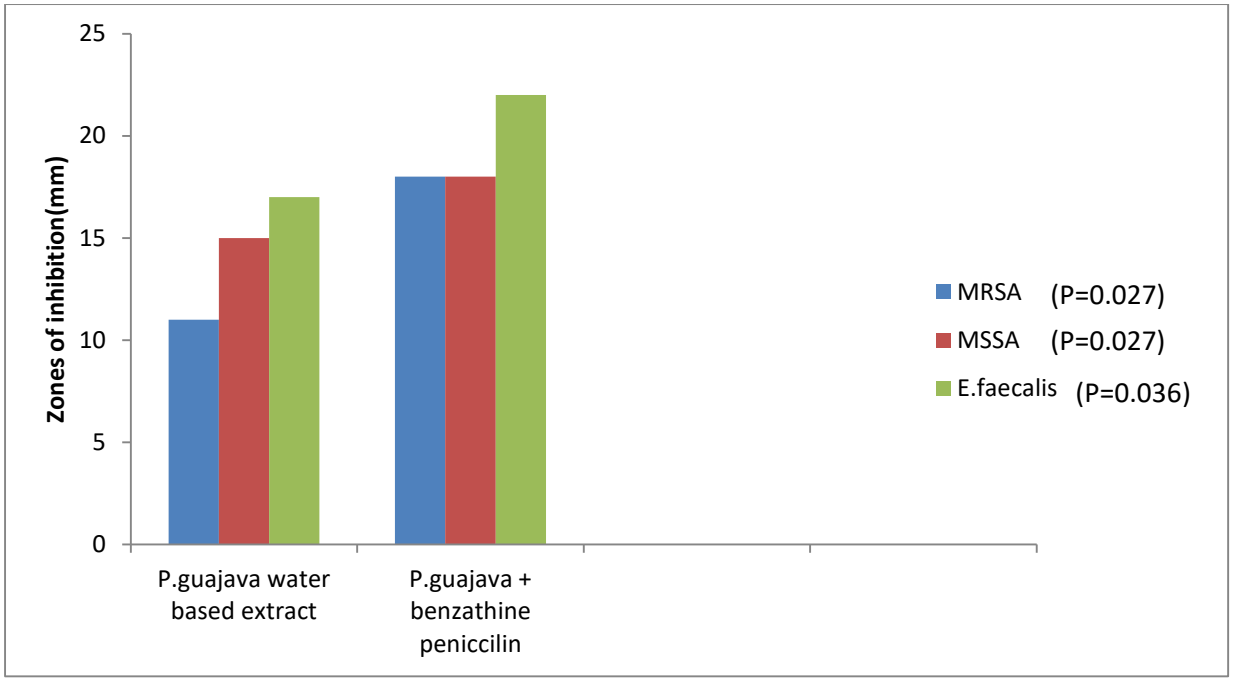


Figure 4.15: Zones of inhibition for a combination of *P. guajava* water-based extract and benzathine penicillin compared to *P. guajava* water-based extract alone on bacteria

4.8.12 Effects of a combination of *P. guajava* water-based extract and benzatine penicillin compared to benzathine penicillin alone on MRSA, MSSA and *E. faecalis*

In terms of MRSA and MSSA, the null hypothesis (H_O) was accepted since $P \geq \alpha$. There was no significant difference in diameter of the zones of inhibition between a combination of *P. guajava* water-based extract and benzathine penicillin compared to benzathine penicillin alone on MRSA and MSSA.

In terms of *E. faecalis,* the alternative hypothesis (H_1) was accepted since $P \leq \alpha$. Thus, there is a significant difference in diameter of zones of inhibition between a combination of *P. guajava* water-based extract and benzathine penicillin compared to benzathine penicillin alone on *E. faecalis* although this difference indicates that there is an antagonistic effect when this combination is used. This was also shown

by the zones of inhibition produced in the Kirby-Bauer Antimicrobial Sensitivity Test (Figure 4.16).

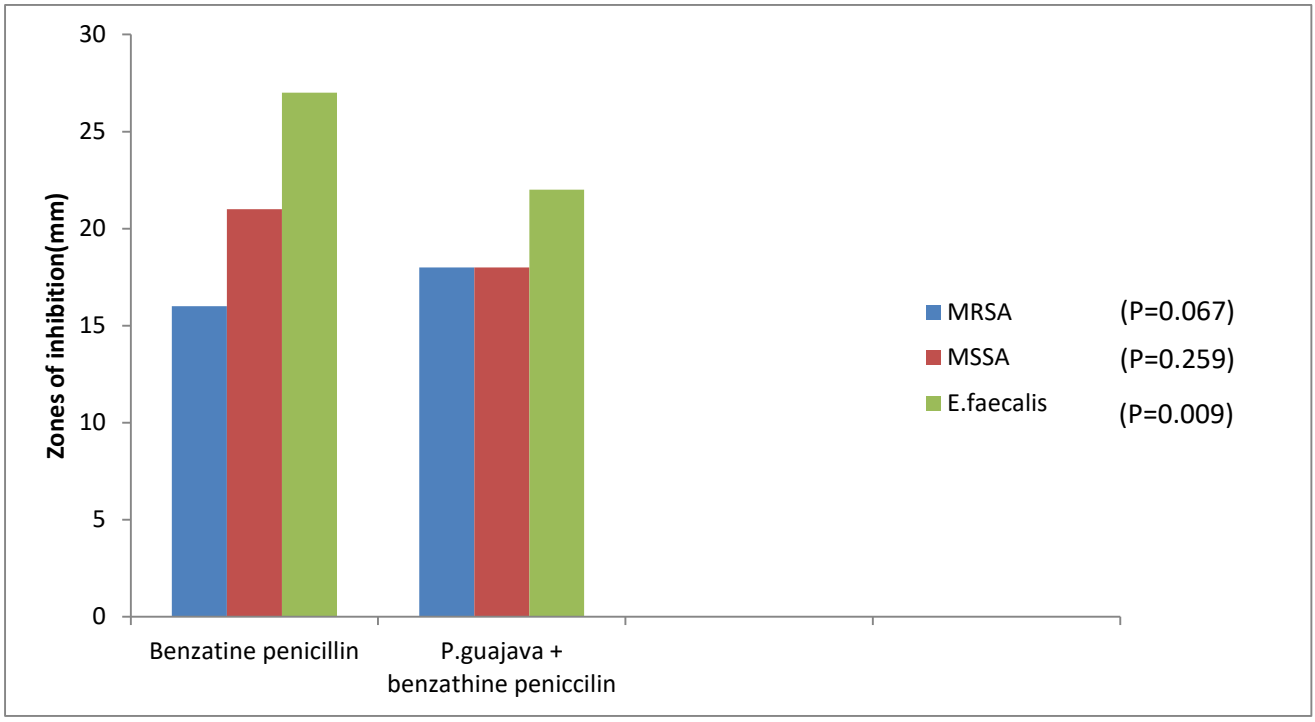


Figure 4.16: Zones of inhibition for a combination of *P. guajava* water-based extract and benzathine penicillin compared to benzathine penicillin alone on bacteria

4.8.13 Effects of a combination of *P. guajava* water-based extract and penicillin V compared to *P. guajava* water-based extract alone on MRSA, MSSA and *E. faecalis*

In terms of MRSA and MSSA, the alternative hypothesis (H_1) was accepted since P ≤ α. There was a significant difference in diameter of zones of inhibition between a combination of *P. guajava* water-based extract and penicillin V compared to *P. guajava* water-based extract alone on MRSA and MSSA.

In terms of *E. faecalis,* the null hypothesis (H_O) was accepted since P ≥ α. There was no significant difference in diameter of the zones of inhibition between a combination of *P. guajava* water-based extract and penicillin V compared to *P. guajava* water-based extract alone on *E. faecalis*. This was also shown by the zones of inhibition produced in the Kirby-Bauer Antimicrobial Sensitivity Test (Figure 4.17).

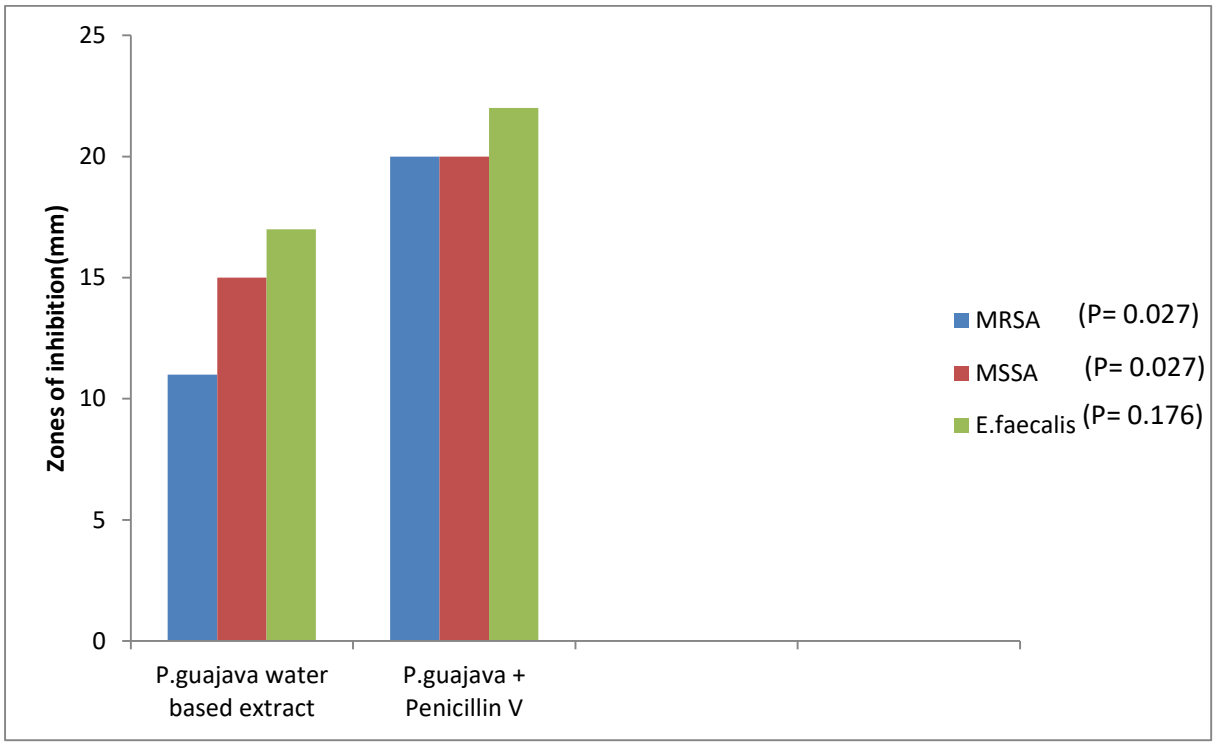


Figure 4.17: Zones of inhibition for a combination of *P. guajava* water-based extract and penicillin V compared to *P. guajava* water-based extract alone on bacteria

4.8.14 Effects of a combination of *P. guajava* water-based extract and penicillin V compared to penicillin V alone on MRSA, MSSA and *E. faecalis*

In terms of MRSA and MSSA, the null hypothesis (H_O) was accepted since P ≥ α. There was no significant difference in diameter of the zones of inhibition between a

combination of *P. guajava* water-based extract and penicillin V compared to penicillin V alone on MRSA and MSSA.

In terms of *E. faecalis,* the alternative hypothesis (H_1) was accepted since $P \leq \alpha$. There was significant difference in diameter of zones of inhibition between a combination of *P. guajava* water-based extract and penicillin V compared to penicillin V alone on *E. faecalis* although this difference indicates an antagonistic effect when this combination is used. This was also shown by the zones of inhibition produced in the Kirby-Bauer Antimicrobial SensitivityTest (Figure 4.18).

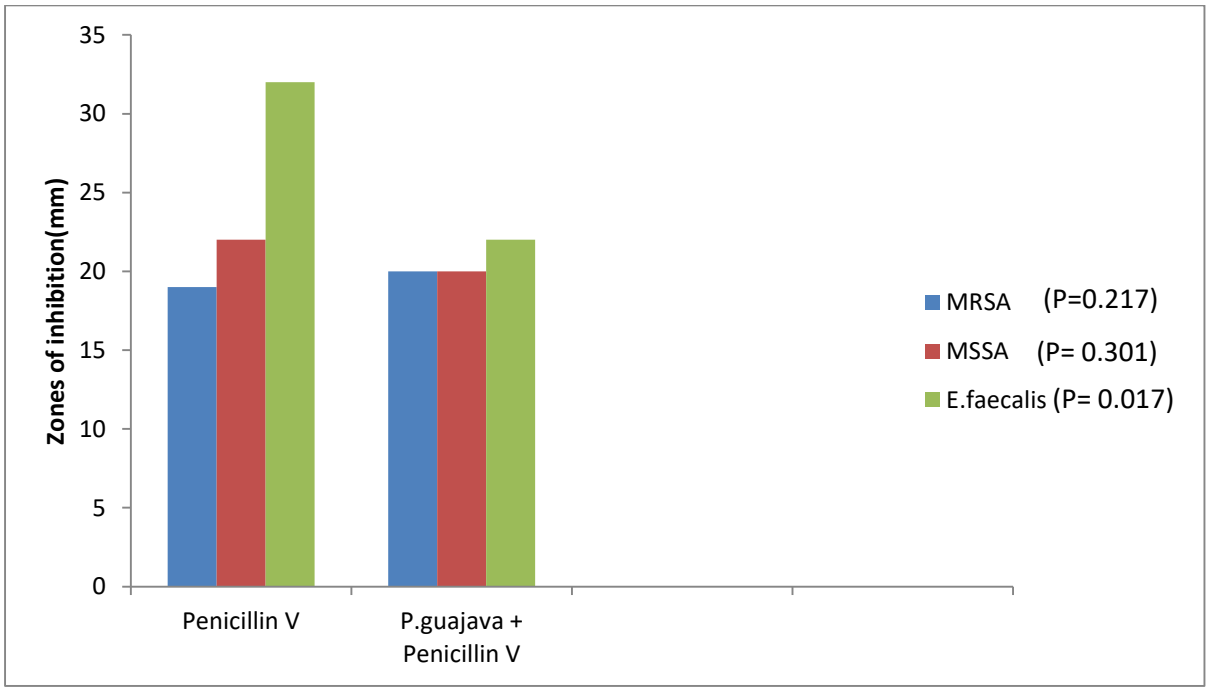


Figure 4.18: Zones of inhibition for a combination of *P. Guajava* water-based extract and penicillin V compared to penicillin V alone on bacteria

4.8.15 Effects of a combination of *P. guajava* water-based extract and penicillin G compared to *P. guajava* water-based extract alone on MRSA, MSSA and *E. faecalis*

In terms of MRSA and MSSA, the alternative hypothesis (H_1) was accepted since P ≤ α. There was a significant difference in diameter of zones of inhibition between a combination of *P. guajava* water-based extract and penicillin G compared to *P. guajava* water-based extract alone on MRSA and MSSA.

In terms of *E. faecalis,* the null hypothesis (H_O) was accepted since P ≥ α. There was no significant difference in diameter of the zones of inhibition between a combination of *P. guajava* water-based extract and penicillin G compared to *P. guajava* water-based extract alone on *E. faecalis*. This was also shown by the zones of inhibition produced in the Kirby-Bauer Antimicrobial Sensitivity Test (Figure 4.19).

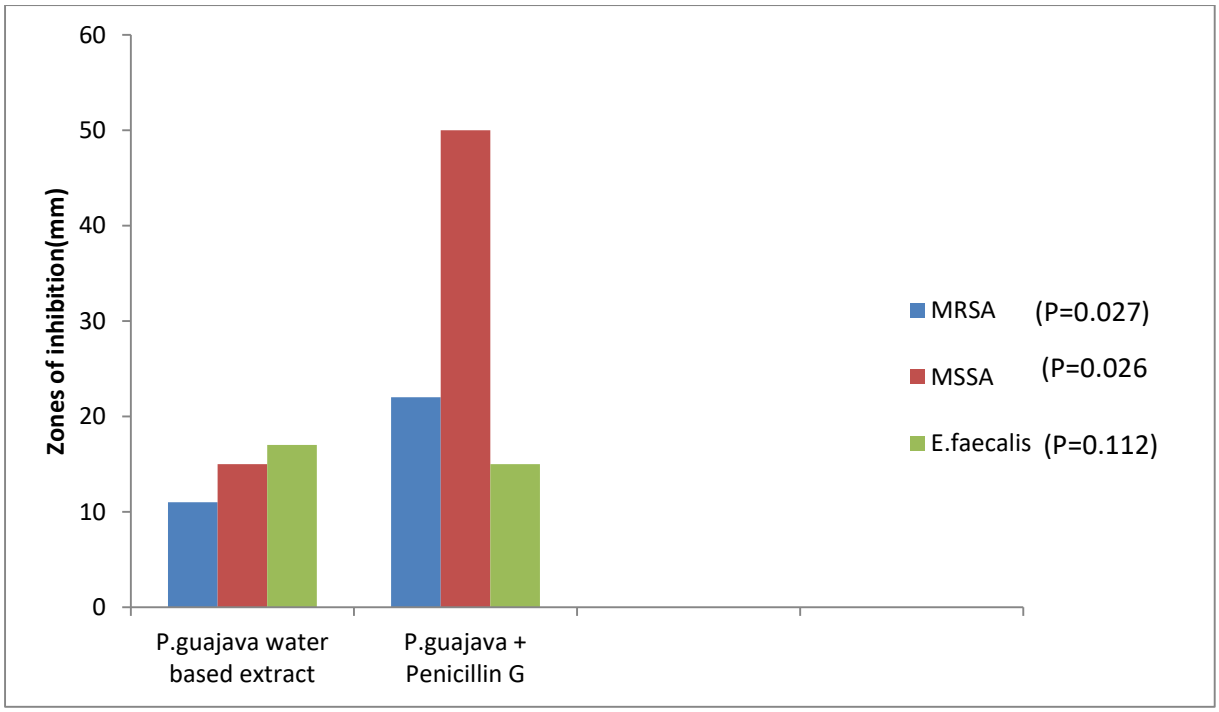


Figure 4.19*:* Zones of inhibition for a combination of *P. guajava* water-based extract and penicillin G compared to *P. guajava* water-based extract alone on bacteria

4.8.16 Effects of a combination of *P. guajava* water-based extract and penicillin G compared to penicillin G alone on MRSA, MSSA and *E. faecalis*

In terms of MRSA, the alternative hypothesis (H_1) was accepted since $P \leq \alpha$. There was a significant difference in diameter of zones of inhibition between a combination of *P. guajava* water-based extract and penicillin G against penicillin G on MRSA although this difference indicates an antagonistic effect when this combination is used.

In terms of MSSA and *E. faecalis,* the alternative hypothesis (H_1) was accepted since $P \leq \alpha$. There was a significant difference in diameter of zones of inhibition between a combination of *P. guajava* water-based extract and penicillin G compared to penicillin G alone on MSSA and *E. faecalis*. This was also shown by the zones of inhibition produced in the Kirby-Bauer Antimicrobial Sensitivity Test (Figure 4.20).

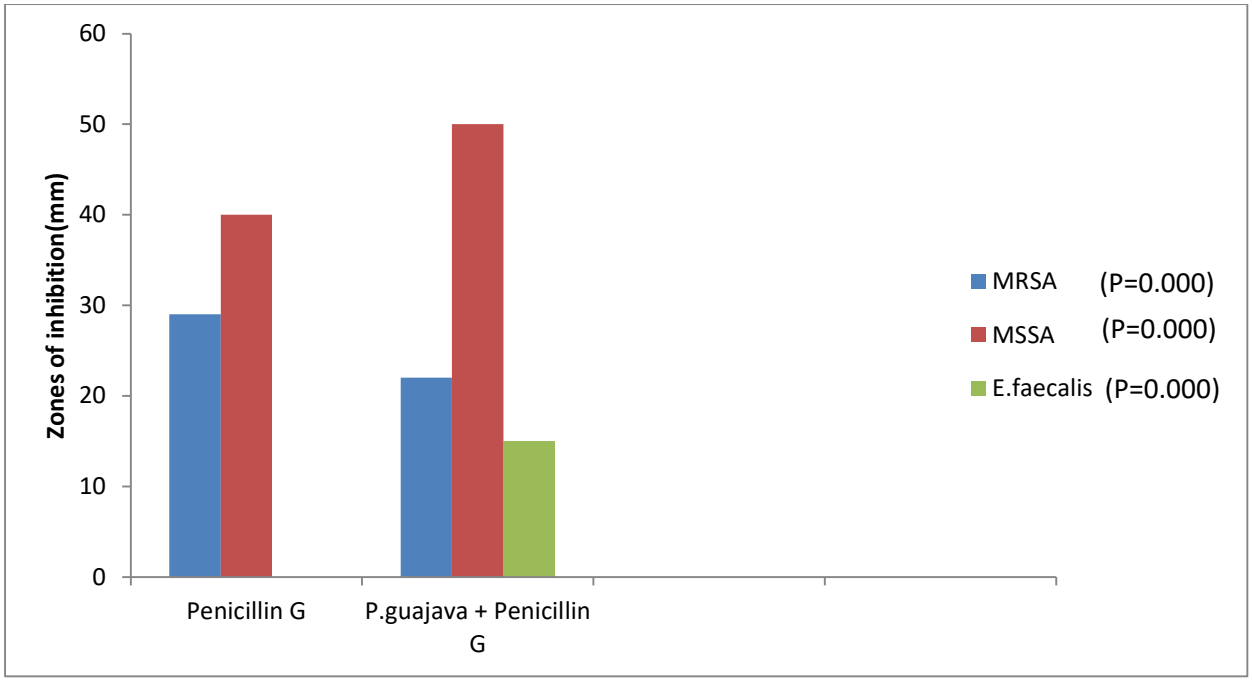
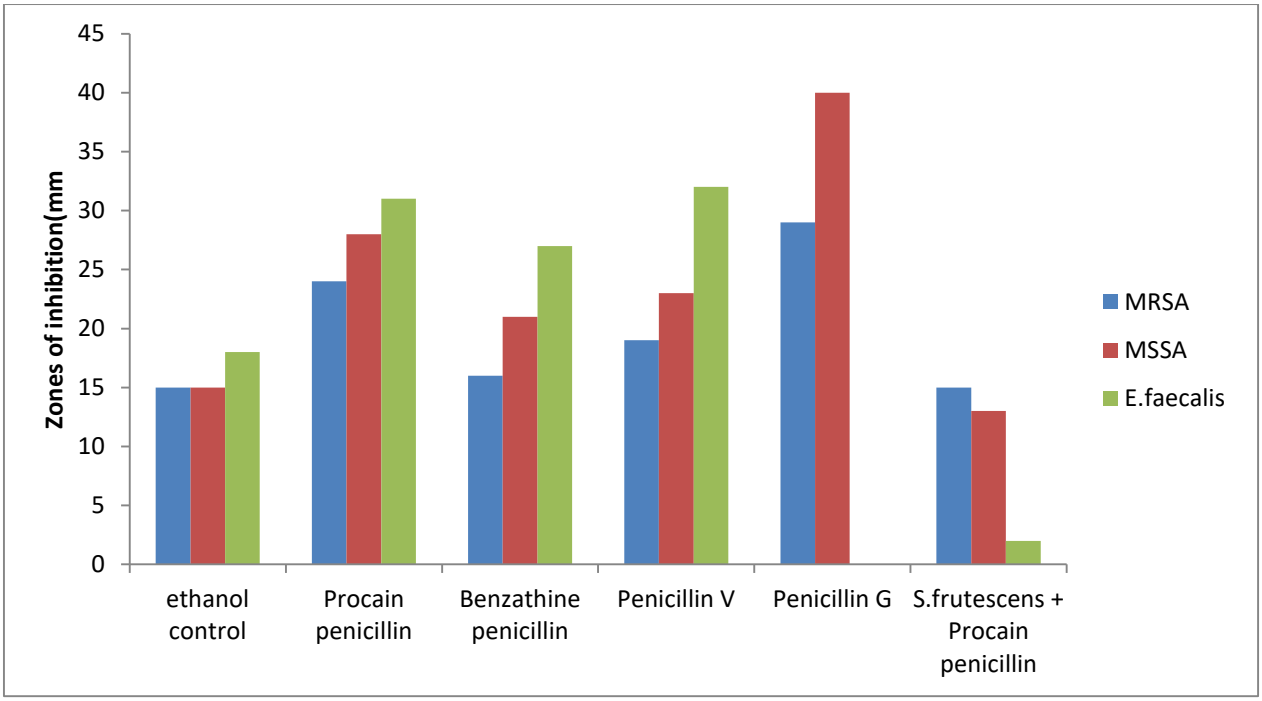


Figure 4.20: Zones of inhibition for a combination of *P. guajava* water-based extract and penicillin G compared to penicillin G alone on bacteria

4.8.17 Effects of a combination of *S. frutescens* ethanol-based extract and penicillins on MRSA, MSSA and *E. faecalis*

The combinations of *S. Frutescens* ethanol-based extract and penicillins were compared with the ethanol control and all the inhibition zones that were less than that of ethanol control were taken as being not effective against respective bacteria. The zones of inhibition that were greater than the ethanol control were then compared with the individual penicillin to establish their synergistic effects. If the zones of inhibition for the combinations were less than the individual penicillins, they were taken as lacking the synergistic effects against respective bacteria. There was no synergistic effect observed. This was also shown by the zones of inhibition mproduced in the Kirby-Bauer Antimicrobial Sensitivity Test (Figure 4.21).



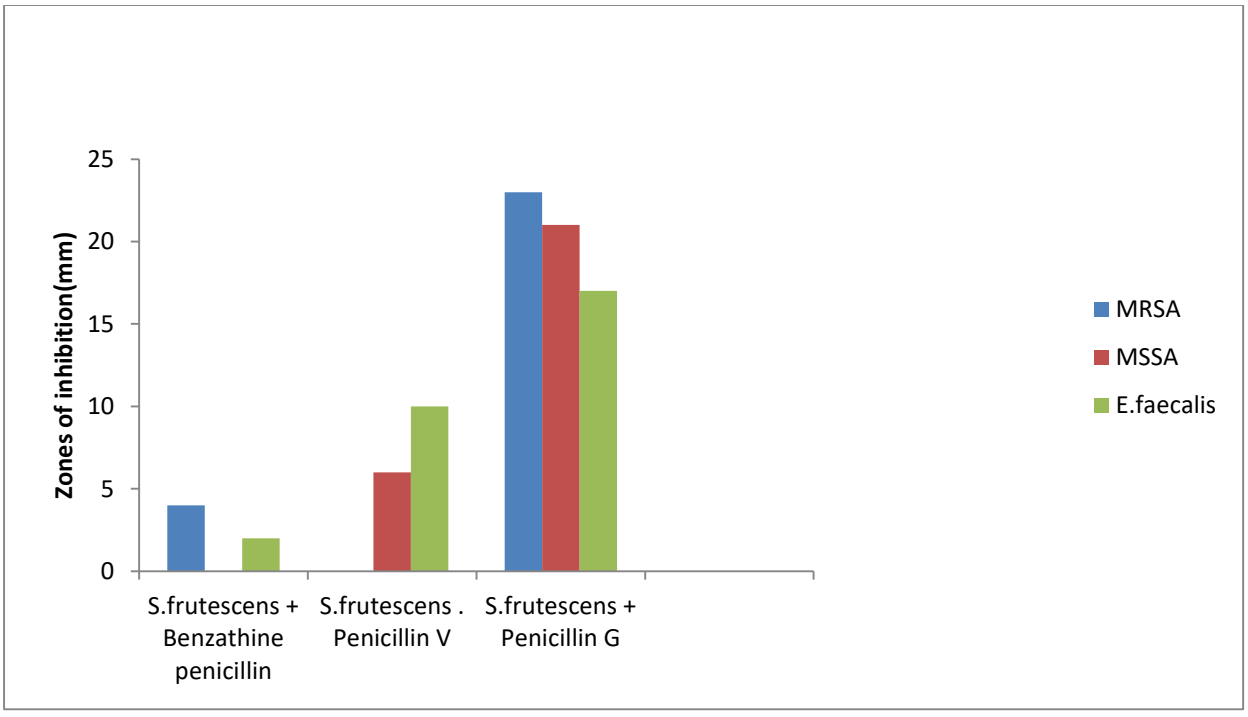
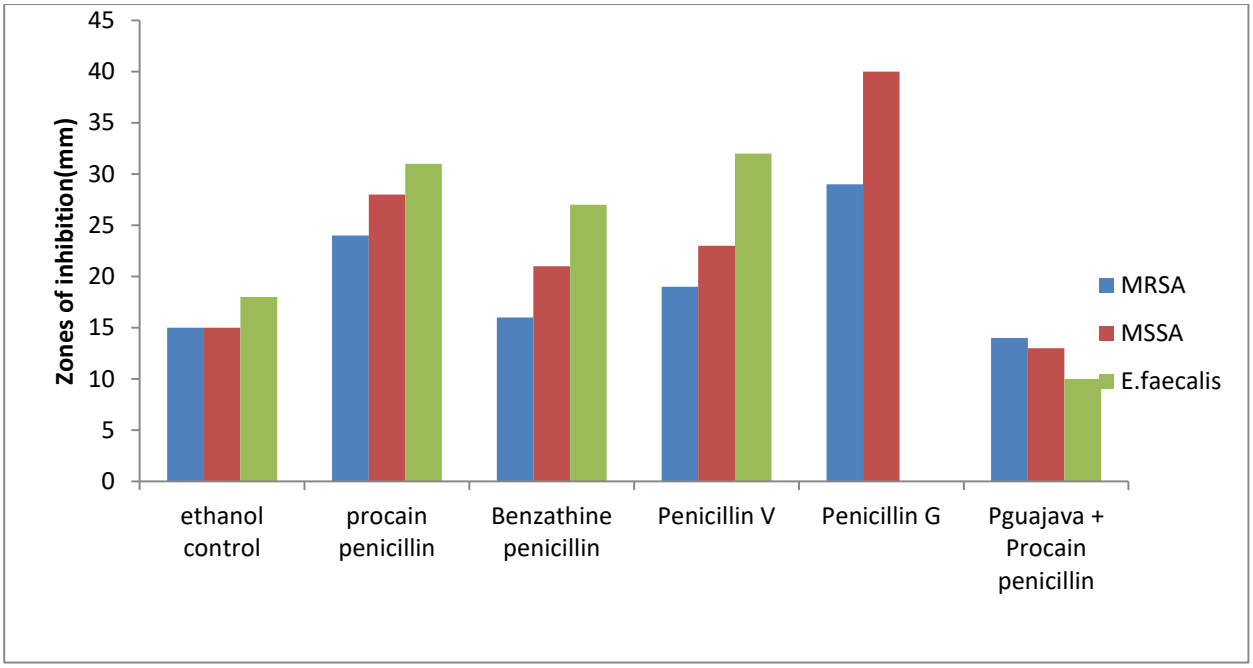


Figure 4.21: Zones of inhibition for a combination of *S. frutescens* ethanol-based extract and penicillins on bacteria

4.8.18 Effects of a combination of *P. guajava* ethanol-based extract and penicillins on MRSA, MSSA and *E. faecalis*

The combination of *P. guajava* ethanol-based extract and penicillins were compared with the ethanol control and all the inhibition zones that were less than that of ethanol control were taken as not effective against respective bacteria. The zones of inhibition that were greater than the ethanol control were then compared with the individual penicillin to establish their synergistic effects. If the zones of inhibition for the combinations were less than the individual penicillins, they were taken as lacking synergistic effects against respective bacteria. There was no synergistic effect observed. This was also shown by the zones of inhibition produced in the Kirby-Bauer Antimicrobial Sensitivity Test (Figure 4.22).



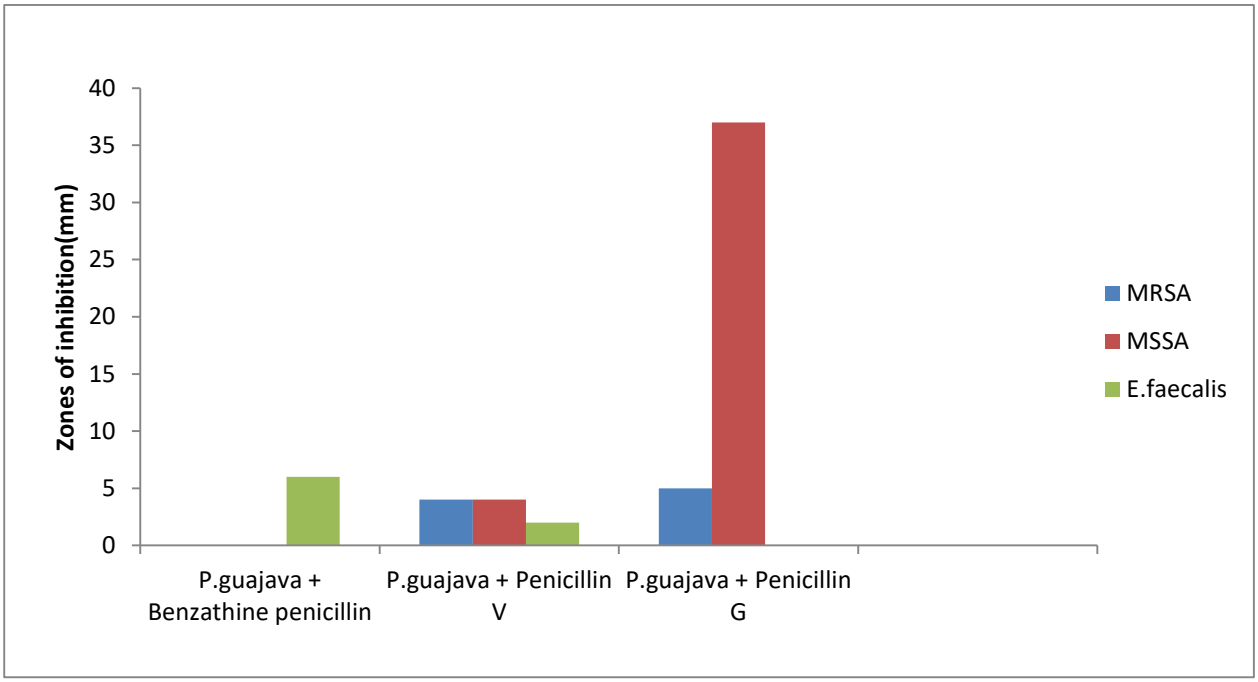


Figure 4.22: Zones of inhibition for a combination of *P. guajava* ethanol-based extract and penicillins on bacteria

4.9 Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) for the combinations between extracts and penicillins (reported as percentage growth)

The extracts that were showing better activity when combined with penicillins rather than individual plant extracts and individual penicillins were further tested for their MICs and the results are presented in the tables below.

4.9.1 Combination of *S. frutescens* water-based extract and benzathine penicillin against MRSA

The MIC and MBC for a 1 in 2 dilution to 1 in 16 dilution of a combination of *S. frutescens* water-based extract and benzathine penicillin against MRSA was 0 % growth when compared to the 100 % growth for *S. frutescens* water-based extract and 0 % growth for benzathine penicillin (Table 4.3).

Table 4.3: Results for a combination of *S. frutescens* water-based extract and benzathine penicillin (1 in 2 to 1 in 16 dilutions) against MRSA

	Growth
S. frutescens water-based extract	100 %
Benzathine penicillin	0 %
S. frutescens+ benzathine penicillin	0 %

4.9.2 Combination of *S. frutescens* water-based extract and benzathine penicillin dilution against *E. faecalis*

The MIC and MBC for a 1 in 2 dilution to 1 in 16 dilution of a combination of *S. frutescens* water-based extract and benzathine penicillin against *E. faecalis* was 0 %

growth when compared to the 0 % growth for *S. frutescens* water-based extract and 0 % growth for benzathine penicillin (Table 4.4).

Table 4.4: Statistical analysis of results for a combination of *S. frutescens* water-based extract and benzathine penicillin (1 in 2 to 1 in 16 dilutions) against *E. faecalis*

	Growth
S. frutescens water-based extract	0 %
Benzathine penicillin	0 %
S. frutescens+benzathine penicillin	0 %

4.9.3 Combination of *S. frutescens* water-based extract and penicillin G (1 in 2 to 1 in 4 dilutions) against MRSA

The MIC and MBC for a 1 in 2 dilution to 1 in 4 dilution of a combination of *S. frutescens* water-based extract and penicillin G against MRSA was 0 % growth when compared to the 100 % growth for *S. frutescens* water-based extract and penicillin G (Table 4.5).

Table 4.5: Results for a combination of *S. frutescens* water-based extract and penicillinG (1 in 2 to 1 in 4 dilutions) against MRSA

	Growth
S. frutescens water-based extract	100 %
Penicillin G	100 %
S. frutescens + penicillinG	0 %

4.9.4 Combination of *S. frutescens* water-based extract and penicillin G (1 in 8 to 1 in16 dilutions) against MRSA

The MIC and MBC for a 1 in 8 dilution to 1 in 16 dilution of a combination of *S. frutescens* water-based extract and penicillin G against MRSA was 100 % growth

when compared to the 100 % growth for *S. frutescens* water-based extract and penicillin G (Table 4.6).

Table 4.6: Results for a combination of *S. frutescens* water-based extract and penicillinG (1 in 8 to 1 in 16 dilutions) against MRSA

	Growth
S. frutescens water-based extract	100 %
Penicillin G	100 %
S. frutescens + penicillin G	100 %

4.9.5 Combination of *S. frutescens* water-based extract and penicillin G (1 in 2 dilution) against MSSA

The MIC and MBC for a 1 in 2 dilution of a combination of *S. frutescens* water-based extract and penicillin G against MSSA was 0 % growth when compared to the 0 % growth for *S. frutescens* water-based extract and 100 % growth for penicillin G (Table 4.7).

Table 4.7: Statistical analysis of results for a combination of *S. frutescens* water-based extract and penicillin G (1 in 2 dilution) against MSSA

	Growth
S. frutescens water-based extract	0 %
Penicillin G	100 %
S. frutescens + penicillin G	0 %

4.9.6 Combination of *S. frutescens* water-based extract and penicillin G (1 in 4 dilution) against MSSA

The MIC and MBC for a 1 in 4 dilution of a combination of *S. frutescens* water-based extract and penicillin G against MSSA was 0 % growth when compared to the

66.7 % growth for *S. frutescens* water-based extract and 100 % growth for penicillin G (Table 4.8).

Table 4.8: Statistical analysis of results for a combination of *S. frutescens* water-based extract and penicillin G (1 in 4 dilution) against MSSA

	Growth
S. frutescens water-based extract	66.7 %
Penicillin G	100 %
S. frutescens + penicillin G	0 %

4.9.7 Combination of *S. frutescens* water-based extract and penicillin G (1 in 8 to 1 in16 dilutions) against MSSA

The MIC and MBC for a 1 in 8 dilution to 1 in 16 dilution of a combination of *S. frutescens* water-based extract and penicillin G against MSSA was 0 % growth when compared to the 100 % growth for *S. frutescens* water-based extract and penicillin G (Table 4.9).

Table 4.9: Results for a combination of *S. frutescens* water-based extract and penicillin G (1 in 8 to 1 in 16 dilutions) against MSSA

	Growth
S. frutescens water-based extract	100 %
Penicillin G	100 %
S. frutescens + penicillin G	0 %

4.9.8 Combination of *S. frutescens* water-based extract and penicillin G (1 in 2 dilution) against *E. faecalis*

The MIC and MBC for a 1 in 2 dilution of a combination of *S. frutescens* water-based extract and penicillin G against *E. faecalis* was 0 % growth when compared to the 0 % growth for *S. frutescens* water-based extract and 100 % growth for penicillin G. (Table 4.10).

Table 4.10: Results for a combination of *S. frutescens* water-based extract and penicillin G (1 in 2 dilution) against *E. faecalis*

	Growth
S. frutescens water-based extract	0 %
Penicillin G	100 %
S. frutescens + penicillin G	0 %

4.9.9 Combination of *S. frutescens* water-based extract and penicillin G (1 in 4 to 1 in16 dilutions) against *E. faecalis*

The MIC and MBC for a 1 in 4 dilution to 1 in 16 dilution of a combination of *S. frutescens* water-based extract and penicillin G against *E. faecalis* was 0 % growth when compared to the 100 % growth for *S. frutescens* water-based extract and 100 % for penicillin G (Table 4.11).

Table 4.11: Results for a combination of *S. frutescens* water-based extract and penicillin G (1 in 4 to 1 in 16 dilutions) against *E. faecalis*

	Growth
S. frutescens water-based extract	100 %
Penicillin G	100 %
S. frutescens + penicillin G	0 %

4.9.10 Combination of *P. guajava* water-based extract and procain penicillin G (1 in 2 dilution) against MRSA

The MIC and MBC for a 1 in 2 dilution of a combination of *P. guajava* water-based extract and procain penicillin against MRSA was 100 % no growth and 0 % growth when compared to the 0 % growth for *P. guajava* water-based extract and 0 % growth for procain penicillin (Table 4.12).

Table 4.12: Results for a combination of *P. guajava* water-based extract and procain penicillin (1 in 2 dilution) against MRSA

	Growth
P. guajava water-based extract	0 %
Procain penicillin	0 %
P. guajava + procain penicillin	0 %

4.9.11 Combination of *P. guajava* water-based extract and procain penicillin (1 in 4 to 1 in 16 dilutions) against MRSA

The MIC and MBC for a 1 in 4 dilution to 1 in 16 dilution of a combination of *P. guajava* water-based extract and procain penicillin against MRSA was 0 % growth when compared to the 100 % growth for *P. guajava* water-based extract and 100 % growth for procain penicillin (Table 4.13).

Table 4.13: Results for a combination of *P. guajava* water-based extract and procain penicillin (1 in 4 to 1 in 16 dilutions) against MRSA

	Growth
P. guajava water-based extract	100 %
Procain penicillin	100 %
P. guajava + procain penicillin	0 %

4.9.12 Combination of *P. guajava* water-based extract and procain penicillin (1 in 2 dilution) against MSSA

The MIC and MBC for a 1 in 2 dilution of a combination of *P. guajava* water-based extract and procain penicillin against MSSA was 0 % growth when compared to the 0 % growth for *P. guajava* water-based extract and 0 % for procain penicillin (Table 4.14).

Table 4.14: Results for a combination of *P. guajava* water-based extract and procain penicillin (1 in 2 dilution) against MSSA

	Growth
P. guajava water-based extract	0 %
Procain penicillin	0 %
P. guajava + procain penicillin	0 %

4.9.13 Combination of *P. guajava* water-based extract and procain penicillin (1 in 4 dilution) against MSSA

The MIC and MBC for a 1 in 4 dilution of a combination of *P. guajava* water-based extract and procain penicillin against MSSA was 0 % growth when compared to the 0 % growth for *P. guajava* water-based extract and 100 % growth for procain penicillin (Table 4.15).

Table 4.15: Results for a combination of *P. guajava* water-based extract and procain penicillin (1 in 4 dilution) against MSSA

	Growth
P. guajava water-based extract	0 %
Procain penicillin	100 %
P. guajava + procain penicillin	0 %

4.9.14 Combination of *P. guajava* water-based extract and procaine penicillin against MSSA

The MIC and MBC for a 1 in 8 dilution of a combination of *P. guajava* water-based extract and procain penicillin against MSSA was 0 % growth when compared to the 100 % growth for *P. guajava* water-based extract and 66.7 % for procain penicillin (Table 4.16).

Table 4.16: Results for a combination of *P. guajava* water-based extract and procain penicillin (1 in 8 dilution) against MSSA

	Growth
P. guajava water-based extract	100 %
Procain penicillin	66.7 %
P. guajava + procain penicillin	0 %

4.9.15 Combination of *P. guajava* water-based extract and procain penicillin against MSSA

The MIC and MBC for a 1 in 16 dilution of a combination of *P. guajava* water-based extract and procain penicillin against MSSA was 0 % growth when compared to the 100 % growth for *P. guajava* water-based extract and 100 % for procain penicillin (Table 4.17).

Table 4.17: Results for a combination of *P. guajava* water-based extract and procain penicillin (1 in 16 dilution) against MSSA

	Growth
P. guajava water-based extract	100 %
Procain penicillin	100 %
P. guajava + procain penicillin	0 %

4.9.16 Combination of *P. guajava* water-based extract and procain penicillin against *E. faecalis*

The MIC and MBC for a 1 in 2 dilution of a combination of *P. guajava* water-based extract and procain penicillin against *E. faecalis* was 0 % growth when compared to the 16.7 % growth for *P. guajava* water-based extract and 0 % growth for procain penicillin (Table 4.18).

Table 4.18: Statistical analysis of results for a combination of *P. guajava* water-based extract and procain penicillin (1 in 2 dilution) against *E. faecalis*

	Growth
P. guajava water-based extract	16.7 %
Procain penicillin	0 %
P. guajava + procain penicillin	0 %

4.9.17 Combination of *P. guajava* water-based extract and procain penicillin against *E. faecalis*

The MIC and MBC for a 1 in 4 dilution to 1 in 8 dilution of a combination of *P. guajava* water-based extract and procain penicillin against *E. faecalis* was 0 % growth when compared to the 100 % growth for *P. guajava* water-based extract and 0 % growth for procain penicillin (Table 4.19).

Table 4.19: Results for a combination of *P. guajava* water-based extract and procain penicillin (1 in 4 to 1 in 8 dilutions) against *E. faecalis*

	Growth
P. guajava water-based extract	100 %
Procain penicillin	0 %
P. guajava + procain penicillin	0 %

4.9.18 Combination of *P. guajava* water-based extract and procain penicillin against *E. faecalis*

The MIC and MBC for a 1 in 16 dilution of a combination of *P. guajava* water-based extract and procain penicillin against *E. faecalis* was 0 % growth when compared to the 100 % growth for *P. guajava* water-based extract and 100 % growth for procain penicillin (Table 4.20).

Table 4.20: Results for a combination of *P. guajava* water-based extract and procain penicillin (1 in 16) dilution against *E. faecalis*

	Growth
P. guajava water-based extract	100 %
Procain penicillin	100 %
P. guajava + procain penicillin	0 %

4.9.19 Combination of *P. guajava* water-based extract and benzathine penicillin against MRSA

The MIC and MBC for a 1 in 2 dilution of a combination of *P. guajava* water-based extract and benzathine penicillin against MRSA was 0 % growth when compared to the 0 % growth for *P. guajava* water-based extract and 0 % growth for benzathine penicillin (Table 4.21).

Table 4.21: Results for a combination of *P. guajava* water-based extract and benzathine penicillin (1 in 2 dilution) against MRSA

	Growth
P. guajava water-based extract	0 %
Benzathine penicillin	0 %
P. guajava + benzathine penicillin	0 %

4.9.20 Combination of *P. guajava* water-based extract and benzathine penicillin (1 in 4) dilution against MRSA

The MIC and MBC for a 1 in 4 dilution of a combination of *P. guajava* water-based extract and benzathine penicillin against MRSA was 0 % growth when compared to the 100 % growth for *P. guajava* water-based extract and 0 % growth for benzathine penicillin (Table 4.22).

Table 4.22: Statistical analysis of results for a combination of *P. guajava* water-based extract and benzathine penicillin (1 in 4) dilution against MRSA

	Growth
P. guajava water-based extract	100 %
Benzathine penicillin	0 %
P. guajava + benzathine penicillin	0 %

4.9.21 Combination of *P. guajava* water-based extract and benzathine penicillin (1 in 8 to 1 in 16 dilution) against MRSA

The MIC and MBC for a 1 in 8 dilution to 1 in 16 dilution of a combination of *P. guajava* water-based extract and benzathine penicillin against MRSA was 100 % growth when compared to the 100 % growth for *P. guajava* water-based extract and 0 % growth for benzathine penicillin (Table 4.23).

Table 4.23: Results for a combination of *P. guajava* water-based extract and benzathine penicillin (1 in 8 to 1 in 16 dilutions) against MRSA

	Growth
P. guajava water-based extract	100 %
Benzathine penicillin	0 %
P. guajava + benzathine penicillin	100 %

4.9.22 Combination of *P. guajava* water-based extract and benzathine penicillin (1 in 2 to 1 in 4 dilutions) against MSSA

The MIC and MBC for a 1 in 2 dilution to 1 in 4 dilution of a combination of *P. guajava* water-based extract and benzathine penicillin against MSSA was 0 % growth when compared to the 0 % growth for *P. guajava* water-based extract and 0 % growth for benzathine penicillin (Table 4.24).

Table 4.24: Results for a combination of *P. guajava* water-based extract and benzathine penicillin (1 in 2 to 1 in 4 dilutions) against MSSA

	Growth
P. guajava water-based extract	0 %
Benzathine penicillin	0 %
P. guajava + benzathine penicillin	0 %

4.9.23 Combination of *P. guajava* water-based extract and benzathine (1 in 8 to 1 in16 dilutions) against MSSA

The MIC and MBC for a 1 in 8 dilution to 1 in 16 dilution of a combination of *P. guajava* water-based extract and benzathine penicillin against MSSA was 100 % growth when compared to the 100 % growth for *P. guajava* water-based extract and 0 % for benzathine penicillin (Table 4.25).

Table 4.25: Results for a combination of *P. guajava* water-based extract and benzathine penicillin (1 in 8 to 1 in 16 dilutions) against MSSA

	Growth
P. guajava water-based extract	100 %
Benzathine penicillin	0 %
P. guajava + benzathine penicillin	100 %

4.9.24 Combination of *P. guajava* water-based extract and benzathine penicillin (1 in 2 dilution) against *E. faecalis*

The MIC and MBC for a 1 in 2 dilution of a combination of *P. guajava* water-based extract and benzathine penicillin against *E. faecalis* was 0 % growth when compared to the 16.7 % growth for *P. guajava* water-based extract and 0 % growth for benzathine penicillin (Table 4.26).

Table 4.26: Results for a combination of *P. guajava* water-based extract and benzathine penicillin (1 in 2 dilution) against *E. faecalis*

	Growth
P. guajava water-based extract	16.7 %
Benzathine penicillin	0 %
P. guajava + benzathine penicillin	0 %

4.9.25 Combination of *P. guajava* water-based extract and benzathine penicillin (1 in 4 to 1 in 16 dilutions) against *E. faecalis*

The MIC and MBC for a 1 in 4 dilution to 1 in 16 dilution of a combination of *P. guajava* water-based extract and benzathine penicillin against *E. faecalis* was 0 % growth when compared to the 100 % growth for *P. guajava* water-based extract and 0 % growth for benzathine penicillin (Table 4.27).

Table 4.27: Results for a combination of *P. guajava* water-based extract and benzathine penicillin (1 in 4 to 1 in 16 dilutions) against *E. faecalis*

	Growth
P. guajava water-based extract	100 %
Benzathine penicillin	0 %
P. guajava + benzathine penicillin	0 %

4.9.26 Combination of *P. guajava* water-based extract and penicillin V against MRSA

The MIC and MBC for a 1 in 2 dilution of a combination of *P. guajava* water-based extract and penicillin V against MRSA was 100 % growth when compared to the 0 % growth for *P. guajava* water-based extract and 0 % for penicillin V (Table 4.28).

Table 4.28: Results for a combination of *P. guajava* water-based extract and penicillin V (1 in 2 dilution) against MRSA

	Growth
P. guajava water-based extract	0 %
Penicillin V	0 %
P. guajava + penicillin V	100 %

4.9.27 Combination of *P. guajava* water-based extract and penicillin V against MRSA

The MIC and MBC for a 1 in 4 dilution to 1 in 16 dilution of a combination of *P. guajava* water-based extract and penicillin V against MRSA was 100 % growth when compared to the 100 % growth for *P. guajava* water-based extract and 0 % growth for penicillin V (Table 4.29).

Table 4.29: Results for a combination of *P. guajava* water-based extract and penicillin V (1 in 4 to 1 in 16 dilutions) against MRSA

	Growth
P. guajava water-based extract	100 %
Penicillin V	0 %
P. guajava + penicillin V	100 %

4.9.28 Combination of *P. guajava* water-based extract and penicillin V dilution against MSSA

The MIC and MBC for a 1 in 2 dilution of a combination of *P. guajava* water-based extract and penicillin V against MSSA was 100 % growth when compared to the 0 % growth for *P. guajava* water-based extract and 33.3 % growth for penicillin V (Table 4.30).

Table 4.30: Results for a combination of *P. guajava* water-based extract and penicillin V (1:1 dilution) against MSSA

	Growth
P. guajava water-based extract	0 %
Penicillin V	33.3 %
P. guajava + penicillin V	100 %

4.9.29 Combination of *P. guajava* water-based extract and penicillin V dilution against MSSA

The MIC and MBC for a 1 in 4 dilution to 1 in 16 dilution of a combination of *P. guajava* water-based extract and penicillin V against MSSA was 100 % growth when compared to the 100 % growth for *P. guajava* water-based extract and 100 % growth for penicillin V (Table 4.31).

Table 4.31: Results for a combination of *P. guajava* water-based extract and penicillin V (1 in 4 to 1 in 16 dilutions) against MSSA

	Growth
P. guajava water-based extract	100 %
Penicillin V	100 %
P. guajava + penicillin V	100 %

4.9.30 Combination of *P. guajava* water-based extract and penicillin V (1 in 2 dilution) against *E. faecalis*

The MIC and MBC for a 1 in 2 dilution of a combination of *P. guajava* water-based extract and penicillin V against *E. faecalis* was 0 % growth when compared to the 16.7 % growth for *P. guajava* water-based extract and 0 % for penicillin V (Table 4.32).

Table 4.32: Results for a combination of *P. guajava* water-based extract and penicillin V (1 in 2 dilution) against *E. faecalis*

	Growth
P. guajava water-based extract	16.7 %
Penicillin V	0 %
P guajava + penicillin V	0 %

4.9.31 Combination of *P. guajava* water-based extract and penicillin V (1 in 4 to 1 in 16 dilutions) against *E. faecalis*

The MIC and MBC for a 1 in 4 dilution to 1 in 16 dilution of a combination of *P. guajava* water-based extract and penicillin V against *E. faecalis* was 0 % growth when compared to the 100 % growth for *P. guajava* water-based extract and 0 % for penicillin V (Table 4.33).

Table 4.33: Results for a combination of *P. guajava* water-based extract and penicillin V (1 in 4 to 1 in 16 dilutions) against *E. faecalis*

	Growth
P. guajava water-based extract	100 %
Penicillin V	0 %
P. guajava + penicillin V	0 %

4.9.32 Combination of *P. guajava* water-based extract and penicillin G (1 in 2 dilution) against MRSA

The MIC and MBC for a 1 in 2 dilution of a combination of *P. guajava* water-based extract and penicillin G against MRSA was 100 % growth when compared to the 0 % growth for *P. guajava* water-based extract and 100 % growth for Penicillin G (Table 4.34).

Table 4.34: Results for a combination of *P. guajava* water-based extract and penicillin G (1 in 2 dilution) against MRSA

	Growth
P. guajava water-based extract	0 %
Penicillin G	100 %
P. guajava + penicillin G	100 %

4.9.33 Combination of *P. guajava* water-based extract and penicillin G (1 in 4 to 1 in 16 dilutions) against MRSA

The MIC and MBC for a 1 in 4 dilution to 1 in 16 dilution of a combination of *P. guajava* water-based extract and penicillin G against MRSA was 100 % growth when compared to the 100 % growth for *P. guajava* water-based extract and 100 % growth for penicillin G (Table 4.35).

Table 4.35: Results for a combination of *P. guajava* water-based extract and penicillin G (1 in 4 to 1 in 16 dilutions) against MRSA

	Growth
P. guajava water-based extract	100 %
Penicillin G	100 %
P. guajava + penicillin G	100 %

4.9.34 Combination of *P. guajava* water-based extract and penicillin G (1 in 2 to 1 in 4 dilutions) against MSSA

The MIC and MBC for a 1 in 2 dilution to 1 in 4 dilution of a combination of *P. guajava* water-based extract and penicillin G against MSSA was 100 % growth when compared to the 0 % growth for *P. guajava* water-based extract and 100 % growth for penicillin G (Table 4.36).

Table 4.36: Results for a combination of *P. guajava* water-based extract and penicillin G (1 in 2 to 1 in 4 dilutions) against MSSA

	Growth
P. guajava water-based extract	0 %
Penicillin G	100 %
P. guajava + penicillin G	100 %

4.9.35 Combination of *P. guajava* water-based extract and penicillin G (1 in 8 to 1 in 16 dilutions) against MSSA

The MIC and MBC for a 1 in 8 dilution to 1 in 16 dilution of a combination of *P. guajava* water-based extract and penicillin G against MSSA was 100 % growth when compared to the 100 % growth for *P. guajava* water-based extract and 100 % growth for penicillin G (Table 4.37).

Table 4.37: Results for a combination of *P. guajava* water-based extract and penicillin G (1 in 8 to 1 in 16 dilutions) against MSSA

	Growth
P. guajava water-based extract	100 %
Penicillin G	100 %
P. guajava + penicillin G	100 %

4.9.36 Combination of *P. guajava* water-based extract and penicillin G (1 in 2 dilution) against *E. faecalis*

The MIC and MBC for a 1 in 2 dilution of a combination of *P. guajava* water-based extract and penicillin G against *E. faecalis* was 100 % growth when compared to the 16.7 % growth for *P. guajava* water-based extract and 100 % growth for penicillin G (Table 4.38).

Table 4.38: Results for a combination of *P. guajava* water-based extract and penicillin G (1 in 2 dilution) against *E. faecalis*

	Growth
P. guajava water-based extract	16.7 %
Penicillin G	100 %
P. guajava + penicillin G	100 %

4.9.37 Combination of *P. guajava* water-based extract and penicillin G (1 in 4 to 1 in 16 dilutions) against *E. faecalis*

The MIC and MBC for a 1 in 4 dilution to 1 in 16 dilution of a combination of *P. guajava* water-based extract and penicillin G against *E. faecalis* was 100 % growth when compared to the 100 % growth for *P. guajava* water-based extract and 100 % growth for penicillin G (Table 4.39).

Table 4.39: Results for a combination of *P. guajava* water-based extract and penicillin G (1 in 4 to 1 in 16 dilutions) against *E. faecalis*

	Growth
P. guajava water-based extract	100 %
Penicillin G	100 %
P. guajava + penicillin G	100 %

4.10 Conclusion

The results in this study indicated that the water extracts of *S. frutescens* and *P. guajava* plants were antibacterial active against *S. aureus* and *E. faecalis*. This is demonstrated by the results as seen in Figure 4.1 up to Figure 4.4. The inhibitory zones were generally larger for *S. frutescens* when compared with *P. guajava* for all bacteria that were tested. The MIC was the same for all water extracts. This is demonstrated by the results as seen in Table 4.1 and Table 4.2. These MICs and MBCs demonstrate that the water extracts for both plants against bacteria in this study are not bacteriostatic or bactericidal when they are diluted.

The ethanol extracts of *S. frutescens* and *P. guajava* plants did not possess any antibacterial activity against *S. aureus* and *E. faecalis*. The inhibitory zones were all less than that of the ethanol control. The MICs and MBCs were not performed since these did not demonstrate any activity against any of the bacteria in this study.

The combination studies demonstrated that an antagonistic effect was observed when *S. frutescens* water-based extract was combined with penicillin G and tested against MRSA and MSSA but a synergistic effect was observed when tested against *E. faecalis*. The synergistic effect was further observed when *P. guajava* water-based extract was combined with procain penicillin and tested against *MRSA*. Synergy was again observed when *P. guajava* water-based extract was combined with benzathine penicillin and tested against MRSA and *E. faecalis*. Synergy was also noted when *P. guajava* water-based extract was combined with penicillin V and tested against *E. faecalis* and lastly it was noted when *P. guajava* water-based

extract was combined with penicillin G and tested against MSSA and *E. faecalis* but there was an antagonistic effect when it was tested against MRSA.

There was no synergistic effect when *P. guajava* ethanol-based extract was combined with penicillins and when *S. frutescens* water-based extract was combined with penicillins and tested against MRSA, MSSA and *E. faecalis.*

CHAPTER 5: DISCUSSION

Alternative natural products of plants could be of interest when considering the increase in the incidence of resistance to antibiotics. Some plants extracts and phytochemicals are known to have antimicrobial properties, which could be significant in therapeutic treatment. In the last few years, various studies have been conducted in different countries demonstrating the efficacy of this type of treatment (Benzizerara *et al*., 2013). Many plants have been evaluated not only for direct antimicrobial activity but also as resistance- modifying agents (Matias *et al.*, 2012).

Al-Saiym *et al.,* (2015), carried out a study on some Jordanian plants and established that the effectiveness of the antibiotics, gentamycin and chloramphenicol against *S. aureus* were allegedly enhanced by the use of plant materials. Ahmad, & Aqil, (2007), likewise stated that crude extracts of Indian medicinal plants revealed synergistic interaction with tetracycline and ciprofloxacin against extended spectrum β-lactamase (ESβL)-producing multidrug-resistant enteric microorganisms. Aiyegoro & Okoh (2009), also observed synergistic interactions between extracts of Brazilian medicinal plants and eight antibiotics on *S. aureus.* The use of *Catha edulis* extracts at sub inhibitory levels, has been reported to reduce the minimum inhibitory concentration (MIC) values of tetracycline, and penicillin G against resistant oral pathogens, *Streptococcus oralis*, *Streptococcus sanguis* and *Fusobacterium nucleatum* (Aiyegoro *et al.,* 2011).

It is common knowledge that many bacterial strains can develop resistance against antibiotics. *S. aureus* and *E. faecalis* are some of the persistent infectious

microorganisms. Therefore, the choice of effective and safe drugs to be used against *S. aureus* and *E. faecalis* is getting reduced day by day. Therefore, attention is needed to develop alternative or combination agent from natural products including medicinal plants.

This experiment was done to screen the effect of *S. frutescens* and *P. guajava* extracts individually and also in combination with procain penicillin, benzathine penicillin, penicillin V and penicillin G to identify systems, which may be used to improve the efficiency of the penicillins against tested MRSA, MSSA and *E. faecalis*.

A total of two extracts for each of the plants were examined against each of the three microbial strains. All microbial strains were Gram-positive. Extracts were found from the leaves of *S. frutescens* and *P. guajava*. The zones of inhibition produced by the ethanol extract of *S. frutescens* and *P. guajava* againsts MRSA, MSSA and *E. faecalis* were smaller than those of ethanol control. Therefore, the ethanol extract of *S. frutescens and P. guajava* were taken as not exhibiting any antibacterial activity against any bacteria in this study. This is shown in Figure 3 and Figure 4.

The water extracts of *S. frutescens* exhibited antibacterial activity against MRSA, MSSA and *E. faecalis*.The significant difference in diameter of zones of inhibition between *S. frutescens* water based extracts and water control on MRSA ($P = 0.002$) demonstrates that there is an existence of antibacterial activity. There is also a significant difference in diameter of zones of inhibition between *S. frutescens* water based extracts and water control on MSSA. ($P = 0.002$), which is indicative of the fact that the *S. frutescens* water extract is antibacterial active on MSSA. The

difference in diameter of zones of inhibition between *S. frutescens* water based extracts and water control on *E. faecalis* is also significant (P = 0.003) and it suggestive of antibacterial activity. This is shown in Figure 1. This finding was in line with that of hexane extract of this plant. The hexane extract for example, was shown to be active against *S. aureus*, *E. faecalis* and *E. coli* (Katerere *et al.,* 2005).

The water extracts of *P. guajava* exhibited antibacterial activity against MRSA, MSSA and *E. faecalis*.The important dissimilarity in diameter of zones of inhibition between *P. guajava* water based extracts and water control on MRSA (p = 0.042) demonstrates that there is an existence of antibacterial activity. There is also an important dissimilarity in diameter of zones of inhibition between *P. guajava* water based extracts and water control on *MSSA.* P value of 0.026 is indicative of the fact that the *P. guajava* water extract is antibacterial active on MSSA. The dissimilarity in diameter of zones of inhibition between *P. guajava* water based extracts and water control on *E. faecalis* is also important (P = 0.042) and it suggestive of antibacterial activity (Figure 2). Lin et al.,2002, demonstrated that this plant can treat diarrhoea. The same plant was also demonstrated by Nsele, 2012, to be antibacterial active. This observation is proving to be highly significant. It serves as the basis for combination studies using *P. guajava.*

The MICs and MBCs were done on the extracts of *P. guajava* and *S. frutescens* that provided zones of inhibition against *S. aureus* and *E. faecalis.* If the zones of inhibition of the extract were smaller than those of the control, the MICs were not done. The MICs were done using the agar dilution technique. The growth or no growth of bacteria indicated the MIC and MBC, respectively.

The zones of inhibition were dissimilar for each plant, demonstrating that the activity of the plants against bacteria is not similar. This was reinforced by the dissimilarity in the MICs and MBCs for *S. frutescens* on MRSA, MSSA *and E. faecalis*. The minimum inhibitory concentration and minimum bactericidal concentration for water based extract showed that the most in effect against MRSA is the neat extract. This was demonstrated by 100 % growth for dilution 1:1 (lowest) up to dilution 1:15 (highest dilution). This means that the antibacterial activity against MRSA is only produced by the neat extract that produced the zone of inhibition during the modified Kirby Bauer method of susceptibility testing. The water based extract against MSSA showed that the most effective dilution is 1:1 with 100 % inhibition of growth and the minimum effective dilution is 1:3 with 33.3 % inhibition of growth. Finally the minimum inhibitory concentration and minimum bactericidal concentration for water based extract against *E. faecalis* showed that the most in effect dilution is 1:1 with 100% inhibition of growth and the minimum in effect dilution is 1:3 with 0 % inhibition of growth (Table 1).

The MIC and MBC for *P. guajava* water based extract against MRSA showed that the most effective dilution is 1:1 with 100 % inhibition of growth and the minimum in effect dilution is 1:3 with 0 % inhibition of growth. The MIC and MBC for *P. guajava* water based extract against MSSA showed that the most effective dilution is 1:3 with 100% inhibition of growth and the minimum effective dilution is 1 in 8 with 0 % inhibition of growth. Finally The MIC and MBC for *P. guajava* water based extract against *E. faecalis* showed that the most effective dilution is 1:1 with 83.3 %

inhibition of growth and the minimum effective dilution is 1:3 with 0% inhibition of growth (Table 2).

Conventionally, greatest plant extracts are made with water. In this study the ethanol extract produced no zones of inhibition; however, water extracts produced substantial inhibition zones. Since the antimicrobial activity was revealed to be great in water extracts, it means that it is likely that most traditional healers do extract the compounds sufficiently, which are responsible for antibacterial activity on MRSA, MSSA and *E. faecalis*. This is an interesting observation since most traditional healers utilise water for extraction. The metabolic properties occurring *in vivo* could also trigger certain composites in the body. These metabolic processes hinge on temperature, pH and other features present *in vivo* but lacking *in vitro*. This may suggest that the plants that are promising should also be tested *in vitro* to fully establish their activity against the micro-organisms used in this study.

The combination studies of water based extract of *S. frutescens* and penicillins were performed to establish the synergistic effects and antagonistic effects between plant extracts and penicillins. The combination studies of water based extract of *P. guajavas* and penicillins were also processed as well. The study also needed to establish whether the ethanol extracts can produce synergistic antibacterial effects when combined with penicillins. Therefore, the combination studies of ethanol extracts were also done. The results were interesting as some of combinations yielded results that are demonstrated synergistic effects and yet other results demonstrated antagonistic effects.

The combination of *S. frutescens* water based extract and procain penicillin did not demonstrate any synergistic or antagonistic effect when compared with the results produced by *S. frutescens* water based extract against MRSA, MSSA and *E. faecalis*. Similarly the synergistic and antagonistic effect was not observed when compared with the results produced by procain penicillin against the bacteria tested in the study. This suggests that this combination is neither synergistic nor antagonistic when tested against MRSA, MSSA and *E*. *faecalis* (Figure 5 and Figure 6).

The combination of *S. frutescens* water based extract and benzathine penicillin did not demonstrate any synergistic or antagonistic effect when compared with the results produced by *S. frutescens* water based extract against MSSA. However, when this combination was compared with the results produced by *S. frutescens* water based extract against MRSA, the synergistic effect was demonstrated but it was not demonstrated when compared with the results produced by benzathine penicillin against MRSA. These results were taken as showing no synergistic effect against MRSA. With regards to *E. faecalis*, this combination did not demonstrate any synergistic effect when compared with the results produced by *S. frutescens* water based extract. However, there was synergy when it was compared with benzathine penicillin. This suggests that this combination has better activity than benzathine penicillin on *E. faecalis* (Figure 7 and Figure 8).

The combination of *S. frutescens* water based extract and penicillin V did not demonstrate any synergistic or antagonistic effect when compared with the results produced by *S. frutescens* water based extract against MRSA, MSSA and *E.*

faecalis. Similarly, the synergistic and antagonistic effect was not observed when compared with the results produces by penicillin V against the bacteria tested in the study. This suggests that this combination is neither synergistic nor antagonistic when tested against MRSA, MSSA and *E. faecalis* (Figure 9 and Figure 10).

The combination of *S. frutescens* water based extract and penicillin G demonstrated a synergistic effect when compared with the results produced by *S. frutescens* water based extract against MRSA, MSSA and *E. faecalis*. However, when this combination was compared with penicillin G, there was an antagonistic effect when tested against MRSA and MSSA. The synergistic effect was produced when this combination was compared with penicillin G and tested against *E. faecalis*. This suggests that this combination is less active on *S. aureus* and more active on *E. faecalis* (Figure 11 and Figure 12).

The combination of *S. frutescens* ethanol based extract and penicillins did not demonstrate any synergistic effect when compared with procain penicillin, benzathine penicillin, penicillin V and penicillin G and tested against MRSA, MSSA and *E. faecalis*. The zones of inhibition of the combinations were less than those of individual penicillins and they were also less than ethanol control (Figure 21).

The combination of *P. guajava* water based extract and procain penicillin demonstrated a synergistic effect when compared with the results produced by *P. guajava* water based extract against MRSA, MSSA and *E. faecalis*. However, when this combination was compared with procain penicillin, the synergistic effect was produced when it was tested against MRSA. There was no synergistic effect when it

was tested against MSSA and *E. faecalis*. This suggests that this combination is more active only on MRSA. This is shown on (Figure 13 and Figure 14).

There was significant difference in diameter of zones of inhibition between a combination of *P. guajava* water based extract and benzathine penicillin against *P. guajava* water based extract on MRSA, MSSA and *E. faecalis*. Similarly there was significant difference in diameter of zones of inhibition between a combination of *P. guajava* water based extract and benzathine penicillin against benzathine penicillin on MRSA and *E. faecalis* but there was no significant difference in zones of inhibition when tested on MSSA. This is also shown by the zones of inhibition (Figure 15 and Figure 16) produced in the Kirby Bauer Antimicrobial Sensitivity Test. This indicates that synergistic effect is produced against MRSA and *E. faecalis.*

There was noteworthy dissimilarity in diameter of zones of inhibition between a combination of *P. guajava* water based extract and penicillin V against *P. guajava* water based extract on MRSA and MSSA but not on *E. faecalis*. Similarly there was significant difference in diameter of zones of inhibition between a combination of *P. guajava* water based extract and penicillin V against penicillin V on *E. faecalis* but there was no significant difference in zones of inhibition when it was tested on MRSA and MSSA. This is also shown by the zones of inhibition (Figure 17 and Figure 18) produced in the Kirby Bauer Antimicrobial Sensitivity Test. This indicates that there is no synergistic effect produced by this combination.

There was an important dissimilarity in diameter of zones of inhibition between a combination of *P. guajava* water based extract and penicillin G against *P. guajava*

water based extract on MRSA and MSSA but not on *E. faecalis*. Similarly there was noteworthy dissimilarity in diameter of zones of inhibition between a combination of *P. guajava* water based extract and penicillin G against penicillin G on *S. aureus* and *E. faecalis*. This is also shown by the zones of inhibition (Figure 19 and Figure 20) produced in the Kirby Bauer Antimicrobial Sensitivity Test. This is suggesting that the synergistic effect is produced by this combination on all three bacteria that was tested.

The idea of synergy between herbal drugs and antibiotics is a novel approach to treating multidrug resistant bacteria. This is due to the increase in antibiotic resistance by bacteria (Bhardwaj *et al*., 2016). The combination of two drugs can be synergistic, additive or antagonistic. The effect is said to be synergistic if the effect of the combination is more than it would be if the concentration of the second drug is replaced by the first drug, whereas antagonistic if combined effect will be less than alone effect. Synergy results in increased killing rate, potentiating of drug, prevention of drug elimination and a better effect in vivo (Bhardwaj *et al*., 2016). Understanding of synergy mechanism may provide a new strategy for the treatment of infectious diseases by reducing the side effects produced by high doses of antibiotics. The herbal extracts and antibiotics are tested for synergistic association against multidrug resistant bacteria (Nscimento *et al*., 2000). This observation is vital tool that can assist in dealing with resistance bacteria.

The combination of *P. guajava* ethanol based extract and penicillins did not demonstrate any synergistic effect when compared with procain penicillin, benzathine penicillin, penicillin V and penicillin G and tested against MRSA, MSSA

and *E. faecalis*. The zones of inhibition of the combinations were less than those of individual penicillins and they were also less than ethanol control (Table 22).

The observation was that the ethanol-based extract of these plants did not possess any synergistic effects while on the other hand some water based extracts possessed the synergistic effects when in combination with penicillins. This could be due to the phytochemical properties and differences among species. It is quite possible that some of the plants that were ineffective in this study do not possess sufficient antibiotic properties, or the plant extracts may have contained antibacterial constituents, but not in sufficient concentrations so as to be effective. They may also contain compounds that act against the penicillins as some combinations produced the antagonistic effects. The balances between the synergic effect and the reduction of the antibacterial activities owing to their binding to each other may result in the antagonistic tendency at certain ratios of the antibiotics (Zhi-Qing Hu *et al*., 2002).

Since the combination of *S. frutescens* water based extract and procain penicillin did not demonstrate any synergistic or antagonistic effect when compared with the results produced by *S. frutescens* water based extract and also compared with benzathine penicillin against MRSA, MSSA and *E. faecalis*, the MIC ‘s and MBC’s were not done.

The MIC and MBC for a combination of S*. frutescens* water based extract and benzathine penicillin showed that the greatest effective combination against MRSA and *E. faecalis* was the undiluted combination. This was demonstrated by 100 % growth for dilution 1:1 dilution (lowest) up to dilution 1:15 (highest dilution). This

means that the synergistic effect against MRSA and *E. faecalis* is only produced by the neat combination that produced the zone of inhibition during the modified Kirby Bauer method of susceptibility testing. This combination did not show any synergistic effect against MSSA.

Since the combination of *S. frutescens* water based extract and penicillin V did not demonstrate any synergistic or antagonistic effect when compared with the results produced by *S. frutescens* water based extract and also compared with penicillin V against MRSA, MSSA and *E. faecalis*, the MIC 's and MBC's were not done.

The MIC and MBC for a combination of *S. frutescens* water based extract and penicillin G indicated that the most synergistic effective dilution against MRSA was 1:3 with 100 % inhibition of growth and the least effective dilution is 1:7 with 0 % inhibition of growth. It further indicated that most synergistic effective dilution against MSSA *and E. faecalis* was 1:15 with 100 % inhibition of growth.

The MIC and MBC for the combination of *P. guajava* water based extract and procain penicillin demonstrated that there was no synergistic effect for a 1:1 dilution as both procain penicillin and the combination had a 100 % inhibition of growth against MRSA. However, the synergistic effect against the same bacteria was shown by a dilution of 1:3 through to 1:15 with a 100 % inhibition of growth for the combination and 0 % inhibition of growth for procain penicillin. Since there was no synergistic effect when it was tested against MSSA and *E. faecalis*, the MIC's and MBC's were not perfomed for the combination of *P. guajava* water based extract and procain penicillin on MSSA and *E. faecalis.*

The MIC and MBC for a combination of *P. guajava* water based extract and benzathine penicillin indicated that the most effective dilution against MRSA and *E. faecalis* was the undiluted combination. This means that the synergistic effect against MRSA and *E. faecalis* is only produced by the neat combination that produced the zone of inhibition during the modified Kirby Bauer method of susceptibility testing.

The MIC and MBC for a combination of *P. guajava* water based extract and benzathine penicillin indicated that the most effective dilution against MRSA, MSSA and *E. faecalis* was the undiluted combination. This means that the synergistic effect against *S. aureus* and *E. faecalis* is only produced by the neat combination that produced the zone of inhibition during the modified Kirby Bauer method of susceptibility testing.

The MIC and MBC for a combination of *P. guajava* water based extract and benzathine penicillin indicated that the most effective dilution against *MRSA* and *E. faecalis* was the undiluted combination. This means that the synergistic effect against MRSA and *E. faecalis* is only produced by the neat combination that produced the zone of inhibition during the modified Kirby Bauer method of susceptibility testing.

The synergic effects between *S. frutescens* or *P. guajva* and penicillins suggest a possible clinical use of these combinations to treat MRSA, MSSA and *E. faecalis* infected patients. However, it is hard to predict either synergic or antagonistic

effects *in vivo* just according to the *in vitro* evidence presented (Zhi-Qing Hu *et al*.,2002). Mostly the studies of drug interaction are carried out *in vitro*. But in human medicine, some of the studies are carried out *in vivo* to determine effect of combined therapy (Bhardwaj *et al*., 2016). The anticancer drugs like Polyphyllin I (rhizome of *Paris polyphyllin*) and evodiamine (*Evodiarutae carpa*) are less effective individually as compared to other anticancer drugs but on combination, they are significantly more effective in cancer patients (Yue *et al*., 2013).

Herbal drugs have great potential as an antimicrobial agent. In combination with each other or with other antimicrobial agents they may of huge value in decreasing use of antibiotics (Bhardwaj *et al*., 2016). The synergistic effects between herbal drugs and antibiotics against resilient bacteria provide a new and another way of treatment of resilient microbes. The synergistic action is of more importance in case where antibiotic(s) is no longer effective as a healing agent. Combinations of herbal drugs in form of *S. frutescens* or *P. guajava* combined with penicillins provide an effective and economical way in combating antibiotic-resistant bacteria.

CHAPTER 6: CONCLUSION and RECOMMENDATIONS

6.1. Conclusion

The first objective was to determine zones of inhibition produced, MIC and MBC, of *P. guajava* and *S. frutescens* extracts on *S. aureus* and *E. faecalis.* The second objective was to compare the antibacterial activity of water and ethanolic extracts for *P. guajava and S. frutescens*. The third objective was to determine the synergistic effects of plant extracts (*P. guajava and S. frutescens)* and Penicillins on *S. aureus* and *E. faecalis*. It is obvious from the results that the traditional plants used in this study have antimicrobial activity. It is also obvious that dissimilar bacteria will not respond in a similar manner to the plant extract, though the plant has antimicrobial activity. This means that some microorganisms are resistant to the antimicrobial activity of plant extracts while others are sensitive. The results additional prove that the plant extracts used in this study produced better activity when used in combination with penicillins. The extraction method is also important for the maximum effect of the plant on microorganisms.

The likelihood for creating antimicrobials from medicinal plants appears to be remunerating as it will prompt the advancement of a phytomedicine to act against microorganisms. Plant-based antimicrobials have gigantic remedial potential as they can fill the need with lesser side effects that are frequently associated with synthetic antimicrobials (Iwu *et al.,* 1999). Continued further investigation of plant-derived antimicrobials is required today. Further research exploration is important to determine the identity of the antibacterial compounds from these plants and furthermore to determine their full range of efficacy. Be that as it may, the present *in*

vitro antimicrobial assessment of some plants forms a primary platform for further phytochemical and pharmacological investigations. Despite the fact that the plant extracts demonstrated promising outcomes, the study was limited by the fact that extraction by utilization of boiled water was not done in this study. Utilizing boiling water for extraction is another strategy that is utilized by traditional healers.

6.2 Recommendations

This work can serve as the bases for future advancements of antimicrobial agents from the traditional plants. The herbs ought to be tried *in vivo* by means of clinical trials and they ought to likewise be tested for their toxicity to cells. Distinctive parts of the plants ought to likewise be tested for antibacterial action to an extensive variety of microorganisms. Trials ought to be run with various stypes of extracts, for example, glycerine, vinegar and acetone to see which is the most effective in extracting the active compounds of the two plants utilized as a part of this study. With the utilization of column chromatography the compounds in each plant can be isolated and made into a powder form. The concentration can be then determined using HPLC. This would then be able to be tried against microorganisms. Lodging of a voucher specimen has an advantage of verifying the plant material used in the experiment at a later stage, should the subsequent review of the experiment by other researchers take place. The literature has indicated that there is synergism between plants and when the plants used in this study were combined with penicillins, synergism was indicated.

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APPENDIX A

Calculation 1: Water-based extraction of *S. frutescens*

The weight of water from the plant was eliminated using the following calculation:

E_3 = 2MD ÷ 100 where M = plant weight (50g) and D = % of drying (74%)

Start weight = 5g

End weight = 1.3g

Water lost weight = 3.7g

Therefore % weight loss = 74%

Substitution in the formula = 2x50x74÷100

= 75ml of distilled water was added to 50g of minced plant

Calculation 2: Water-based extraction of *P. guajava*

The weight of water from the plant was eliminated using the following calculation:

E_3 = 2MD ÷ 100 where M = plant weight (45g) and D = % of drying (67.5%)

Start weight = 4g

End weight = 1.35g

Water lost weight = 2.7g

Therefore % weight loss = 67.5%

Substitution in the formula = 2x45x67.5÷100

= 60.80ml of distilled water was added to 45g of minced plant

Calculation 3: Ethanol tincture preparation of *S. frutescens*

The weight of water from the plant was eliminated using the following calculation:

E_3 = 2MD ÷ 100where M = plant weight (50g) and D = % of drying (74%)

Start weight = 5g

End weight = 1.3g

Water lost weight = 3.7g

Therefore % weight loss = 74%

Substitution in the formula = 2x50x74÷100

= 75ml of 96% ethanol was added to 50g of minced plant

Calculation 4: Ethanol tincture preparation of *P. guajava*

The weight of water from the plant was eliminated using the following calculation:

E_3 = 2MD ÷ 100 where M = plant weight (50g) and D = % of drying (74%)

Start weight = 5g

End weight = 1.3g

Water lost weight = 3.7g

Therefore % weight loss = 74%

Substitution in the formula = 2x50x74÷100

= 60.80ml of 96% ethanol was added to 45g of minced plant

APPENDIX B

Preparation of culture media

Mueller-Hinton agar (Oxoid):

38 g was weighed out into three separate one litre glass bottles. Distilled water was added until the one litre mark of each bottle was reached using a measuring cylinder. This was mixed until the powder was completely dissolved. Bottles were sterilised by autoclaving for 15 minutes at 121 ^{o}C. The agar was poured into plates to solidify. It was then kept at 4 ^{o}C until use.

Preparation of nutrient broth

40 g of nutrient broth powder was weighed into a one litre glass bottle. Distilled water was added until the one litre mark was reached. This was mixed until the powder was completely dissolved. This was then dispensed into bijou bottles before autoclaving. Bijou bottles were sterilised by autoclaving for 15 minutes at 121 ^{o}C. it was kept at 4 ^{0}C until use.

Preparation of Nutrient agar slopes

Twenty eight grams of Nutrient agar powder was weighed into a one litre glass bottle. Distilled water was added until the one litre mark was reached. This was mixed until the powder had completely dissolved. Ten millilitres was dispensed into MacCathy bottles before autoclaving. These were sterilised by autoclaving for 15 minutes at 121^{o}C. They were then allowed to slope before setting.