# CHEMICAL COMPOSITION AND ANTIMICROBIAL ACTIVITIY OF DIFFERENT ROOT EXTRACTS OF *Hermannia geniculata* AGAINST HUMAN PATHOGENS OF MEDICAL IMPORTANCE

BY

**PHEELLO JEREMIA MOJAU**
2001130273

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**SUPERVISOR: DR. A.O.T. ASHAFA**

## DECLARATION

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Signature:……..

Date

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## DEDICATION

This dissertation is dedicated to my late father, Tumo Solomon Mojau, my mother, Tshokolo Gloria Mojau, My wife, Matshidiso and my children Refilwe, Junior and Tebello for their unconditional love.

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LIST OF ABBREVIATIONS AND SYMBOLS

| | |
|---|---|
| **µl** | - Microlitre |
| **mg/ml** | - Milligram per millilitre |
| ± | - More or less |

**A**

| | |
|---|---|
| ATCC | - American Type Culture Collection |
| *A. flavus* | - *Aspergillus flavus* |
| *A. fumigates* | - *Aspergillus fumigates* |
| *A. niger* | - *Aspergillus niger* |

B

| | |
|---|---|
| *B. dermatididis* | - *Blastomyces dermatitidis* |

**C**

| | |
|---|---|
| *C. albicans* | - *Candida albicans* |
| *C. dubliniensis* | - *Candida dubliniensi* |
| *C. glabrata* | - *Candida dubliniensi* |
| *C. immitis* | - *Coccidiodes immitis* |

| | |
|---|---|
| *C. krusei* | - *Candida krusei* |
| *C. neoformans* | - *Cryptococcus neoformans* |
| *C. posadasii* | - *Coccidiodes posadasii* |
| *C. rugosa* | - *Candida rugosa* |
| *C. tropicalis* | - *Candida tropicalis* |

**D**

| | |
|---|---|
| DNA | - Deoxyribonucleic Acid |

**E**

| | |
|---|---|
| *E. coli* | - *Escherichia coli* |
| *E. floccosusm* | - *Epidermophyton floccosusm* |

**G**

| | |
|---|---|
| GC-MS | - Gas Chromatography Mass Spectrometry |
| GI | - Gastrointestinal |
| G | - Grams |

**H**

| | |
|---|---|
| *H. althaeifolia* | - *Hermannia althaefolia* |
| *H. capsulatum* | - *Histoplasma capsulatum* |
| *H. incana* | - *Hermannia incana* |

| | |
|---|---|
| *H. geniculata* | - *Hermannia geniculata* |
| *H. salviifolia* | - *Hermannia salviifolia* |
| H | - Hour |
| HIV | - Human Immunodeficiency Virus |
| **I** | |
| IR | - Infrared |
| **K** | |
| *K. pneumoniae* | - *Klebsiella pneumoniae* |
| **M** | |
| *M. canis* | - *Microsporum canis* |
| *M. gypseum* | - *Microsporum gypseum* |
| MIC | - Minimum Inhibitory Concentration |
| mL | - Millilitre |
| MBC | - Maximum Bactericidal Concentration |
| **N** | |
| *N. meningitides* | - *Neisseria meningitidis* |
| NMR | - Nuclear Magnetic Resonance |
| **O** | |
| $O_2$ | - Oxygen |

**S**

| | |
|---|---|
| *S. typhi* | - *Salmonella typhi* |
| *S. typhimurium* | - *Salmonella typhimurium* |
| *S. aereus* | - *Staphylococcus aureus* |
| *S. faecalis* | - *Streptococcus faecalis* |
| *S. flexneri* | - *Shigella flexneri* |
| *Sp.* | - Species |

**T**

| | |
|---|---|
| *T. mentagrophytes* | - *Trichophyton mentagrophytes* |
| *T. mucoides* | - *Trichophyton mucoides* |
| *T. rubrum* | - *Trichophyton rubrum* |

**U**

| | |
|---|---|
| UTI | - Urinary Tract Infection |
| UV | - Ultra-violet light |

**W**

| | |
|---|---|
| WHO | - World Health Organisation |

## ABSTRACT

*Hermannia geniculata* has been used widely as traditional medicine for treatment against infectious human pathogens. The aim of the study was to determine the antibacterial and antifungal activities of *H. geniculata* root extracts and their fractions against 16 microbial strains. The dried plant materials were extracted separately in 150 ml of methanol, acetone, ethanol, water and 150 ml (50/50) of hydro-ethanol. Acetone extract inhibited the growth of microorganisms with minimum inhibitory concentration (MIC) values of 1.56 mg/ml against all the tested strains except for *Salmonella typhimurium* and *Candida rugosa* at the concentration of 6.25 mg/ml. The ethanol, hydro-ethanol and methanol extracts inhibited bacterial growth with MIC values ranging from3.13 mg/ml to 12.50 mg/ml, while water extract had MIC of 12.50 mg/ml against all tested bacterial and fungal strains. Acetone extract had maximum bactericidal concentration (MBC) values ranging from 1.56 to 3.13 mg/ml against most microorganisms. Butanol fraction of acetone extract had MIC of 0.78 mg/ml against *Staphylococcus aureus* (OK2b) and *Staphylococcus aureus* (ATCC 6538), whilst the ethyl acetate had the lowest MBC of 1.56 mg/ ml against *S. aureus* (OK2b), *S. aureus* (ATCC 6538), and *Streptococcus faecalis*. The extracts and their respective fractions displayed similar inhibitory properties which are indications that either the crude extract or their fractions could be used to manage infections associated with bacteria and fungi.

# CHAPTER ONE

# INTRODUCTION AND LITERATURE REVIEW

## Background on the Evolution of Traditional Medicine Use

Since the dawn of history, people have relied on the use of plants to support most of their fundamental needs, including food, clothing, medicines and shelter. Many of these plants are used as food and medicines for both humans and animals species, but it has been alleged that only ten percent of plants have been thoroughly scientifically investigated (Cowan, 1999). Plants of medicinal importance have a promising future but in most cases the information around their medical activities is scanty as they have not been thoroughly investigated. It is believed that their knowledge of medical properties could be decisive in the treatment of present or future diseases (Rasool Hassan, 2012). Over the past few years, herbal medicine has become a topic of universal significance, making an impact on both health and international trade. Medicinal plants continue to contribute a critical role in the healthcare system of enormous proportions of the world's population (Akerele, 1988). Both developing and industrialised nations have seen an increase in the recognition and development of the medicinal and economic use of these plants (WHO, 1998).

Medicinal plants are used worldwide and have a rapidly growing economic significance. In developing countries, traditional medicines are often the only accessible and affordable treatment available. In Africa, 80% of the population uses traditional medicine as the primary health care system (Fisher and Ward, 1994). Traditional medicine usage is gaining more respect by national governments and health care providers. Due to poor communications means, poverty, ignorance and unavailability of modern health facilities, most of the rural people are forced to practice traditional medicines for their common ailments (Khan, 2002). The current belief that drugs that come in capsules or pills are the only medicines that can be trusted and

used have created an impression that medicinal plants are not effective. Even so a lot of these pills that are taken and used in daily lives come from plants. Medicinal plants are often used as raw materials for extraction of different active ingredients which is used in the synthesis of different drugs (Rasool Hassan, 2012).

**Traditional Medicine Use in Southern Africa**

Southern part of Africa has one of the richest plant diversity in the world. Most of these plant species have been implicated in traditional medicine of the region for several hundreds of years (Lewu and Afolayan, 2009). Approximately 27 million South Africans depend on traditional medicine for their basic health care needs (Street et al., 2009). Number of factors can be apportioned to the reliance of such a large portion of the population; accessibility to the plants, affordability and the level of extensive knowledge and expertise amongst the local communities (Grundy and Wynberg, 2001). In the past, the field of medicine was dominated by traditional knowledge and most indigenous healers across ethnic and racial populations of the world are not keen to accurately share their experience with outsiders. As a result, there is a great gap in knowledge between modern medicine and traditional healing. The development of Phytomedicine within the last few decades with specific reference to South Africa has rapidly bridged that gap (Van Wyk and Gericke, 2000)

Approximately 72% of Black population of South Africa (about 26.6 million) use traditional medicine. These consumers are from a variety of age categories, education levels, religions and occupations. The diverse number of consumers is an indication that traditional medicine is a common practice across most sectors of Black African population, and that traditional medicine use is not restricted to needy, rural and illiterate users (Mander et al., 2007).

The most popular form of traditional medicine is herbs, and they are highly lucrative in the international market place. Annual revenues in Western Europe reached US$5 billion between 2003 and 2004. Herbal medicine revenue in Brazil was US$160 million in 2007 (WHO, 2008). Notwithstanding, the use of herbal medicine is increasingly becoming mainstream with retail sales of herbal products in Australia estimated to be $200 million (Wohlmuth et al., 2003). The global use of herbal medicines has increased in the past decade reaching annual sales in excess of 60 billion U.S. dollars and is expected to reach 55 trillion U.S. dollars by 2050. Although most herbal remedies are consumed by adults, a growing proportion is consumed by children of all ages. Two recent surveys report that up to 20% of children who are scheduled for elective surgery consume herbal medicine (Lerman, 2005).

According to Mander and Le Breton (2005), there are up to 100 million traditional-remedy consumers in southern Africa and as many as 500,000 traditional healers. Up to 700,000 tonnes of plant material is consumed annually with an estimated value of as much as 150 million US dollars per annum. The trade in traditional medicines forms part of a multimillion-rand 'hidden economy' in southern Africa (Cunningham 1989). Stimulated by high population growth rates, rapid urbanization and the important cultural value placed on traditional medicines this trade is now greater than at any time in the past. At national level, it is estimated that annually 20,000 tonnes of material from over 700 plant species are traded, with a value of approximately R 270 million (US$ 60 million) (Mander 2004). The use and trade of plants for medicine is no longer confined to traditional healers, but has entered both the informal and formal entrepreneurial sectors of the South-African economy, resulting in an increase in the number of herbal gatherers and traders (Cocks et al. 2004).

Indigenous plants constitute the pre-dominant source of medicine for traditional healers, with at least 771 plant species recorded in the trade in South Africa. It is projected that 20 000 tonnes of indigenous plants are harvested from grasslands, forests woodlands and

thickets in eastern South Africa every year, with a small portion being cultivated (Mander et al., 2007).

## Bacteriology

Lots of infections and diseases are believed to be caused by bacteria. It is therefore pertinent to include a brief review of bacteriology in an attempt to understand the antimicrobial actions of the plant extracts.

### Structure of Bacteria

Bacteria are microscopic organisms whose single cells have neither a membrane-enclosed nucleus nor other membrane-enclosed organelles like mitochondria and chloroplasts. Biologists think they closely resemble the first organisms to evolve on earth. Too small to see with un-aided eye, majority of bacteria range from 0.20 to 2.0 micrometres (μm) in diameter and from 2 to 8μm in length (Talaro and Talaro, 1996).

Bacteria are in most cases simple in form and exhibit one of the three basic structures: Bacillus (straight and rod-shaped), coccus (spherical-shaped), and spirollus (long and helical shaped), also called spirochetes (Tortora *et al.*, 1994). Large portion of clinically significant bacteria are classified as either Gram positive or negative based on their morphology.

### Bacterial diseases

Millions of bacteria normally live on the skin, in the intestines, and on the genitalia. The vast majority of bacteria do not cause disease, and many bacteria are actually helpful and even necessary for good health. Harmful bacteria that cause bacterial infections and disease are called pathogenic bacteria. Bacterial diseases result when pathogenic bacteria get into the body and begin to reproduce and crowd out healthy bacteria, or grow in tissues that are normally

sterile. Harmful bacteria may also emit toxins that damage the body. Common pathogenic bacteria and the types of bacterial diseases they cause include:

- *Escherichia coli* and Salmonella cause food poisoning.
- *Helicobacter pylori* cause gastritis and ulcers.
- *Neisseria gonorrhoeae* causes the sexually transmitted disease gonorrhoea.
- *Neisseria meningitidis* causes meningitis.
- *Staphylococcus aureus* causes a variety of infections in the body, including boils, cellulitis, abscesses, wound infections, toxic shock syndrome, pneumonia, and food poisoning.
- Streptococcal bacteria cause a number of infections in the body, including pneumonia, meningitis, ear infections, and strep throat.

Bacterial diseases are contagious and can result in many serious or life-threatening complications such as blood poisoning (bacteremia or septicemia), kidney failure, and toxic shock syndrome (http://www.healthgrades.com/conditions/bacterial-diseases)

Untreated septicemia can quickly progress to sepsis, which is a serious complication of an infection characterized by inflammation throughout the body. This inflammation can cause blood clots which block oxygen from reaching vital organs, resulting in organ failure and death in some cases. It is caused by a bacterial infection (typically severe) in another part of the body. Urinary tract infections, lung infections, and infections in the abdominal area are all potential causes of septicemia. Bacteria from these infections enter the bloodstream and multiply, causing immediate symptoms. If left untreated, it can be fatal. One complication of septicemia is a serious drop in blood pressure, called septic shock. Toxins released by the bacteria in the

bloodstream can cause extremely low blood flow, which may result in organ or tissue damage. Septic shock is a medical emergency (O'Connell, 2012).

**Mycology**

Mycology is the study of fungi; their genetic and biochemical properties, as well as their taxonomy. Pathogenic fungi have the ability to actively attack and invade tissues (Hawksworth, 1974). Bauman, 2007). The study also focuses on the impact of fungi on human health in some way. Surprisingly, the causative relationship of fungi to human health was known before the pioneering work of Pasteur and Koch with pathogenic bacteria. Fungi are omnipresent in the environment, and infection due to fungal pathogens has become more frequent (Walsh and Groll, 1999; Fleming *et al.*, 2002). During the early years, mycology was really the study of dermatophytes (tinea and ringworm fungi), with Raimond Sabouraud (1864-1938) being the most well-known name in the field. Sabouraud's agar to date remains the most famous name in the formation for growing fungi.

It has been estimated that there are between 250,000 and 1.5 million species of fungi on this planet, and about 70,000 of these species have been described. Fortunately, only about 300 of these species cause human infection, and of these about 30 species are seen regularly (Davis1994).

The search for novel antifungal agents relies mainly on ethnobotanical information and ethnopharmacologic exploration. The medicinal knowledge of North American First Nations peoples has been shown to be a valid resource. Studies have revealed a high degree of correlation between traditional medicinal uses and laboratory analysis (McCutcheon *et al.*, 1994; Bergeron *et al.*, 1996; Jones *et al.*, 2000).

Fungal diseases can also be classified broadly on the basis of causative agents; these diseases differ in nature, causative agents, and distribution (Khan et al., 2010).

**Antibiotics**

After Bayarski: an antibiotic is a drug that kills or inhibits the growth of bacteria. It is a one class of antimicrobials, a larger group that includes anti-viral, anti-fungal, and anti-parasitic drugs. They are chemicals produced by or derived from microorganisms (i.e. bacteria and fungi). The first antibiotic was discovered by Alexander Fleming in 1928.

Antibiotics are among the most frequently prescribed medications in modern medicine. Some antibiotic are "bactericidal", meaning their role is to kill bacteria. Other antibiotics are "bacteriostatic", meaning their role is to stop bacteria from multiplying.

Some antibiotics can be used to treat a wide range of infections and are known as "broad-spectrum" antibiotics.

S.A Waksman introduced the term "antibiotic" in 1942. In forties to sixties, the term "antibiotic" was clearly contrasted from the term "chemotherapeutic drug": Antibiotics were natural drugs produced by several fungi or bacteria. These drugs were man-made substances. However the distinctions were abolished after chemical synthesis of some antibiotics has been realized and the new drugs have developed form the natural products with binding various side chains to the basic structure.

These drugs have been used effectively to control infection by curing clinical symptoms and \or illnesses and basically have been reported to reduce infections (Molefe et al., 2012).

Previous researches have focused on what motivates inaccurate demand for and use of antibiotics, and have tended to ignore existing views and practices that could form resources for reducing inappropriate use (Norris et al., 2009). These includes among other things understanding of the negative consequences of antibiotic use, lay people's reluctance to use antibiotics and the strategies they use to avoid antibiotics. These could establish a significant basis for developing educational and health promotion interventions that are relevant and acceptable to the focused population and new could result in culturally rooted norms about antibiotics and their use (Wilson et al., 1999 and Arroll et al., 1999).

**Antibiotics side effects**

Antibiotics can literally save lives and are effective in treating illnesses caused by bacterial infections. However, like all drugs, they have the potential to cause unwanted side effects. A lot of these side effects can make life unpleasant while the drug is being taken. The most common side effects are diarrhoea, nausea, vomiting. Fungal infections of the mouth, digestive tract and vagina can also occur with antibodies, because they cause destruction of the protective "good" bacteria in the body (that assists to prevent overgrowth of any one organism), as well as the "bad" ones, responsible for the treatment of the infection.

**Antibiotic resistance**

The clinically useful antibiotics now in use have major setbacks. Apart from the narrow spectrum of antimicrobial activity many of them have been found to be neurotoxic, nephrotoxic, ototoxic or hypersensitive and few others have debilitating effects on the liver and are associated with bone marrow depression (chong and Pagano, 1997) and significantly; infectious pathogens have developed resistance to all known antibiotics (Aiyegoro and Okoh, 2009).

Antibiotic resistance poses a stern threat to public health in both developing and developed countries. This is mostly attributed to in accurate prescriptions and use of antibiotics for conditions for which they are ineffective (such as upper tract respiratory infections caused by viruses). Knowledge and understanding of antibiotics is significant because they are crucial determinant of inaccurate use. Patients may either access antibiotics directly without the prescription; although this is not legal in most countries, it is a common practise (Okeke et al., 2005).

The only time antibiotic can be qualified as safe is on the condition that it is effective selectively on microorganism. The structure or the enzyme processes of prokaryotic cells that are affected by antibiotics are not present in a human prokaryotic cell. Even though antibiotics, deemed relatively safe drugs, they exhibit a frequent occurrence of harmful effects, which is attributed to the frequent prescriptions, as well as to non-rational use (Laurence and Bennett, 1992)

The origin of antibiotic resistance extends way back in evolutionary terms and reflects the attack and the counter attack of complex microbial flora in order to determine ecological niches to survive. Early treatment draw backs with antibiotics represented an important clinical problem because other types of agents, with different cellular targets, were available.

Salmonellosis which is an infection caused by Salmonella bacteria, often confined to the gastrointestinal tract and is often a self-limiting disease. Most people who get infected by *Salmonella typhimurium* experience moderate gastrointestinal illness involving diarrhoea, chills, abdominal cramps, fever, head and body aches, nausea, and vomiting (Honish, 1999). Infections are in most cases self-limiting, and antimicrobial treatment is not recommended for uncomplicated illnesses (Aserkoff and bennet, 1969; Gill and Hammer, 2001). Nonetheless, extraintestinal infection can occur, particularly in very young, elderly and

immunocompromised patients (Angulo and Swerdlow, 1995; Thuluvath and McKendrik, 1998). In these cases effective antimicrobial treatment is necessary (Cruchaga et al., 2001). Salmonelloses have been reported to be season dependent and occur more in the winter than summer and in mostly referred to as gastroenteritis or diarrhoea. Likewise more cases of diarrhoea caused by enterobacteria especially *E. coli*, occurring more during wet season than dry season (Olowe et al., 2003). Multidrug-resistant (MDR) strains of *Salmonella* are now encountered frequently and the rates of multidrug-resistance have escalated considerably in recent years. Even worse, some variants of *Salmonella* have developed multidrug-resistance as an integral part of the genetic material of the organism and are therefore likely to retain their drug-resistant genes even when antimicrobial drugs are no longer used, a situation where other resistant strains would typically lose their resistance. Many of the *Salmonella typhimurium* strains isolated in a study in western part of Nigeria were resistant to drugs like streptomycin, amoxicillin, tetracycline, ampicillin, kanamycin and chloramphenicol (Olowe et al., 2007).

Since the antibiotics have been discovered their uses as chemotherapeutic agents, there was a belief in the medicinal fraternity that this would lead to eradication of infectious diseases. However, diseases and disease agents that were once thought to have been controlled by antibiotics are returning in new forms resistant to antibiotic therapies (Levy and Marshall, 2004).

Resistance to antimicrobial agents typically occurs as a result of four main mechanisms namely enzymatic inactivation of the drug (Davies, 1994), alteration of target sites (Spratt, 1994), reduced cellular uptake, and extrusion by efflux. It has also been reported that chemical modifications could be important in antibiotic resistance, though exclusion from the cell of unaltered antibiotic represents the primary means denying the antibiotic access to its targets and this is believed to enhance resistance even in situations where modification is the main mechanism (Li et al., 1994).

Plants have traditionally provided a source of hope for novel drug compounds, as plant herbal mixtures have made large contributions to human health and well-being (Iwu et al., 1999). Owing to their popular use as remedies for a lot of infectious diseases, searches for substances with antimicrobial activity in plants are frequent (Shibata et al., 2005; Betoni et al., 2006). Plants have been found to be rich in a wide variety of secondary metabolites, such as tannins, terpenoids, alkaloids, and flavonoids, which have been found *in vitro* to have antimicrobial properties (Cowan, 1999; Lewis and Ausubel, 2006). The observation that plant derived compounds is generally weak compared to bacterial or fungal produced antibiotics and that these compounds often show considerable activity against Gram-positive bacteria than Gram-negative species has been made by many (Nostro et al., 2000; Gibbons, 2004). This observation lead Tegos et al. (2002) hypothesizing that; plants produce compound that can be effective antimicrobials if they find their way into the cell of the pathogen especially across the double membrane barrier of Gram negative bacteria.

**Antimicrobial Activity of Medicinal Plants**

Medicinal plants have been found useful as antimicrobial agents (Prescott et al., 2002), the medicinal actions of plants are unique to a specific plant species or groups, consistent with the concept that the combination of secondary products in a specific plant is taxonomically discrete (Parikh et al., 2005).

The usage of crude extracts of parts of plants and phytochemicals, of known antimicrobial properties, can play a pivotal role in the therapeutic treatments. Few years ago, numerous studies have been conducted in different countries to prove such efficiency. A lot of plants have been used because of their antimicrobial traits, which are attributed to the secondary metabolites synthesized by the plants (Wu et al., 1999).

Medicinal plants are renewable in nature unlike the synthetic drugs that are obtained from non-renewable sources of fundamental raw materials such as fossil sources and petrochemicals (Samanta et al., 2000). Because of all these advantages, plants continue to be a major source of new lead compounds. Nowadays, the inappropriate use of commercial antimicrobial drugs has caused multiple drug resistance in human pathogenic microorganisms (Aliero et al., 2008). This situation forced scientists to search for new and effective antimicrobial agents to replace the current regiments (Jacquelyn G.B, 2002).

Plants antimicrobials have been found to be cooperative enhancers in a sense that though they may not have antimicrobials properties alone, but when they are taken concurrently with standard drugs they enhance the effect of that drug (Kamatou et al., 2006). The cooperative effect from the synergy of antibiotic and plant extracts against resistant bacteria results in the new choices for the treatment of infectious diseases. This effect allows the use of the respective antibiotic when it no longer effective on its own during therapeutic treatment (Nascimento et al., 2000).

**Primary and Secondary Metabolites from Medieinal Plants**

Since the beginning of time, it is estimated that 80% of individuals use traditional medicine, which comprise of chemical compounds derived from medicinal plants. These compounds are classified into primary and secondary metabolites (Vinoth et al., 2011). Primary metabolites are fundamentally required for growth and developments of plants such as proteins, sugars, lipids. Secondary metabolites are not involved directly and they have been utilized as biocatalysts which are synthesized during secondary metabolism of plants are potential source of drugs. The most important ones are saponin, alkaloids, tannins, flavonoids and cardiac glycosides (Lingarao and Savithramma, 2011). Phytochemical screening process is used to identify these compounds available in the plant extracts derived from any part of the plants like

roots, bark, leaves etc .Phytochemicals and plant extract usage, both with known antimicrobial properties, can be of pivotal importance in therapeutic treatments (Gislene et al., 2000).

**Major Groups of Antimicrobial Compounds from Plants**

Plants have an almost unrestricted ability to synthesize aromatic substances, most of which are phenols or their oxygen-substituted derivatives (Geissman, 1963). Most are secondary metabolites, of which at least 12,000 have been isolated, a number estimated to be below 10% of the total (Schultes, 1978). In most instances, these substances serve as plant defense mechanisms against predation by microorganisms, insects, and herbivores. Some, such as terpenoids, give plants their odours; others (quinones and tannins) are responsible for plant pigment. Many compounds are responsible for plant flavour (e.g., the terpenoid capsaicin from chili peppers), and some of the same herbs and spices used by humans to season food yield useful medicinal compounds.

**Southern African Distribution of *Hermannia* genus**

Southern Africa has nearly 150 species, including some of those found further north in Africa. The greatest diversity is within Cape Province and Namibia, but there are relatively few species within the southern coastal areas of Cape Province (Cape Floristic Province). The other South African provinces have between 18 (Gauteng) and 34 (Free State) species. There are 8 species in Lesotho, 20 in Botswana and 13 in Swaziland. There are perhaps 20 species in southern tropical Africa, of which 12 occur in Zimbabwe, 3 in Zambia. Mozambique has 6 species, 4 of these are also shared with Zimbabwe. At least 6 species occur in Angola. The majority of the remaining species are presumably to be found in Natal, Transvaal, the Orange Free State, Namibia and Angola. Madagascar has a single species (*Hermannia exappendiculata*) which is shared with East and North East Africa (Leistner, 2000).

**Medicinal Properties of *Hermannia* genus**

The genus *Hermannia* has been used traditionally by people of diverse cultures for the treatment of fever, cough, respiratory diseases such as asthma, wounds, burns, eczema, stomachache. This plant is also used as purgative, diaphoretic, for heartburn, flatulence in pregnant women, colic and haemorrhoids (Essop *et al.*, 2008).

In addition, the Xhosa use a decoction of the root of *H. incana* for dysuria; while a decoction of the root of *H. salviifolia* is utilized as an old-fashioned European household remedy for convulsions (Watt and Breyer-Brandwijk, 1962).

*Hermannia incana* is used as an emetic and the leaf sap extracted in cold water, is used to treat stomach-ache and diarrhoea, having purgative and diaphoretic effects. Decoctions of

the whole plant are taken to soothe coughs. However, no other studies relating to the chemical composition of this species have earlier been reported (Van Wyk *et al.*, 1997).

*Hermannia geniculata* is a species under the genus *Hermannia* of the subfamily *Byttnerioideae* and tribe *Hermanieae* of the family *Malvaceae* (previously *Sterculiaceae*). The wide diversity of species in a restricted geographical region is suggestive of a recent origin and diversification of the species. The lack of reported variation in chromosome counts may be further evidence in favour of this interpretation, or may reflect a limited sampling of the species of the genus. On the other hand the genus seems less derived that the other genera of the tribe (for example in the presence of 5-locular ovaries with pluri-ovulate locules, which is a widespread condition in *Byttneroideae*, whereas the other genera show reduction in both the number of locules and ovules).

**Morphology of *Hermannia geniculata***

*Hermannia* is a genus of small shrubs, ranging from upright to sprawling prostrate shrublets. They are characterized by the presence of minute glandular or star-like hairs on the leaves and stems. The stems often have a dark grey bark. Leaves are alternate and entire, lobed or incised. Flowers consist of 5 petals which are slightly or very strongly spirally twisted into an upended rose. Most *Hermannia* species have a thick woody stem and root, forming an underground stem, which enables the plants to survive dry periods and fires. In the veld, the plants appear woody, some species being very palatable to stock and browsed down to the main branches.

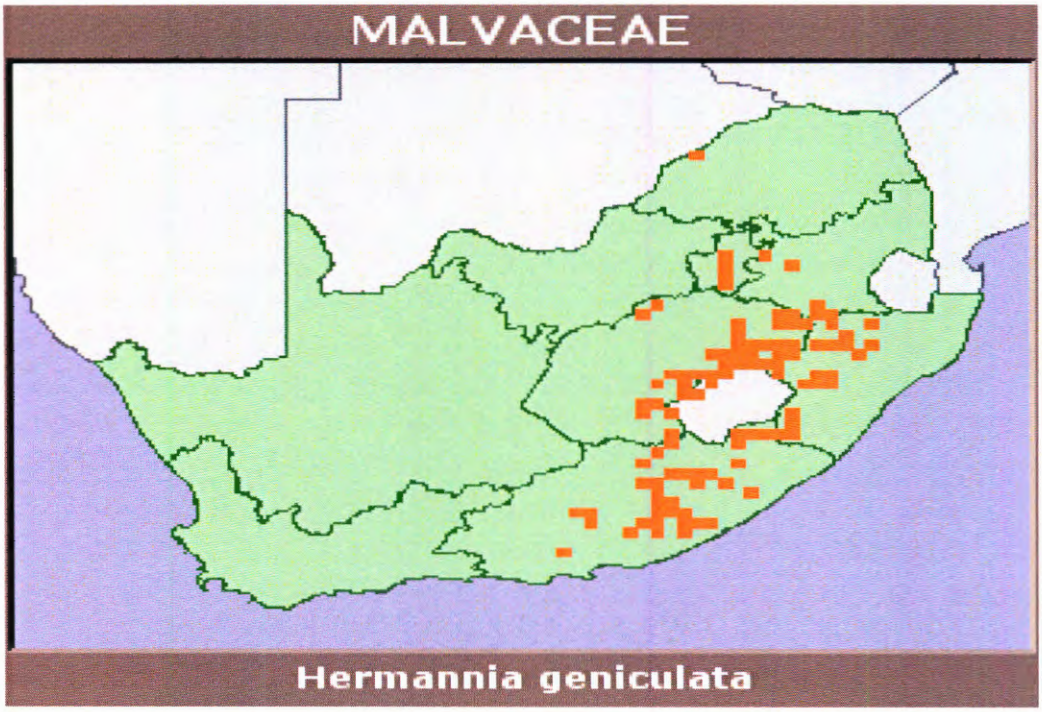



Figure 1. Distribution map of *H. geniculata* in South Africa (redlist.sanbi.org)

*Hermannia geniculata* is a decumbent, leaves petiolate, elliptic-oblong, obtuse, sub-cordate at base, corrugated and first pubescent, but grows glabrous on the upper side, stipules membranous, broadly ovate. The Basotho tribe of the Eastern Cape Province of South Africa use the plant as the traditional medicine. The dry root material is chopped, boiled in water and taken three times daily to ameliorate blood sugar disorders. It is also used in the management of diarrhoea, heartburn, stomach disorder and flatulency called "leletha" in pregnant Sotho women (Kazeem and Ashafa, 2015)

Figure 2. *H. geniculata* growing on a rocky hill (www.colinpatersonjones.co.za)

**Aim of the study**

The aim of this study is to investigate the chemical composition and antimicrobial activity of different extracts of *Hermannia geniculata*.

**Speeific objectives**

- To determine the antibacterial and antimycotic activities of different root extracts of *H. geniculata*
- To identify chemical compounds responsible for antimicrobial activity using GCMS
- To validate the folkloric claims of the plant as a natural antibiotic

# CHAPTER TWO

# RESEARCH METHODOLOGY

## Introduction

The current chapter discusses the materials and methods used in the sampling of *Hermannia geniculata* plant materials, as well as performance of antimicrobial activity screening of the respective extracts of plants using four testing methods, analysis of chemical compounds responsible for inhibition of bacteria and fungi using Gas Chromatography Mass Spectrometry (GCMS)

## Plant collection and identification

Fresh roots of *Hermannia geniculata* were collected from vegetation along Wetsi café at Monontsha village, Qwaqwa, Eastern Free State Province, South Africa. The roots were thereafter authenticated and a Voucher Specimen (Mojamed/1/2013/Qhb) was prepared and deposited at the Herbarium of Plant Sciences Department, University of the Free State, Qwaqwa Campus, South Africa.

## Extract preparation

The fresh roots were cut into smaller pieces and washed under running water to remove all debris dried in an Ecotherm oven at 40°C. Dried plant materials were then powdered with the help of Waring laboratory blender (Labcon, Durban, South Africa).

Powdered plant materials (10 g each) were extracted separately in methanol (150 ml), Ethanol (150 ml), Acetone (150 ml), hydro-ethanol (150 ml: 50/50) and water (150 ml), the

plant in different solvents were put on a Labcon platform shaker for 24 h at the speed of 120rpm.

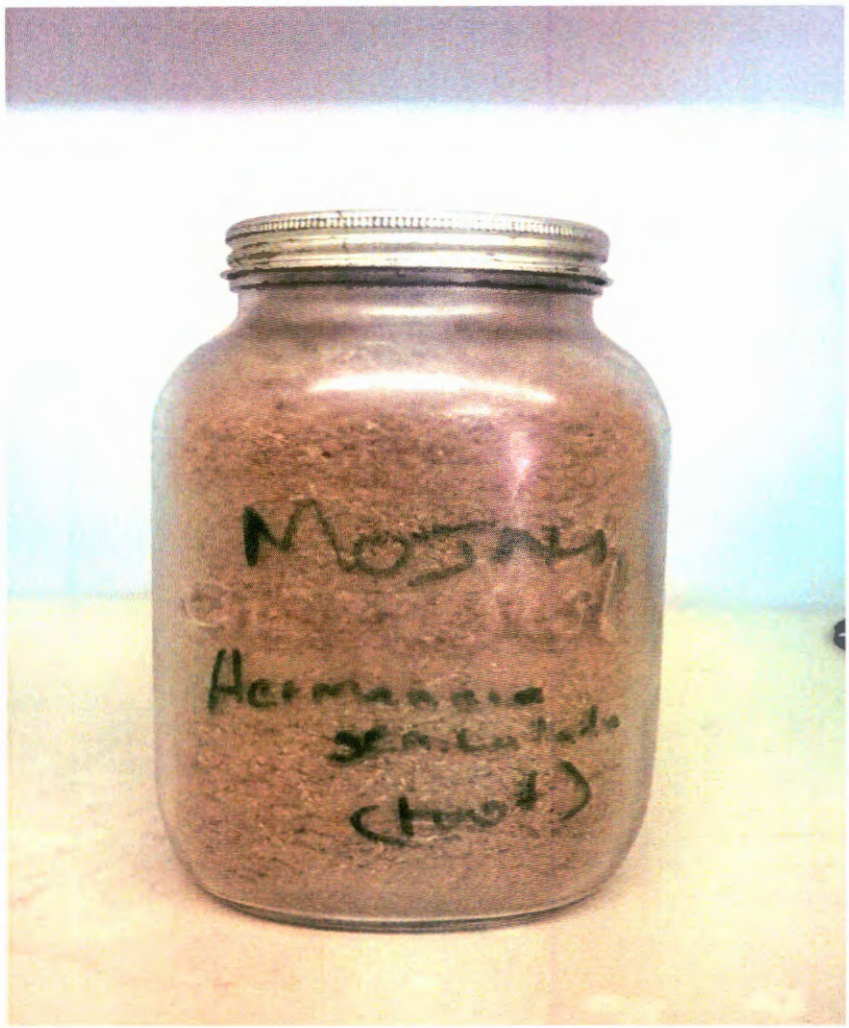


Figure 3. Powdered *H. geniculata* root (Mojau, 2014)

Figure 4. Filtration of extracts (Mojau, 2014)

Extracts were filtered using Whatman no. 1 filter paper (fig 4.) and each filtrate was concentrated to dryness under reduced pressure at 40°Celcius using rotary evaporator-Cole Parmer (fig 5.)

Figure 5. Concentration of filtrate to obtain crude extract (Mojau, 2014)

Finally, extracts were dried to yield ethanol extract (1.2 g), methanol (2 g), acetone (1 g), hydro-ethanol (2.5 g), and water (1 g). Each extract was re-suspended in its respective solvent to make a 50 mg/ml stock solution.

**Fractionation of the extract**

The crude extract of acetone and ethanol, that showed the most antibacterial and Antimycotic activity were subjected to bio-guided fractionation by solubilisation in water and sequential partition with (for acetone extract) hexane (4 x 400mL) ethyl acetate (7 x 400 mL), chloroform (5 x 400 mL), dichloromethane (5 x 400 mL) and n-butanol (5 x 400 mL) (fig 6).

Figure 6. Partition fractioning with hexane (Mojau, 2014)

Ethyl acetate fraction of acetone extract showed the most inhibition of bacterial and fungal activity; as a result it was re-partitioned with hexane (4 x 400 mL), ethyl acetate (4 x 400 mL), chloroform (2 x 400 mL) and dichloromethane (1 x 100 mL). Each fraction thus

obtained was evaporated to dryness under reduced pressure and subjected to bioassay (antibacterial and Antimycotic activity)

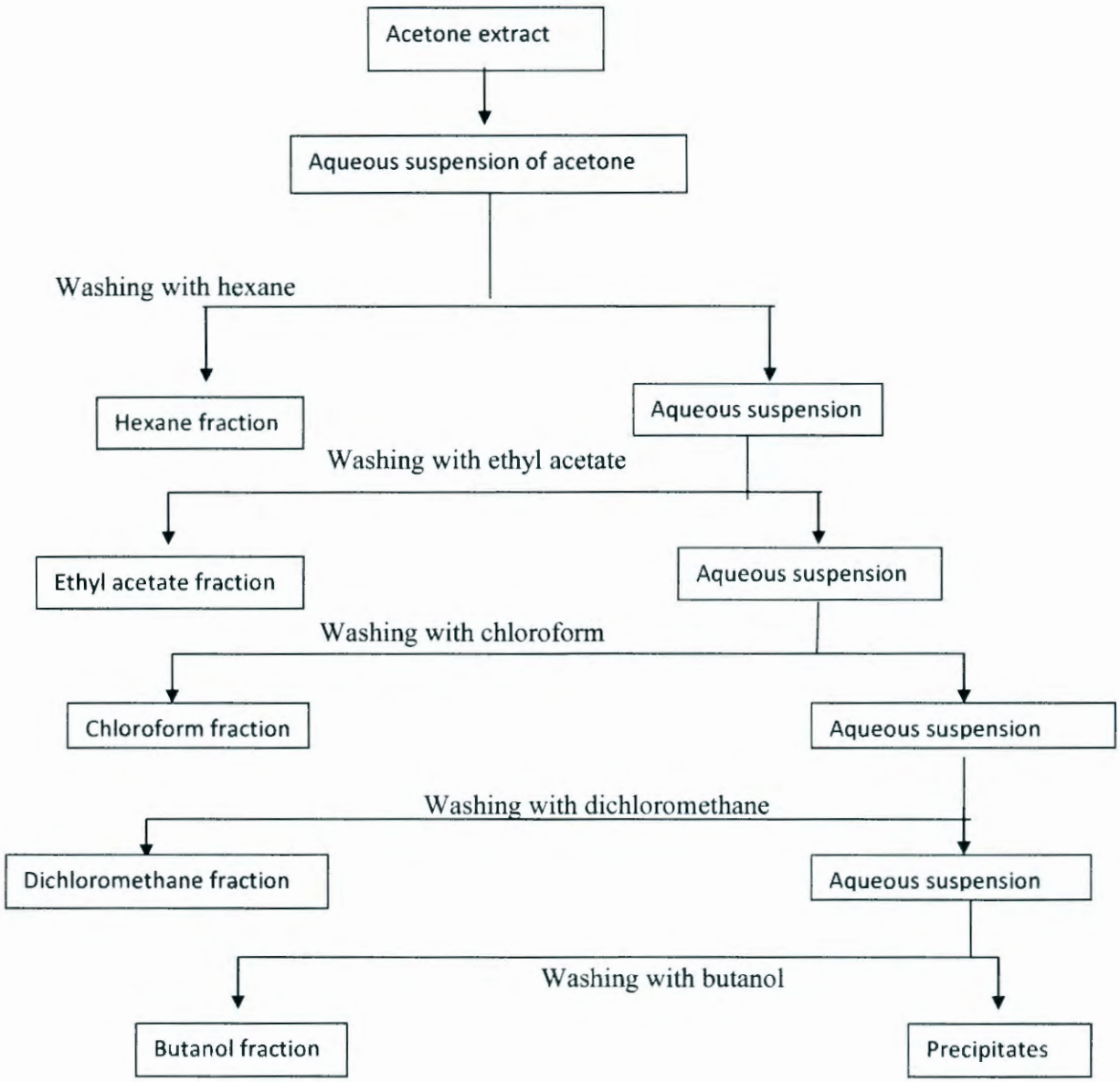



Figure 7. Scheme of acetone fractions preparation

Hexane and ethyl acetate fractions were dissolved in 50% acetone, chloroform, dichloromethane and butanol fractions were dissolved in 50% methanol.

Ethyl acetate fraction of acetone extract was repartitioned since it showed highest antimicrobial activity against most microorganisms (Figure 8.)

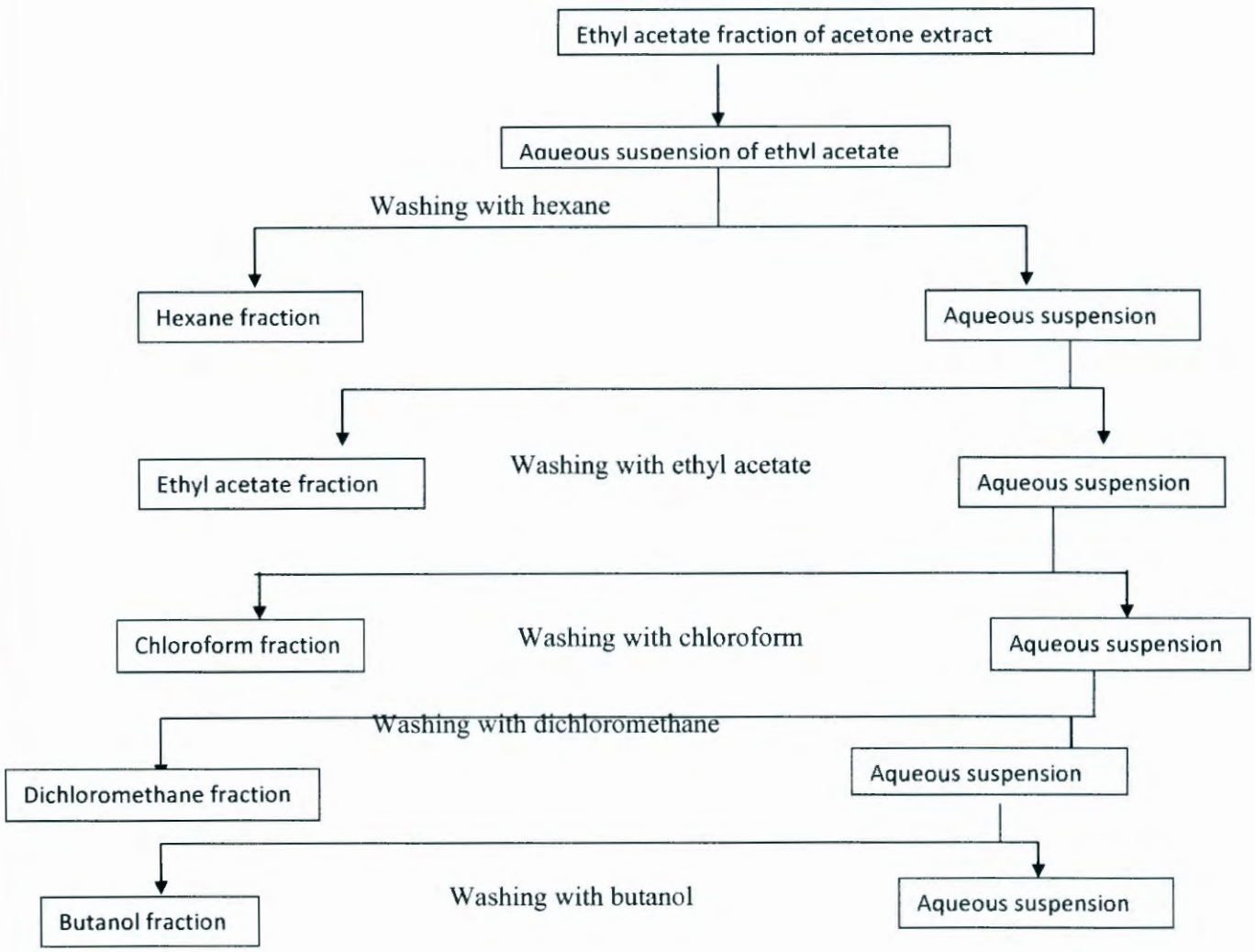



Figure 8. Ethyl acetate fraction of acetone extract partitioning

**Analysis of Chemical Compounds**

The acetone extract and its fractions showed most of the antimicrobial activity against microorganisms and were further subjected to gas chromatography mass spectrometer (GCMS) for identification of chemical compounds that might be responsible for inhibition of microorganisms' growth. The extract and fractions were analyzed using Hewlett Packard 6890 Gas Chromatograph linked with Hewlett Packard 5973 mass spectrometry system and equipped with HP5-MS capillary column (30 m x 0.25 mm, film thickness 0.25 µm, Agilent Technologies Wilmington, DE, USA). The oven temperature was programmed from 50 - 250°C at a rate of 5°C/min and pressure at 16.0 kPa. The ion source was set at 200°C with ionization voltage of 70 Ev and interface temperature of 250°C. Helium was used as the carrier gas. Spectra were analyzed using Hewlett-Packard Enhanced Chem Station G1701 programme for windows.

The components of the extract and its fractions were identified by matching their spectra and retention indices with those of Wiley 275 library (Wiley, New York) in the computer library and literature (Shibamoto, 1987). Percentage composition was calculated using the summation of the peak areas of the total extract composition.

**Microorganisms**

The bacterial cultures used in this study consisted of four Gram-positive viz. *Staphylococcus aureus* (ATCC6538), *Bacillus pumilis* (ATCC14884), these were reference isolates. *Staphylococcus aereus* (OK2a), *Staphylococcus aereus* (OK2b), clinical isolates from KwaZulu Natal Province in South Africa and *Streptococcus faecalis* (laboratory isolate) and eight Gram-negative strains *Escherichia coli* (ATCC8739), *Shigella sonnei* (ATCC29930), *Klebsiella pneumoniae* (ATCC13047), these were reference isolates; *Shigella flexneri* (KZN),

a clinical isolate; *Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella typhi* and *Salmonella typhimurium* (all laboratory isolates).

Four species of fungi (*Candida rugosa, Candida neoformans, Candida albicans* and *Trichophyton mucoides*) were used for antimycotic investigation.

Each organism was maintained on nutrient broth and was recovered for testing by subculturing in nutrient broth for 24 h (fig 9).

Figure 9. Microorganisms inside freshly prepared nutrient broth (Mojau, 2014)

Before use, each bacterial culture was diluted 1:100 with fresh sterile nutrient broth (Afolayan and Meyer, 1997). Using 96-well microplates, 100 µl of sterile water was added into every well. Microplates were labelled appropriately. 100 µl of extract was added into well A. Mixing was repeated with 2-fold serial dilution. Then 100 µl was taken from well A to well B, until well H where 100 µl from well H (final well) was discarded. Appropriate control was

prepared using 100 µl of water, dilute bacteria and no extract was added. After inoculation, the microplates were incubated at 37°C for 24 h. Each treatment was performed in duplicates, and complete inhibition of bacterial growth was required for an extract to be declared bioactive. This concentration was regarded as minimum inhibitory concentration (MIC)

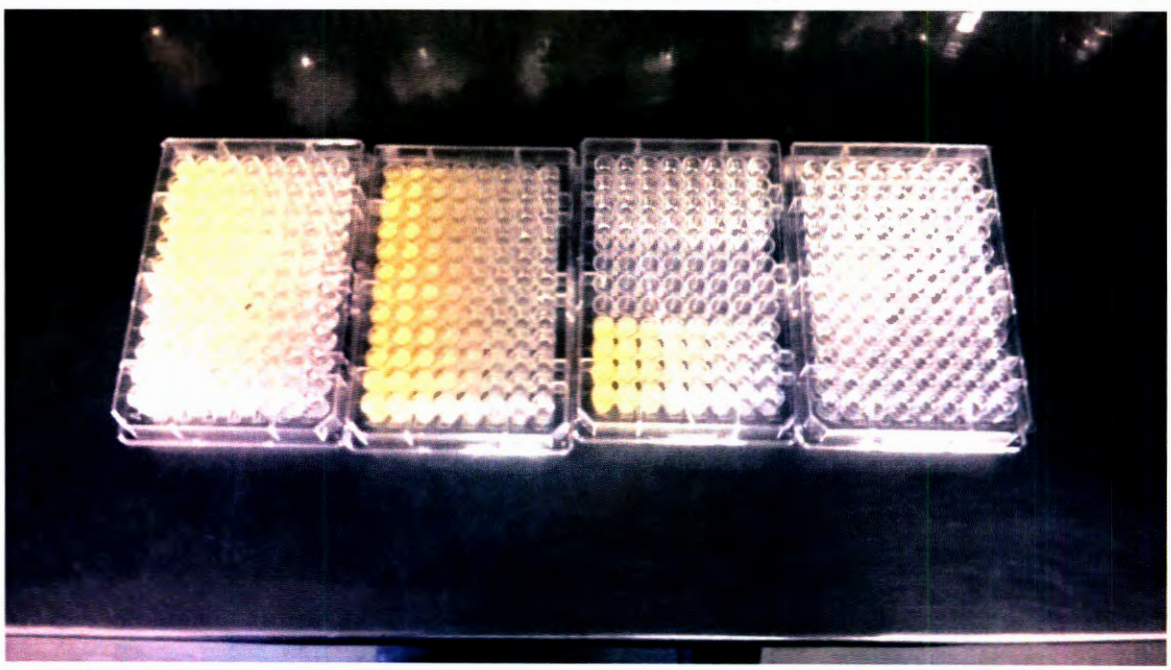


Figure 10. Microplates containing *H. geniculata* acetone extract and bacterial cultures (Mojau, 2014)

After 24 h of incubation of the microplates, MIC was detected following addition of (40 µl) of 0.2 mg/ml iodonitrotetrazolium chloride (INT, Sigma-Aldrich, USA) in all wells and incubated at 37°C for 30 minutes. Microbial growth were determined by observing the change of colour of iodonitrotetrazolium chloride (INT) in the microplate wells reduced to pinkish-red indicating that the microorganisms are active and a clear solution in the well indicates inhibition of growth by the extract.

# CHAPTER THREE

# RESULTS

## Minimum Inhibitory Concentration (MIC) against Bacterial Strains

MIC values of the extracts were determined as the lowest concentration that completely inhibited bacterial growth after 24 h of incubation at 37°C. MIC values of methanol, ethanol, acetone, water and hydro-ethanol extracts from the roots of *Hermannia geniculata* against tested bacteria are presented in table1. The acetone extract inhibited growth of bacteria at MIC values ranging between 1.56 to 6.25 mg/ml. The ethanol extract inhibited bacterial growth at MIC values ranging between 0.38 to 3.13 mg/ml. Ethanol extract showed more inhibition of Gram-negative strains. Hydro-ethanol and methanol extracts showed moderate activity at concentrations between 1.56 and 3.13 mg/ml, while there was a moderated activity shown by water extract at high concentration of 6.25 mg/ml. The acetone extract showed the highest consistent activity at the low concentration of 1.56 against all bacterial strain except the Gram-negative *Salmonella typhimurium* which was inhibited at the concentration of 6.25 mg/ml.

## Minimum Inhibitory Concentration (MIC) against Fungal Strains

Minimal inhibitory concentration (MIC) values of methanol, ethanol, acetone, water and hydro-ethanol extracts from the roots of *Hermannia geniculata* against tested fungal strains are presented in table1. The acetone extract showed the highest antimycotic activity at lowest concentrations of 1.56 mg/ml for *C. rugosa, C. albicans* and *T. mucoides*. While the ethanol extract showed the highest antimycotic activity at lowest concentrations of 1.56 mg/ml for *C. rugosa, C. Neoformans* and *T. Mucoides*. Hydro-ethanol extract showed antimycotic activity against *C. rugosa* at concentration of 3.13 mg/ml and all other fungal strains were inhibited at

1.56 mg/ml concentration. Methanol and water extract showed inhibition of fungal activity at concentrations of 3.13 and 6.25 mg/ml respectively

Table1. Antimicrobial activity of *Hermannia geniculata* root extracts showing minimum inhibitory concentration.

| | | | Extract (mg\ML$^{-1}$) | | | | |
|---|---|---|---|---|---|---|---|
| **Organism** | **Ref. no** | **G +\-** | **Acet** | **EtoH** | **EtOH+ $H_2O$** | **MeOH** | **Water** |
| **Bacterial strains** | | | | | | | |
| *E. coli* | ATCC 8739 | - | 1.56 | 3.13 | 3.13 | 3.13 | 6.25 |
| *K. pneumoniae* | | - | 3.13 | 0.78 | 3.13 | 3.13 | 6.25 |
| *K. pneumoniae* | ATCC 13047 | - | 3.13 | 0.38 | 3.13 | 3.13 | 6.25 |
| *P. aeruginosa* | | - | 1.56 | 0.78 | 3.13 | 3.13 | 6.25 |
| *S faecalis* | | + | 1.56 | 1.56 | 3.13 | 3.13 | 6.25 |
| *S. aereus* | OK2a | + | 1.56 | 3.13 | 3.13 | 3.13 | 6.25 |
| *S. aereus* | OK2b | + | 1.56 | 3.13 | 3.13 | 3.13 | 6.25 |
| *S. aureus* | ATCC 6538 | + | 1.56 | 3.13 | 3.13 | 3.13 | 6.25 |
| *S. typhi* | | - | 1.56 | 1.56 | 3.13 | 3.13 | 6.25 |
| *S. flexneri* | KZN | - | 1.56 | 3.13 | 3.13 | 3.13 | 6.25 |
| *S. sonnei* | ATCC 29930 | - | 1.56 | 3.13 | 3.13 | 3.13 | 6.25 |
| *S. typhimurium* | | - | 6.25 | 0.78 | 3.13 | 3.13 | 6.25 |
| **Fungal strains** | | | | | | | |
| *C. albicans* | | | 1.56 | 6.25 | 1.56 | 3.13 | 6.25 |
| *C. neoformans* | | | 6.25 | 1.56 | 1.56 | 3.13 | 6.25 |
| *C. rugosa* | | | 156 | 1.56 | 3.13 | 3.13 | 6.25 |
| *T. mucoides* | | | 156 | 1.56 | 1.56 | 3.13 | 6.25 |

Key: G = Gram reaction, Acet = Acetone, EtOH = Ethanol, MeOH = Methanol

**Minimum Bactericidal Concentration (MBC) against Bacterial Strains**

The MBC values of methanol, ethanol, acetone, water and hydro-ethanol extracts from the roots of *Hermannia geniculata* against tested bacteria are given in Table 2. After 48 h of incubation at 37°C, the MBC was determined as the concentration that exhibited no growth of bacterial strains. The lowest MBC (1.56 mg/ml) was obtained from acetone extract against Gram-negative strains of *E. coli; S. Sonnei* (both reference isolates) and Gram-positive *S. Aereus* (clinical isolate from KZN). However, most of the bacterial strains exhibited growth after 48 h at concentration levels ranging from 1.56 – 3.13 mg/ml except for *S. typhi* at 6.25 mg/ml. The MBC of ethanol extract after 48 h was at 3.13mg/ml against all bacterial strains except *S. flexneri* at 6.25 mg/ml. Hydro-ethanol and methanol extracts MBC concentrations ranged from 3.13 – 12.50 mg/ml. Water extract recorded MBC concentration of 12.50 mg/ml. These results show that for hydro-ethanol, methanol and water extracts, the microorganisms were only bacteriostatic and not completely dead.

Table 2: Antimicrobial activity of *Hermannia geniculata* root extracts showing minimum bactericidal concentration

| | | | Extract (mg\ML$^{-1}$) | | | | |
|---|---|---|---|---|---|---|---|
| **Organisms** | **Ref.no** | **G+\-** | **Acet** | **EtoH** | **EtOH + $H_2O$** | **MeOH** | **Water** |
| **Bacterial strains** | | | | | | | |
| *E. coli* | ATCC 8739 | - | 1.56 | 3.13 | 3.13 | 6.25 | 12.50 |
| *K. pneumoniae* | | - | 3.13 | 3.13 | 12.50 | 12.50 | 12.50 |
| *K. pneumoniae* | ATCC 13047 | - | 3.13 | 3.13 | 12.50 | 12.50 | 12.50 |
| *P. aeruginosa* | | - | 3.13 | 3.13 | 3.13 | 6.25 | 12.50 |
| *S faecalis* | | + | 3.13 | 3.13 | 6.25 | 6.25 | 12.50 |
| *S. aereus* | OK2a | + | 1.56 | 3.13 | 3.13 | 12.50 | 12.50 |
| *S. aereus* | OK2b | + | 3.13 | 3.13 | 3.13 | 12.50 | 12.50 |
| *S. aureus* | ATCC 6538 | + | 3.13 | 3.13 | 3.13 | 12.50 | 12.50 |
| *S. typhi* | | - | 6.25 | 3.13 | 3.13 | 12.50 | 12.50 |
| *S. flexneri* | KZN | - | 3.13 | 6.25 | 12.50 | 12.50 | 12.50 |
| *S. sonnei* | ATCC 29930 | - | 1.56 | 3.13 | 3.13 | 12.50 | 12.50 |
| *S. typhimurium* | | - | 6.25 | 3.13 | 12.50 | 6.25 | 12.50 |
| **Fungal strains** | | | | | | | |
| *C. albicans* | | | 1.56 | 3.13 | 12.50 | 3.13 | 12.50 |
| *C. neoformans* | | | 12.5 | 3.13 | 6.25 | 3.13 | 12.50 |
| *C. rugosa* | | | 1.56 | 3.13 | 12.50 | 3.13 | 12.50 |
| *T. mucoides* | | | 1.56 | 3.13 | 6.25 | 3.13 | 12.50 |

Key: G = Gram reaction, Acet = Acetone, EtOH = Ethanol, MeOH = Methanol

**Minimum Bactericidal Concentration (MBC) against Fungal Strains**

The MBC values of methanol, ethanol, acetone, water and hydro-ethanol extracts from the roots of *Hermannia geniculata* against the tested fungi is given in Table 2. The lowest MBC (1.56 mg/ml) was obtained from acetone extract. The acetone extract exhibited no growth of *C. rugosa, C. albicans* and *T. Mucoides* at the lowest concentration of 1.56 mg/ml after 48 h, except for *C. neoformans* at the concentration of 12.50 mg/ml suggesting it was bacteriostatic after 24 h. Ethanol and methanol extracts had MBC concentration of 3.13 mg/ml for all fungal strains. Hydro-ethanol and water extracts concentrations ranged from 6.25-12.50 mg/ml.

**Antimicrobial activities of fractions from the acetone extract of *H. geniculata* against bacterial strains**

**Minimum inhibitory concentrations (MIC) of acetone extract fraetions against bacterial strains**

The antibacterial activity of acetone extract of *H. geniculata* (hexane, ethyl acetate, chloroform, dichloromethane, butanol and water) was carried out against 12 bacterial strains used in the study. The results showed that four of six fractions i.e. ethyl acetate, chloroform, dichloromethane and butanol had antibacterial activity against all bacterial at low concentrations (Table 3). Ethyl acetate had the highest antibacterial activity at concentration of 1.56 mg/ml for all bacterial strains. The chloroform fraction had concentration of 3.13 mg/ml against all bacterial strains while dichloromethane fraction had a concentration of 6.25 mg/ml against *P. aeruginosa*, *S, typhi* and *S. typhimurium*. Hexane fraction had the concentration of 6.25 mg/ml for most bacterial strains except for gram-negative *E. coli*, which showed no inhibition whereas *S. typhi* was inhibited at the highest concentration of 12.50 mg/ml. Water fraction had inhibition at the highest concentration of 12.50 mg/ml. Butanol fraction had the

lowest concentration of antibacterial activity at 0.78 mg/ml against *S. aureus* and *S. aereus* (clinical isolate). Table 3.

**Minimum Inhibitory Concentration (MIC) of acetone extract fractions against *fungal strains***

The results showed that four of six fractions i.e. ethyl acetate, chloroform, dichloromethane and butanol had antifungal activity against all fungi at low concentrations (Table 3). Ethyl acetate showed highest inhibition at the lowest concentration of 1.56 mg/ml against all fungal strains. The chloroform, dichloromethane and butanol fractions had concentration of 3.13 mg/ml, this might suggest that both might have extracted almost similar compounds as their polarities are close to each other in terms of specific gravity density. Butanol had highest antibacterial activity at the lowest concentration of 1.56 mg/ml against *C. neoformans*, but all other strains were at the concentration of 3.13 mg/ml. Water and hexane fractions had the concentration raging between 6.25 – 12.50 mg/ml.

Table 3: Antimicrobial activities of *Hermannia geniculata* acetone root extract fractions showing minimum inhibitory concentration

| | | | Extract (mg\ML$^{-1}$) | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Organism** | **Ref.no** | **G+\-** | **HEX** | **EA** | **CF** | **DCM** | **WATER** | **BL** |
| **Bacterial strains** | | | | | | | | |
| *E. coli* | ATCC 8739 | - | N\A | 1.56 | 3.13 | 3.13 | 6.25 | 1.56 |
| *K. pneumoniae* | | - | 6.25 | 1.56 | 3.13 | 3.13 | 6.25 | 3.13 |
| *K. pneumoniae* | ATCC 13047 | - | 6.25 | 1.56 | 3.13 | 3.13 | 6.25 | 3.13 |
| *P. aeruginosa* | | - | 6.25 | 1.56 | 3.13 | 6.25 | 6.25 | 3.13 |
| *S faecalis* | | + | 6.25 | 1.56 | 3.13 | 3.13 | 6.25 | 1.56 |
| *S. aereus* | OK2a | + | 6.25 | 1.56 | 3.13 | 3.13 | 6.25 | 1.56 |
| *S. aereus* | OK2b | + | 6.25 | 1.56 | 3.13 | 3.13 | 6.25 | 0.78 |
| *S. aureus* | ATCC 6538 | + | 6.25 | 1.56 | 3.13 | 3.13 | 6.25 | 0.78 |
| *S. typhi* | | - | 12.5 | 1.56 | 3.13 | 6.25 | 6.25 | 3.13 |
| *S. flexneri* | KZN | - | 6.25 | 1.56 | 3.13 | 3.13 | 6.25 | 1.56 |
| *S. sonnei* | ATCC 29930 | - | 6.25 | 1.56 | 3.13 | 3.13 | 6.25 | 1.56 |
| *S. typhimurium* | | - | 6.25 | 1.56 | 3.13 | 6.25 | 6.25 | 6.25 |
| **Fungal strains** | | | | | | | | |
| *C. albicans* | | | 6.25 | 1.56 | 3.13 | 3.13 | 6.25 | 3.13 |
| *C. neoformans* | | | 12.5 | 1.56 | 3.13 | 3.13 | 6.25 | 1.56 |
| *C. rugosa* | | | 12.5 | 1.56 | 3.13 | 3.13 | 6.25 | 3.13 |
| *T. mucoides* | | | 6.25 | 1.56 | 3.13 | 3.13 | 6.25 | 3.13 |

Key: G = Gram reaction, Hex = Hexane, E.A = Ethyl acetate, CF = Chloroform, DCM = Dichloromethane, BL = Butanol

**Minimum Bactericidal Concentration (MBC) of acetone extract fractions against bacterial strains**

After 48 h of inoculation, most of the fractions showed a noticeable growth in most of the bacterial strains. These implied that after 24 h of inoculation, the microorganisms were only bacteriostatic. Only the butanol fraction showed consistent level of antibacterial activity compared to all other fractions. Butanol fraction showed broad-based antimicrobial activity at relatively low concentrations but ethyl acetate showed highest activity for some bacterial strains. Dichloromethane showed no inhibition against all microorganisms, except for *S. sonnei* at the concentration of 6.25 mg/ml. Hexane showed some antibacterial activity against bacterial strain which varied from 6.25 - 12.50 mg/ml, *E. coli, K. Pneumoniae* and *S. typhi* were not inhibited. Chloroform fraction showed antibacterial activity at concentrations ranging between 3.13 - 12.50 mg/ml except for *P. aeruginosa, S. typhi* and *S. typhimurium* which were not inhibited. Water showed some activity for all microorganisms at the highest concentration of 12.50 mg/ml, except for *E. coli* which showed no inhibition.

**Minimum bactericidal concentration (MBC) of acetone fraction against *fungal strains***

The butanol fraction had the highest activity at the lowest level of 3.13 mg/ml for all the fungal strains. Ethyl acetate had the antifungal activity at the concentration of 6.25 mg/ml. Hexane and Chloroform concentrations ranging from 6.25 mg/ml to 12.50 mg/ml. Dichloromethane and water had a concentration of 12.50 mg/ml for all the fungal strains (Table 4).

Table 4: Antimicrobial activity of *Hermannia geniculata* root extracts - Partitioning Fractions for Acetone extract showing minimum bactericidal concentration

| | | | Extract (mg\ML$^{-1}$) | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Organism** | **Ref.no** | **G+\-** | **HEX** | **E A** | **CF** | **DCM** | **WATER** | **BL** |
| **Bacterial strains** | | | | | | | | |
| *E. coli* | ATCC 8739 | - | N\A | 3.13 | 12.5 | N\A | N\A | 3.13 |
| *K. pneumoniae* | ATCC 13047 | - | N\A | 1.56 | 6.25 | N\A | 12.5 | 3.13 |
| *K. pneumoniae* | | - | 6.25 | 1.56 | 3.13 | N\A | 12.5 | 3.13 |
| *P. aeruginosa* | | - | 12.5 | 1.56 | N\A | N\A | 12.5 | 3.13 |
| *S. aereus* | OK2a | + | 6.25 | 3.13 | 6.25 | N\A | 12.5 | 3.13 |
| *S. aereus* | OK2b | + | 6.25 | 1.56 | 6.25 | N\A | 12.5 | 3.13 |
| *S. faecalis* | | + | 12.5 | 1.56 | 6.25 | N\A | 12.5 | 3.13 |
| *S. flexneri* | KZN | - | 6.25 | 1.56 | 6.25 | N\A | 12.5 | 3.13 |
| *S. typhi* | | - | N\A | 1.56 | N\A | N\A | 12.5 | 3.13 |
| *S. typhimurium* | | - | 12.5 | 3.13 | N\A | N\A | 12.5 | 3.13 |
| *S. aureus* | ATCC 6538 | + | 12.5 | 1.56 | 6.25 | N\A | 12.5 | 3.13 |
| *S. sonnei* | ATCC 29930 | - | 6.25 | 3.13 | 6.25 | 12.5 | 12.5 | 3.13 |
| **Fungal strains** | | | | | | | | |
| *C. albicans* | | | 6.25 | 6.25 | 6.25 | 12.5 | 12.5 | 3.13 |
| *C. neoformans* | | | 12.5 | 6.25 | 12.5 | 12.5 | 12.5 | 3.13 |
| *C. rugosa* | | | 12.5 | 6.25 | 12.5 | 12.5 | 12.5 | 3.13 |
| *T. mucoides* | | | 12.5 | 6.25 | 6.25 | 12.5 | 12.5 | 3.13 |

Key: G = Gram reaction, Hex = Hexane, E.A = Ethyl acetate, CF = Chloroform, DCM = Dichloromethane, BL = Butanol

**Minimum inhibitory concentration (MIC) of ethyl acetate fraction of acetone fraction against bacterial strains**

Antibacterial activity of all phases of *H. geniculata* fraction partitioning of ethyl acetate with hexane, ethyl acetate, chloroform, butanol and water was carried out against 12 bacterial strains used in the study.

The results showed that three of five partition fractions i.e. ethyl acetate, chloroform, and butanol had antibacterial activity against different micro- organisms (Table 4). Ethyl acetate showed moderate level of antibacterial activity against most bacterial strains at the concentration of 5.00mg/ml, except for *S. typhi* and *S. typhimurium* where there was not inhibition of growth. *E. coli* and *S. aereus* were both inhibited at the highest concentration of 10.00mg/ml. Chloroform showed inhibition at the highest concentration of 10.00mg/ml for most microorganisms except for *K. pneumoniae* at the concentration of 5.00mg/ml. Clinical isolates, gram-positive *S. aereus (*OK2a) and *S. flexneri (*KZN) were not inhibited. Butanol fraction showed the highest antibacterial activity at the lowest concentration of 2.50 mg/ml for most microorganisms, except for gram-positive strain of *S. aereus* (ATCC 6538) and gram-negative strains of *K. Pneumoniae* (ATCC 13047), *S. typhimurium* and a clinical isolate of *S. flexneri (*KZN) at the concentration of 5.00mg/ml. Hexane and water did not inhibit any of the microorganisms. See Table 5.

**Minimum bactericidal concentration (MBC) of ethyl acetate fraction of acetone fraction against bacterial strains**

After 48 h of inoculation, most of the fractions have shown a noticeable growth against most bacterial strains. These implied that after 24 h of inoculation, the mieroorganisms were only bacteriostatic. Only the butanol fraction showed consistent level of antibacterial activity compared to all other fractions. Butanol had the highest antibacterial activity at the lowest concentration of 6.25 mg/ml. This was noticed for most microorganisms except for *E. coli* and *S. aureus* which were inhibited at 12.50 mg/ml. *S. typhimurium* strain was no longer inhibited, this implied that after 24hours of inoculation this microorganism was only bacteriostatic. Ethyl acetate showed inhibition of bacterial activity to most microorganisms; however, this was at the highest concentration of 12.50 mg/ml, except for clinical isolate *S. aereus* (OK2a), *K. pneumoniae, S. typhi* and *S. typhimurium* which were not inhibited. Chloroform fraction could not show most antibacterial activity after 48 h, this was evident as most of the bacterial strains were no longer inhibited, except for *E. coli, S. aureus, P. aeruginosa* and *S. typhimurium* which were inhibited at the concentration of 12.50 mg/ml. Table 6.

**Minimum inhibitory concentration (MIC) of ethyl acetate fraction of acetone fraction against fungal strains**

After 48h, butanol and chloroform showed consistent antifungal activity against microorganisms at the concentration of 3.13 mg/ml, except for *C. rugosa* which had the concentration of 6.25 mg/ml. Ethyl acetate had shown level of highest antifungal activity

against *T. mucoides* at the concentration of 3.13 mg/ml and all other microorganisms were inhibited at 6.25 mg/ml. See Table 5.

**Minimum bactericidal concentration (MBC) of ethyl acetate fraction of acetone fraction against fungal strains**

Antifungal activity of all phases was carried out against 4 fungal strains used in the study. After 48h, the results showed consistent antifungal inhibition of ethyl acetate and chloroform fraction at the concentration of 6.25 and 12.50mg/ml respectively. Hexane and water did not inhibit any of the fungal strains. Butanol inhibited all the fungal strains at the concentration of 6.25 mg/ml, except for *C. neoformans* at the concentration of 3.13 mg/ml. See Table 6.

Table 5: Partitioning Fractions of Ethyl Acetate fraction showing minimum inhibitory concentration

| | | | Extract (mg\ML$^{-1}$) | | | | |
|---|---|---|---|---|---|---|---|
| **organism** | **Ref.no** | **G+\-** | **HEX** | **E A** | **CF** | **WATER** | **BL** |
| **Bacterial strains** | | | | | | | |
| *E. coli* | ATCC 8739 | - | N/A | 12.5 | 12.50 | N/A | 3.13 |
| *K. pneumoniae* | | - | N/A | 6.25 | 6.25 | N/A | 6.25 |
| *K. pneumoniae* | ATCC 13047 | - | N/A | 6.25 | 12.50 | N/A | 3.13 |
| *P. aeruginosa* | | - | N/A | 6.25 | 12.50 | N/A | 3.13 |
| *S faecalis* | | + | N/A | 6.25 | 12.50 | N/A | 3.13 |
| *S. aereus* | OK2a | + | N/A | 6.25 | N/A | N/A | 3.13 |
| *S. aereus* | OK2b | + | N/A | 6.25 | 12.50 | N/A | 3.13 |
| *S. aureus* | ATCC 6538 | + | N/A | 12.5 | 12.50 | N/A | 6.25 |
| *S. typhi* | | - | N/A | N/A | 12.50 | N/A | 3.13 |
| *S. flexneri* | KZN | - | N/A | 6.25 | N/A | N/A | 6.25 |
| *S. sonnei* | ATCC 29930 | - | N/A | 6.25 | 12.50 | N/A | 3.13 |
| *S. typhimurium* | | - | N/A | N/A | 12.50 | N/A | 6.25 |
| **Fungal strains** | | | | | | | |
| *C. albicans* | | | N/A | 6.25 | 3.13 | N/A | 3.13 |
| *C. neoformans* | | | N/A | 6.25 | 3.13 | N/A | 3.13 |
| *C. rugosa* | | | N/A | 6.25 | 6.25 | N/A | 3.13 |
| *T. mucoides* | | | N/A | 3.13 | 3.13 | N/A | 3.13 |

Key: G = Gram reaction, Hex = Hexane, E.A = Ethyl acetate, CF = Chloroform, DCM = Dichloromethane, BL = Butanol

Table 6: Partitioning Fractions of Ethyl Acetate fraction showing minimum bactericidal concentration

| | | | Extract (mg\ML$^{-1}$) | | | | |
|---|---|---|---|---|---|---|---|
| **Organisms** | **Ref.no** | **G +\-** | **HEX** | **E A** | **CF** | **WATER** | **BL** |
| **Bacterial strains** | | | | | | | |
| *E. coli* | ATCC 8739 | - | N/A | 12.5 | 12.5 | N/A | 12.5 |
| *K. pneumoniae* | ATCC 13047 | - | N/A | N/A | N/A | N/A | 6.25 |
| *K. pneumoniae* | | - | N/A | 12.5 | N/A | N/A | 6.25 |
| *P. aeruginosa* | | - | N/A | 12.5 | 12.5 | N/A | 6.25 |
| *S. aereus* | OK2a | + | N/A | N/A | N/A | N/A | 6.25 |
| *S. aereus* | OK2b | + | N/A | 12.5 | N/A | N/A | 6.25 |
| *S. aureus* | ATCC 6538 | + | N/A | 12.5 | 12.5 | N/A | 12.5 |
| *S. faecalis* | | + | N/A | 12.5 | N/A | N/A | 6.25 |
| *S. flexneri* | KZN | - | N/A | 12.5 | N/A | N/A | 6.25 |
| *S. sonnei* | ATCC 29930 | - | N/A | 12.5 | N/A | N/A | 6.25 |
| *S. typhi* | | - | N/A | N/A | N/A | N/A | 6.25 |
| *S. typhimurium* | | - | N/A | N/A | 12.5 | N/A | N\A |
| **Fungal strains** | | | | | | | |
| *C. albicans* | | | N/A | 6.25 | 12.5 | N/A | 6.25 |
| *C. neoformans* | | | N/A | 6.25 | 12.5 | N/A | 3.13 |
| *C. rugosa* | | | N/A | 6.25 | 12.5 | N/A | 6.25 |
| *T .mucoides* | | | N/A | 6.25 | 12.5 | N/A | 6.25 |

Key: G = Gram reaction, Hex = Hexane, E.A = Ethyl acetate, CF = Chloroform, DCM = Dichloromethane, BL = Butanol

**Minimum inhibitory concentration (MIC) of ethanol extract against bacterial strains**

After 24 h of incubation, the ethanol extract had MIC values that demonstrated the highest antimicrobial activity against the gram negative *E. coli* (ATCC 8739) at the lowest concentration of 0.78 mg/ml. All other microorganisms had MIC values of 3.13 mg/ml, except for *S. sonnei* which was not inhibited (Table 7).

**Minimum inhibitory concentration (MIC) of ethanol extract against fungal strains**

The ethanol extract showed antimicrobial activity against tested fungi in which *C. neoformans* and *T. mucoides* were inhibited at the concentration of 3.13 mg/ml while C. *albicans* and *C. rugosa* were inhibited at lower concentration level of 6.25 mg/ml.

**Minimum bactericidal concentration (MBC) of ethanol extract against bacterial strains**

After 48 h of incubation, *K. pneumoniae* showed inhibition with the MBC value of 6.5 mg/ml. *E. coli* which had MIC value of 0.78 mg/ml had shown inhibition levels of 12.5 mg/ml (Table 8).

**Minimum bactericidal concentration (MBC) of ethanol extract against fungal strains**

Only *C. neoformans* was inhibited at the MBC value of 6.25 mg/ml, other fungal strains were no longer inhibited after 48 h.

**Minimum inhibitory concentration (MIC) of ethanol fractions against bacterial strains**

The results are depicted in Table 7. Ethyl acetate recorded the highest antibacterial activity against *S. typhi*, at the lowest concentration of 0.19 mg/ml followed by chloroform at 0.78 mg/ml. Chloroform had the highest overall inhibition against all bacterial strains at the MIC value of 0.78 mg/ml. Butanol showed antibacterial activity with MIC values ranging between 1.56-3.13 mg/ml. Hexane was not active against *E. coli* and *S. sonnei* , which were both inhibited by Ethyl acetate and chloroform at the lowest concentration of 0.78 mg/ml. Water extract showed moderate activity against *E. coli* at the concentration of 6.25 mg/ml

**Minimum inhibitory concentration (MIC) of ethanol fractions against fungal strains**

Ethyl acetate fraction exhibited the highest antifungal activity with the lowest MIC values. Both *C. neoformans* and *T. mucoides* were inhibited at the concentration of 0.78 mg/ml. Chloroform and butanol fractions showed inhibition of growth against all fungal strains at the concentration of 3.13 mg/ml. Water fraction showed the lowest activity at the highest concentration of 12.5 mg/ml.

**Minimum bactericidal concentration (MBC) of ethanol fraction against bacterial strains**

The results showed that no microorganism was inhibited by hexane fraction after 48 h. Ethyl acetate inhibited *S. sonnei* at the concentration of 1.56 mg/ml, whereas chloroform showed inhibition at 3.13 mg/ml. Other solvents and water fraction had MBC values of 12.5 mg/ml. Dichloromethane and water fractions had the lowest antibacterial activity at the highest concentration of 12.5 mg/ml.

**Minimum bactericidal concentration (MBC) of ethanol fraction against fungal strains**

*C. albicans* and *C. neoformans* were inhibited by ethyl acetate at the concentration of 1.56 mg/ml while other fungal strains were inhibited at MBC value of 3.13mg/ml. Butanol and chloroform had antifungal activity against all tested microorganisms at the concentration of 6.25 mg/ml (Table 8).

Table 7: Antimicrobial activity of *Hermannia geniculata* ethanol root extracts and its fractions showing minimum inhibitory concentration

| | | | Extract (mg\ML$^{-1}$) | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Organism** | **Ref.no** | **G+\-** | **EtOH** | **HEX** | **E A** | **CF** | **DCM** | **WATER** | **BL** |
| **Bacterial strain** | | | | | | | | | |
| *E. coli* | ATCC 8739 | - | 0.78 | N/A | 0.78 | 0.78 | 3.13 | 6.25 | 1.56 |
| *K. pneumoniae* | ATCC 13047 | - | 3.13 | 3.13 | 0.78 | 0.78 | 12.5 | 12.5 | 3.13 |
| *K. pneumoniae* | | - | 3.13 | 3.13 | 1.56 | 0.78 | 12.5 | 12.5 | 3.13 |
| *P. aeruginosa* | | - | 3.13 | 12.5 | 1.56 | 0.78 | 12.5 | 12.5 | 3.13 |
| *S. typhi* | | - | 3.13 | N/A | 0.19 | 0.78 | 6.25 | 12.5 | 3.13 |
| *S. typhimurium* | | - | 3.13 | 12.5 | 3.13 | 0.78 | 12.5 | 12.5 | 3.13 |
| *S. flexneri* | KZN | - | 3.13 | 12.5 | 1.56 | 0.78 | 12.5 | 12.5 | 1.56 |
| *S. sonnei* | ATCC 29930 | - | N/A | N/A | 0.78 | 0.78 | 3.13 | 12.5 | 1.56 |
| *S. aereus* | OK2a | + | 3.13 | 12.5 | 0.38 | 0.78 | 12.5 | 12.5 | 1.56 |
| *S. aereus* | OK2b | + | 3.13 | 1.56 | 0.78 | 0.78 | 12.5 | 12.5 | 3.13 |
| *S. aureus* | ATCC 6538 | + | 3.13 | 12.5 | 1.56 | 0.78 | 6.25 | 12.5 | 1.56 |
| *S. faecalis* | | + | 3.13 | 12.5 | 1.56 | 0.78 | 12.5 | 12.5 | 3.13 |
| **Fungal strains** | | | | | | | | | |
| C. *albicans* | | | 6.25 | 3.13 | 0.19 | 3.13 | 6.25 | 12.5 | 3.13 |
| *C. neoformans* | | | 3.13 | 12.5 | 0.78 | 3.13 | 6.25 | 12.5 | 3.13 |
| *C. rugosa* | | | 6.25 | 12.5 | 1.56 | 3.13 | 12.5 | 12.5 | 3.13 |
| *T. mucoides* | | | 3.13 | 12.5 | 0.78 | 3.13 | 12.5 | 12.5 | 3.13 |

Key: G = Gram reaction, Hex = Hexane, E.A = Ethyl acetate, CF = Chloroform, DCM = Dichloromethane, BL = Butanol

Table 8: Antimicrobial activity of *Hermannia geniculata* ethanol root extract and its fractions showing minimum bactericidal concentration

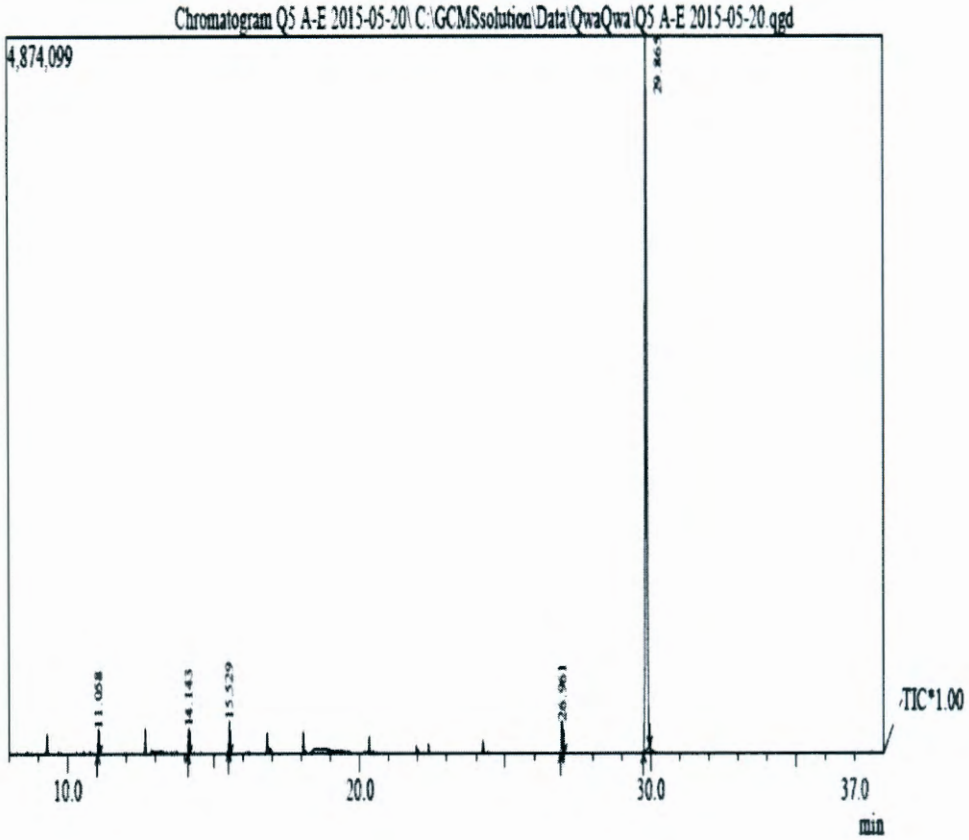
| | | | Extract (mg\ML$^{-1}$) | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Organism** | **Ref.no** | **G +\-** | **EtOH** | **HEX** | **E A** | **CF** | **DCM** | **WATER** | **BL** |
| **Bacterial strains** | | | | | | | | | |
| *E. coli* | ATCC 8739 | - | 12.5 | N/A | 3.13 | 0.78 | 12.5 | 12.5 | 12.5 |
| *K. pneumoniae* | ATCC 13047 | - | N/A | N/A | 3.13 | 3.13 | 12.5 | 12.5 | 12.5 |
| *K. pneumoniae* | | - | 6.25 | N/A | 3.13 | 3.13 | 12.5 | 12.5 | 12.5 |
| *P. aeruginosa* | | - | N/A | N/A | 3.13 | 12.5 | 12.5 | 12.5 | 1.56 |
| *S. typhi* | | - | N/A | N/A | 6.25 | 6.25 | 12.5 | 12.5 | 6.25 |
| *S. typhimurium* | | - | N/A | N/A | 12.5 | 0.78 | 12.5 | 12.5 | 6.25 |
| *S. flexneri* | KZN | - | N/A | N/A | 6.25 | 3.13 | 12.5 | 12.5 | 12.5 |
| *S. sonnei* | ATCC 29930 | - | N/A | N/A | 1.56 | 3.13 | 12.5 | 12.5 | 12.5 |
| *S. aereus* | OK2a | + | N/A | N/A | 3.13 | 3.13 | 12.5 | 12.5 | 12.5 |
| *S. aereus* | OK2b | + | N/A | N/A | 3.13 | 3.13 | 12.5 | 12.5 | 12.5 |
| *S. aureus* | ATCC 6538 | + | N/A | N/A | 3.13 | 0.78 | 12.5 | 12.5 | 12.5 |
| *S. faecalis* | | + | N/A | N/A | 6.25 | 3.13 | 12.5 | 12.5 | 12.5 |
| **Fungal strains** | | | | | | | | | |
| *C. albicans* | | | N/A | N/A | 1.56 | 6.25 | 12.5 | 12.5 | 6.25 |
| *C. neoformans* | | | 6.25 | N/A | 1.56 | 6.25 | 12.5 | 12.5 | 6.25 |
| *C. rugosa* | | | N/A | N/A | 3.13 | 6.25 | 12.5 | 12.5 | 6.25 |
| *T. mucoides* | | | N/A | N/A | 3.13 | 6.25 | 12.5 | 12.5 | 6.25 |

Legend: G = Grams, Hex = Hexane, E.A = Ethyl acetate, CF = Chloroform, DCM = Dichloromethane, BL = Butanol

**GCMS analysis for acetone extract and fractions**

Table 9: Acetone extract fraction compounds

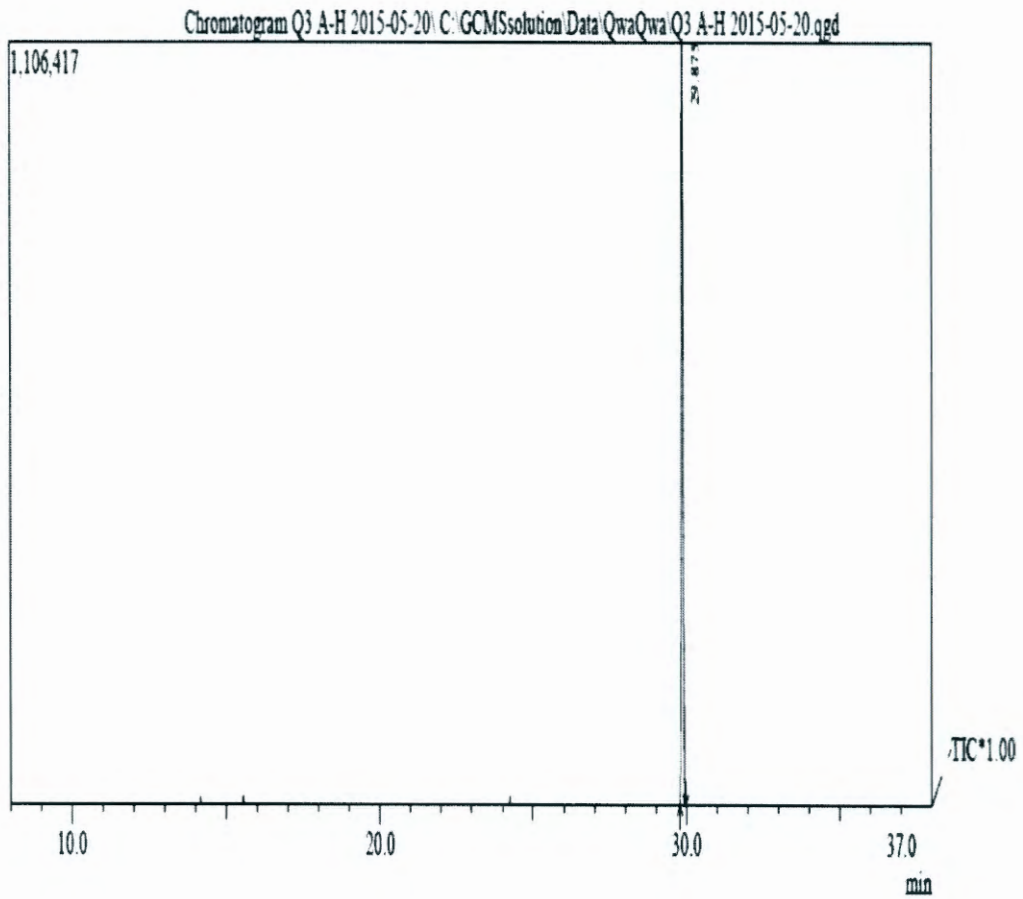
| Peak no # | Retention time | Area % | Compound name |
|---|---|---|---|
| 1. | 11.058 | 1.32 | Dodecane |
| 3. | 15.529 | 1.71+ 1.36 | Pentadecane |
| 4. | 26.961 | 3.41 | 4,6'-Biazulenyl |
| 5. | 29.865 | 92.20 | 1,2-Benzenedicarboxylic acid |




Chromatogram.1 Acetone extract compounds

Table 10: Acetone- Hexane fraction compounds

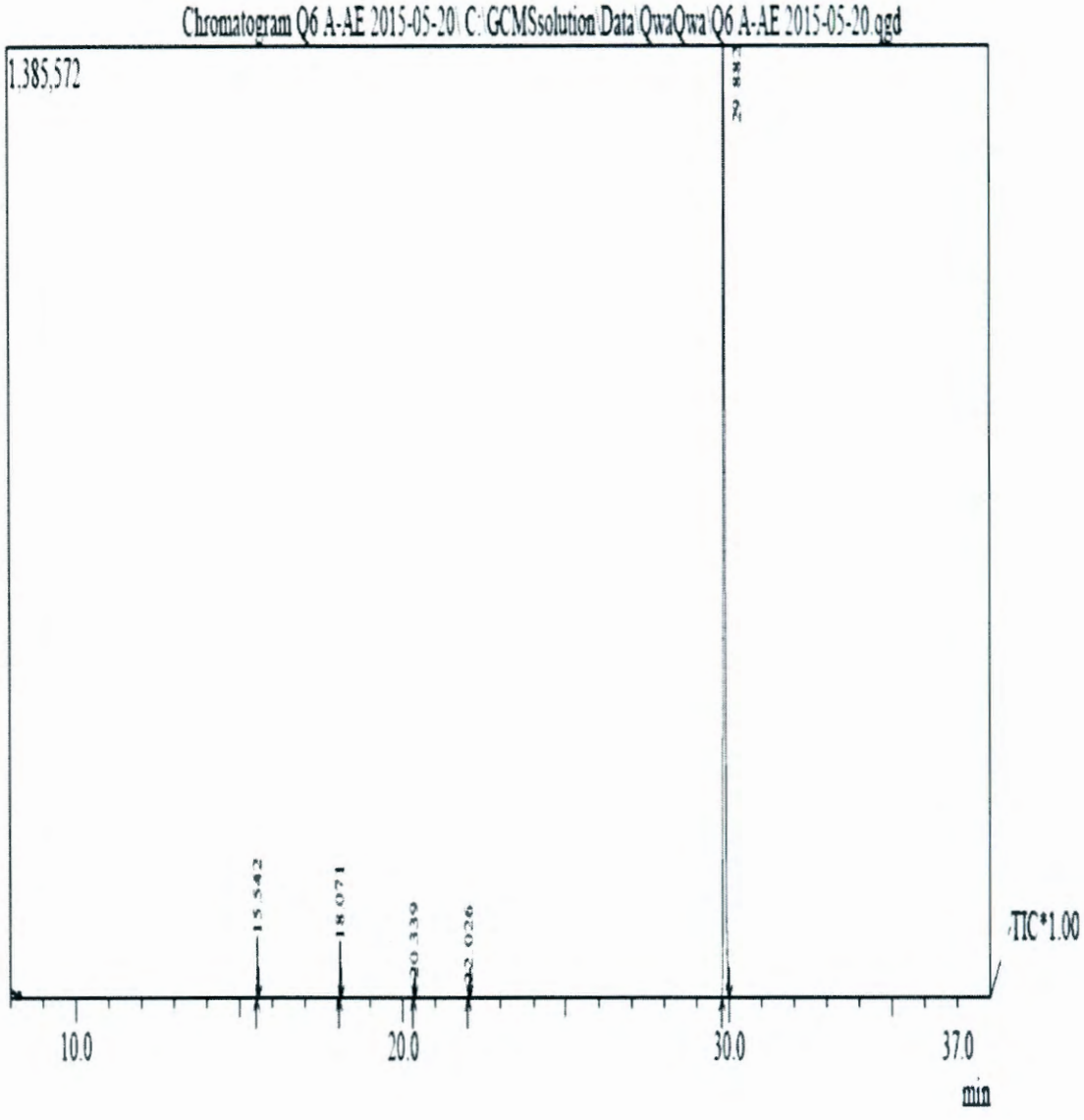
| Peak no # | Retention time | Area % | Compound name |
|---|---|---|---|
| 1. | 29.875 | 100.00 | 1,2-benzenedicarboxylic acid |




Chromatogram 2. Acetone – Hexane fraction

Table 11: Acetone extract - Ethyl acetate fraction

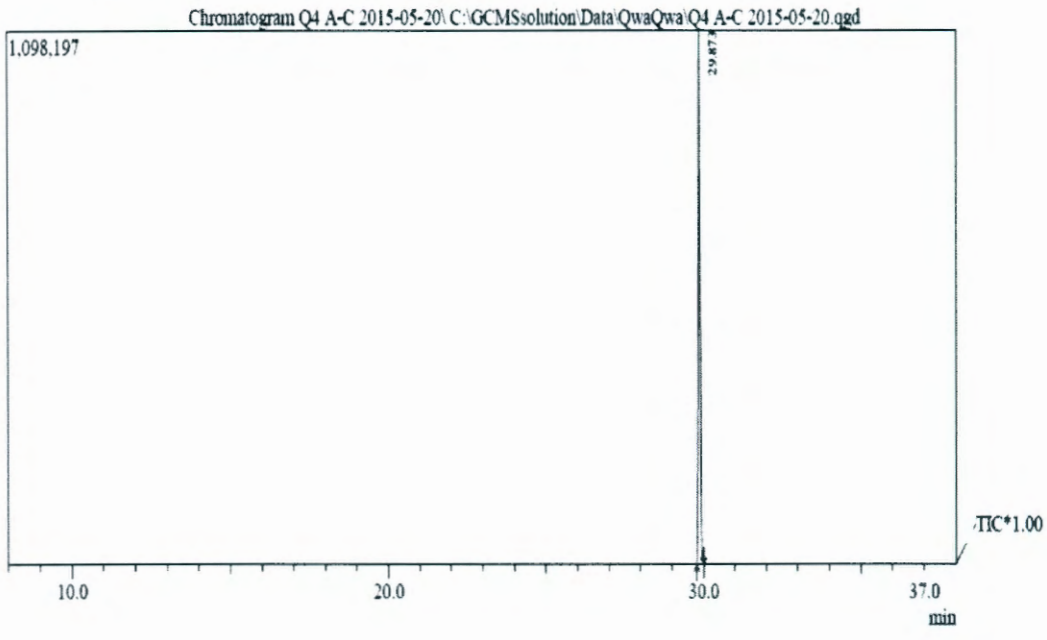
| Peak no # | Retention time | Area % | Compound name |
|---|---|---|---|
| 1 | 15.542 | 2.39 | (3r*,4s*)-3-ethyl-4-methyltetrahydrofuran-3-ol |
| 2 | 18.071 | 2.22 | pentadecane |
| 3 | 20.339 | 0.55 | 1-methylbutyl nitrite |
| 4 | 22.026 | 0.56 | 2-propanone |
| 5 | 29.882 | 94.28 | 1,2-benzenedicarboxylic acid |




Chromatogram 3. Acetone extract – Ethyl acetate fraction

Table 12: Acetone – Chloroform fraction compounds

| Peak no # | Retention time | Area % | Compound name |
|---|---|---|---|
| 1 | 29.873 | 100.00 | 1,2-benzenedicarboxylic acid |




Chromatogram 4. Acetone extract – Chloroform fraction

Table 13: Acetone – Dichloromethane fraction compounds

| Peak no # | Retention time | Area % | Compound name |
|---|---|---|---|
| 4 | 29.860 | 99.11 | 1,2-benzenedicarboxylic acid |

Chromatogram 5. Acetone- Dichloromethane

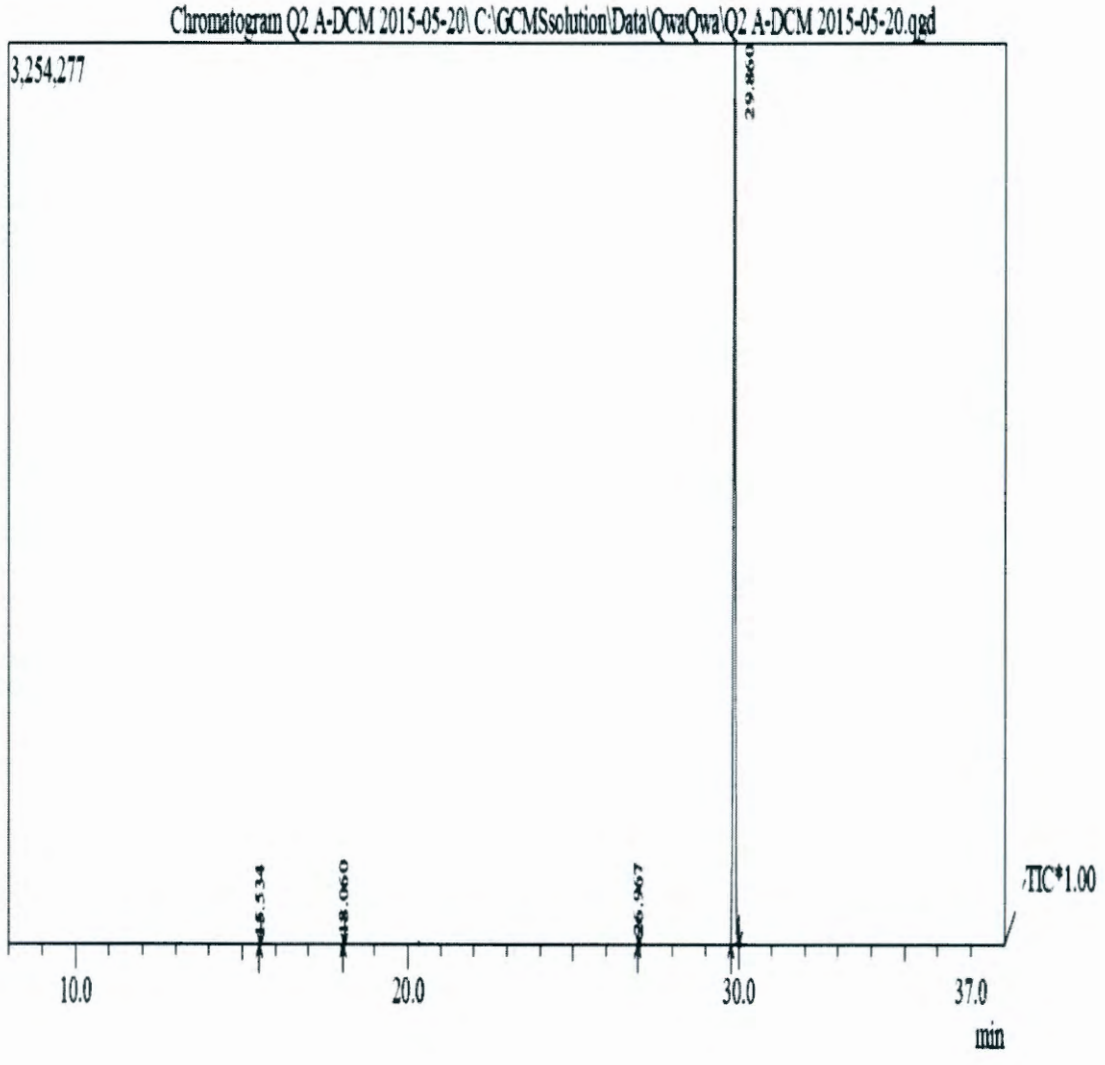
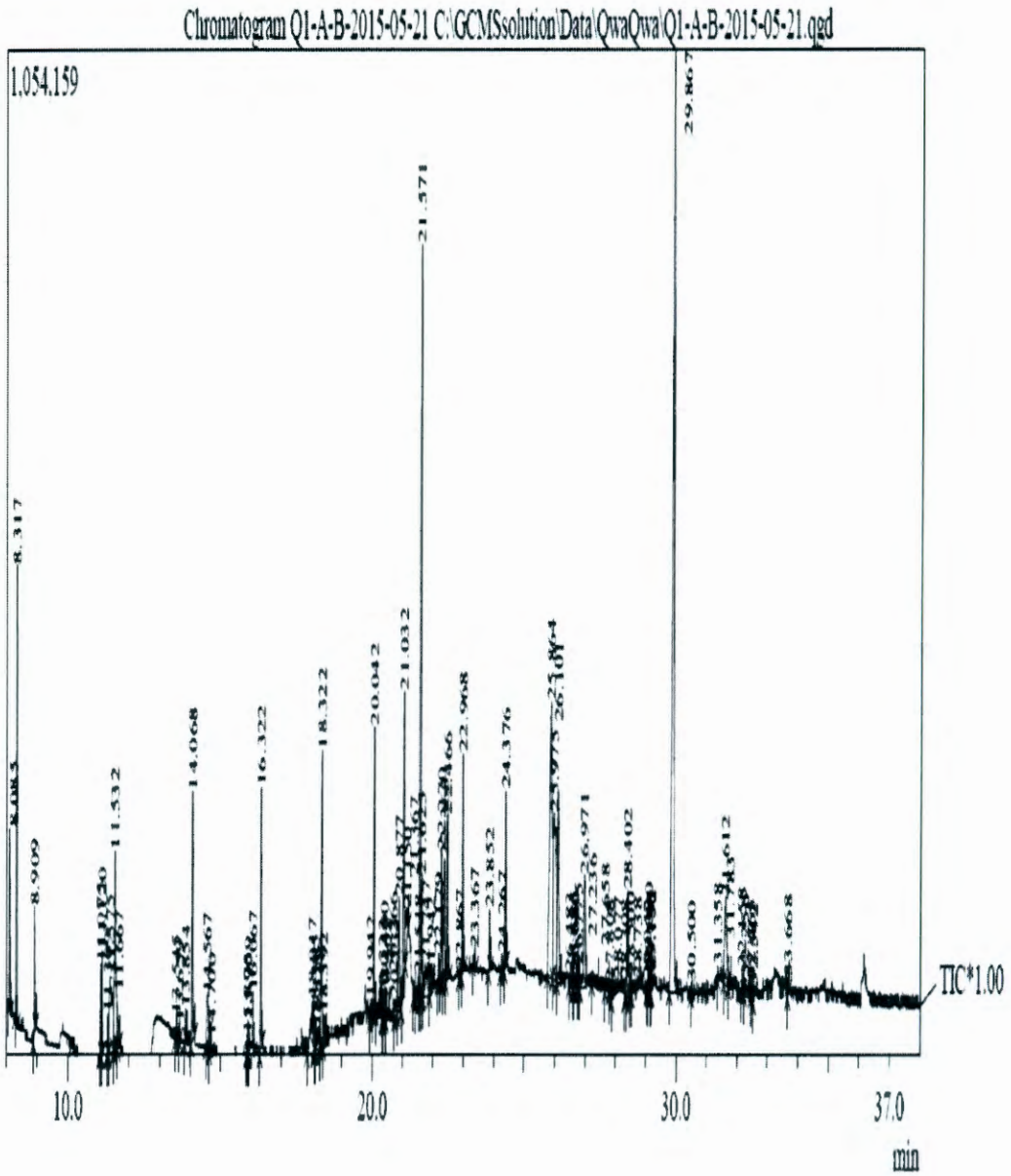



Table 14: Acetone – Butanol fraction compounds

| Peak no # | Retention time | Area % | Compound name |
|---|---|---|---|
| 1 | 8.085 | 1.35 | 5-isopropyl-5-phenyl-.gamma.butyrolactone |
| 2 | 8.317 | 3.17 | disiloxane |
| 3 | 8.909 | 1.01 | e / z -3-{3'-(3",3"-dimethyl-1"-butynyl) benzothiophen-2'-yl) -2-oxiranecarbonitrile |
| 4 | 11.075 | 0.58 | 1h-1,2,4-triazolium |
| 5 | 11.120 | 0.67 | stannan |
| 7 | 11.375 | 2.54 | 2-methyl-1,3 |
| 8 | 11.532 | 2.92 | cyclopentasiloxane |
| 13 | 14.068 | 2.34 | cyclohexasiloxane |
| 14 | 14.567 | 1.11 | 2,3-indandione |
| 18 | 16.067 | 0.85 | 1,4-bis[(trimethylsilyl)carbonyl]benzene |
| 19 | 16.322 | 2.13 | 3-butoxy-1,1,1,7,7,7-hexamethyl-3,5,5-tris(trimethylsiloxy)tetrasiloxane |
| 20 | 18.017 | 1.46 | 3,4-dihydro-4-(1,3-dioxolan-2-yl)-5,7-dimethoxy-1(2h)-benzopyran-2-one |

| | | | |
|---|---|---|---|
| 22 | 18.158 | 0.74 | d-ribitol, 1,4-anhydro-1-c-(2,6-diamino-3-pyridinyl)-2,3-o-(1-methylethylidene)-, (1s)- |
| 23 | 18.322 | 2.73 | benzoic acid, 2,5-bis(trimethylsiloxy)-, |
| 24 | 18.392 | 0.60 | (+-)-1-(acetoxy)-2-(1-bromoethyl)-3-methoxyanthraquinone |
| 26 | 20.042 | 2.03 | 1,1,1,5,7,7,7-heptamethyl-3,3-bis(trimethylsiloxy)tetrasiloxane |
| 32 | 20.877 | 1.48 | benzyl 4-hydroxybenzoate $$ benzyl p-hydroxybenzoate |
| 33 | 21.032 | 3.04 | 2h,6h-benzo[1,2-b:5,4-b']dipyran-6-one |
| 34 | 21.150 | 0.77 | 3-c-benzyloxymethyl-3-deoxy-1,2-o-isopropylidene-6-o-(4-methoxybenzyl)-.alpha.-d-ribo-hexofuranos-5-ulose |
| 37 | 21.571 | 7.44 | benzene |
| 38 | 21.625 | 1.09 | 1,2-bis(3'-methoxyphenyl)ethane |
| 39 | 21.747 | 1.31 | 5-methyl-1,3,2,4-dithiadiszolium chloride |
| 42 | 22.270 | 1.37 | benzene |

| | | | |
|---|---|---|---|
| 43 | 22.363 | 1.72 | 2-phenylthiazolo[5,4-e]-1,2,4-[3-t-butyl]triazine |
| 44 | 22.466 | 1.59 | 1-ethyl-2,4,6-triphenyl-3,5-bis(trimethylsiyl)benzene |
| 46 | 22.968 | 1.55 | benzoic acid, |
| 48 | 23.852 | 0.52 | 3,3',4,4'-tetrakis[(trimethylsilyl)ethynyl]-2,2'-bithiophene |
| 50 | 24.376 | 1.81 | 1h-purin-6-amine |
| 51 | 25.864 | 7.68 | quinoline-3-carbonitrile |
| 52 | 25.975 | 4.82 | quinoline-3-carbonitrile |
| 53 | 26.101 | 2.86 | eicosamethylcyclodecasiloxane |
| 58 | 26.971 | 2.27 | 1,2'-binaphthalene (cas) 1,2'-binaphthyl |
| 64 | 28.402 | 1.80 | eicosamethylcyclodecasiloxane |
| 71 | 29.867 | 18.28 | 1,2-benzenedicarboxylic acid, |
| 74 | 31.612 | 2.12 | hexadecamethylcyclooctasiloxane |




Chromatogram 6. Acetone extract – Butanol fraction

**Analysis of GCMS results**

GCMS identified a number of chemical compounds from acetone extract and its respective fractions. However, only compounds that constituted 0.5 or more area percentage were recorded.

For all the identified chemical compounds, there was a chemical compound that was identified for every extract, 1,2-Benzenedicarboxylic acid. For Acetone extract, this compound constituted area percentage of 92% of all the identified compounds after the retention time of 29.865 minutes. Hexane and Chloroform had the same chemical compound identified at the area percentage of 100% in the retention times of 29.875 and 29.873 minutes respectively. Dichloromethane had this compound identified at the retention time of 29.860 minutes with the area percentage of 99.11%. Ethyl acetate identified this compound in the retention time of 29.882 with the area percentage of 94.28. Butanol fraction which had many chemical compounds that had the area percentages of more than 0.5%, had the 1,2-Benezenedicarboxylic acid identified at the retention time of 29.867 minutes with the area percentage of 18.28%.

## CHAPTER 4. DISCUSSION, CONCLUSION AND RECOMMENDATIONS

Microorganisms are the concealed enemies to the mankind. They are small but cause a very profound damage in human body as well as other living organisms. The agents, which have capacity to kill microbes or minimize their multiplication, are called the antimicrobial agents or drugs. The last two decades have seen an increase in the investigations of plants as source of human disease management (Dash et al., 2011) and more natural antimicrobials have driven scientists to investigate the effectiveness of inhibitory compounds such as extracts from plants (Nasar-Abbas and Halkman, 2004)

The antibacterial potency of *H geniculata* against *Escherichia coli, Staphylococcus aureus, Shigella sonnei, Staphylococcus aereus,* and *Shigella flexneri* is noteworthy, because all these bacteria have been implicated as causative agents of diarrhoea. *Shigella flexneri* causes bacillary dysentery, an invasive human colonic mucosa (Perdomo et al., 1994). In recent years, widely used and inexpensive antimicrobials have been reported to be no longer effective against *Shigella* as it has become progressively resistant (Bennish et al., 1992). *Escherichia coli* which causes diarrhoea infection in both developing and developed countries accounts for high rates of mortality in newborn children (Radu et al., 2001). The antibacterial activities of *H. geniculata* particularly at low concentrations suggest that it could be useful for treatment of diarrhoea caused by enteropathogenic strains of *Escherichia coli.*

The leaf extract of *Sennia siamae* was shown to have antibacterial activity against *S. typhi* in which case the ethanol extract demonstrated the highest activity followed by acetone (Doughari and Okafor, 2008), the similar results was obtained in this study where *S. typhi* was inhibited by ethanol extract at the low concentration of 1.56 mg/ml. This high activity by the organic solvents may be attributed to the realisation that the phytoconstituents are more soluble in them than the aqueous solvents: particularly acetone which is known to be selective for tannins (Marjorie, 1999). This was also observed in this study regarding the water extract that had the inhibition at the concentration of 6.25 mg/ml against all microorganisms for MIC.

Acetone extract of Coriander produced zone of inhibition against *E. coli* and *Shigella dysentiriae*, but *S. typhi* was not sensitive to acetone extract (Dash et al., 2011), similarly in this study acetone extract had inhibited *E. coli*, *Shigella flexneri* KZN (a clinical isolate) and *Shigella sonnei* ATCC 29930. It was observed in this study that *S. typhi* was inhibited by the acetone extract *at* low concentration of 1.56 mg/ml.

The acetone and ethanol extracts of the toot and stem bark of *Cassia aereh* (Del.) showed the highest antimicrobial activity against *E. coli, K. pneumoniae, S. aureus* and *S. typhi* (De, et al., 2009). It is noteworthy that in similar studies acetone extract had higher antimicrobial activities; hence they were both partitioned in this study to further investigate their potency against microorganisms.

The results of this study revealed that acetone fractions showed significant antimicrobial activity as evidenced by consistent inhibition of microorganisms at low concentration compared to ethanol. Similar results were shown against *Verbena officinalis* plant in which acetone fraction had high zone of inhibition and lower MIC value (Mengiste et al., 2015).

It was therefore necessary in this study to identify the phytochemical properties of the Acetone extract and its fractions. The many compounds identified had a certain chemical present in the extract and all its fractions at higher concentrations 1,2-Benzenedicarboxylic acid, this suggests that this compound might be involved in some way for the activity of acetone extract in the inhibition of microorganisms. However, further studies need to be undertaken to isolate it and investigate the safe toxicity properties of it as it was reported to be toxic to animals.

The fields of ethnopharmacology and ethnobotany, is progressing steadily in South Africa. Ethnopharmacologists, botanists and microbiologists are searching the earth for phytochemicals which could be developed for the treatment of infectious diseases (Tanaka *et*

*al.*, 2006) especially in light of the emergence of drug-resistant microorganisms and the need to produce more effective agents against microorganisms.

It has been shown that *in vitro* screening methods could provide the needed fundamental observations necessary to select crude plant extracts with potentially useful properties for further chemical and pharmacological investigations (Mathekaga and Meyer, 1998).

Modern scientific evaluation of plants and herbs is mainly focused with validating the traditional use of plants and identifying the active components of extracts and preparations. As a result, continued examination of traditional plant medicines is required to establish the scientific basis for activity, efficacy and safety (Palombo, 2006).

In this study, it was shown that antimicrobial properties of *H. geniculata* cannot be overlooked. This was seen with the ability of *H. geniculata* to successfully inhibit the growth of microorganisms of human pathogenic importance with its different root extracts. Since this plant is used in the traditional Basotho medicine for different stomach ailments, the results of this study validates that this plant holds hope.

Different extracts of the plants were made and it was found that the water extracts did not perform well in this study. Literature supports this finding as reported by Meyer and Afolayan (1995) as well as Masika and Afolayan (2002), stating that the choice of acetone and methanol as solvents for extraction and the exclusion of water extracts in their analysis was based on their previous observation and other reports that water extracts of plants generally showed little or no antimicrobial activities

It was observed that acetone extract was able to inhibit both gram positive microorganisms at the lowest concentration of 1.56 mg\ml. However, partitioning of fractions

did not improve the inhibition of microorganisms' growth, where only butanol fraction had highest antimicrobial activity at the concentration of 3.13 mg\ml. This may imply that these compounds are responsible for antimicrobial activity present in this plants work in synergy.

However, the identification of the 1,2-Benzenedicarboxylic acid and its toxicity suggests that more research needs to be done on the safety and toxicity of the plant. Especially since there is no previous report on the safety or toxicological evaluation of this significant plant in literature.

It is thus concluded that *Hermannia geniculata* does possess antimicrobial properties for at least certain microbes and that this activity is largely dependent on the extract used. However, further studies are required in order to gain more clarity as to the specificity and biochemical mechanisms responsible for the antimicrobial properties of this plant. The possible loss of volatile components present in the extracts during drying and evaporation might have produced results different from those obtained in studies where other methods of extract drying were employed.

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