ANTIMICROBIAL ACTIVITIES OF MEDICINAL PLANTS USED BY INDIGENOUS PEOPLE OF MOGALAKWENA AND MOOKGOPONG MUNICIPALITIES IN THE WATERBERG DISTRICT LIMPOPO PROVINCE SOUTH AFRICA FOR THE TREATMENT OF FOOD-BORNE PATHOGENS

by

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Declarations:

I declare that “ANTIMICROBIAL ACTIVITIES OF MEDICINAL PLANTS USED BY INDIGENOUS PEOPLE OF MOGALAKWENA AND MOOKGOPONG MUNICIPALITIES IN THE WATERBERG DISTRICT LIMPOPO PROVINCE SOUTH AFRICA FOR THE TREATMENT OF FOOD-BORNE PATHOGENS” is my work and all resources used have been acknowledged by means of complete references.

Signed by: Agnes Maluleke

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Date: March 2023

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Dedication

This work is dedicated to my dear son, Ikenna Osizigbo, my partner Beneth Osizigbo and my family John Maluleke, Rebecca Maluleke, Thandi Maluleke, Catherine Maluleke and Kgothatso Baloyi. I would not have completed this work without their help and support.

Abstract

Food-borne diseases (FBD) are a main concern globally, around 250 different FBD having been described to date and bacteria contributing to two third of FBD outbreaks. Among the leading bacteria involved in FBD are *Salmonella typhi* and *Escherichia coli,* which are accountable for majority of global deaths of FBD. In Africa the condition is worse, about 70% of mortality is caused by food related bacterial diarrhoea. Symptoms of FBD vary widely, however diarrhoea and vomiting are the most common symptoms. It is estimated that 43 000 South Africans die of diarrhoeal disease every year, while private and public health care spend over R73 million per annum due to diarrhoea. However, in the rural communities of Southern Africa dependence on medicinal plants as remedies for diarrhoea and vomiting is still on the rise.

Almost 80% of the South African population use medicinal plants to meet their primary health care needs; however, few medicinal plants in South Africa have been scientifically tested. Medicinal plants can inhibit the growth of wide range of pathogenic microorganisms. About fifty percent (50%) of all drugs in clinical use in the world are derived from natural products. There are large number of plant species that are of medicinal use that have not been tested for antimicrobial activities. Some bacteria's have developed resistance against antibiotics currently in use, making it difficult to treat patients successfully. Medicinal plant extracts offer great potential in developing new drugs which might be effective in treating infections currently difficult to treat. The use of medicinal plants in dates back to over 4000 years ago, the knowledge acquired since then is through trial and errors. Every community in South Africa have different medicinal plants used to treat the same disease. Hence, it is important to work with the experienced traditional healers to acquire information on medicinal plants used to treat diarrhoeal pathogens.

A questionnaire was used to collect the data, the antimicrobial activities of the five species with the highest frequency were evaluated against *Salmonella typhi* and *Escherichia coli*. They were further subjected to phytochemical tests. The study revealed 19 plants species belonging to 12 families were used to treat diarrhoeal food borne pathogens in the study area. *Poulzozia mixta*, *Sclerocarya birrea*, *Psidium guajava*, *Ozoroa reticulata* and *Punica granatum* were the most mentioned plant species.

Dried ground plant materials were each extracted using distilled water and 70% ethanol. Distilled water extract had higher percentage yield over the ethanol extract. Ethanol extract showed higher antimicrobial activities over the aqueous extract. *Punica granatum* fruitpeels was the most effective medicinal plant against *Escherichia coli* on both aqueous and ethanol extract, when using agar disc diffusion method. However, it also exhibited antimicrobial activities against *Salmonella typhi*. During phytochemical test *Punica granatum* fruitpeels had positive results for saponins and tannins. Saponins are known to have anti-inflammatory, antiviral and antimicrobial activities. Pharmaceuticals use tannins as antidiarrheal, diuretics, stomach pain, anti-inflammatory, antiseptic and antioxidant. In their antidiarrheal activity, tannins are responsible for increasing colonic water and electrolyte reabsorption. The results obtained points to the value of untested medicinal plants which can have significant contribution to the pharmaceutical industry.

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$	Dollar
%	Percentage
ATCC	American Type Culture Collection
BSC	Biosafety Cabinet
C5	5 Carbon atoms
CO_2	Carbon dioxide
DALYs	Disability adjusted life years
DNA	Deoxyribonucleic Acid
E. coli	*Escherichia coli*
Ec	*Escherichia coli*
FBD	Food borne diseases
g	gram
G Force	Force of Gravity
GHGs	Greenhouse gases
H	hour
IUCN	International Union for Conservation of Nature
Kg	Kilograms
L	Litter
mbar	millibar
mg	milligram
MHa	Mueller Hinton agar

min	minutes
ml	millilitre
mm	millimetre
N. Sotho	Northern Sotho
O_3	Ozone
ºC	Degree Celsius
PH	Potential hydrogen
R	Rand
RCF	Relative Centrifugal Force
rpm	revolutions per minute
S. typhi	*Salmonella typhi*
SANBI	South African National Biodiversity Institute
UNICEF	United Nations Children's Fund
US	United State
UV	Ultra violet
WHO	World Health Organisations
µl	microliter

Chapter 1

1.1 Introduction

Few medicinal plants in South Africa have been scientifically tested; however, almost 80% of the South African population use “medicinal plants to meet their primary health care needs” (Street and Prinsloo, 2012). Medicinal plants can “inhibit the growth of wide-range of pathogenic microorganisms” (Akthar *et al.,* 2013). Medicinal plants are recognised as a rich source of antimicrobial agents. There are many medicinal plants that still need to be tested for antimicrobial activities (Mahesh and Satish, 2008). Antibiotics-resistant bacteria make it difficult to treat patients successfully world-wide (Wendakoon *et al.,* 2012). Medicinal plant extracts offer great potential in developing new drugs which might be effective in treating infections currently difficult to treat (Wendakoon *et al*., 2012). Medicinal plant parts used for the extraction of raw drugs includes; fruits, flowers, twigs, stem and roots (Mahesh and Satish, 2008).

Considering the potential of plants as a source of antimicrobial drugs, the local flora has to be screened for antimicrobial properties (Mahesh and Satish, 2008) against food-borne diseases (FBD). Food-borne diseases are a main concern globally, with around 250 different FBD having been described to date and bacteria contributing to two third of FBD outbreaks (Le Loir *et al*., 2003). Amongst the leading bacteria involved in FBD are *Salmonella typhi (S. typhi)* and *Escherichia coli (E. coli)* which are accountable for majority of global deaths. In Africa the condition is worse, about 70% of mortality is caused by food related bacterial diarrhoea (WHO, 2007 – 2015).

1.2 Background

Medicinal plants are broadly defined as all plants that have medicinal properties and which have been proven as useful drugs according to western standards, furthermore they are known to have the ability to alleviate or cure illnesses (Akerele *et al.,* 1991). According to the Holy Bible, plants have been in existence before mankind, the use of plants as medicine was first recorded over 5000 years ago (Lall, 2018). In the African continent the use of plants as medicine was first recorded 4000 years ago, it was the only medicinal assistance and remains dominant in certain areas at the estimated use

of 80% (Lall, 2018). The knowledge acquired since then is through trial and errors, which form the root in which modern medicine is birthed (Jamshidi-Kia *et al.,* 2018).

All cultures across the world have used plants as a source of medicine, with China and India being the two leading nations (Srivastava *et al.,* 1997). Medicinal plants are the most preferred treatment in Africa as it is easily accessible, affordable, culturally and spiritually acceptable by many and most traditional healers also offer counselling (Mahomoodally, 2013). According to Moyo *et al*., (2015) “80% of population in African continent depends on medicinal plants for their primary health care”. According to Williams *et al.,* (2013) in South Africa 72% of Black South Africans including those in urban areas use medicinal plants despite the availability and accessibility of western medicine. Limpopo Province is predominantly rural with high level of unemployment rate; it is with this reason that the communities are heavily depended on medicinal plants to treat different kinds of illnesses (Matotoka and Masoko, 2017). There are few health care facilities in Waterberg district that are not easily accessible by most communities living in Mogalakwena and Mookgopong municipalities, hence medicinal plants remain the only first aid they can depend on.

The increase in the use of medicinal plants is also due to the believe that plants are closer to nature and safe to use than western medicine, they are believed to be a cheaper method of treatment, easily accessible and they show less side effects than western medicines (Yimer *et al*., 2019 and Umair *et al.*, 2019). Medicinal plants can be used to treat or prevent the progression of diseases that are either microbial or parasitic infections (Jamshidi-Kia *et al.,* 2018 and Mustafa *et al.,* 2017). Medicinal plants are prepared for use in various ways such as powders, decoctions, infusions, or as poultice (Umair *et al.,* 2019). Medicinal plants have a significant contribution in the discovery of western medicines, there are many early medicines which are “derived from plants such as Aspirin (from willow bark), Digoxin (from Foxglove), Morphine (from Opium poppy), Quinine (from Cinchona skin) and Pilocarpine (from Maranham Jaborandi)” some of which are still in use to date (Mustafa *et al.,* 2017 and Jamshidi-Kia *et al.,* 2018).

The knowledge of medicinal plants still lies in the hands of traditional healers who are aging and dying. There is a loss of indigenous medicinal knowledge as the young generation expected to succeed them do not show interest as well as lack of

documentation (Kayombo *et al.,* 2013 and Moyo *et al*., 2015). Half of the medicine used in pharmaceutical industry is produced from natural products, 25% are vascular plants (van Wyk and Prinsloo, 2018). Medicinal properties of most plants are yet to be studied and researchers have increased interest in studying plants in a quest to search for new drugs (Tomlinson and Akerele, 1998). Medicinal plant extracts have the capability to stop the growth of a variety of pathogenic microorganisms (Akthar *et al*., 2013).

1.3 Problem statement

Globally, around 600 million people become sick every year from ingesting contaminated foods. Of these, approximately 400 000 die of whom 130 000 are under five years old children (WHO, 2007-2015). In Africa, diarrhoeal food-borne bacteria are responsible for about 70% deaths (WHO, 2007 – 2015). Since the discovery of antimicrobial drugs, the control of bacterial infection remained extraordinarily effective, but over time some pathogens have become resistant to many of the effective drugs (Moyo *et al.,* 2012).

According to Ventola (2015), the overuse and misuse of antibiotic drugs causes the bacteria to develop resistance on the once-effective medications. The development of drug resistant bacteria and unexpected side effects to certain antibiotics has encouraged the search for new antimicrobial plants extracts (Moyo *et al.,* 2012). Medicinal plants are a source of medicine used widely as an alternative healing tool to prevent and treat numerous illnesses (Kaur and Mondal, 2014).

The increase of wild medicinal plants use in many places has resulted in intensive harvesting and over exploitation; posing a severe threat to biodiversity and ecosystems (Alves and Rosa, 2007). There is a concern that many medicinal plants are on the edge of extinction. Plant samples collected today may in future be found to combat dread disease, but there is no guarantee that the plants will still exist (Srivastava *et al.,* 1996). The use of non-renewable plant parts such as bark as a source of medicinal plants trade can lead to the death of the plants. The effectiveness and safety of many medicinal plants haven't been scientifically tested and their use remains a concern (Ekor, 2014).

Correct identification of medicinal plants poses a serious challenge because similar plant species can be called by different names in different communities (Mamedov, 2012). Beliefs, myths and practices by indigenous people will determine access to the specimens required for study. Many traditional healers do not share their knowledge with anyone not approved by their ancestors. Some medicinal plants require rituals to be performed before harvest and the harvest of which can only be done by specific individuals.

The development of plant-derived drugs started with the development of chemistry, isolation, purification, and characterization of plant active compounds (Shakya, 2016). The choice of which plant species to use depends on the historical use by humans, it is assumed to be safe compared to the ones which were never used by humans (Katiyar *et al.,* 2012), however this might be a limitation in discovering new drugs. The cost of introducing new drugs is between US $ 300 million and $1000 million and there are challenges of intellectual property rights as the information might be from indigenous people. This makes it difficult for the pharmaceutical industries to take the risk in developing new drugs but rather manufacture what they know (Saha and Bhattacharya, 2011). Other challenges are: if there is a small population of the plant species involved, then supply becomes a concern; there is also the threat of extinction by deforestation and land degradation in general as well as global warming (Krause and Tobin, 2013)

1.4 Justification of the study and its benefits.

In many developing countries, medicinal plants have not been studied scientifically and documented. The skills and knowledge is still in the hands of traditional healers which is either lost or passed on to the next generation by word of mouth. The purpose of this research was to study and document medicinal plants used by indigenous people in Mogalakwena and Mookgopong municipalities in treating bacterial food-borne diseases, to analyse the antimicrobial activities of the five most commonly used plant species and also conduct phytochemical analysis of the selected species.

Indigenous people have acquired a vast knowledge over decades on the use of medicinal plants endemic to the area in which they live. It is however very unfortunate

that due to socioeconomic stress as well as the lure of the city life, majority of people leave the rural areas at a young age to the city in pursuit of a better life. This poses a threat to medicinal plants, because the information is not handed over to the young generation. It is foreseen that medicinal plants knowledge of indigenous people is in danger of becoming extinct, because traditional healers pass on the knowledge from one generation to another orally (Mahwasane *et al.,* 2013).

Most medicinal plants used by indigenous people have proven to be very effective in treating different kinds of illnesses (Mahomoodally, 2013). It is very important that scientists put more effort in researching, testing and documenting this rare knowledge for future generations. Medicinal plants are the only hope to cure some of the diseases believed not to have cure. The conservation of this scarce knowledge and the important role it plays in sustaining life, will in turn eradicate poverty and support development in the communities.

1.5 Aims and objectives

The main aim of this research was:

To identify and verify five medicinal plants which are commonly known to be effective in treating diarrhoeal food-borne pathogens (*S. typhi* and *E. coli* O157:H7) by indigenous people of Mogalakwena and Mookgopong municipalities in Limpopo Province South Africa. To investigate antimicrobial activities and analyse phytochemicals of plant specimen found to be effective. Preserve and conserve the knowledge of indigenous people on medicinal plants use for future generation.

Objectives of the research were:

- To collect five medicinal plant species commonly used by indigenous people in Mogalakwena and Mookgopong municipalities against diarrhoeal food-borne pathogens.
- To screen them for antimicrobial activities.
- To isolate and characterize chemical components which are responsible for antimicrobial activity.

1.6 Research questions

The research questions are outlined below:

- Are the commonly used medicinal plants effective in treating diarrhoeal food-borne bacteria?
- What are the other medicinal plants used to treat diarrhoeal food-borne diseases?
- Do the commonly used medicinal plants contain the phytochemicals responsible for antidiarrheal and antimicrobial activities against food borne pathogens?

Chapter 2: Literature review

2.1 Introduction.

Worldwide there are 500 000 plant species identified (Corlett, 2016), of which 77 000 plants are used in healthcare (Rajeswara *et al.*, 2012). It is estimated that 700 million kilograms(kg) of plants are used yearly to the value of almost 4-billion-rand (Street and Prinsloo, 2013). There are over 3 000 medicinal plants traded in markets world-wide, around 900 are cultivated in different countries and particularly in developing countries (Rajeswara *et al.,* 2012). It was estimated by International Union for Conservation of Nature (IUCN) that 70% of the world plants species are threatened with extinction, 15 000 of which are medicinal plants (Rajeswara *et al.*, 2012).

In 2009, South Africa completed the IUCN Red List assessments of 20 456 indigenous vascular plant taxa (Raimondo, 2011). Approximately 2 062 indigenous plant species were recorded for traditional medicine use in South Africa, of which 82 species are threatened with extinction and 100 species are of conservation concern (Williams *et al*., 2013). South Africa has an estimated 200 000 traditional healers compared to the 25 000 medical doctors and they use about 3000 plants for traditional healing (Hübsch *et al.*, 2014). There are two categories of traditional healers, diviner and herbalists (Mahwasane *et al.*, 2013). “The diviner uses bones and the spirits of the ancestors to diagnose and prescribe medicine for different physiological, psychiatric and spiritual conditions” (Mokgobi, 2014). “Herbalist is a person who grows, sells, collects, or specializes in the use of herbs, especially medicinal plants” (Hoffman, 2003).

South Africa is known to have one of the richest plant biodiversity in the world which is decreasing at a shocking rate, and there are over half a million people in the country who are directly involved in the trade of medicinal plants (Xego *et al.,* 2016). The local trade in South African medicinal plants is estimated at 20 000 tons a year and amounting to R270 million, involving 574 species (Xego *et al.,* 2016). Medicinal plant parts used includes; fruits, flowers, twigs, stem, bark and roots (Mahesh and Satish, 2008). A large number of medicinal plants used are harvested from the wild and it is estimated that 80% of the wild plants from which the material is harvested will die due

to harvesting practices (Diederrichs, 2006). A limited number of South African medicinal plants have been scientifically studied; researching and exploiting the chemical treasures contained in the floral kingdom can contribute to growing the economy and job creation in South Africa (Street and Prinsloo, 2012).

The increasing use of wild medicinal plants in many places resulted in intensive harvesting and over exploitation posing a serious threat to biodiversity (Street and Prinsloo, 2012). To maintain wild medicinal plants species, the only option for many species is cultivation at large scale (Chen *et al.,* 2016).

2.2 Over-harvesting and poor harvesting techniques.

Most of the medicinal plants used are harvested from the communal land (Semenya and Maroyi, 2019). Communal land is a land that is offered to the local community to sustain their livestock, to gather firewood, access medicinal plants and to meet other socio economic needs. There is no strict management of the available resources, which leads to over exploitation and degradation of the resources (Benin and Pender, 2002). Due to poor management of communal lands, over-harvesting, habitat destruction and harvesting methods are a major concerns leading to medicinal plant scarcity, at times extinction of some species (Xego *et al.,* 2016).

The increased demand for medicinal plants is met by destructive harvesting practices which involve unselective collection of species, ring-barking of stem bark, frequent collection and collection of roots by uprooting whole species amongst the others (Semenya and Maroyi, 2019). The consumption of medicinal plants by the local community is very minimal; however, commercialization to meet worldwide increased interest on medicinal plants particularly by pharmaceutical companies has caused high demand in African plant species predominantly in South Africa due to its species richness (Louw *et al.,* 2002). The South African medicinal plant parts used range from whole plant, bulb, roots, bark, fruits and seeds which have long shelf life; however, other countries in Africa predominantly use leaves the harvest of which is unlikely to have adverse effect on the plant (Grace *et al.,* 2002).

51% of medicinal plants sold in traditional medicine markets in South Africa are exclusively tree bark which is not harvested in a sustainable manner, many trees suffer from ring barking and eventually die from it (Geldenhuys and Williams, 2005). Some herbalists preferably use underground parts such as bulbs, tubers and roots, because they believe the underground parts have high healing properties and they can be stored for longer period for later use (Louw *et al.,* 2002). Many believe that medicinal plants have less harmful effects than modern drugs leading to increased demand which are met by overharvesting resulting in many plant species becoming threatened, vulnerable, rare, endangered and even extinct (Pandey and Savita, 2017). The general guidelines of medicinal plants method of collection, harvest times and sustainable harvesting are listed in Table 2.1. Following the outlined guideline can save medicinal plants from extinction.

Table 2. 1: Guidelines of medicinal plants method of collection, harvest times and sustainable harvesting (Pandey and Savita, 2017).

Plant parts	Harvest time and method of collection
Bulbs	Harvesting should be in late autumn, after the plant has flowered and bear fruits; bulbs needs to be dug from a significant distance from the main plant. This allows old matured bulbs to be collected and young small bulbs are left to redevelop.
Bark	The best time to collect bark is from autumn after leaf fall until before leaves develop in spring. Bark needs to be removed in a vertical long strips using flexible thin knife. Bark should not be collected from the same tree until the tree recover fully. Frequent collection leads to ring barking (cutting a bark around the whole tree forming a closed circle) and ultimately the tree die.
Root and rhizomes	Harvesting of annual plants just before flowering. Biennials, after first year growth during autumn or winter. Perennials, after two or three years of growth during autumn or winter. Avoid cutting the tap root, dig the root at least 30cm from the main stem. Only collect lateral roots, not all roots.

Leaves	Leaves should be harvested in dry weather, when plant is flowering. Leaves should be plucked individually, avoid leaf striping and using sharp equipment's. The best time to collect is early morning, it offers high quality product in some plants (solanaceous leaves). The ideal time to harvest leaves is before or at the beginning of plant flowering.
Flowers	Ideally flowers are to be collected during dry weather and in early hours of the day after morning moisture clears out. Care needs to be taken when harvesting the flowers, so that main stem is not damaged. Harvest flowers carefully without damaging plant main stem. When harvesting is done once the flower have just opened or shortly afterwards, the aroma of the flower is captured.
Seeds and fruits	Seeds collection is ought to be done when fruits are fully grown and ripe, preferably before they overripe to avoid seed dispersal. Fruits in the forest areas should be collected in some trees and others should be left to regenerate. No cutting of shrubs or tree branches for ease collection of fruits and seeds.
Annual herbs/ whole plant	Ideally harvesting of annual herbs should be done at the start of flowering. Never harvest whole population in a specific area. Enough population should be left to regenerate to facilitate for future collections.

Over harvesting of medicinal plants causes habitat destruction, as endemic species are cleared new species takeover. Habitat destruction greatly affects the plant community structure, composition and function (Botha *et al.,* 2004).

2.3 The effects of habitat destruction on medicinal plants.

In addition to over harvesting, habitat destruction is also caused by overgrazing, fire, agricultural purpose, firewood extraction, construction or human settlement (Zenebe *et al.,* 2012 and Gafna *et al.*, 2017).

Fire can be used as a management tool in different vegetation types. Fire affects medicinal plants in different ways, fire tolerant species will thrive after burning and fire intolerant species will not survive veld fire thereby reducing availability of the medicinal plants (Gafna *et al.*, 2017). After veld fire a lot of dead trees are used as firewood. Fire also opens up the thicket, making some areas accessible for firewood collection. There are still poor rural communities in South Africa without electricity supply, they mainly use firewood in their household and also use it to generate income. Firewood collection may affect the availability of medicinal plants, some of the plants species which are commonly used as firewood and they also have medicinal value, the collection of firewood decreases the availability for medicinal purposes (Gafna *et al.,* 2017). Some woody species have multiple uses such as *Catha edulis* (Bushman's tea) and *Rapanea melanophloeos* (Cape beech) they are both used for medicinal purpose (to treat respiratory diseases), used as firewood and to make furniture (Botha *et al.*, 2004). Global warming also has a significant effect on the decline of plants, it can also cause extinction of important medicine plants species (Grabherr, 2009).

2.4 The effects of global warming and climate change on medicinal plants.

The term "global warming and climate change" are often used interchangeably. According to Das *et al.,* (2016) "Climate change refers to any significant change in measures of climate such as temperature, precipitation, or wind over an extended period of time (decades or longer). Global warming refers to an increase in the temperature of the atmosphere that can contribute to change in global climate patterns". Climate change particularly global warming can cause extinction of plants species which are important to local people relying on them for traditional medicine (Grabherr, 2009).

Science already proved that the value of medicinal plants resides in their secondary metabolites which are produced during stressful conditions, while competing with other plants species (Luseba *et al.*, 2011). According to Mishra (2016), there are various environmental factors that affects plant growth and secondary metabolites such as “temperature, humidity, light intensity, water, minerals and carbon dioxide”. Mishra (2016) evaluates the impact of climate change on medicinal plants in Table 2.2.

Table 2. 2: The impact of climate change on medicinal plants is assed under the following topics (Mishra, 2016):

Phenological changes	The progression of global warming will have an effect on the arrival of spring and the growing season length.
Shifting Ranges	Climate change may cause endemic species to become extinct throughout the world due to challenges related to migratory and habitat loss.
Effect of elevated CO_2 on productivity and quality of medicinal plants	Increased CO_2 levels will ultimately increase the weight of fresh leaf, roots and shoots.
Effect of elevated ozone levels	The production of plants’ secondary chemicals may be altered by the changes in O_3 concentrations.
Effect of ultraviolet radiation	Ultraviolet radiation can cause molecular and cellular damage on proteins, DNA and other biopolymers. It can also affect plant growth and development resulting in changes in vegetative or reproductive biomass, height, leaf characteristics, and flowering time.
Climate warming vs. secondary metabolite production	Generally, the increase in volatile organic compounds is detected, however more studies are required.

Effect on threats to medicinal plants species	If the species that co-exist no longer occur in the same space at the same time, they both may be driven to extinction. Medicinal plants and other fragile plant communities may be eliminated and replaced by pests, diseases and invasive alien species. Plant species that require specific habitat requirements and with long growth periods are more likely to be threatened with extinction.
Adaptation measures for climate change and global warming	Putting measures in place to conserve endangered fauna and flora will reduce the future vulnerabilities of medicinal plants to climate change. These can be done by cultivation of medicinal plants. The key of medicinal plants adaptation in a natural environment depends on maintaining their genetic diversity. By documenting and promoting the traditional knowledge of local people can help with the adaptation of medicinal plants to climate change impacts. In turn involving the local communities in ecotourism by promoting the traditional art and craft while improving their livelihood and income generation.
Mitigation measures to reduce emission of CO_2/GHGs	The mitigation actions comprise of conservation of resources, organic farming, mulching, etc. Perennial medicinal plants such as trees and shrubs have potential valuable mitigation through carbon restoration.

As plants are faced with various threats, cultivation offers a promising future for medicinal plants.

2.5 Cultivation, a promising future of medicinal plants.

South Africa has numerous initiatives in attempt to develop propagation and sustainable production methodologies for continuous sustenance of medicinal plants. However, funding and coordination efforts compete for limited resources with housing, sanitation, education, health care and crime prevention (van Wyk and Prinsloo, 2018). Considering the demand for medicinal plants; cultivation, changing harvesting practice and protection against the existing threats could be the important strategy for conserving medicinal plants (Prakash *et al.*, 2015). There can be lots of benefits in commercialization of plants through cultivation; it can generate food, medicine and income to the community (Akankwasah *et al.*, 2012).

Most importantly, cultivation should be done in such a way that it prevents environmental degradation and loss of genetic diversity (Prakash *et al.*, 2015). There are few species cultivated and some other species are not cultivated, there are few concerns to be addressed for cultivation to be successful. It is believed that cultivated medicinal plants are qualitatively inferior when compared with wild gathered specimens (Schippmann *et al.,* 2002). For example, in China the similarity in appearance of the shape of wild ginseng roots to the human body symbolizes the strength and potency of the root, the ginseng that lacks this shape is unpopular (Nair *et al.*, 2012).

Many believe that active ingredients in cultivated plants are lower due to fast growth rate compared to slow growing wild plants (Hishe *et al.,* 2016). It is assumed that cultivated plants are likely to be different in their properties from those gathered from their natural habitats; however, certain values in plants can be enhanced under controlled conditions of cultivation (Schippmann *et al.*, 2002). Cultivation of medicinal plants can improve the local people's economic condition and reduce pressure on the wild population (Phondani *et al.*, 2011).

Despite all challenges observed and possible solutions to secure the future of medicinal plants there is still a lot of work to be done. Considering the potential of plants as a source of antimicrobial drugs, the local flora has to be screened for antimicrobial properties (Mahesh and Satish, 2008) against ailments such as food-borne diseases (FBD).

2.6 Food-borne diseases.

Food-borne diseases (FBD) are a major concern world-wide. The term FBD is normally used to indicate gastrointestinal complications that happen after recent ingestion of specific food or drinks (Dharma *et al.*, 2013). Food-borne diseases can be caused by bacteria, viruses, parasites, toxins and chemicals. It is estimated that 600 million people become sick every year from ingesting contaminated food, 420 000 people die annually world-wide including 125 000 children under the age of five (WHO, 2007-2015). The largest health impact is in Africa, which loses up to 1,300 disability-adjusted life years (DALYs) per 100,000 people, followed by Asia at 710 DALYs per 100,000 people. The lowest is Canada, Cuba and the United States which lose approximately 35 DALYs per 100,000 people (WHO, 2007-2015).

Food-borne diseases are a significant global concern and around 250 different FBD have been described to date with bacteria contributing to two thirds of FBD outbreaks (Le Loir *et al*., 2003). The predominant bacteria involved in food-borne diseases are *Staphylococcus aureus, Clostridium perfringens, Salmonella typhi, Bacillus cereus, Campylobacter* spp., *Listeria monocytogenes* and *Escherichia coli* (Dharma *et al.,* 2013). Amongst the leading bacteria involved in FBD are *Salmonella typhi* and *Escherichia coli*, which account for the majority of global deaths from FBD. In Africa, the condition is worse, where about 70% of mortality is caused by food-related bacterial diarrhoea (WHO, 2007 – 2015). Symptoms vary widely; however, diarrhoea and vomiting are the most common symptoms (Le Loir *et al.*, 2003).

Food-borne illnesses are often associated with diarrhoea and vomiting. Diarrhoea is defined as having watery stools at least three times per day. Diarrhoeal cause of death is dehydration as a result of loss of electrolytes in the stools (Appidi *et al.*, 2008). "Vomiting is described as a forceful expulsion of the contents of the stomach via the mouth or sometimes the nose, also known of as emesis" (UNICEF/WHO 2009). In the rural communities of Southern Africa dependence on medicinal plants as remedies for diarrhoea and vomiting is still on the rise (van Vuuren *et al.*, 2015).

The study focused on bacterial diarrhoea associated with *Escherichia coli* O157:H7 (Ali *et al.,* 2014) and *Salmonella typhi*.

2.6.1. *Salmonella typhi.*

Salmonella typhi (*S. typhi)* is a Gram-negative, motile, non-spore forming rod and the intestinal tract of birds and other animals are the initial source of the bacterium (Dharma *et al.*, 2013). People are infected by *S. typhi* by ingesting contaminated foods such as beef products, poultry, eggs, egg products and water. The main source of infection for *Salmonella* is from people working in food-processing plants, restaurants and canning processes (Willey *et al.*, 2008). The symptoms include abdominal pain, cramps, diarrhoea, nausea, vomiting and fever, which usually occur within 12 to 72 hours and can continue for several more days (Dharma *et al.*, 2013).

2.6.2*. Escherichia coli* O157:H7.

Escherichia coli (E. coli) is a Gram-negative, facultative anaerobic, rod-shaped bacterium and is commonly found in the digestive tract of humans and animals (Dharma *et al.*, 2013). There are many strains of *E. coli* and most of them are harmless, but some can cause bloody diarrhoea, urinary tract infections, severe anaemia or kidney failure, which can lead to death (Dharma *et al.*, 2013). Infection with *E. coli* may follow touching and ingesting infected faeces or water either from people or animals. *E. coli* can also infect meat during processing (Willey *et al.*, 2008).

To prevent infection, contaminated meat needs to be cooked above 71°C. *E. coli* can also be found on unpasteurized dairy products, raw fruits and vegetables, and unpasteurized juices (Willey *et al.*, 2008). People may become carriers if they do not disinfect their hands properly after using the toilet and can spread the bacteria especially those working in food processing industries (Willey *et al.*, 2008). One can also be infected with *E. coli* by touching the hands of infected person and not disinfecting your hands properly thereafter.

2.7 Medicinal plants commonly used to treat diarrhoeal food-borne bacteria.

It is estimated that 43 000 South Africans die of diarrhoeal disease every year, while private and public health care spend over R73 million per annum due to diarrhoea (Appidi *et al.*, 2008). “Majority of the communities in the rural areas of Africa rely on medicinal plants for basic health care (Mongalo and Makhafola, 2018)”. Different cultural groups use different medicinal plants to cure the same disease. Table 2.3 contains a list of some of the medicinal plants used in different parts of South Africa to treat diarrhoeal pathogens and vomiting. There is a need to collect more data on medicinal plants used, as many pathogens causing diarrhoea have developed resistance against antibiotics used (Madikizela, 2012).

Table 2. 3: Medicinal plants used in South Africa to treat diarrhoeal pathogens and vomiting.

Scientific name	**Family**	**Growth form**	**Parts used**	**Preparation**
Sclerocarya birrea	Anacardiaceae	Tree	Bark	Bark decoctions administered orally.
Psidium guajava	Myrtaceae	Shrub	Leaves, roots, bark	Bark, roots and leaves decoctions administered orally.
Elephantorrhiza elephantina	Fabaceae	Underground tree	Roots	Roots decoctions administered orally.
Prunus persica	Rosaceae	Tree	Leaves	Leaves decoctions

				administered orally.
Ziziphus mucronata	Rhamnaceae	Tree	Roots, leaves, bark, fruits	Whole plant decoctions administered orally.
Mangifera indica	Anacardiaceae	Tree	Bark, leaves	Bark and leaves decoctions administered orally.
Pelargonium luridum	Geraniaceae	Herb	Roots, leaves	Roots and leaves decoctions administered orally.
Schotia brachypetala	Fabaceae	Tree	Bark, roots	Bark and roots decoctions administered orally.
Ximenia caffra	Olacaceae	Tree	Roots, leaves	Roots and leaves decoctions administered orally.
Terminalia sericea	Combretaceae	Tree	Bark, roots, leaves	Bark, roots and leaves decoctions administered orally.

Strychnos spinosa	Loganiaceae	Tree	Bark	Bark decoctions administered orally.
Peltophorum africanum	Fabaceae	Tree	Leaves and roots	Roots and leaves decoctions administered orally.
Grewia flava	Malvaceae	Shrub	Roots	Roots decoctions administered orally.
Punica granatum	Lythraceae	Shrub	Roots	Roots decoctions administered orally.
Cassampelos capensis	Menispermaceae	Shrub	Roots, leaves	Roots and leaves decoctions administered orally
Bulbine asphodeloides	Asphodelaceae	Succulent herb	Tuber	Tuber decoctions administered orally
Vangueria infausta	Rubiaceae	Tree	Roots, leaves	Roots and leaves infusion administered orally

Vachellia karroo	Fabaceae	Tree	Bark	Bark decoctions administered orally
Waltheria indica	Malvaceae	Herb	Whole plant	Whole plant decoctions administered orally
Pappea capensis	Sapindaceae	Tree	Leaves, bark	Bark and leaves decoctions administered orally
Gymnosporia senegalensis	Celastraceae	Shrub	Roots, leaves	Roots and leaves decoctions administered orally
Lippia javanica	Verbenaceae	Shrub	Leaves, twigs	Leaves and twigs decoctions administered orally
Colophospermum mopane	Fabaceae	Tree	Bark	Bark decoctions administered orally
Dichrostachys cinerea	Fabaceae	Shrub	Leaves	Leaves decoctions

				administered orally
Gymnosporia buxifolia	Celastraceae	Shrub	Bark	Bark decoctions administered orally

Source: Olajuyigbe and Afolayan (2012), Madikizela (2012), Mongalo and Makhafola (2018).

Medicinal plants have phytochemicals known as secondary metabolites which also protect the plants against microbial infections or infestations by pests, these active ingredients pose therapeutic properties that are considered as medicine (Shakya, 2016).

2.8 Phytochemicals.

Phytochemicals are also defined as natural non-essential chemical compounds which are found in plants; they give plants a flavour, are also responsible for the colour and aroma of plants (Chukwuebuka and Chinenye, 2015). Phytochemicals protects the plants from harsh environmental conditions such as UV light, pollution, extreme temperatures, osmotic stress, extreme pH, drought, salinity, insufficient nutrients, dehydration, protects the plants from attack by microorganisms, defend plants from herbivores or invading insects or competitor plants, attract pollinators, natural predators and symbiotic organisms (Leonov *et al.,* 2015 and Saxena *et al.*, 2013).

Phytochemicals are not only produced by plants, but also non-pathogenic bacteria endophytes and fungi living in the plants (Leonov *et al.,* 2015). Scientists estimated that there are more than 10,000 different phytochemicals with the possibility to affect different kinds of diseases (Chukwuebuka and Chinenye, 2015). Phytochemicals “can be used to treat chronic as well as infectious diseases” (Ingle *et al.*, 2017) and

“possess antitumor, anticancer, antimicrobial, anti-inflammatory, analgesic, anaesthetic, antioxidant, neuroprotective and antiplatelet activity” (Tsuchiya, 2015).

According to Leonov *et al.,* (2015) phytochemicals are divided into the following major classes:

1) Phenolic compounds, including flavonoids, phenolic acids, hydroxycinnamic acids, lignans, tyrosol esters, stilbenoids and alkylresorcinols;
2) Terpenes, including carotenoids, monoterpenes, saponins, some modified lipid species and triterpenoids;
3) Betalains, including betacyanins and betaxanthins;
4) Polysulfides;
5) Organosulfur compounds;
6) Indole compounds;
7) Protease inhibitors;
8) Oxalic and anacardic organic acids;
9) Modified purines;
10) Guinones and;
11) Polyamines.

Phytochemicals accumulate in different plants parts such as leaves, roots, seeds, stems, fruits and flowers; they have important properties to prevent and fight diseases, they are not needed nutrients to sustain human body (Saxena *et al.*, 2013). They are mostly studied for their antibacterial activity against multidrug-resistant Gram-negative and Gram-positive bacteria (Barbieri *et al.*, 2017). It has been established that the anti-diarrhoeal activity of plants is because the plants contain the following phytochemicals: tannins, alkaloids, saponins, flavonoids and terpenoids (Njume *et al.*, 2012). As active antidiarrheal agents, tannins and flavonoids are responsible for increasing colonic

water and electrolyte reabsorption; others act by inhibiting intestinal motility (Palombo, 2006).

2.8.1. Tannins

Tannins are known to have antibacterial (Hisanori *et al.*, 2001), antitumor and antiviral activities (Kumari and Jain, 2012). Pharmaceuticals also uses tannins as antidiarrheal, diuretics, stomach pain, anti-inflammatory, antiseptic and antioxidant (Saxena *et al.*, 2013). In their antidiarrheal activity, tannins are responsible for increasing colonic water and electrolyte reabsorption (Palombo, 2006). They precipitate microbial protein making nutritional protein unavailable for them (Iqbal *et al.*, 2015*).* Tannins are classified into four major groups: Gallotannins, ellagitannins, complex tannins, and condensed tannins (Saxena *et al.*, 2013). From a biological point of view, the importance of tannin in plants is to repel predators (Haslam, 1989).

2.8.2. Alkaloids.

Alkaloids are organic nitrogenous bases found mostly in plants; one or more nitrogen atoms are present, normally as primary, secondary or tertiary amines (El-Sakka, 2010). They have been historically used in medicine and they are produced by different organisms such as animals, plants, fungi and bacteria (Barbieri *et al.*, 2017). Alkaloids protect the plant from herbivores, microbial attack, mechanical damage or stress (Roberts and Wink, 1998). Clinical use of plant-derived alkaloids includes the analgesics morphine and codeine, as a muscle relaxant, antibiotics, anticancer agent, antiarrhythmic, pupil dilator and sedative (Doughari, 2012). Alkaloids have an antispasmodic, antimalarial, analgesic, diuretic activity, antiasthma, anti-hypertensive, anti-tumour and anti-virulence (Shakya, 2016 and Barbieri *et al.*, 2017). According to Saxena *et al.,* (2013) “almost all alkaloids have bitter taste”.

2.8.3. Saponins.

Saponins are so named because they form a stable foam in water (Saxena *et al.*, 2013). Saponins are a group including compounds that are glycosylated steroids, triterpenoids, and steroid alkaloids very common in a large number of plants (Francis *et al.*, 2002). Most saponins are known to be “antimicrobial, inhibit mould, and to

protect plants from insect attack" (Saxena *et al.*, 2013). Saponins have hypolipidemic, anticancer activity and are also necessary for activity of cardiac glycosides (Doughari, 2012). Saponins show haemolytic activity, have bitter taste and are toxic to fish (Hostettmann and Marston, 2005). Saponins have antimicrobial activity and play a role in plant disease resistance (Haralampidis *et al.*, 2002). Saponins are reported to have anti-inflammatory, antiviral, plant defence activities (Shakya, 2016).

2.8.4. Flavonoids

Flavonoids are water-soluble polyphenolic compounds, there are more than 8000 flavonoids identified (Alseekh *et al.*, 2020). In plants, flavonoids are responsible for UV protection, pigmentation, stimulation of nitrogen-fixing nodules and disease resistance (Goldberg, 2003). In their antidiarrheal activity, flavonoids are responsible for increasing colonic water and electrolyte reabsorption (Palombo, 2006). Phenols and flavonoids have antiallergic, antibacterial properties, antimicrobial, cytotoxicity, anti-inflammatory, antitumor activities and have powerful antioxidants (Shakya, 2016 and Saxena *et al.*, 2013).

2.8.5. Terpenoids.

"Terpenoids function as phytoalexins in plant direct defence, or as signals in indirect defence responses which involves natural enemies and herbivores; volatile terpenes are produced by lots of plants to attract insects' pollinators or expel animals using them as food. While some plants produce less volatile strongly bitter-tasting terpenes to protect them from being eaten by animals and terpenes also play an important role as signal compounds and growth regulators of plants" (Saxena *et al.*, 2013). There are more than 25 000 terpenoids; they vary in structure and are classified according to the number of C5 isoprenoid units (Goldberg, 2003).

Terpenoids are widely used as anti-inflammatory agents (Ramirez, 2016). Terpenoids are known for their antiviral, anthelmintic, antibacterial, anticancer, antimalarial, anti-inflammatory properties (Shakya, 2016). A class of terpenes called monoterpenes are found in the essential oils extracted from many plants and it consist of two isoprene units. These essential oils possess good antibacterial effects against both gram-positive and gram-negative bacteria (Barbieri *et al.*, 2017).

CHAPTER 3: RESEARCH DESIGN AND METHODOLOGY

3.1 BACKGROUND

The study was based on quantitative and qualitative research methods. Ethics committee approval was obtained before the study commenced. Verbal permission was granted by the community leaders during the community meeting. A questionnaire was developed in which 30 traditional healers were randomly selected throughout the study area to participate in the study as per the ethics committee recommendation. The chosen participants provided information on medicinal plants used to treat diarrhoeal food-borne diseases. They participated in their own free will and they were not paid; however, they had to sign a consent form. Five commonly used medicinal plant parts were collected for the study and processed in the laboratories at UNISA Florida campus.

3.2 STUDY AREA

The study was conducted in South Africa in Limpopo province. Limpopo is one of South Africa's nine provinces, sharing borders with Botswana, Zimbabwe and Mozambique (Figure 3.1). The province lies within the great curve of the Limpopo River. It is known for its true bushveld, majestic mountains, primitive indigenous forests, patches of farmland and well-preserved wilderness including the northern section of Kruger National Park. The population of Limpopo consists of different ethnic groups distinguished by culture, language and race. The population consists of 5 803 900 people of which 52.9% are Northern Sotho (Sepedi) speaking, 16.9% speaks Xitsonga and 16.7% are Tshivenda speaking (www.gov.za/about-sa/south-africas-provinces) .

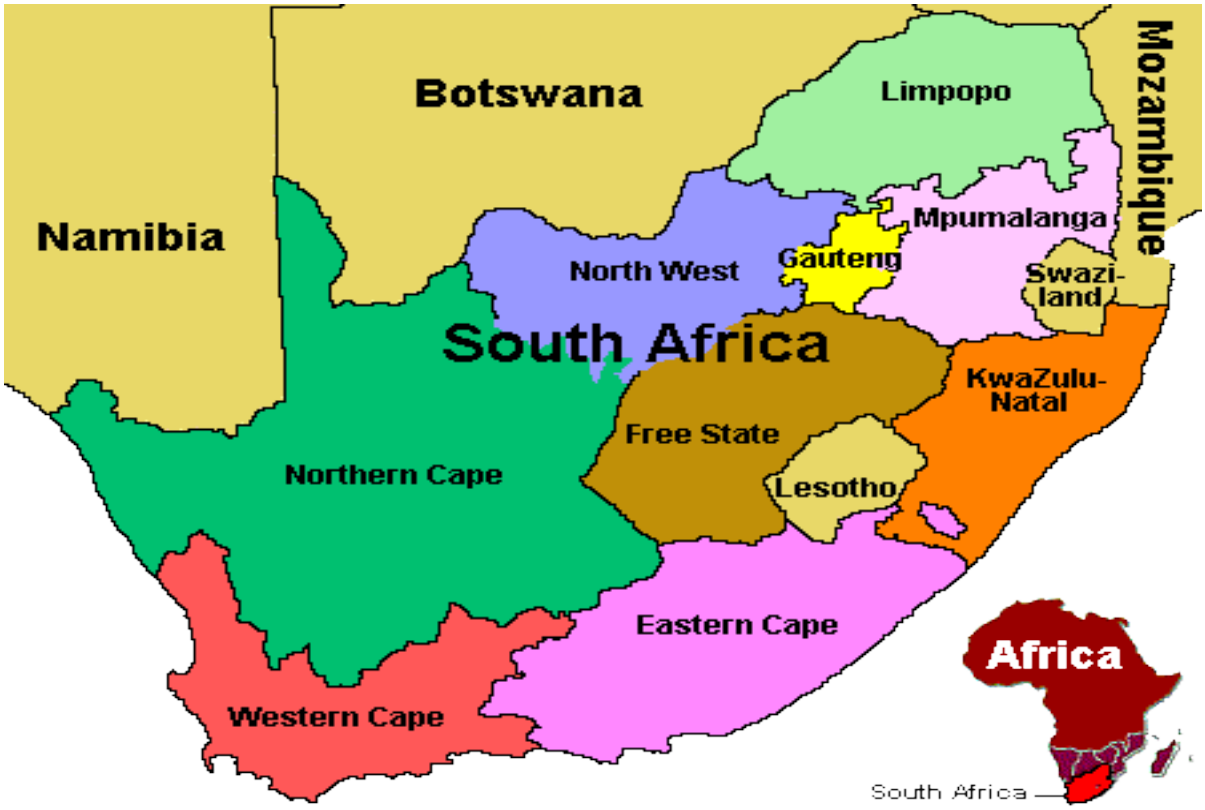


Figure 3. 1 Map of South Africa showing Limpopo province in the north-eastern part of the country
(Source : http://buzzsouthafrica.com/wp-content/uploads/political-south-africa-map.gif)

Limpopo province is divided into five districts (Figure 3.2). The study focused on Waterberg district which is divided into 6 municipalities (Figure 3.3).

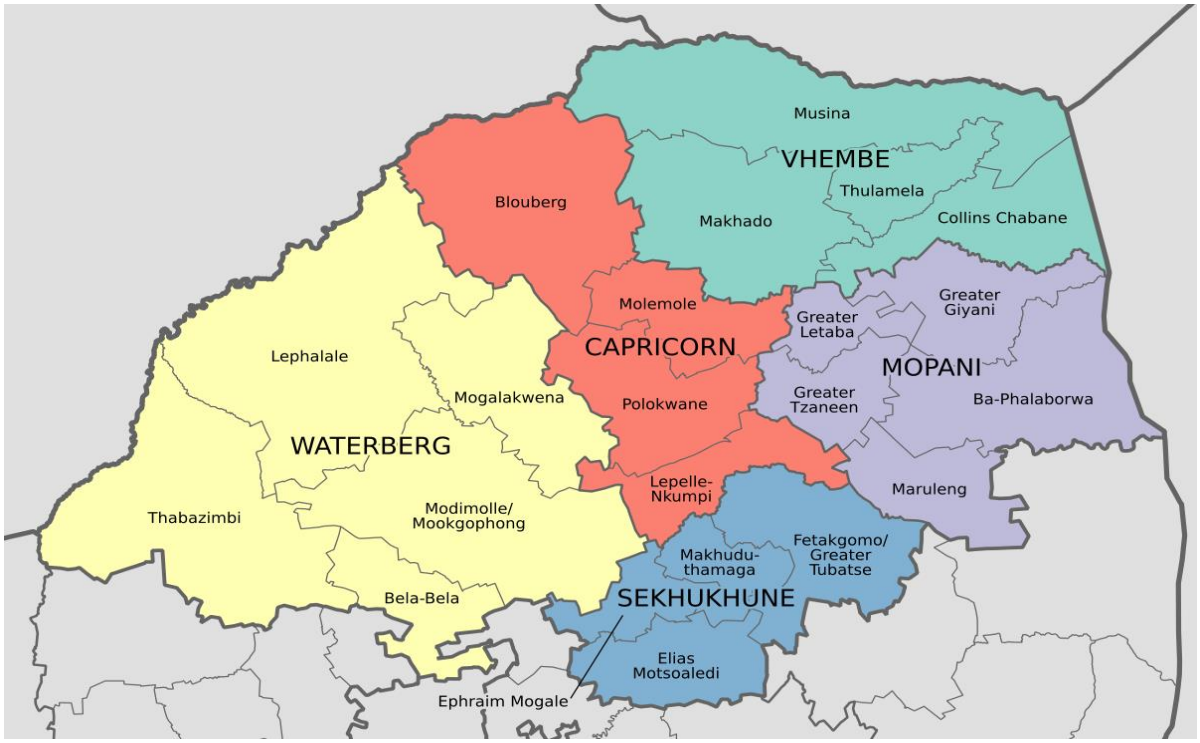


Figure 3. 2 Map showing 5 districts in Limpopo province
(Vhembe, Mopani, Capricorn, Waterberg and Sekhukhune)

(Source: https://en.wikipedia.org/wiki/List of municipalities in Limpopo).

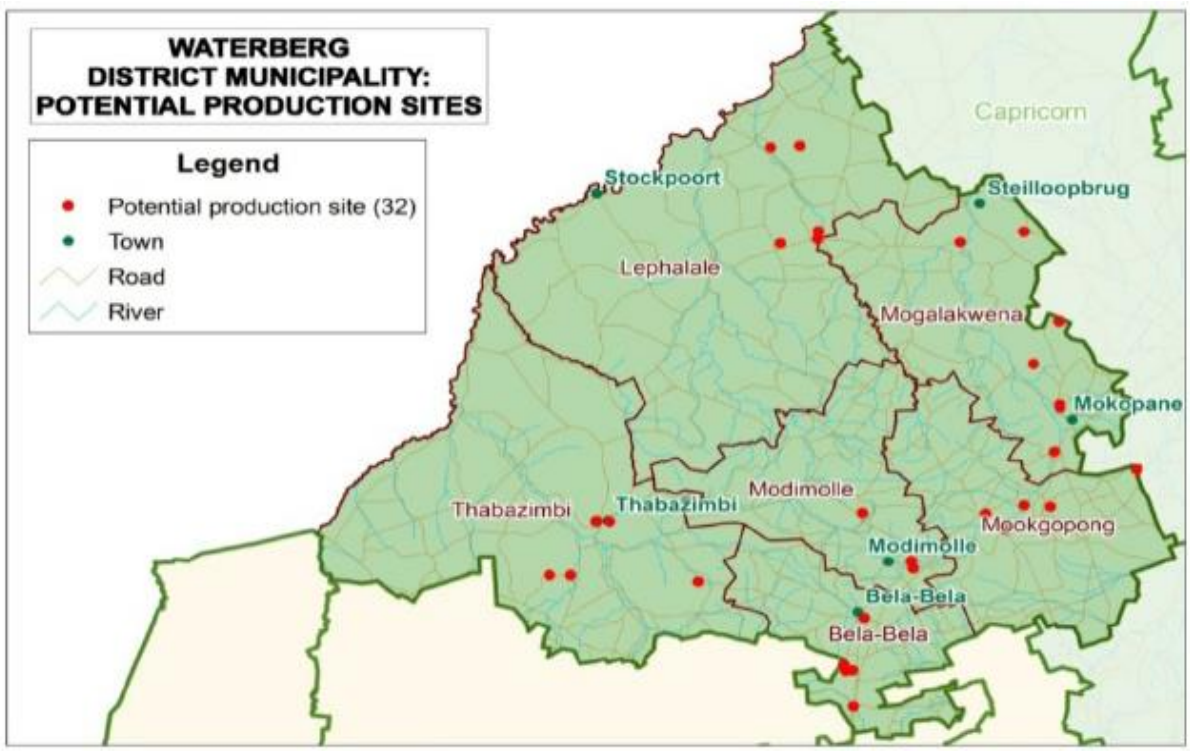


Figure 3. 3 Map showing Waterberg district with 6 municipalities
(Lephalale, Mogalakwena, Mookgopong, Modimolle, Thabazimbi and Bela-Bela) *(Source: https://www.researchgate.net/Map-of-Waterberg-District-indicating-selected-potential-production-sites-shown-as-red_fig1_269687226)*

The study was conducted in the Mogalakwena and Mookgopong municipalities in the Waterberg district. The large population in this part of Limpopo Province is Bapedi (Northern Sotho speaking people) and a small population of other ethnic groups such as Ndebele, Tsonga, Afrikaans and English. Although the district is populated by several ethnic groups, they all share a deep-rooted attachment to their specific traditions and cultural values. Mogalakwena and Mookgopong municipalities were chosen for the study because the traditional healers in the area still adhere to the old age traditional beliefs and customs in their everyday practice.

3.3 ETHNOBOTANICAL SURVEY OF PLANTS USED TO TREAT DIARRHOEA

3.3.1 Data collection using questionnaire

Data was collected in June and July 2019 in agreement with Unisa Research Ethics Policy. Participants at Mogalakwena and Mookgopong municipalities in the Waterberg district were randomly selected regardless of their ethnic groups. Thirty (30) participants (traditional healers) with either formal (diviner) or non-formal training (herbalists) in medicinal plants use were selected for the study throughout the study area. Participants were only included in the study if they had successfully treated at least one patient with diarrhoea and vomiting as major symptoms of food-borne pathogens. The participant information sheet (Appendix 1) was read to all participant and they all had to sign a consent form (Appendix 2) to participate in the study in accordance with CAES Health Research Ethics Committee recommendations.

Two volunteer assistants helped with collecting data and supervision. It was thoroughly explained to the volunteers; the purpose of the study, aims and objectives. They were also trained on the sections of the questionnaire and the importance of capturing data accurately. A structured questionnaire was used for data collection and the responses were recorded on the questionnaire form (Appendix 3) Gallardo-Casas *et al.,* (2012) and Uddin *et al.*, (2014). Participants were interviewed by data collectors; they were observed processing medicinal plants for use or storage to be used later.

A mixture of both open-ended questions and closed-ended questions were used for the study to gather required information for further analyses. Confidentiality and respect for the participants were the key factors in ensuring that questionnaire survey research was successful. Each participant was informed of this before the

questionnaire survey was administered. A sample size for questionnaire comprised of 30 participants aged between 18 – 85 years old. The age group between 18 – 85 was chosen because participants are regarded as mature, have a better understanding and most can read and write. The questionnaire was written in English and translated into the language the participants felt comfortable with during data collection. The duration of the interview was estimated to be between 15-30 minutes per person.

The questionnaire addressed the following aspects:

- Knowledge of the participant on medicinal plants used to treat diarrhoea and vomiting as major symptoms of food-borne pathogens;
- Preparation of the medicinal plants used;
- Administration of the medicinal plants.

Inclusion Criteria

Individuals were included in the study if they met the following criteria: they were 18 years of age or older, they had some background knowledge of medicinal plants used to treat diarrhoea and vomiting as symptoms of food-borne pathogens, and used the plants successfully. Individuals were only allowed to participate if they read and signed the informed consent form.

Exclusion Criteria

Individuals were excluded from the study if they were younger than 18 years of age and had no background knowledge and/or experience of medicinal plants used to treat diarrhoea and vomiting as symptoms of food-borne pathogens.

3.3.2 Collection of plant material

Plant specimen collection permit was obtained from Department of Economic Development, Environment & Tourism in Limpopo Province before collecting plant specimens. Permission to collect plant specimen in private properties were also granted. Specimen samples of different plant species commonly used to treat food-borne diseases by indigenous people in Mogalakwena and Mookgopong

municipalities were collected and used for the study. Specimen from the following five most commonly used medicinal plants were collected, based on the information provided by the traditional healers: *Psidium guajava* roots, bark and leaves*, Punica granatum* fruit peels*, Sclerocarya birrea* bark*, Ozoroa reticulata* roots and *Pouzolzia mixta* roots*.* Small branches of plant species mentioned were collected for identification confirmation by a Botanist at the Johannesburg Botanical Garden.

3.4 THE FIVE PLANT SPECIES MOST MENTIONED DURING THE QUESTIONARE SURVEY

3.4.1 *Psidium guajava*

Family: Myrtaceae

Common names: Guava

Description

Psidium guajava (Figure 3.4) is an evergreen tree that grows up to 10m and it usually grows in warm areas in wide range of soil type and vegetation types from forest, grass land, bushveld, wetland to road side and can invade forest margins (Boon, 2010). It starts producing fruits from the age of 4 years; the bark is smooth, has copper colour that peels off in flakes exposing the underlining green layer (Parle and Broka, 2010). Dharani (2011) describe the leaves, flowers and fruits. Leaves are oval shaped and oppositely arranged, hard, thick, sunken veins above and raised below. Flowers are white in colour and arranged as single or grouped up to three (3). The fruit is fleshy with many seeds, green and turns yellow when ripe.

(a)

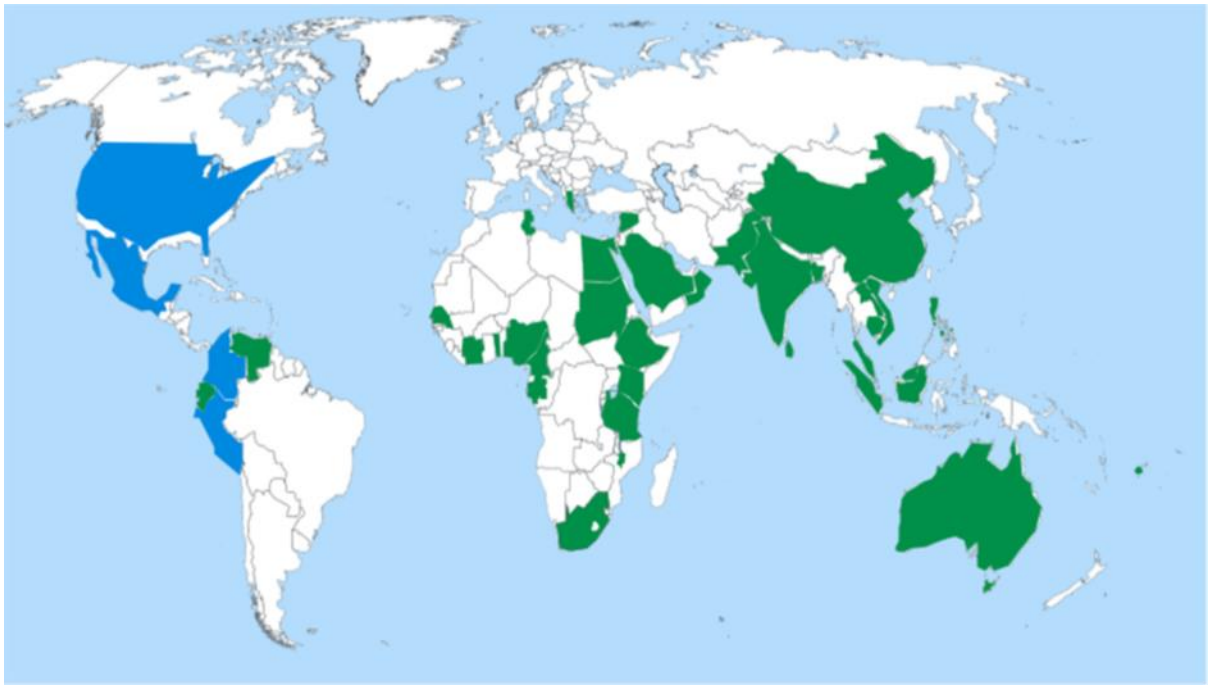
(b)

(c)

Figure 3. 4 *Psidium guajava* L.
a- mature plant, b- bark and c- leaves and flowers.

Distribution

Psidium guajava is from tropical America (Figure 3.5) and is extensively cultivated worldwide for its fruits (Dharani, 2011).



Native Introduced

Figure 3. 5 *Psidium guajava* distribution map (Ugbogu *et al.,* 2022)

Uses

According to Parle and Broka (2010), *Psidium guajava* leaves and bark are used to treat vomiting, diarrhea, dysentery, sore throat, irregular menstrual cycles, stomach pain, vertigo, mouth sores and bleeding gums. Leaves and roots are used to treat diarrhea, indigestion, dysentery and stomach disorder.

3.4.2 *Punica gratanum* L.

Family: Lythraceae

Common name: Pomegranate

Description

Punica gratanum (Figure 3.6) is a small tree or a shrub that belongs to the family Lythraceae and is commonly known as pomegranate (Zarfeshany *et al.*, 2014). Shaygannia *et al.,* (2016) describe pomegranate as a “shrub that can grow up to five meters with evergreen glossy smooth dark green leaves and unequal thorny branches. Bark of old branches is grey and new branches have red-brown bark. Flowers are orange-red, and can be solitary or grouped in two or three at the end of the branch.

Flowers are self-pollinated or by insects. Fruits are round with crown at the base. Fruits are covered with tough leathery skin that is deep pink or red outside and yellow inside. Inside the fruit is separated by a white spongy and bitter membranes. It is divided into sections packed with sacks filled with red, pink or whitish sweet acidic juice. There is soft or hard angular seed in each sac". According to Kumari and Jain, (2012) the "high temperature is essential for the best fruit flavour. Pomegranate can bear fruits a year after planting, the fruit takes 5-7 months to mature after bloom. The fruits can only be picked when mature before they crack(overripe)".

(a)

(b) (c)

Figure 3. 6 *Punica gratanum*.
a- mature plant (Source: https://www.csbe.org/pomegranate-punica-granatum), b - fruits (Source: https://www.kew.org/plants/pomegranate) and c- leaves and flowers (Source: https://www.csbe.org/pomegranate-punica-granatum).

Distribution

Punica gratanum is endemic from Iranian plateau, Himalayas in north Pakistan and India (Chandra *et al.,* 2010), however today there are about 500 cultivators globally (Figure 3.7) Loizzo *et al.,* (2019) due to its ability to adapt to different climate conditions.

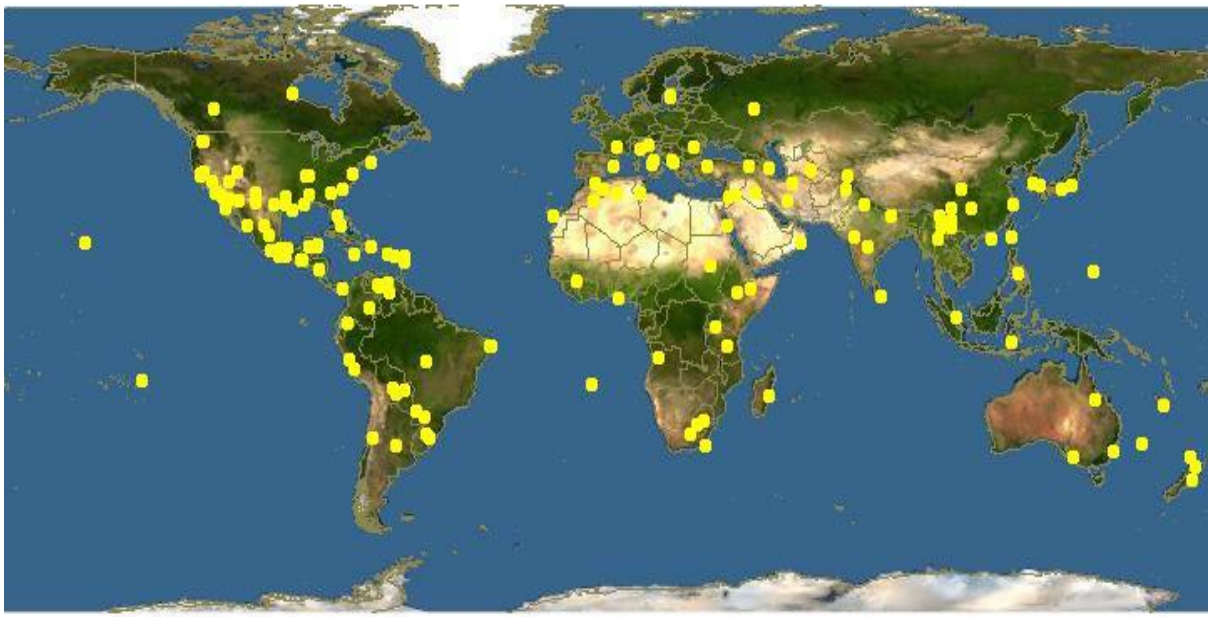


Figure 3. 7 *Punica gratanum* distribution map
(https://www.discoverlife.org/20/q?search=Punica+granatum)

Uses

Punica gratanum has several uses which include baking, beverages, cooking and treatment of different diseases in several cultures (Loizzo *et al*., 2019). According to Zarfeshany *et al.,* (2014), *Punica gratanum* juice has antioxidant, anti-inflammatory, antiproliferative, antiatherogenic effect and the fruitpeels (rind) is used to treat diarrhea and stomach ache. *Punica gratanum* is also used to treat sore throat, coughs, urinary infections, digestive disorder, skin disorder, arthritis and expelling tapeworms (Kumari and Jain, 2012).

3.4.3 *Sclerocarya birrea* (A. Rich.) Hochst.

Family: Anacardiaceae

Common names: Marula (English); Morula (Northern Sotho); Mufula (Tshivenda); ukanyi (Tsonga)

Description

Sclerocarya birrea (Figure 3.8) is a medium sized tree that grows up to 15m in height and it grows naturally in open woodland, on sandy to loam soil (SANBI-Plantsafrica.com). It can grow for over 100 years and start producing fruits from 7 years old (Mokgolodi *et al.,* 2011). It has upright single stem and round crown with male and female flowers growing on separate trees (SANBI- Plantsafrica.com). The bark is mottled grey and peels in disk shaped flakes (Maroid and Abdelwahab, 2012). It flowers from September to December and produces fruits from January to March. Male flowers produce pollen and female flowers produce fruits. The fruit is oval shaped with white flesh around the seed that contains juice, pale green with thick soft skin, usually fall off the tree unripe and turn yellow when ripe (Mokgolodi *et al.*, 2011). The fruit has hard brown seed that has soft white kernel inside (Maroid and Abdelwahab, 2012). *Sclerocarya birrea* has pinnately compound leaves that are paired with one terminal leaf at the end. Leaf margins are entire, the leaf blade is shiny dark green above and lighter green below (Harley *et al.*, 2022).

(a)

(b)

(c)

Figure 3. 8 *Sclerocarya birrea*.

a- mature tree, b- main stem with bark and c- fruits

Distribution

Sclerocarya birrea is endemic to Africa and has been introduced to Australia, India, Israel, Mauritius, Oman and Réunion (Figure 3.9) Mokgolodi *et al.,* (2011).

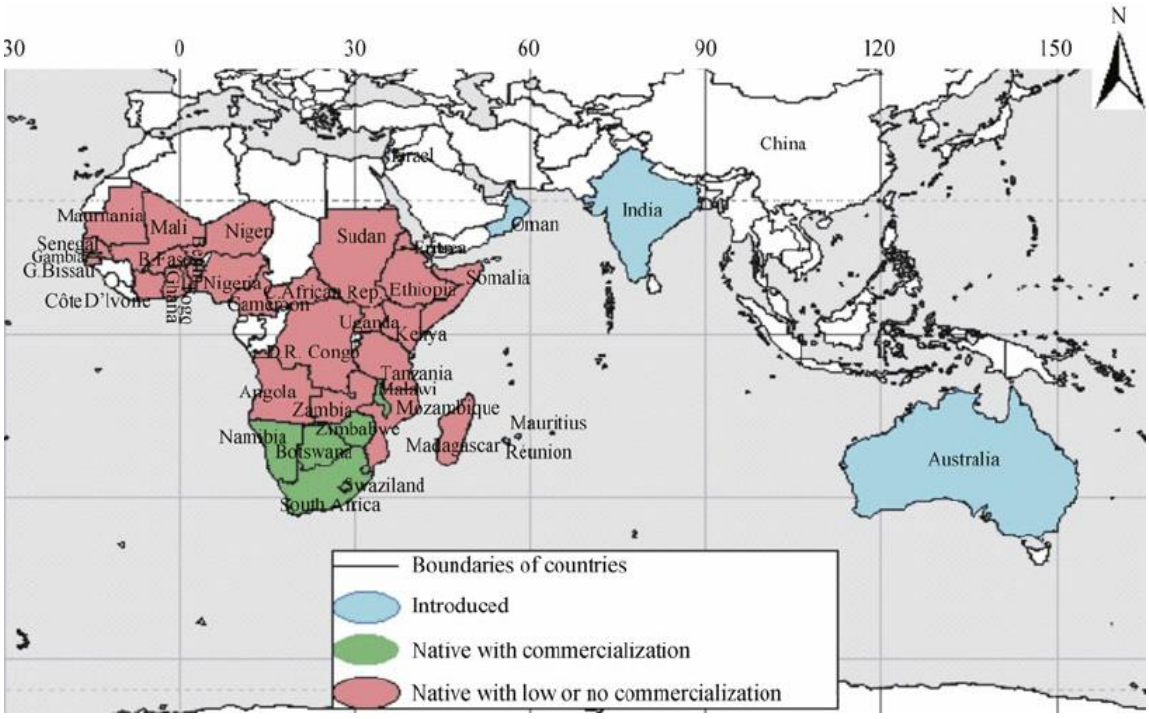


Figure 3. 9 Distribution of *Sclerocarya birrea* (Mokgolodi *et al.,* 2011)

Uses

Different plant parts of *Sclerocarya birrea* are used to treat various illnesses. "The leaves and fruits are used to treat coughs, diabetes, dysentery, scorpion and snake bites, malaria, inflammations, and hypertension" (Harley *et al.,* 2022). "Leaves are also used to treat gonorrhoea" (SANBI- Plantsafrica.com). "Powdered bark infusion is used on pregnant women to determine the gender of an unborn baby" (Mokgolodi *et al.,* 2011). "Bark decoction is used to treat dysentery, diarrhoea, rheumatism, haemorrhoids and has malaria prophylaxis" (SANBI- Plantsafrica.com). "Roots are used to treat sore eyes, pharyngitis, goitre, and splenomegaly" (Harley *et al.*, 2022).

3.4.4 *Ozoroa reticulata* (Baker f.) Engl.

Family: Anacardiaceae

Common name: Tarberry Resin-tree (English); Teerbessieharpuisboom (Afrikaans); Monoko (N. Sotho); Xinugu mafi (Tsonga).

Description

According to Palgrave (2002) *Ozoroa reticulata* (Figure 3.10) is a tree that can grow up to 15m high, grows in savannah vegetation in rocky areas. Bark is rough, dark grey to brown, produces small square flakes, branches are covered in dense hair and are yellow to red in colour. Leaves are spirally arranged or in whorls of 3, are different in shapes and sizes, the upper part of the leaf is green and densely covered with hair, underneath it has yellow like rustic colour with hair and leaf margins are entire. The tree flowers from November to February and the flowers are creamy green. It produces flattened kidney shaped fruits that turn black when ripe and have wrinkles.

(a) (b)

Figure 3. 10 *Ozoroa reticulata*
a- Leaf arrangement (Source: https://www.malawiflora.com/speciesdata/image-display.php?species_id=136590&image_id=3) and b- Leaves and flowers during growing season (Source: https://www.malawiflora.com/speciesdata/image-display.php?species_id=136590&image_id=4).

Distribution

According to Palgrave (2002) *Ozoroa reticulata* is found in high population in Namibia, Botswana and Zimbabwe. Small population found throughout South Africa (Figure 3.11).

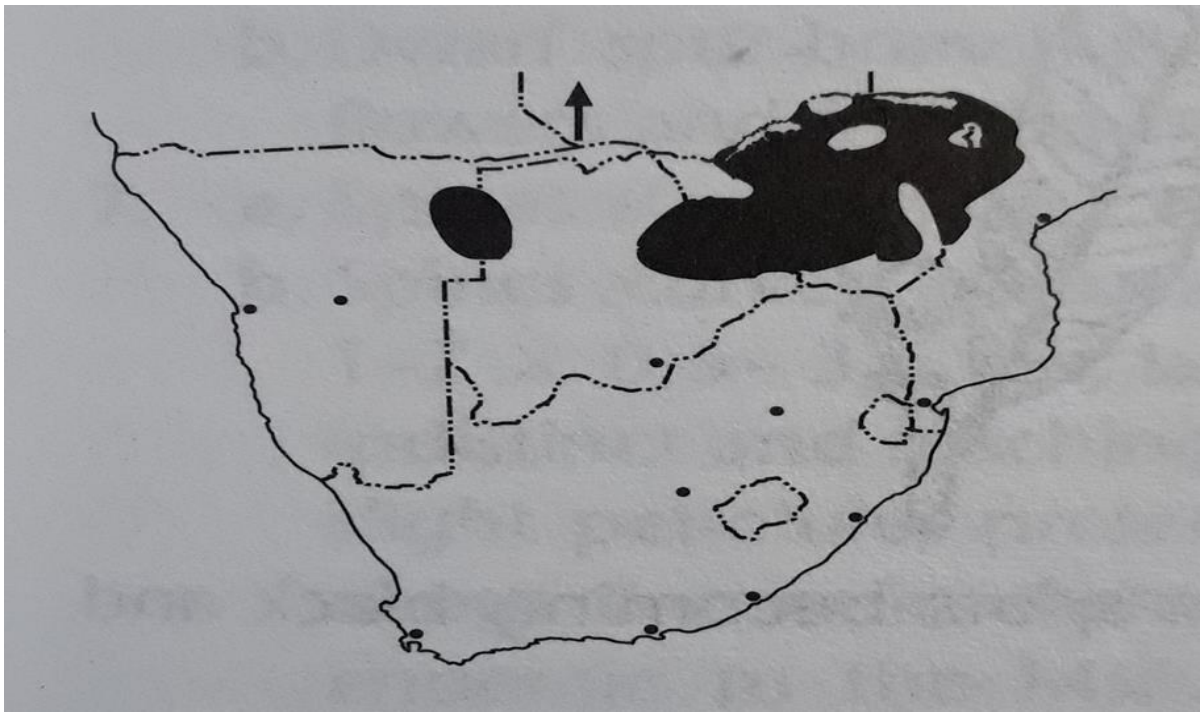


Figure 3. 11 *Ozoroa reticulata* distribution map (Palgrave, 2002)

Uses

Ozoroa reticulata “leaf and bark infusion is used to treat diarrhoea, kidney, liver, ulcer, throat infection, schistosomiasis and chest pain. Bark and leaf paste is applied on the skin to treat different skin infection and diseases. Roots infusion is taken by woman to increase lactation after child birth” (Katsukunya *et al.,* 2021).

3.4.5 *Poulzozia mixta* Sohms

Family: Urticaceae

Common name: Soap-nettle (English); Seepnetel, Wildebraam (Afrikaans); Isikukuku (IsiNdebele); Nthadzwa (Setswana); Muthanzwa, Murovhadembe (Tshivenda); Nthadzwa (Xitsonga)

Description

According to Boon (2010), *Poulzozia mixta* (Figure 3.12) is a multi-stemmed shrub that can grow up to 4m. It occurs in savannah preferably in hot dry areas, rocky hills or near wetlands. The bark is grey, brown or red/pink brown, and smooth, has watery latex and the main stem has hairs that sting. Leaves are simple, round to ovate, and spirally arranged. The base has three veins, the upper part of the leaf is dark green and rough, underneath it is silver grey and has sting hair that can stick to each other and on clothes. Regarding flowers; male and female flowers occur separate on the same tree, they are green-white, small, cluster around auxiliary bud and flower from November to December. Fruits are small and orange red in colour.

(a)

(b)

(c)

Figure 3. 12 *Poulzozia mixta*.
a- mature plant, b- leaves colour and arrangements and c- flower arrangements and colour (Source: https://pza.sanbi.org/pouzolzia-mixta).

Distribution

Pouzolzia mixta (SANBI- Plantsafrica.com) occur in KwaZulu-Natal, Mpumalanga,

Gauteng, North-West and Limpopo, Swaziland, Botswana, Zimbabwe, and Malawi (Figure 3.13).

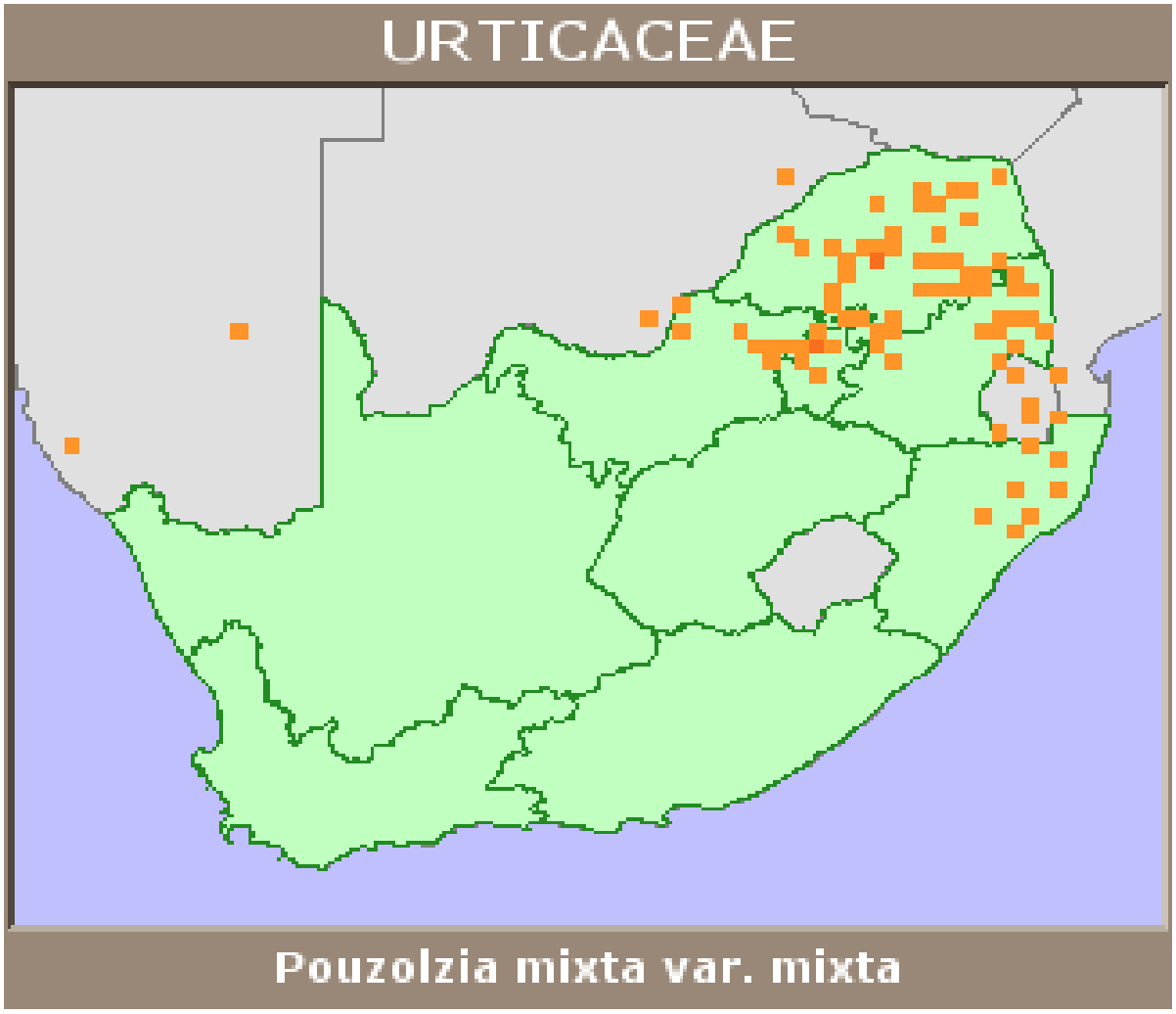


Figure 3. 13 *Pouzolzia mixta* distribution map
*(*Source *: http://redlist.sanbi.org/species.php?species=4021-2).*

Uses

Pouzolzia mixta roots are used to treat diarrhea and dysentery (Appidi *et al.*, 2008). Roots are also used to treat infertility in women, expel retained placenta, treat painful uterus and as contraceptives (Sewani-Rusike, 2013).

3.5 PREPARATION OF COLLECTED PLANT SAMPLES

Instruments and reagents

Grinding machine/ Blender, distilled water and airtight containers.

Preparation of the plant samples

Medicinal plant parts used for the study included roots, bark, leaves and fruit peels. Fresh plant parts were washed under running tap water, rinsed with distilled water, air dried for two weeks (drying under the shade), ground into a fine powder using a blender (Figure 3.14) and stored in clearly labelled airtight containers for later use and analysis.

(a)

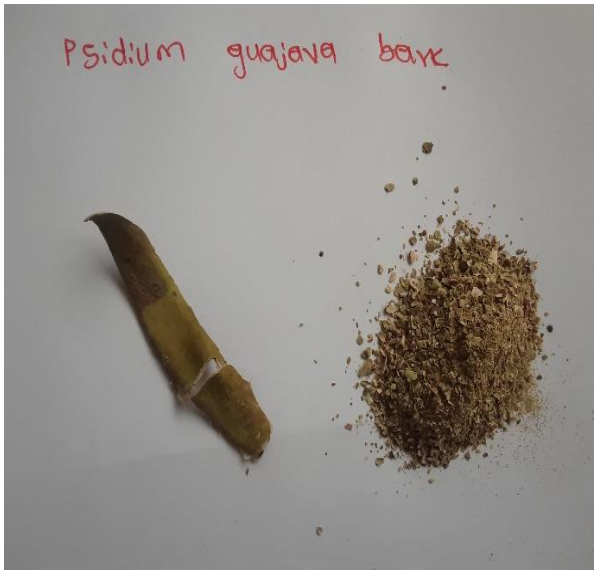
(b)

(c)

(d)

(e)

(f)



(g)

(h)

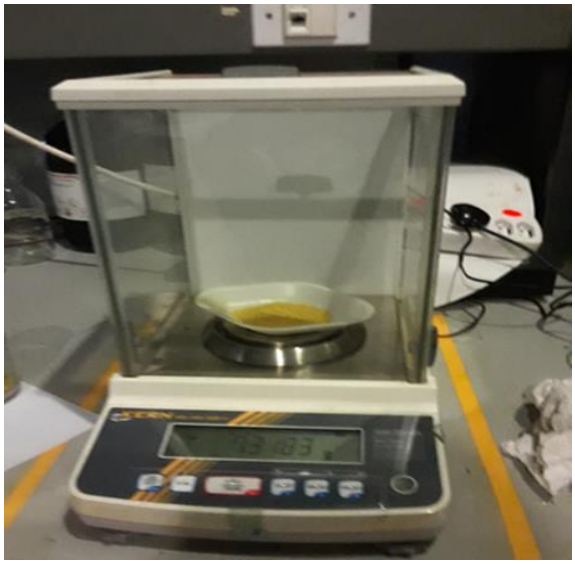
Figure 3. 14 Plants specimen collected for the study
a- *Pouzolzia mixta* roots, b- *Sclerocarya birrea* bark, c- *Punica granatum* fruit peels, d- *Ozoroa reticulata* roots, e- *Psidium guajava* roots, f- *Psidium guajava* leaves, g- *Psidium guajava* bark and h- grinding machine/blender.

3.5. EXTRACTION OF PLANT COMPOUNDS

3.5.1. Aqueous extraction:

Instruments and reagents

Powdered plant material, distilled water, spatula, scale, weighing boat, Erlenmeyer flask, water bath, whatman filter paper, centrifuge machine, Schott Duran bottles, freezer, latex gloves and freeze dryer (Figure 3.15).



(a)

(b)

(c)

(d)

Figure 3. 15 Main instruments used to prepare aqueous plants extracts.
a- Scale, b- Water bath, c- Freeze dryer and d- centrifuge machine.

Preparation of the plant extract

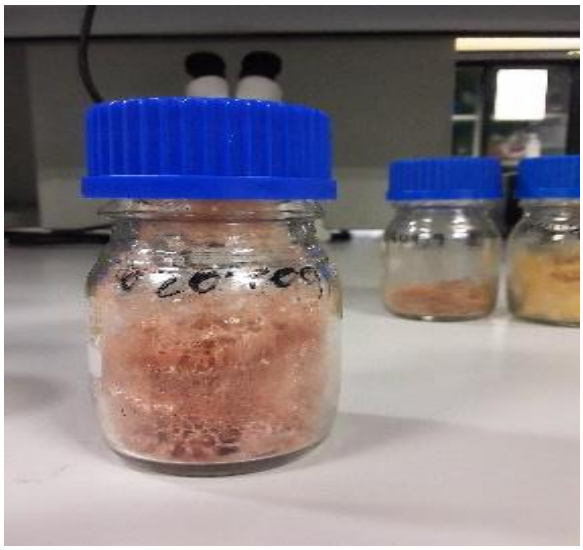
The aqueous plant extracts were prepared as described by (Parekh *et al.,* 2005). Aqueous extraction was chosen as many traditional healers commonly use water to prepare medicinal plants. 10g of air-dried powdered plant material was placed in the Erlenmeyer flask, 100ml distilled water was added, and the mixture was boiled for 6 hours in the water bath at 100ºC. Every 2 hours it was taken out, left to cool at room temperature, filtered through whatman filter paper and centrifuged at 5000 RCF or G-

force for 15 min. After 6 hours, the plant extract was left to cool at room temperature in the 100ml Schott Duran bottles, then placed in the freezer.

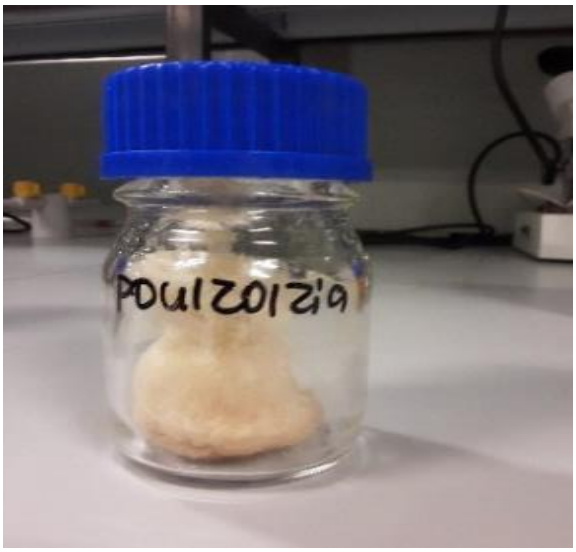
After freezing the lid was taken off, a latex glove was tied on to prevent loss of plant extract during freeze drying process and a few holes were made for evaporation. The extract was frozen in the freezer so that the water in the plant material could solidify first, before being put in the freeze dryer. The extracts were placed in the Labconco Freezone Benchtop freeze dryer. They were freeze dried at a vapor pressure of 0.036mbar and temperature of -50ºC for 3 days (Figure 3.16). Freeze drying is the fast evaporation process and most of the phytochemicals are preserved using this method (Azwanida, 2015). The extracts were then subjected to antimicrobial studies.

(a)

(b)

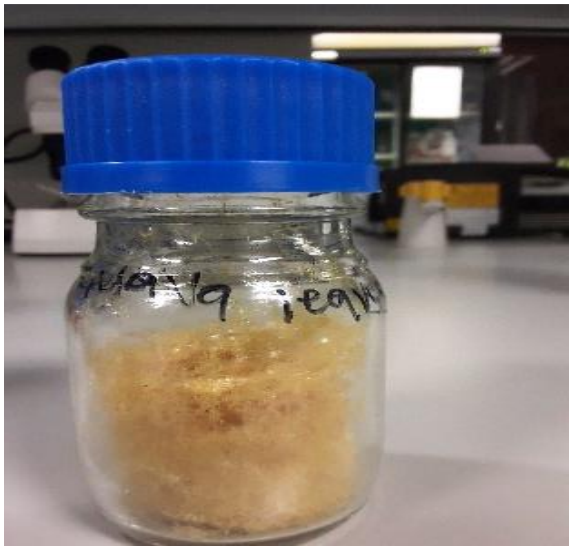


(c)



(d)

(e)



(f)

(g)

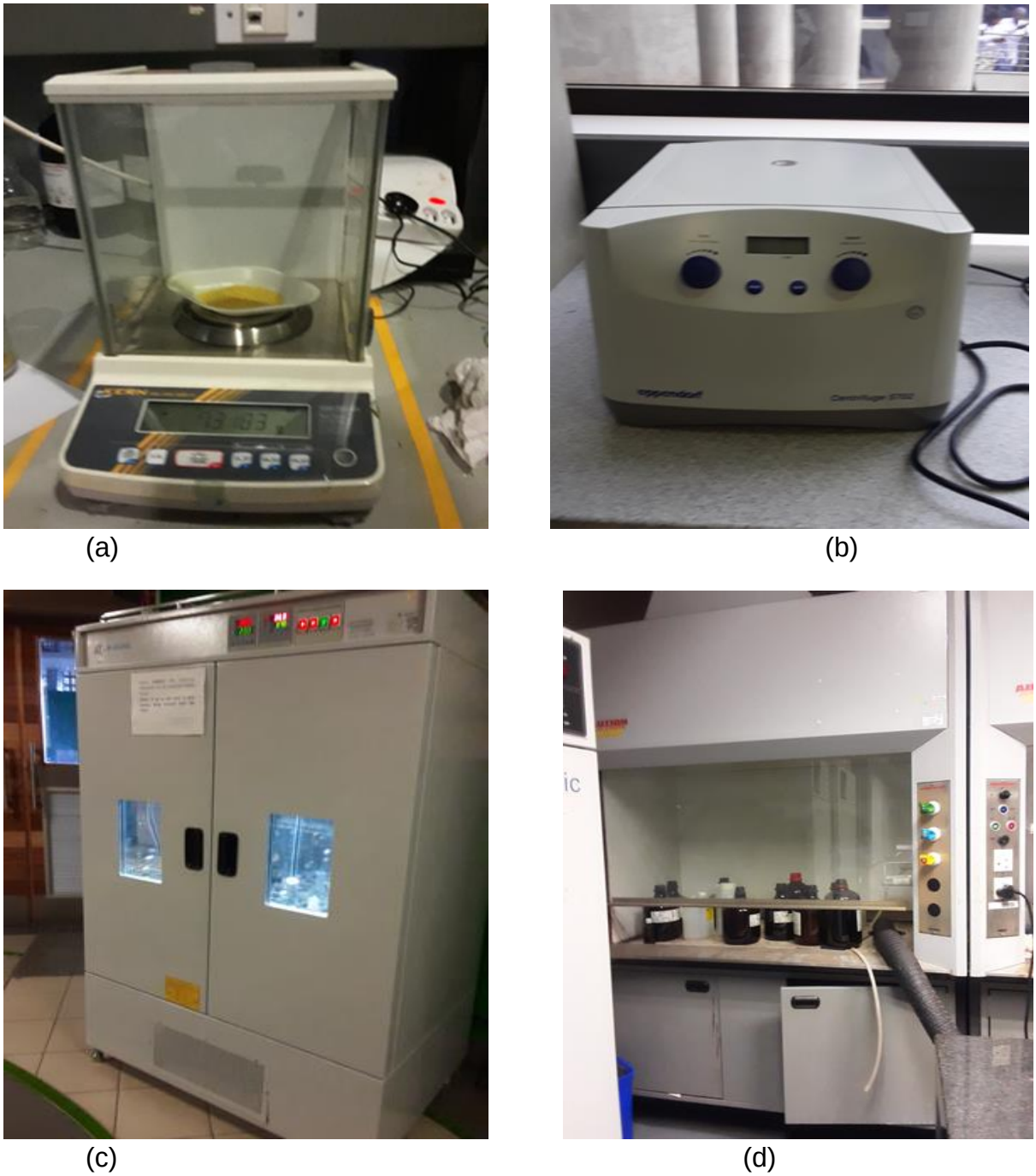
(h)

Figure 3. 16 Aqueous plant extracts after freeze drying.
a- all seven specimens. b- *Punica granatum* fruit peels, c- *Ozoroa reticulata* roots, d- *Pouzolzia mixta* roots, e- *Sclerocarya birrea* bark, f- *Psidium guajava* leaves, g- *Psidium guajava* bark and h- *Psidium guajava* roots.

3.5.2. Solvent extraction:

Instruments and reagents

Powdered plant material, 70% ethanol, spatula, scale, weighing boat, Erlenmeyer flask, aluminium foil, rotary shaker, whatman filter paper, centrifuge machine, glass beakers and biosafety cabinet (Figure 3.17).



(a) (b)

(c) (d)

Figure 3. 17 Main instruments used to prepare ethanol plants extracts.
a- scale, b- centrifuge machine, c- rotary shaker and d- biosafety cabinet.

Preparation of the plant extract

The ethanol plant extracts were prepared as described by (Parekh *et al.*, 2005). 70% Ethanol was used for extraction. Ethanol is reasonably safe for human ingestion hence it was a selected extraction solvent for this study (Wendakoon *et al.*, 2012). 10 g of air-dried powder plant material was placed in the Erlenmeyer flask, 100ml ethanol was

added, the flask was closed with aluminium foil to prevent spillage and then kept in a rotary shaker at 190-220 rpm for 24hours. The extract was filtered through a filter paper and centrifuged at 2800 RCF or G-force for 15minutes. The supernatant was collected into a glass beaker and the solvent was evaporated in the biosafety cabinet (BSC) (Figure 3.18). The extracts were weighed and subjected to antimicrobial studies.

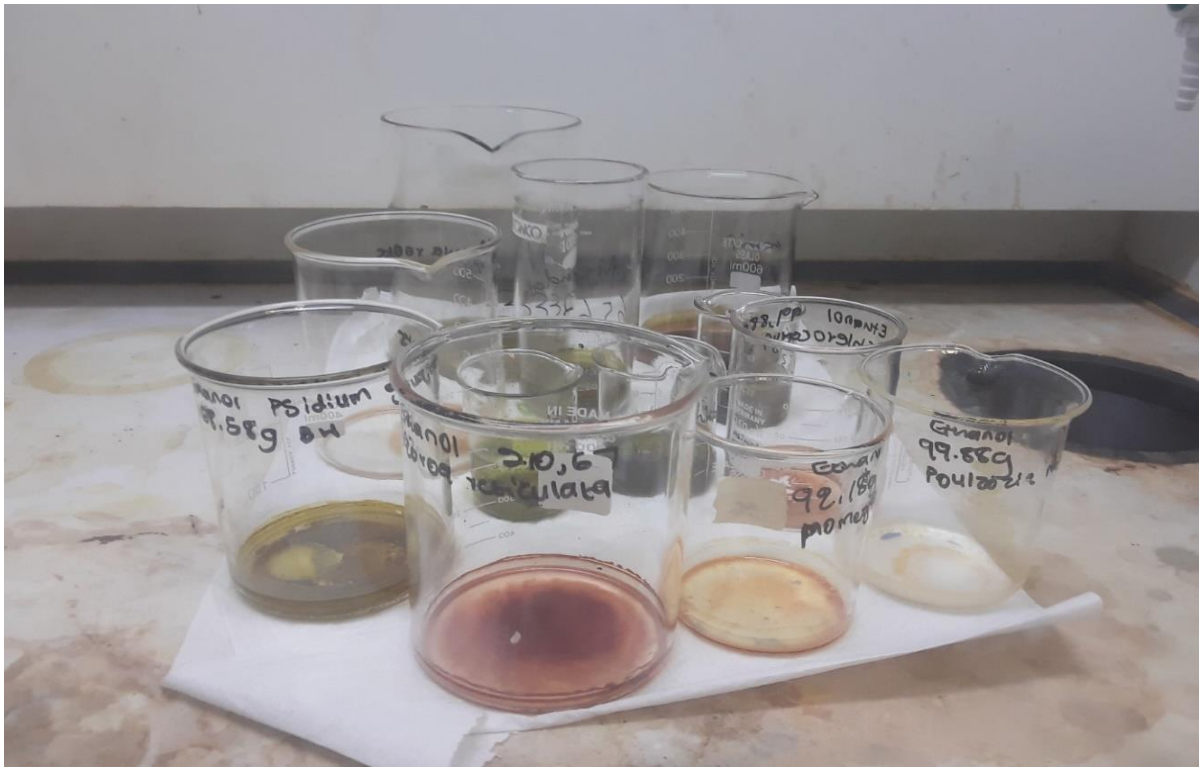


Figure 3. 18 Solvent extracts (70% ethanol) under biosafety cabinet

3.6 PREPARATION OF BACTERIA CULTURE

Microorganisms used were *Salmonella typhi* (ATCC 6539) and *Escherichia coli* (ATCC 25922). These are amongst the leading bacteria involved in diarrheal food borne diseases. A pure cell suspension of *Salmonella typhi* and *Escherichia coli* O157:H7 were obtained from LANCET Laboratories.

The stock bacteria culture was used to prepare a new bacteria culture by preparing a fresh nutrient agar plate, once solidified it was seeded with stock bacteria culture and incubated for 24hours at 37ºC. Isolated colony was picked and inoculated into 3ml to 5ml freshly prepared sterile broth. The inoculated medium was incubated at 37ºC for

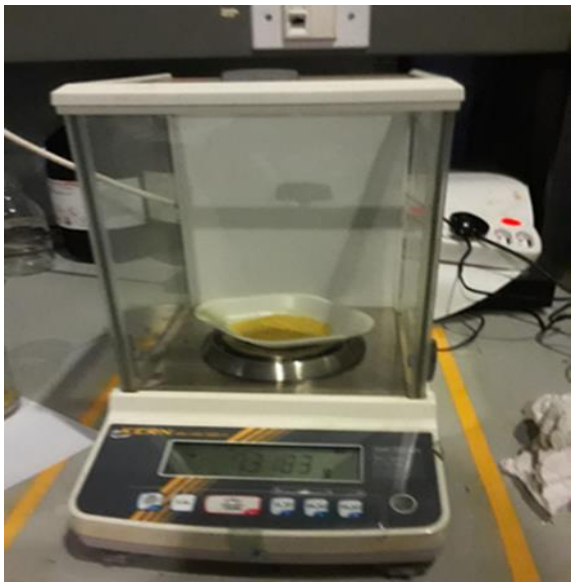
18hours. Due to the increased number of bacteria, the broth becomes cloudy after incubation. The cell suspension was stored at 4ºC and for long term use it was stored at -20ºC.

3.7 DETERMINING ANTIMICROBIAL ACTIVITY

3.7.1 Mueller Hinton Agar plate preparation

Instruments and reagents

Mueller Hinton agar powder, spatula, scale, weighing boat, glass beakers, Schott Duran bottle, hotplate, glass stirring rod, distilled water, fume hood, autoclave machine and sterile petri dish (Figure 3.19).



(a)

(b)

(c)

(d)

Figure 3. 19 **Main instruments used to prepare Mueller Hinton agar plates.**
a- Scale, b- hotplate, c- fume hood and d- autoclave machine (Source: Afromedics.co.za).

Mueller Hinton agar (MHa) plates were prepared by weighing 38g of MHa powder into a glass beaker, 1L of distilled water was then added. The mixture (agar) was placed on a hotplate and bring to boil while stirring with glass stirring rod until completely dissolved. The agar was transferred into a Schott Duran bottle and the lid was not closed tight. The agar was sterilized by autoclaving for 15 minutes at 121ºC. The agar was left to cool to 50ºC. The agar was swirled and 20ml was transferred into each sterile petri dish. Agar plates were left in a fume hood to solidify.

3.7.2. Agar disk diffusion method

Instruments and reagents

Agar plates, cell suspension of *Salmonella typhi* and *Escherichia coli*, distilled water, 70% ethanol, sterile cotton swap, fume hood, 6mm sterile filter paper discs, glass beaker, forceps, Bunsen burner, pipette, plants extract, weighing boat, scale, incubator (Figure 3.20).

(a) **(b)**

Figure 3. 20 Main instruments used for agar disk diffusion method.
a- fume hood and b- incubator.

Agar disk diffusion method was used to determine the antimicrobial activities for both aqueous and solvent (70% ethanol) extract of five different plant species using Mueller Hinton Agar as described by (Parekh *et al.*, 2005) with minor adjustments. Agar plates were divided using a permanent marker and clearly labelled. A lawn of the test microorganism (*Salmonella typhi* and *Escherichia coli*) were prepared by dipping a sterile cotton swab into a cell suspension and streaked on the surface of the agar under a fume hood. The bacterial agar plates were incubated at 37°C for 24 hours in the desk top incubator.

After 24 hours' incubation, the bacterial growth was observed on the agar plates. Plant extract for each plant specimen was prepared by dissolving 100mg of aqueous plant extract into 1ml of distilled water. Hundred milligram (100mg) of ethanol plant extract was dissolved into 1ml of 70% ethanol. Six millimeter (6mm) sterile filter paper discs were soaked with plant specimen and placed on the surface of the bacterial agar plate. Forceps were used to pick and place the disks onto the bacterial agar plate. However, forceps were sterilized after each use by dipping it into 100% ethanol and pass it through open flame. When the flame died off, it was used for the next disk.

The bacterial agar plates with plant extract disk were incubated at 37°C for 24 hours in the desk top incubator. Active antimicrobial agent diffused in the bacterial agar and inhibited the growth of the microbial strain tested. The antibacterial activity was assessed by measuring the diameter in millimeters (mm) of inhibition zone. Inhibition zone is the area around the plant extract disc where bacteria are unable to grow. Antibacterial potential of different plant species was assessed by comparing diameter of the inhibition zone.

3.7.3 Agar-well diffusion assay

Instruments and reagents

Agar plates, cell suspension of *Salmonella typhi* and *Escherichia coli*, distilled water, plants extract, 70% ethanol, sterile cotton swap, glass beaker, pipette, weighing boat, scale, pipette tips, fume hood and incubator (Figure 3.21).

(a) (b)

Figure 3. 21 Main instruments used for agar well diffusion method.
a- fume hood and b- incubator.

Agar-well diffusion assay was used to determine the antimicrobial activities for both aqueous and solvent (70% ethanol) extract of five different plant species using Mueller Hinton Agar as described by (Parekh *et al.*, 2005) with minor adjustments. Agar plates were divided using a permanent marker and clearly labelled. Holes with a diameter of 6mm were punched aseptically on the agar using the back of white sterile pipette tip. A lawn of the test microorganism (*Salmonella typhi* and *Escherichia coli*) were prepared by dipping a sterile cotton swab into a cell suspension and streaked on the surface of the agar. The bacterial agar plates were incubated at 37°C for 24 hours in the desk top incubator.

After 24 hours' incubation, the bacterial growth was observed on the agar plates. Plant extract for each plant specimen was prepared by dissolving 100mg of aqueous plant extract into 1ml of distilled water. Hundred milligram (100mg) of ethanol plant extract was dissolved into 1ml of 70% ethanol. Each well was filled with a volume (20–100 µL) of a specific plant extract.

The bacterial agar plates were incubated at 37°C for 24 hours in the desk top incubator. Active antimicrobial agent diffused in the bacterial agar and inhibited the

growth of the microbial strain tested. The antibacterial activity was assessed by measuring the diameter in millimeters (mm) of inhibition zone. Inhibition zone is the area around the plant extract disc where bacteria are unable to grow. Antibacterial potential of different plant species was assessed by comparing diameter of the inhibition zone.

3.8. PHYTOCHEMICAL ANALYSIS

Instruments and reagents:

Dry ground plant specimens, weighing boat, scale, spatula, conical flask, distilled water, whatman filter paper, glass beakers and hotplate (Figure 3.22).

Figure 3. 22 Hotplate.

Experimental method

The phytochemical analysis was performed for the five most commonly used medicinal plants chosen for the study. The standard procedure was used for screening of the phytochemicals as described by (Vishnu *et al.*, 2019, Vinoth *et al.*, 2012, Vijisaral and Arumugam, 2013). The aqueous extracts were tested for the presence of tannins, alkaloids, saponins, flavonoids and terpenoids by using the procedures outlined below.

Decoction was prepared by weighing 20g of dry ground plant specimen into a glass beaker in which 200ml of distilled water was added and boiled for 15 minutes. The

mixture was then allowed to cool for 10 minutes at room temperature. The decoction was filtered using whatman filter paper and used to test for the presence of tannins, alkaloids, saponins, flavonoids and terpenoids.

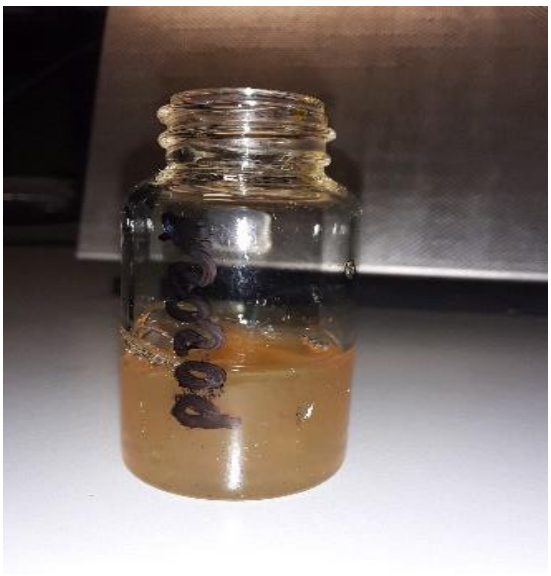
3.8.1 Tannins

The ratio of 2.5 ml of the decoction to 10ml of distilled water was added into a transparent glass vial and 3-4 drops of 10% ferric chloride solution was then added. A blue or green colour observed indicated the presence of tannin (Figure 3.23). The colour differed depending on the type of tannin.

(a)

(b)

(c)



(d)

(e)

(f)

(g)

Figure 3. 23 Tannin test.
a- *Ozoroa reticulata* roots, b- *Punica granatum* fruit peels, c- *Sclerocarya birrea bark*, d- *Pouzolzia mixta* roots, e- *Psidium guajava* roots, f- *Psidium guajava* bark and g- *Psidium guajava* leaves.

3.8.2 Alkaloids

The ratio of 2.5 ml of the decoction to 10ml of distilled water was added into a transparent glass vial and few drops of picric acid solution was added slowly. The mixture was shaken and colour change was observed. An orange to red coloration indicated the presence of alkaloids (Figure 3.24).

(a)

(b)

(c)

(d)

(e)

(f)

(g)

Figure 3. 24 Alkaloids test.
a- *Ozoroa reticulata* roots, b- *Punica granatum* fruit peels, c- *Sclerocarya birrea* bark, d- *Pouzolzia mixta* roots, e- *Psidium guajava* roots, f- *Psidium guajava* leaves and g- *Psidium guajava* bark.

3.8.3 Saponins

Saponins were detected using the froth test. The ratio of 2.5 ml of the decoction to 10ml of distilled water was added into a transparent glass vial. The vial was closed

with a lid and shaken vigorously for about 30 seconds. It was then allowed to stand for half an hour. Honeycomb froth indicated the presence of saponins (Figure 3.25)

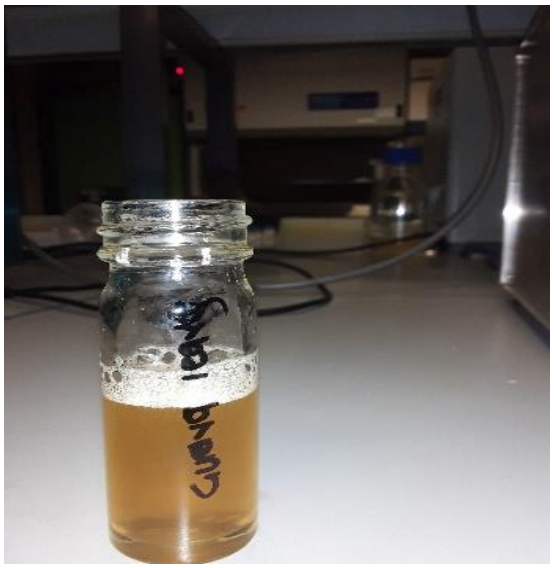
(a)

(b)

(c)

(d)

(e)



(f)

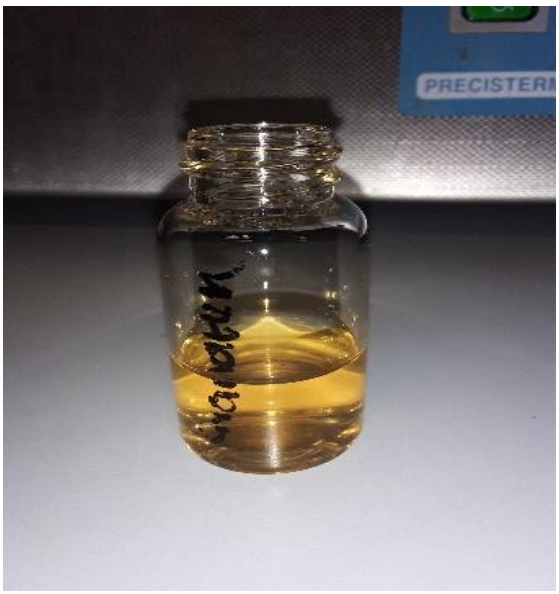
(g)

Figure 3. 25 Detecting saponins using the froth test.
a- *Ozoroa reticulata* roots, b- *Punica granatum* fruit peels, c- *Sclerocarya birrea* bark, d- *Pouzolzia mixta* roots, e- *Psidium guajava* roots, f- *Psidium guajava* leaves and g- *Psidium guajava* bark.

3.8.4. Flavonoids

The ratio of 2.5 ml of the decoction to 10ml of distilled water was added into a transparent glass vial. Every 4ml of the decoction was treated with 1.5 ml of 50% methanol solution. The solution was warmed, 5 to 6 drops of concentrated hydrochloric acid was added and red colour was observed for flavonoids and orange colour for flavones (Figure 3.26)

(a)



(b)

(c)

(d)

(e)

(f)

(g)

Figure 3. 26 Flavonoids test.
a- *Ozoroa reticulata* roots, b- *Punica granatum* fruit peels, c- *Sclerocarya birrea* bark, d- *Pouzolzia mixta* roots, e- *Psidium guajava* roots, f- *Psidium guajava* leaves and g- *Psidium guajava* bark.

3.8.5 Terpenoids

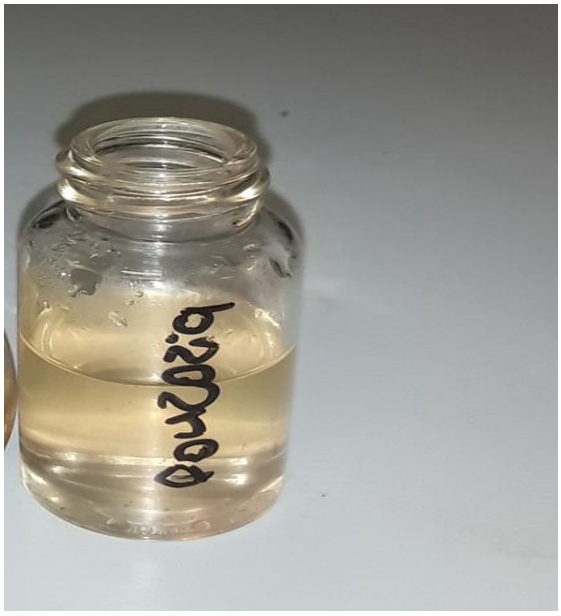
The ratio of 2.5 ml of the decoction to 10ml of distilled water was added into a transparent glass vial. Every 4ml of the decoction was mixed with 0.5ml of acetic anhydride and 0.5ml of chloroform. Drops of concentrated sulphuric acid were added

slowly. The mixture was shaken; the reddish brown colour was formed to indicate the presence of terpenoids (Figure 3.27).

(a)

(b)

(c)



(d)

(e)

(f)

(g)

Figure 3. 27 Terpenoids test.

a- Ozoroa reticulata roots, b- Punica granatum fruit peels, c- Sclerocarya birrea bark, d- Pouzolzia mixta roots, e- Psidium guajava roots, f- Psidium guajava leaves and g- Psidium guajava bark.

Chapter 4: Results

4.1 Medicinal plants used to treat diarrhoea

The communities in Mogalakwena and Mookgopong municipalities use different plant species to treat various illnesses and they have rich knowledge in the use of medicinal plants. Of the 30 community members consulted, 16 (53%) were Tsonga speaking and 14 (47%) were Northern Sotho speaking i.e. Sepedi speaking (Figure 4.1). The age of participants ranged between 27 years to 83 years of age, 70% of which were diviners and 30% were herbalists (Table 4.1).

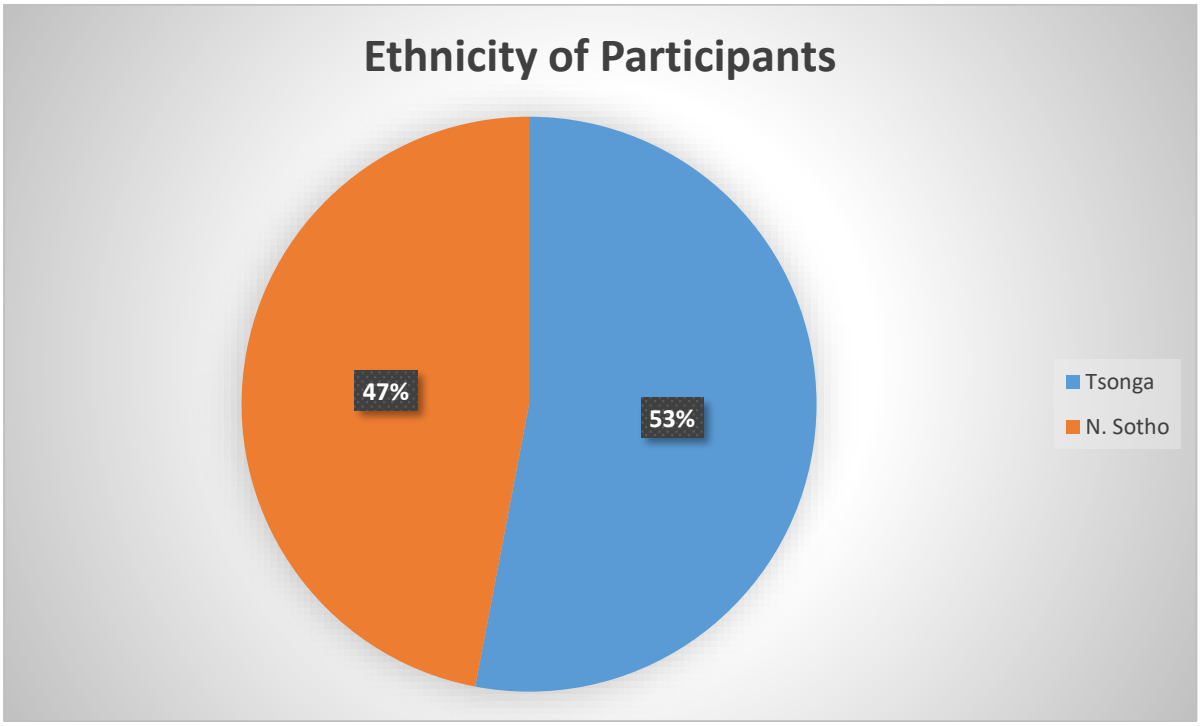


Figure 4. 1 Percentage of participants according to their ethnicity.

Table 4. 1 Participants population structure of the data collected from the thirty traditional healers participated in the study of medicinal plants used to treat foodborne pathogens in Mogalakwena and Mookgopong Municipalities in the Waterberg district in Limpopo province South Africa in June and July 2019.

Participants category	Males	Females	Age groups			
			18-30	31-40	41-60	Above 60
Diviners	9	12	2	5	5	9
Herbalists	5	4	-	1	3	5
Percentage	46.67%	53.33%	6.67%	20.00%	26.67%	46.67%

4.1.1 Other medicinal uses of the 19 reported plant species

A total of 19 plant species were mentioned during questionnaire interviews (Table 4.2), belonging to 12 different plant families (Figure 4.2). Some plants species were mixed together to treat diarrheal food borne pathogens either during preparation or were prepared separate but used simultaneously (Table 4.3). According to the participants, *Poulzozia mixta* is commonly used to treat diarrhea in infants and children making it very popular to many mothers.

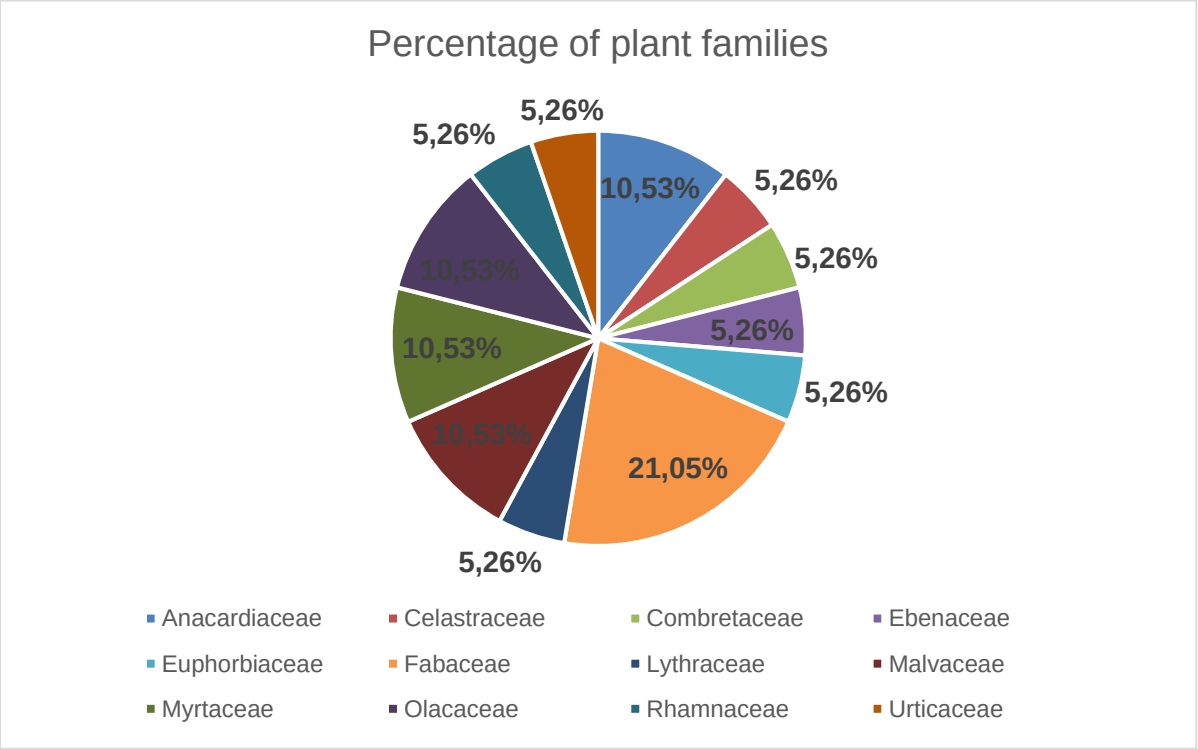


Figure 4. 2 Percentage of plants families of the data collected from the thirty traditional healers participated in the study of medicinal plants used to treat foodborne pathogens in Mogalakwena and Mookgopong Municipalities in the Waterberg district in Limpopo province South Africa in June and July 2019.

Table 4. 2 Plants species used to treat diarrheal and vomiting foodborne pathogens, information collected from 30 participants in Mogalakwena and Mookgopong municipalities.

Family	Scientific name	Common name in the study area	Growth form	Conservation status	Parts used	Parts used, uses and preparation methods by participants,	Other medicinal uses by other sources	References
Urticaceae	*Poulzozia mixta* Sohms	Nthadzwa (N. Sotho); Xirheti (Tsonga)	Shrub	Least Concern	Roots	Soak roots in tap water for two to three days and drink for few days to stop diarrhea in infants.	Roots are used to treat diarrhea and dysentery. Roots also used to treat infertility in woman. Expel retained placenta. Treat painful uterus and as contraceptives.	Appidi *et al.* (2008) and Sewani-Rusike (2013)
Anacardiaceae	*Sclerocarya birrea* (A. Rich.) Hochst.	Morula (N. Sotho); Nkanyi (Tsonga)	Tree	Least Concern	Bark	Boil the bark, store decoction and drink for few days. Decoction used to treat abdominal pain, diarrhea and is also used to change the gender of unborn child. A mixture of a bark and roots is used as laxative.	Bark used to treat stomach pain, diarrhea, dysentery, fever and malaria. Bark, leaves and roots used to treat diarrhea and stomach pain. Roots used to treat sore eyes, pharyngitis, goitre, and splenomegaly.	Chauke *et al.* (2015) and de Wet *et al.* (2010)
Myrtaceae	*Psidium guajava*	Guava (N.	Tree	Least	Roots,	Boil either roots	Leaves used to	de Wet *et al.*

	L.	Sotho and Tsonga)		concern	bark and leaves	or bark or leaves or mix all together, store decoction and drink for few days. Decoction used to treat stomach pain and diarrhea. Leaves treat diabetes, helps with weight loss and treat cancer.	treat diarrhea, diabetes, fever, cough, ulcers, boils, wounds and malaria.	(2010), van Wyk *et al.* (2005) and van Wyk and Wink (2004)
Anacardiaceae	*Ozoroa reticulata* (Baker f.) Engl.	Monoko (N. Sotho); Xinugu mafi (Tsonga)	Tree	Least Concern	Roots	Boil the roots, store decoction and drink for few days. Decoction used to treat abdominal pain and diarrhea.	Roots and bark used to treat diarrhoea, kidney, liver, ulcer, throat infection and chest pain. Bark and leaf paste is applied skin to treat skin infections and diseases. Roots infusion taken by woman to increase lactation after child birth.	Katsukunya *et al.* (2021)
Lythraceae	*Punica granatum* L.	Granat (N. Sotho and Tsonga)	Tree	Least Concern	Dry fruit peels	Finely grind the fruit peels and mix with soft porridge or drink	Fruit peels used to treat diarrhea and stomach pain. Root bark	Mongalo and Makhafola, (2018), van Wyk and

						with cold water. Fruit peels used to treat stomach pain and diarrhea.	used to treat internal parasites.	Wink (2004) and van Wyk *et al.* (2005)
Rhamnaceae	*Ziziphus mucronata* Willd.	Mphasamhala (Tsonga); Mokgalô, (N. Sotho	Tree	Least Concern	Roots	Boil the roots, store decoction and drink for few days. Roots decoction used to treat diarrhea, abdominal pain, diabetes and snake bite.	Roots used to treat diabetes, STI, infertility and erectile dysfunction. Bark, leaves and roots used to treat diarrhea and dysentery. Warm bark infusion used to treat cough and chest pain. Roots and leaves used to treat boils, sores and granular swelling.	Chauke *et al.* (2015), Olajuyigbe and Afolayan (2012) and van Wyk *et al.* (2005)
Fabaceae	*Schotia brachypetala* Sond.	Nwabilombe (Tsonga); Molope (N. Sotho)	Tree	Least Concern	Roots and bark	Boil either roots or bark, store decoction and drink for few days. Decoction used to treat nausea, diarrhea, heartburn and hangover.	Stem, bark and roots used to treat stomach illnesses, diarrhea, dysentery, heart burn, hangover and nervous heart condition.	Chauke *et al.* (2015) and de Wet *et al.* (2010), Mongalo and Makhafola (2018), Olajuyigbe and Afolayan (2012)
Combretaceae	*Combretum*	Mokgwethe (N.	Tree	Least	Roots	Boil the roots,	Leaves used as	Palgrave

	molle R.Br. ex G. Don	Sotho); Xipapa (Tsonga)		Concern		store decoction and drink for few days. Decoction used to treat abdominal pain, diarrhea and headache.	antiseptic for wounds and treat fever. Leaves and roots used as antidote for snakebite. Roots used to induce abortion and relief constipation. Bark used for stomach pain.	(2002)
Malvaceae	*Dombeya rotundifolia* Hochst.	Mohlabaphala (N. Sotho); Mbewula (Tsonga)	Tree	Least Concern	Roots	Boil the roots, store decoction and drink for few days. Decoction used to treat abdominal pain and diarrhea.	Treat diarrhea, dysentery, hemorrhoids, chest pain, in pregnant woman used for nausea and for delayed labour.	van Wyk *et al.* (2005).
Fabaceae	*Indigofera daleoides* Benth. ex Harv.	Indigo (Tsonga and N. Sotho)	Perennial herb	Least Concern	Roots	Boil the roots, store decoction and drink for few days. Decoction used to treat stomach pain and diarrhea.	Whole plant used to treat diarrhea.	Gerometta *et al.* (2020)
Myrtaceae	*Syzygium cordatum* Hochst. ex Krauss	Muhlwa (N. Sotho) and Mooi (Tsonga)	Tree	Least Concern	Bark	Boil the bark, store decoction and drink for few days. Decoction used to treat stomach pain, diarrhea and	Bark, leaves and roots used to treat stomach pain, diarrhea, respiratory illness and tuberculosis.	van Wyk *et al.* (2005)

						persistent cough.		
Euphorbiaceae	*Tragia dioica* Sond.	Mmabetane (N. Sotho) and Mbebetani (Tsonga)	Forb	Not listed	Roots	Boil the roots, store decoction and drink for few days. Decoction used to treat nausea, stops the vomiting and treat diabetes.	Leaves used to treat tuberculosis	Semenya and Maroyi (2018)
Fabaceae	*Elephantorrhiza elephantina* (Burch.) Skeels	Mositsane (N. Sotho) Nhwenyane (Tsonga)	Forb	Least Concern	Roots	Boil the roots, store decoction and drink for few days. Decoction used to treat abdominal pain, diarrhea and STI.	Roots used to treat diarrhea, dysentery, stomach disorder, ulcer, hemorrhoids and eye infection.	Maroyi (2016), Mongalo and Makhafola (2018)
Malvaceae	*Grewia bicolor* Juss.	Guguna (Tsonga); Kukuna (N.Sotho)	Shrub	Least Concern	Roots	Boil the roots, store decoction and drink for few days. Decoction used to treat diarrhea, snakebites and chest pain.	Leaves used to treat tuberculosis	Semenya and Maroyi (2018)
Celastraceae	*Gymnosporia senegalensis* (Lam.) Loes	Se/mophatho (N Sotho); xihlangwa (Tsonga)	Shrub	Least Concern	Roots	Boil the roots, store decoction and drink for few days. Decoction used to treat diarrhea, lung infection, heavy menstruation and prevent	Roots used to relive chest pain, treat blood cough and snakebite. Leaves used to treat snakebite.	Palgrave (2002)

						abortion.		
Ebenaceae	*Diospyros mespiliformis* Hochst. ex A.DC.	Mgula (Tsonga); Mogula (N. Sotho)	Tree	Least Concern	Roots	Boil the roots, store decoction and drink for few days. Decoction used to treat diarrhea, stomach pain, internal parasites and works as antibiotics for other diseases.	Leaves, twigs and bark used to treat leprosy, ringworm, fever, dysentery and wounds.	Palgrave (2002)
Fabaceae	*Dichrostachys cinerea* (L.) Wight & Arn.	Moretshe (N. Sotho) and Mtetemba (Tsonga)	Tree	Least Concern	Roots	Boil the roots, store decoction and drink for few days. Decoction used to treat abdominal pain, diarrhea, ease the pain on the insects and snake bites.	Roots used to treat STI, infertility and erectile dysfunction. Treat Sprain, inflammation and wounds.	Chauke *et al.* (2015), Zenebe *et al.* (2012) and Maroyi (2016)
Olacaceae	*Ximenia americana* L.	Motshidi (N Sotho); Ntsengele (Tsonga)	Shrub	Least Concern	Roots	Boil the roots, store decoction and drink for few days. Decoction used to treat diarrhea, abdominal pain, mental illness, fever and bilharzia	Leaves used to stop vomiting, get rid of leech, treat tonsils.	Zenebe *et al.* (2012)
Olacaceae	*Ximenia caffra* Sond.	Morokologa (N. Sotho)	Shrub	Least Concern	Roots	Boil the roots, store decoction	Roots used to treat STI, leaves	Chauke *et al.* (2015) and

						and drink for few days. Decoction used to treat diarrhea, abdominal pain, infertility and nausea during pregnancy.	used to treat eye pain and bilharzia. Roots used to treat diarrhea.	Maroyi (2016)

Table 4. 3 Medicinal plants used in combination to treat diarrheal food borne pathogens by participants Mogalakwena and Mookgopong municipalities.

Plant Species	Family	Growth form	Parts used	other medicinal plants added
Sclerocarya birrea	Anacardiaceae	Tree	Bark	*Ziziphus mucronata*
Ziziphus mucronata	Rhamnaceae	Tree	Roots	*Sclerocarya birrea*
Elephantorrhiza elephantina	Fabaceae	Forb (underground tree)	Roots	*Grewia bicolor*
Schotia brachypetala	Fabaceae	Tree	Roots and bark	*Schotia brachypetala, Combretum molle* and *Dombeya rotundifolia*
Combretum molle	Combretaceae	Tree	Roots	
Dombeya rotundifolia	Malvaceae	Tree	Roots	

The 19 mentioned plants species were dominated by 10 trees (52.63%), followed by 6 shrubs (31.58%), 2 forbs (10.52%) and 1 herb (5.26%) (Figure 4.3). The plant parts used for treatment were 89.47% roots, 10.53% bark, 5.26% leaves and 5.26% fruitpeels or rind (Figure 4.4).

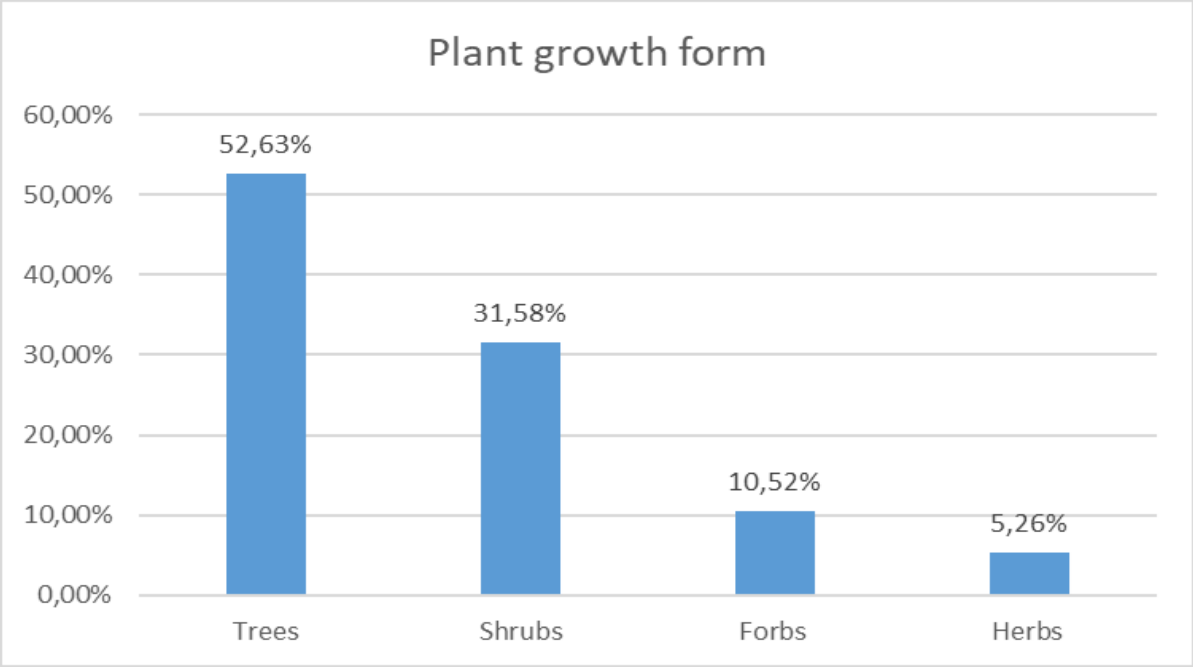


Figure 4. 3 Percentage of plant parts used by thirty traditional healers participated in the study of medicinal plants used to treat foodborne pathogens in Mogalakwena and Mookgopong Municipalities in the Waterberg district in Limpopo province South Africa in June and July 2019.

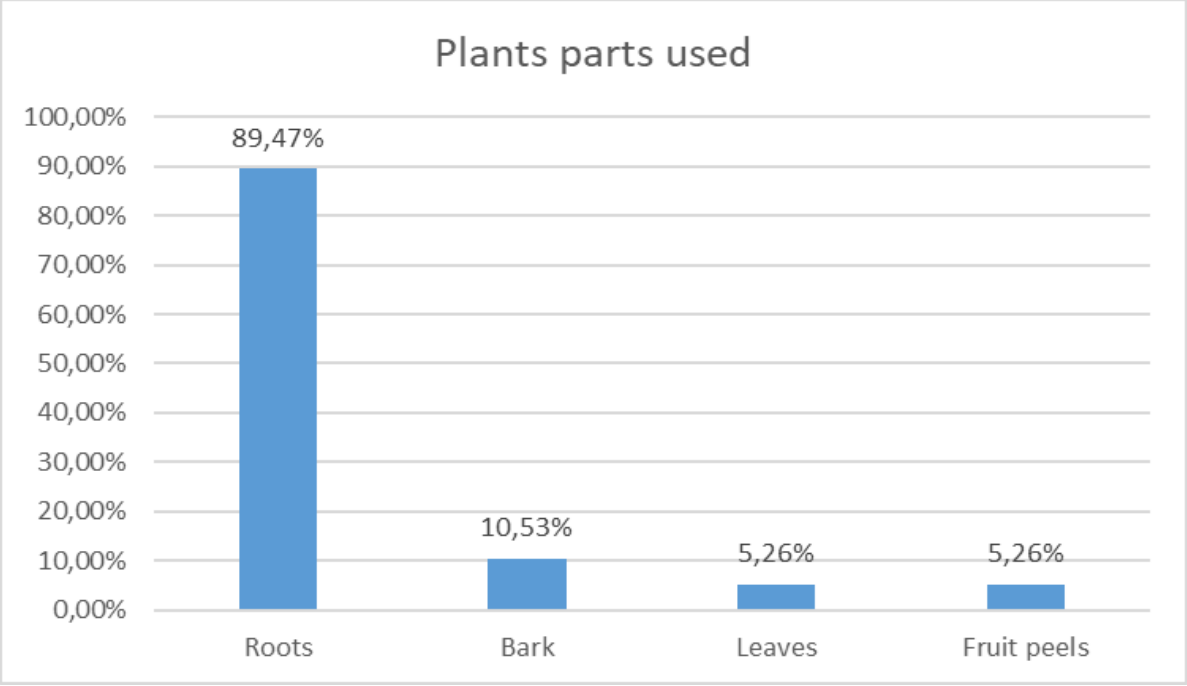


Figure 4. 4 Plant parts used by thirty traditional healers participated in the study of medicinal plants used to treat foodborne pathogens in Mogalakwena and Mookgopong Municipalities in the Waterberg district in Limpopo province South Africa in June and July 2019.

4.1.2 Description of medicinal plants commonly used to treat diarrhoea

Five medicinal plants with the highest frequency (i.e. the most mentioned) (Figure 4.5) were selected for further laboratory study; these are *Poulzozia mixta* 86.67%, *Sclerocarya birrea* 76.67%, *Psidium guajava* 66.67%, *Ozoroa reticulata* 66.67% and *Punica granatum* 63.33%.

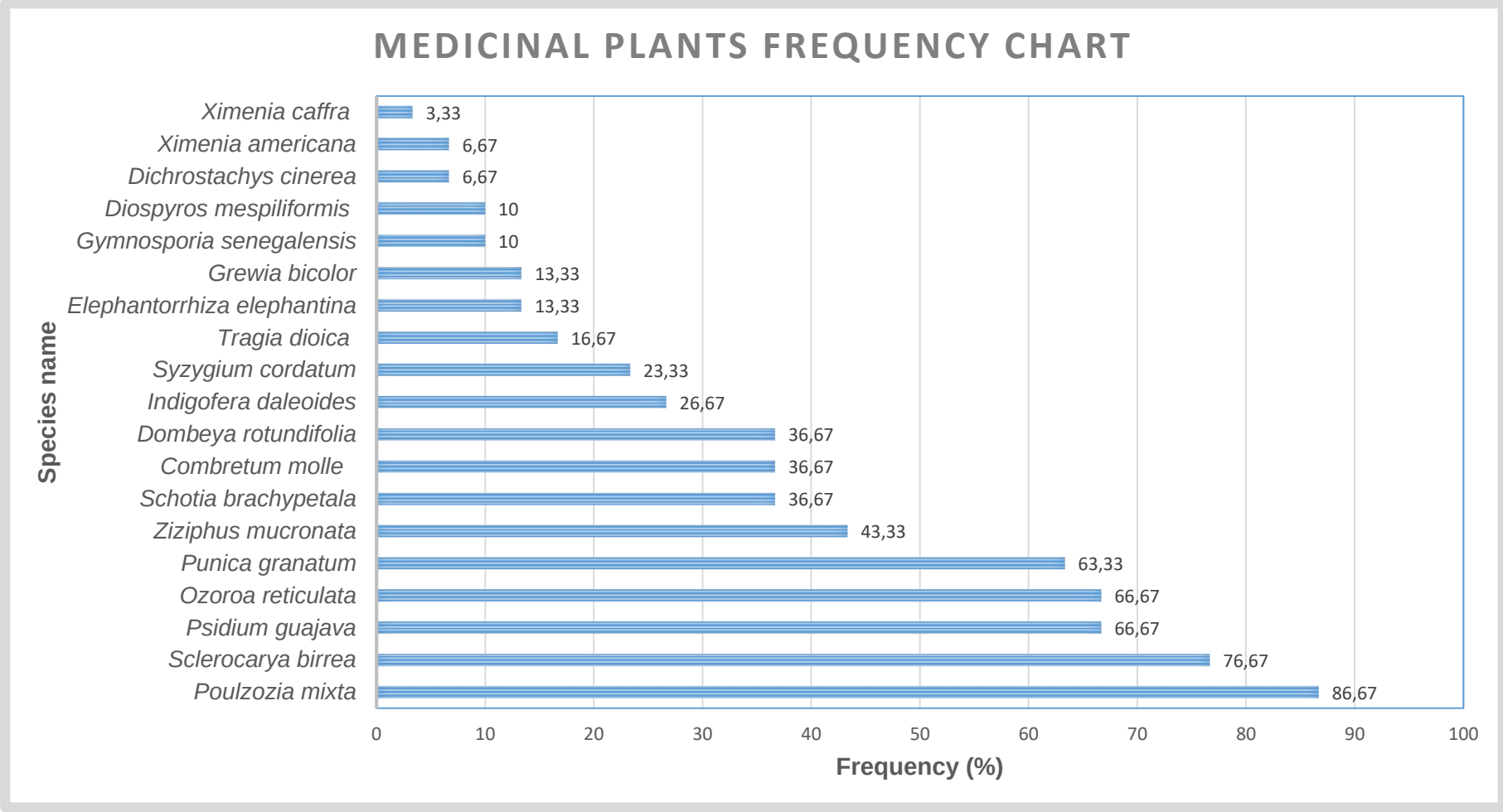


Figure 4. 5 Percentage frequency of plant species names collected from thirty traditional healers participated in the study of medicinal plants used to treat foodborne pathogens in Mogalakwena and Mookgopong Municipalities in the Waterberg district in Limpopo province South Africa in June and July 2019.

4.2 Anti-microbial screening

4.2.1 Yield comparisons between aqueous and ethanol extractions

Researchers commonly use water, ethanol, methanol and acetone for medicinal plants extraction (Wendakoon *et al.*, 2012); however, ethanol and water are reasonably safe for human ingestion hence they were selected extraction solvent for this study. Furthermore, majority of traditional healers commonly use aqueous extraction method for medicinal plants that are usually ingested.

Table 4.4 indicates the percentage yield of aqueous and 70% ethanol extracts for all five plant species. It was observed that the percentage yield of aqueous extracts is higher than the ethanol extracts in all five plants species. The highest percentage yield was obtained from *Psidium guajava* leaves on both aqueous extracts (7.52%) and ethanol extracts (5.09%). *Ozoroa reticulata roots* had the lowest percentage yield on aqueous extracts (5.37%) and *Psidium guajava* bark had the lowest percentage yield on ethanol extracts (3.04%).

Table 4. 4 Aqueous and solvent (70% ethanol) extracts of five commonly used medicinal plants.

Plant species	**Aqueous extracts**			**Ethanol extracts**	
	Colour of the extract	**Recovered mass in grams (g)**	**Percentage yield**	**Recovered mass in grams (g)**	**Percentage yield**
Psidium guajava roots	Dark brown	0.618g	6.18%	0.466g	4.66%
Psidium guajava leaves	Very light brown	0.752g	7.52%	0.509g	5.09%
Psidium guajava bark	Light brown	0.539g	5.39%	0.304g	3.04%
Punica granatum	Yellow	0.656g	6.56%	0.468g	4.68%

fruit peels					
Sclerocarya birrea stem bark	Dark brown	0.689g	6.89%	0.482g	4.82%
Ozoroa reticulata roots	Dark brown	0.537g	5.37%	0.312g	3.12%
Pouzolzia mixta roots	Cream white	0.583g	5.83%	0.377g	3.77%

Percentage yield = $\frac{\text{Recovered mass}}{\text{Initial mass}} \times 100$

4.2.2 The effect of plant extracts against *Salmonella typhi* and *Escherichia coli*.

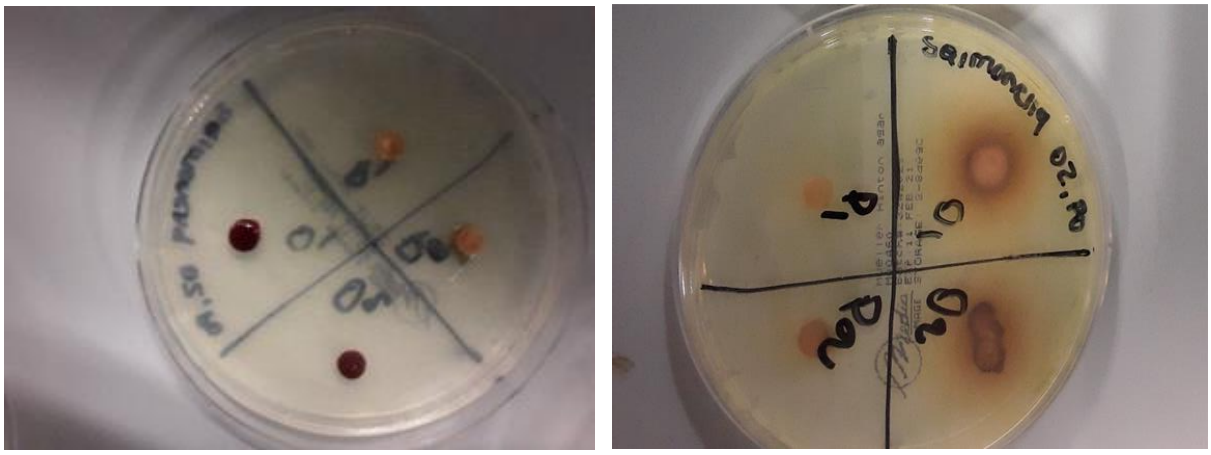
4.2.2.1 Disc diffusion assay

Antimicrobial activity was observed in aqueous extracts of the five plant species against *Salmonella typhi* and *Escherichia coli* using agar disc diffusion method (Table 4.5) and (Figure 4.6). The maximum inhibition zone against *Escherichia coli* was 12mm by *Punica granatum* fruit peels and the maximum inhibition zone against *Salmonella typhi* was 9mm by *Ozoroa reticulata roots*, *Psidium guajava* leaves and bark. No antimicrobial activities were observed from *Pouzolzia mixta* roots and *Psidium guajava* roots on both *Salmonella typhi* and *Escherichia coli*.

Antimicrobial activity was observed in ethanol extracts of the five plant species against *Salmonella typhi* and *Escherichia coli* using agar disc diffusion method (Table 4.5) and (Figure 4.6). The maximum inhibition zone against *Salmonella typhi* is 12mm by *Ozoroa reticulata roots.* No antimicrobial activities were observed from *Pouzolzia mixta* roots and *Psidium guajava* roots. The maximum inhibition zone against *Escherichia coli* was 13mm by *Punica granatum* fruit peels. No antimicrobial activity observed from *Pouzolzia mixta* roots.

Table 4. 5 Inhibition zone for aqueous and ethanol extracts against *Salmonella typhi* and *Escherichia coli* (agar disk diffusion method).

Plant species	Inhibition zone for aqueous extracts (mm)		Inhibition zone for ethanol extracts (mm)	
	Salmonella typhi	*Escherichia coli*	*Salmonella typhi*	*Escherichia coli*
Psidium guajava roots	6	6	6	8
Psidium guajava leaves	9	9	10	10
Psidium guajava bark	9	9	9	9
Punica granatum fruit peels	8	12	10	13
Sclerocarya birrea stem bark	7	10	7	11
Ozoroa reticulata roots	9	8	12	9
Pouzolzia mixta roots	6	6	6	6



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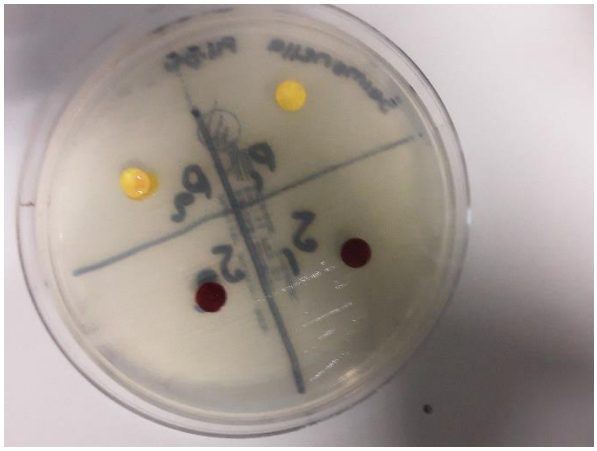

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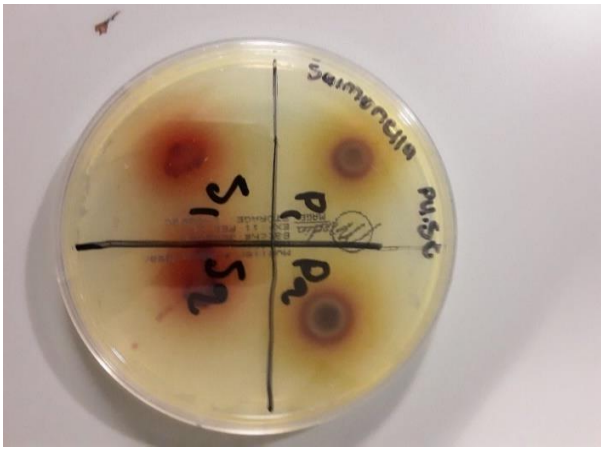

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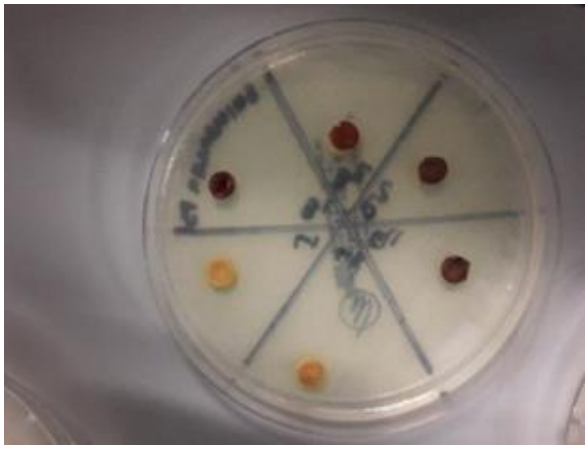

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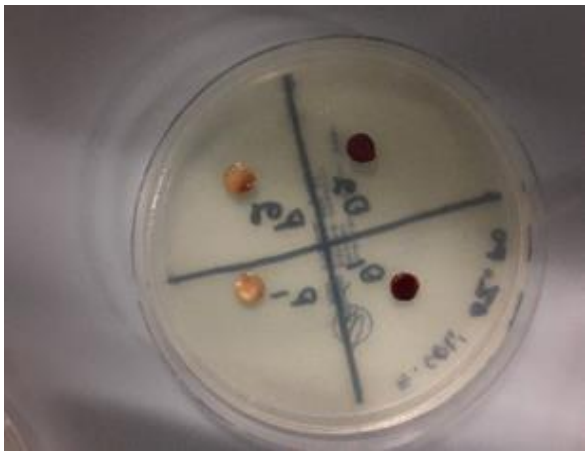

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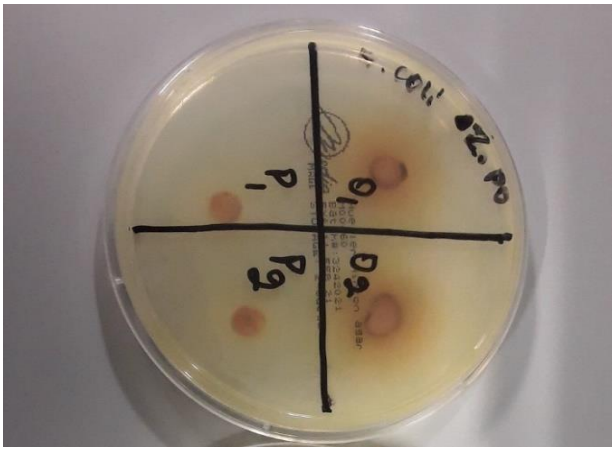

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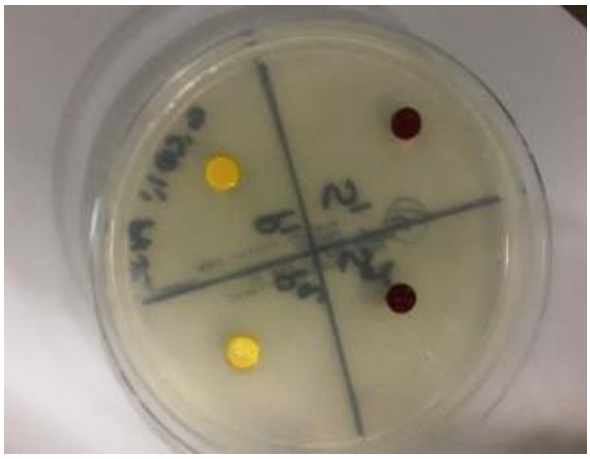

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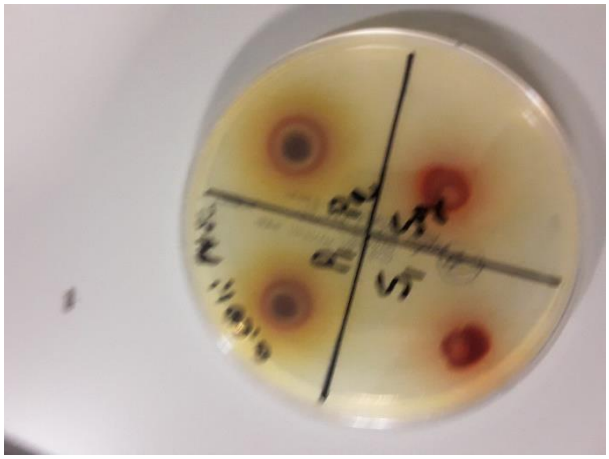

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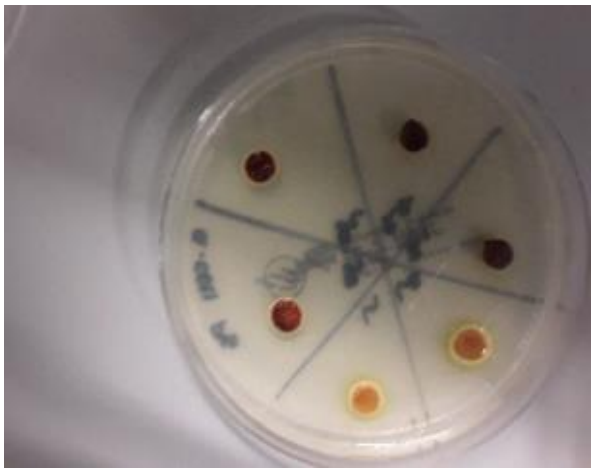

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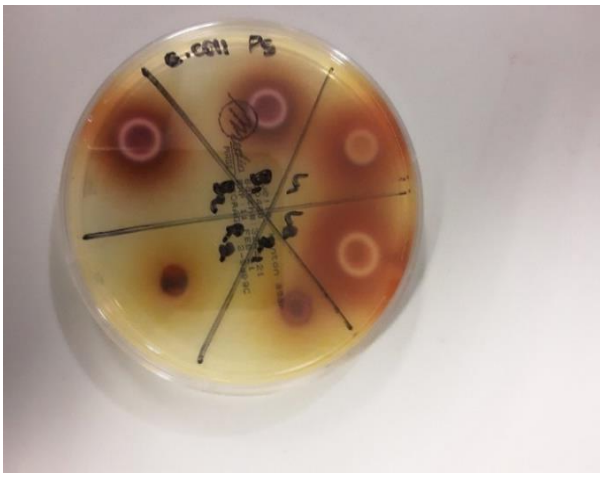


(l)

Figure 4. 6 *Salmonella typhi* and *Escherichia coli* agar disk diffusion method pre- and post-incubation.
***Salmonella typhi* aqueous and ethanol extracts: a**- pre- and **b**- post-incubation *Ozoroa reticulata roots* and *Pouzolzia mixta* roots, **c**- pre and **d**- post-incubation *Punica granatum* fruit peels and *Sclerocarya birrea* stem bark, **e**- pre- and **f**- post-incubation *Psidium guajava* roots, leaves and bark.

***Escherichia coli* aqueous and ethanol extracts: g**- pre- and **h**- post-incubation *Ozoroa reticulata roots* and *Pouzolzia mixta* roots, **i**- pre and **j**- post-incubation *Punica granatum* fruit peels and *Sclerocarya birrea* stem bark, **k**- pre and **l**- post-incubation *Psidium guajava* roots, leaves and bark.

4.2.2.2 Agar-well diffusion assay

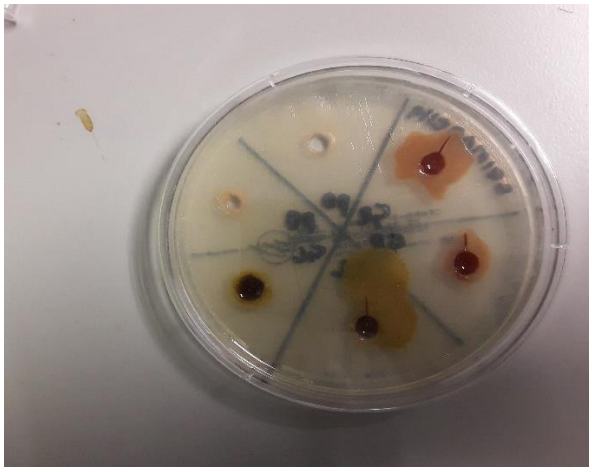
There was observation of antimicrobial activity in aqueous extracts of the five plant species against *Salmonella typhi* and *Escherichia coli* using agar well diffusion method (Table 4.6) and (Figure 4.7). The maximum inhibition zone against *Escherichia coli* was 17mm by *Punica granatum* fruit peels and *Psidium guajava* leaves. The maximum inhibition zone against *Salmonella typhi* was 16mm by *Sclerocarya birrea* stem bark. No antimicrobial activities observed from *Pouzolzia mixta* roots on both *Salmonella typhi* and *Escherichia coli*.

There was observation of antimicrobial activity in ethanol extracts of the five plant species against *Salmonella typhi* and *Escherichia coli* using agar well diffusion method (Table 4.6) and (Figure 4.7). The maximum inhibition zone against *Salmonella typhi* was 18mm by *Psidium guajava* roots and minimum inhibition zone of 7mm by *Pouzolzia mixta* roots. The maximum inhibition zone against *Escherichia coli* was 16mm by *Punica granatum* fruit peels. No antimicrobial activities observed from *Pouzolzia mixta* roots.

Table 4. 6 Inhibition zone for aqueous and ethanol extracts against *Salmonella typhi* and *Escherichia coli* (agar-well diffusion method).

Plant species	**Inhibition zone for aqueous extracts (mm)**		**Inhibition zone for ethanol extracts (mm)**	
	Salmonella typhi	*Escherichia coli*	*Salmonella typhi*	*Escherichia coli*
Psidium guajava roots	11	12	18	13
Psidium guajava leaves	11	17	11	13
Psidium guajava bark	12	14	11	15

Punica granatum fruit peels	14	17	15	16
Sclerocarya birrea stem bark	16	14	17	13
Ozoroa reticulata roots	9	11	8	14
Pouzolzia mixta roots	6	6	7	6



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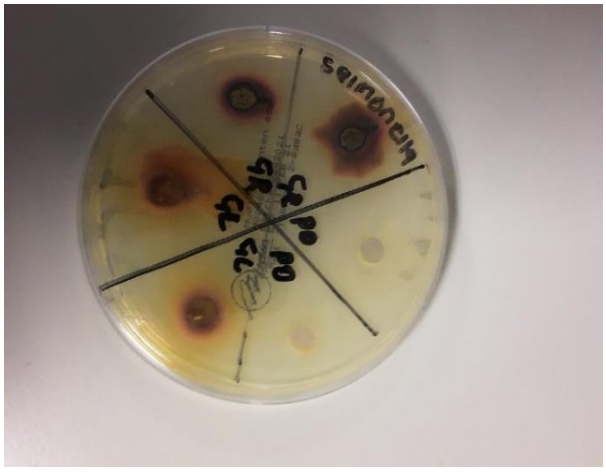

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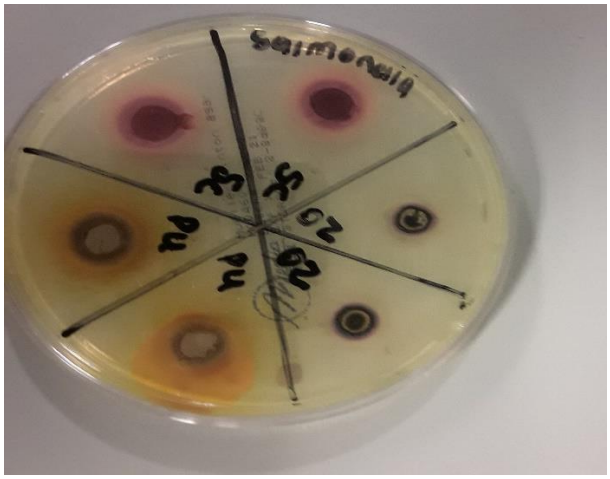
(c)



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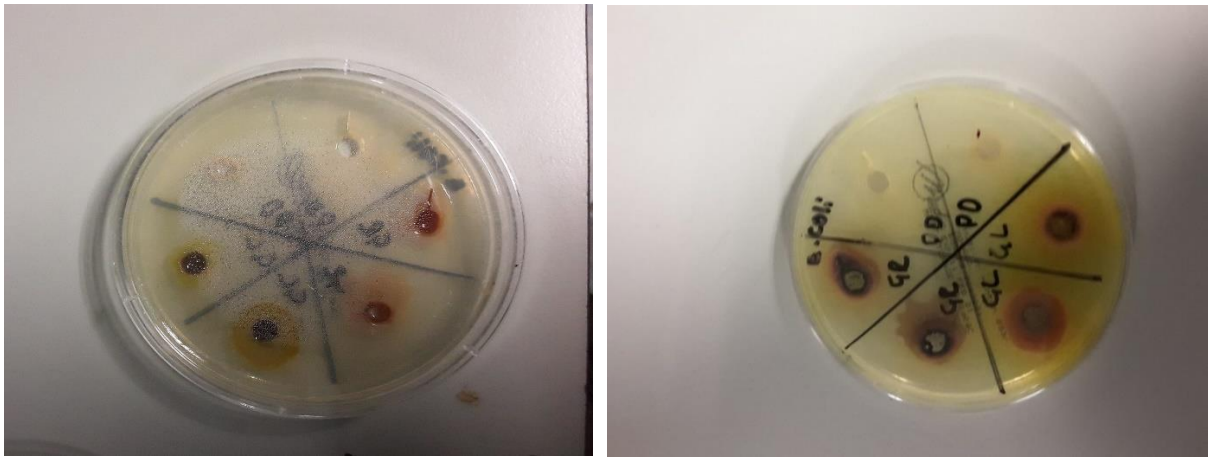

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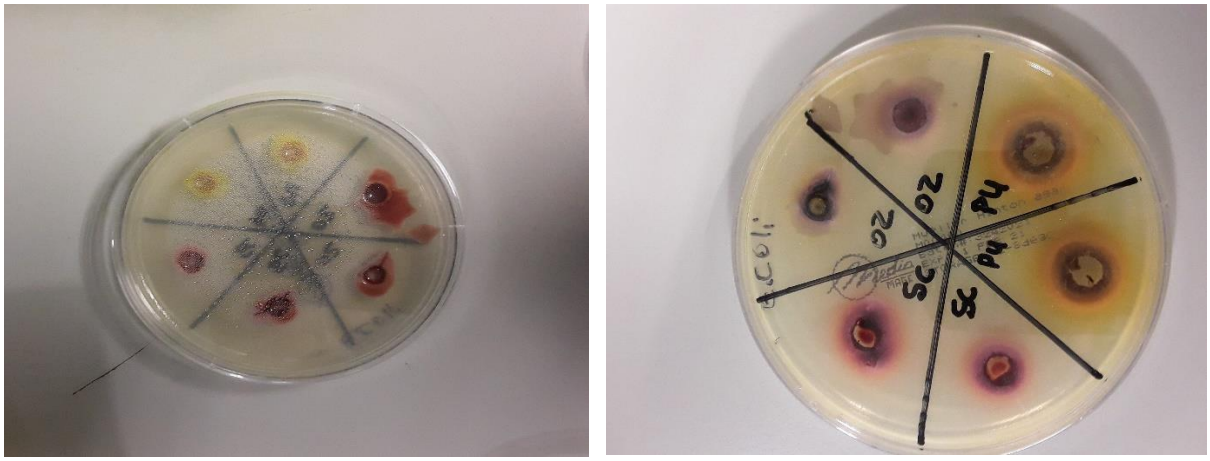

(g) (h)

Figure 4. 7 *Salmonella typhi* and *Escherichia coli* agar-well diffusion method pre- and post-incubation.

***Salmonella typhi* aqueous and ethanol extracts: a**- pre- and **b**- post-incubation *Psidium guajava* roots, leaves and *Pouzolzia mixta* roots, **c**- pre and **d**- post-incubation *Ozoroa reticulata roots*, *Punica granatum* fruit peels and *Sclerocarya birrea* stem bark.

***Escherichia coli* aqueous and ethanol extracts: e**- pre- and **f**- post-incubation Pouzolzia *mixta* roots, *Psidium guajava* roots and leaves, **g**- pre- and **h**- post-incubation *Ozoroa reticulata roots*, *Punica granatum* fruit peels and *Sclerocarya birrea* stem bark.

4.3 Phytochemical analyses

A decoction was prepared for each of the five test plant species and used to test the presence of the five selected phytochemicals, the results are displayed in table 4.7. All the selected five plant specimen indicated the presence of saponins. *Pouzolzia mixta* roots is the only plant species that indicated the absence of tannin out of the five selected plant species. *Psidium guajava* leaves, *Punica granatum* fruit peels and *Pouzolzia mixta* roots indicated the absence of alkaloids. *Punica granatum* fruit peels and *Pouzolzia mixta* roots indicated the absence of flavonoids and terpenoids.

Table 4. 7 Phytochemical test results

Plant species	Saponins	Tannins	Alkaloids	Flavonoids	Terpenoids
***Psidium guajava* roots**	+	+	+	+	+
***Psidium guajava* leaves**	+	+	_	+	+
***Psidium guajava* bark**	+	+	+	+	+
***Punica granatum* fruit peels**	+	+	_	_	_
***Sclerocarya birrea* stem bark**	+	+	+	+	+
Ozoroa reticulata roots	+	+	+	+	+
Pouzolzia mixta roots	+	_	_	_	_

CHAPTER 5: DISCUSSION AND CONCLUSION

Communal lands are the main source of wild medicinal plants for the communities in the study area, the increased demand for medicinal plants is met by destructive harvesting practices which involve harvesting plants species to local extinction, ring-barking of stem bark, frequent collection denying the plant opportunity to regrow and collection of roots by uprooting the whole plant species. Some once effective medicinal plants no longer occur in the study area; locals are left with no choice but to seek alternative replacement.

All community members testified on how certain effective plant species have been harvested out of existence. It was also observed during data collection that harvesting techniques is a major threat to indigenous medicinal plants in the community grazing land. The communities where data was collected are surrounded by open savanna vegetation. Annual rainfall is between 495mm and 599mm, with maximum temperature of just over 30ºC and reaching a low temperature of 6ºC in winter. Herbs and tubers are harvested out of existence in certain areas by uprooting the whole plant during rainy season by few individuals with vast knowledge and experience. Majority of study participants use woody species as they survive all seasons and are easy to identify. They also prefer to use roots over other plant parts as they believe roots have more medical potency than the rest of the plant parts and are not affected by other environmental elements and pollutants.

During data collection, a large piece of land used by the community in Tshamahanzi village for food crop was discovered to have valuable minerals. The community leaders were allocating the land to the community for human settlement and for food crop. This was followed by clearing of natural vegetation including plants that are of medicinal value. As the community grows, habitat destruction becomes more prominent caused by overgrazing, unplanned fires, clearing natural vegetation for agricultural purpose, collecting firewood and construction or human settlement. There

is a rapid decline of medicinal plants in the communal lands, the plants seen today might not be found tomorrow due to lack of management.

Over 70% of participants were over the age 41 years old, and this gave the impression that the young generation do not have interest learning about medicinal plants use. The study area is dominated by Northern Sotho and Tsonga speaking people, and hence the study participants belonged to these two ethnic groups. The majority of participants were female; many households in the study area are led by women and among other challenges they are confronted with treating loved one's ill-health. This forces women to learn about medicinal plants used to treat different illnesses. Some plant species were mentioned/are used more frequently than others to treat food-borne illnesses. The high frequency use of certain species might be the indication of common source of information and might also be influenced by the community beliefs. The least used medicinal plants were used by traditional healers who were trained from other provinces.

Of the 19 plants species mentioned by participants, 15 have been reported to treat diarrheal related diseases by other studies. *Grewia bicolor, Dichrostachys cinerea, Gymnosporia senegalensis* and *Tragia dioica* are reported by other authors to treat other illnesses that are not associated with food borne pathogens.

Not all five commonly used medicinal plants in Mogalakwena and Mookgopong municipalities have antimicrobial activities against the two selected bacteria. *Pouzolzia mixta* on both aqueous and ethanol extract indicated no antimicrobial activities against *Salmonella typhi* and *Escherichia coli*. Traditional healers prepare *Pouzolzia mixta* by soaking the roots in cold water for three days before use and water becomes slimy. With aqueous extraction the mixture turned into a porridge making it even difficult to filter; while, ethanol extract appeared watery. Hence, it is suspected that the extraction method used might have a significant effect on the results attained. It was also observed that ethanol extraction had higher inhibition zone than the aqueous extraction. It was observed that the inhibition zone for agar well method is higher on both aqueous and ethanol extract. It might be due to high concentration used, as a lot

of extract was used to fill up a well since it was seeping through to the bottom of the agar while filling up. Phytochemical analysis of aqueous extract of *Pouzolzia mixta roots* was positive on saponins only; tannins, alkaloids, flavonoids and terpenoids were negative.

In this study, the pathogens responsible for diarrheal food-borne diseases (*Salmonella typhi* and *Escherichia coli*) were challenged with five selected plant species extracted using aqueous and ethanol solvents. Antimicrobial activity was observed from *Sclerocarya birrea* using the agar disc and agar well diffusion method. Abdallah *et al. (*2021), Manzo *et al.* (2017) and Komolafe (2014) also reported antimicrobial activity from the same plant species using different antimicrobial test methods, suggesting that *Sclerocarya birrea* has potential to be used as anti-diarrheal foodborne agent. *Sclerocarya birrea* tested positive to all five selected phytochemicals test done (saponins, tannins, alkaloids, flavonoids and terpenoids). The results obtained are in agreement with the study done by (Komolafe, 2014) using aqueous extracts. Manzo *et al.,* (2017) used ethanol extract to test phytochemicals and obtained negative results on alkaloids and positive results on saponins, tannins and flavonoids.

Antimicrobial activity was observed from *Punica granatum* fruit peels. Khan and Hanee (2011) also observed antimicrobial activity using ethanol and aqueous extracts of *Punica granatum* peels against *Escherichia coli* and other gram-negative bacteria using agar well diffusion method. Dahham *et al.,* (2010) also reported antimicrobial activities using methanol and aqueous extracts of *Punica granatum* peels against *Escherichia coli* and other gram-negative and positive bacteria using agar well diffusion method. Phytochemicals test was performed against the aqueous extract of *Punica granatum* fruit peels, the test results were positive for saponins and tannins. No results or negative results were observed on alkaloids, flavonoids and terpenoids. Khan and Hanee (2011), Chebaibi and Filali (2013), and Dahham *et al.* (2010) also observed positive phytochemical test results against saponins and tannins. However, they are all in contradiction with the negative results observed from flavonoids test. The findings of Chebaibi and Filali (2013) agree with the negative results obtained from alkaloids test.

Psidium guajava bark and leaves proved to have antimicrobial activity, however; *Psidium guajava* roots only showed antimicrobial activities in ethanol extract on agar well method against *Escherichia coli*. Positive results obtained from *Psidium guajava* roots agar well could be due to high concentration of the extract used, as a lot was used to fill up the leaking well. Sanches *et al.,* (2005), Growther and Sukirtha (2018), and Oncho *et al.,* (2021) also observed antimicrobial activities of *Psidium guajava* bark and leaves using different methods. Sanches *et al.,* (2005) attest to lack of antimicrobial activity in *Psidium guajava* roots. Aqueous extract of *Psidium guajava* roots, bark and leaves tested positive to the phytochemicals test done (saponins, tannins, alkaloids, flavonoids and terpenoids). However, Psidium *guajava* leaves tested negative on alkaloids. This results are supported by Oncho *et al.,* (2021), although ethanol extracts were used to perform the test.

Ozoroa reticulata tested positive for all phytochemicals tests done (saponins, tannins, alkaloids, flavonoids and terpenoids). Katsukunya *et al.,* (2021) also obtained positive results on the mentioned phytochemicals. Antimicrobial activity was observed on *Ozoroa reticulata*. This species has not attracted attention of researchers; however, (Katsukunya *et al.,* 2021) tested it against a gram positive bacteria *Staphylococcus aureus* and observed antimicrobial activities.

In conclusion, the extraction and antimicrobial analyses methods used might have presented some limitations to the study. For example, a challenge with agar well diffusion method was that the plant extract seeped through to the bottom of the agar making it difficult to read the results. Hence, agar disc diffusion method is highly recommended for this kind of study. Furthermore, traditional healers either mix together some of the plants species or plant parts during preparation or prepare them separately but use them at the same time. Isolating them for experimental purposes might not be a good representation of the outcome observed by the community. The preparation method of *Pouzolzia mixta* roots used for experimental purposes is not the same as what the community uses. Hence, it comes highly recommended by the community but did not indicate antimicrobial activities against *Salmonella typhi* and

Escherichia coli. *Pouzolzia mixta* roots might not have antimicrobial activities or is effective against is effective against pathogenic microorganisms not included in the study. It would have been more beneficial to test all the plants species mentioned during the study against the two selected bacteria. There is high possibility that some of the not commonly used medicinal plants are more effective than the most commonly used ones. Therefore, further studies need to be conducted in this regard.

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Appendix 1

PARTICIPANT INFORMATION SHEET

Ethics clearance reference number: 2018/CAES/171
Research permission reference number: REC-170616-051

03 December 2018

Title: Antimicrobial activities of medicinal plants used by indigenous people of Mogalakwena and Mookgopong municipalities in the Waterberg district Limpopo province South Africa for the treatment of food-borne pathogens.

Dear Prospective Participant
My name is Agnes Maluleke and I am doing research with Dr Mamokete N.V. Dingaan, a senior lecture in the Department of Life and Consumer Sciences towards MSc Life Sciences at the University of South Africa. We are inviting you to participate in a study entitled Antimicrobial activities of medicinal plants used by indigenous people of Mogalakwena and Mookgopong municipalities in the Waterberg district Limpopo province South Africa for the treatment of food-borne pathogens.

WHAT IS THE PURPOSE OF THE STUDY?
The purpose of this research is to study and document commonly used medicinal plants by indigenous people in Mogalakwena and Mookgopong municipalities in treating bacterial food-borne diseases. Most medicinal plants used by indigenous people have proven to be very effective in treating different kinds of illnesses. Indigenous people have acquired a vast knowledge over decades on the use of medicinal plants endemic to the area in which they live. The skills and knowledge is still in the hands of traditional healers which is either lost or passed on to the next generation by word of mouth. It is however very unfortunate that due to socioeconomic stress, the majority of people leaves the rural areas at a young age to the city in pursuit of a better life. This poses a threat to medicinal plants, because the information is not handed over to the young generation. Medicinal plants are the only hope to cure some of the diseases believed not to have cure. It is very important that scientists put more effort in researching, testing and documenting this rare knowledge for future generation. The conservation of this scarce knowledge and the important role it plays in sustaining life, will in turn eradicate poverty and support development in the community.

WHY AM I BEING INVITED TO PARTICIPATE?
Participants at Mogalakwena and Mookgopong municipalities in the Waterberg district will be randomly selected regardless of their ethnic groups. An estimated 20

participants (traditional healers) with either formal (diviner) or non-formal training (herbalists) in medicinal plants use will be selected for the study throughout the study area. Participants will only be included in the study if they have effectively treated at least one patient infected with food-borne pathogens in the past, presented with the symptoms of vomiting and diarrhoea. Participants should be aged between 18 – 65 years old. The age group between 18 – 65 is chosen because they are mature and have a better understanding and most are able to read and write. The questionnaire will be prepared in English and translated into the language the participants feel comfortable with during data collection. Consent and information of who will participate in the study is obtained from the tribal office.

WHAT IS THE NATURE OF MY PARTICIPATION IN THIS STUDY?

The study involves observing participants preparing medicinal plants for use, structured interviews which consists of a mixture of both open-ended questions and closed-ended questions. The duration of the interview and observations is estimated to be between 30-45 minutes per participant.

CAN I WITHDRAW FROM THIS STUDY EVEN AFTER HAVING AGREED TO PARTICIPATE?

Participating in this study is voluntary and you are under no obligation to consent to participation. If you do decide to take part, you will be given this information sheet to read and be asked to sign a written consent form. You are free to withdraw at any time during the interview and without giving a reason. Every questionnaire form is given a unique number and your names will not be written on the form to ensure confidentiality, hence it will not be possible to withdraw after questioners are submitted.

WHAT ARE THE POTENTIAL BENEFITS OF TAKING PART IN THIS STUDY?

Due to our current lifestyle we are moving away from the use of medicinal plants. They are fast substituted by the use of synthetic drugs. Traditionally there are lots of medicinal plants used to treat some illnesses effectively. There is an urgent need to promote medicinal plants especially when their effectiveness is scientifically proven. It is important for the community to be aware that they are seating with treasure in their garden and nearby natural habitat. This will encourage them to return to nature, protect plant and their natural habitat and promote them globally.

Where it is scientifically proven that certain vegetation contains plants of great medicinal value, the land use planning and development initiatives in the area will focus on strategies that could alleviate the major threats affecting medicinal plant resources in the landscape and encourage their cultivation to enhance their availability and complement ex-and in-situ conservation. Based on the outcome of the study, certain medicinal plants might prove to be highly effective in treating food borne diseases. This will encourage cultivation at large scale, which will in turn elevate poverty.

ARE THEIR ANY NEGATIVE CONSEQUENCES FOR ME IF I PARTICIPATE IN THE RESEARCH PROJECT?

The relationship between traditional healing and western medicine practices is characterized by mistrust, tension and conflict. Because of that patients are unlikely to disclose their visit to traditional healers. This study is will assist in bridging the gap between western medicine and traditional healing practices by providing evidence of the effectiveness of medicinal plants used to treat food-borne diseases. It also ensures confidentiality by identifying participants using unique numbers not their names.

Sharing of medicinal plants knowledge may have negative effect on species publicized to be effective, depending on the population size, the type of plant part harvested and the volume required for use.

Despite the benefits from the use of traditional medicines, there are concerns about the exploitation of traditional medicines, the need to preserve the rights of holders of knowledge and ensure that they receive a fair share of benefits. South Africa has legislations in place to protect indigenous knowledge.

WILL THE INFORMATION THAT I CONVEY TO THE RESEARCHER AND MY IDENTITY BE KEPT CONFIDENTIAL?

You have the right to insist that your name will not be recorder anywhere and that no one, apart from the researcher and identified members of the research team, will know about your involvement in this research. Your name will not be recorded anywhere and no one will be able to connect you to the answers you give. The questionnaire with your answers will be given a unique number and you will be referred to in this way in the data, any publications, or other research reporting methods such as conference proceedings.

Your answers may be reviewed by people responsible for making sure that research is done properly, including the transcriber, external coder, and members of the Research Ethics Review Committee. Otherwise, records that identify you will be available only to people working on the study, unless you give permission for other people to see the records. Your anonymous data may be used for other purposes, such as a research report, journal articles and/or conference proceedings. However, your privacy will be protected in any publication of the information by identifying you with a unique number.

Focus group is a group of individuals usually 6-12 people brought together in a room to engage in a guided discussion of a topic. While every effort will be made by the researcher to ensure that you will not be connected to the information that you share during the focus group, I cannot guarantee that other participants in the focus group will treat information confidentially. I shall, however, encourage all participants to do so. For this reason, I advise you not to disclose personally sensitive information in the focus group.

HOW WILL THE RESEARCHER(S) PROTECT THE SECURITY OF DATA?

Hard copies of your answers will be stored by the researcher for a period of five years in a locked cupboard/filing cabinet at UNISA department of Life Sciences for future research or academic purposes; electronic information will be stored on a password protected computer. Future use of the stored data will be subject to further Research Ethics Review and approval if applicable. Information will be destroyed if necessary e.g. hard copies will be shredded and electronic copies will be permanently deleted from the hard drive of the computer.

WILL I RECEIVE PAYMENT OR ANY INCENTIVES FOR PARTICIPATING IN THIS STUDY?

Participation in this study is voluntary; participants will not be paid to participate in the study.

HAS THE STUDY RECEIVED ETHICS APPROVAL

This study has received written approval from the Research Ethics Review Committee of the College of Agriculture and Environmental Sciences, Unisa. A copy of the approval letter can be obtained from the researcher if you so wish.

HOW WILL I BE INFORMED OF THE FINDINGS/RESULTS OF THE RESEARCH?

If you would like to be informed of the final research findings, please contact Agnes Maluleke on 078 915 3691 or send your email to 35969180@mylife.unisa.ac.za. The findings will be accessible from January 2020 on the completion of the study.
Should you require any further information or want to contact the researcher about any aspect of this study, please contact Agnes Maluleke on 078 915 3691 or send your email to 35969180@mylife.unisa.ac.za.

Should you have concerns about the way in which the research has been conducted, you may contact Dr Mamokete Dingaan, Senior Lecturer, Department of Life and Consumer Sciences, College of Agriculture and Environmental Sciences, Tel: 011 471 3580, E-mail: dingam@unisa.ac.za. Contact the research ethics chairperson of the CAES General Ethics Review Committee, Prof EL Kempen on 011-471-2241 or kempeel@unisa.ac.za if you have any ethical concerns.

Thank you for taking time to read this information sheet and for participating in this study.

Thank you.
Agnes Maluleke

Appendix 2

CONSENT TO PARTICIPATE IN THIS STUDY

I, **……** (Participant name), confirm that the person asking my consent to take part in this research has told me about the nature, procedure, potential benefits and anticipated inconvenience of participation.

I have read (or had explained to me) and understood the study as explained in the information sheet.

I have had sufficient opportunity to ask questions and am prepared to participate in the study.

I understand that my participation is voluntary and that I am free to withdraw at any time without penalty (if applicable).

I am aware that the findings of this study will be processed into a research report, journal publications and/or conference proceedings, but that my participation will be kept confidential unless otherwise specified.

I agree to the data collection method used.

I have received a signed copy of the informed consent agreement.

Participant Name & Surname……. (Please print)

Participant Signature……..Date……..

Researcher's Name & Surname……..(Please print)

Researcher's signature……..Date……

Appendix 3

Questionnaire of medicinal plants used by indigenous people in Limpopo province South Africa to treat food-borne diseases.

Section A

Serial number:……. Date:……

Participant information

Age:…… Gender: F( ) M( )

Race: African( ) White( ) Coloured( ) Asian( ) Other( ) If other specify:……

Occupation: Diviner ( ) Herbalist ( ) Specify years of experience:……

Village name:…….. District:……

Local language:…….....

Plant use information

Plant type:…… Plant common name:……..

Habitat type:…….. Scientific name:……

Parts of plant used: Fruits ( ) Seeds ( ) Flowers ( ) Leaves ( ) Roots ( ) Root bark ( )

Stem ( ) Stem bark ( )

Give brief details of parts used:……

Knowledge about toxicity:…….

Smell: Yes ( ) No ( ) give details:…….

Latex: Absent ( ) Present ( ) give details:……

Colour:……

Disease(s)/symptoms you use the plant against.……

Do you mix it with other plants? Yes ( ) No ( ) If yes specify:…….

How a plant is used: Fresh ( ) Dried ( ) Powdered ( )

Method of preparation for use: Extraction with water, cold ( ) Hot ( ) Boiled ( )

Extraction with local gin ( ) other ( ) If other please specify:……

Mode of administration:……

Dosage:…… Duration of treatment:……..

Status of plant: Wild ( ) or Cultivated ( )

Any other comments:……

Section B.

Have you ever used any of these five medicinal plants in the past to treat diarrhoea and/or vomiting?

Common name	Form	Yes/No	If yes Which plant part
Punica granatum - Pomegranate (English) and Phomegiranete (Tsonga)	Shrub or small tree	Yes/No	
Ziziphus mucronata - Buffalo thorn (English); Mphasamhala (Tsonga) and Mokgalô, Moonaona (N Sotho);	Tree	Yes/No	
Psidium guajava - Guava (English) and Mugwava(Tsonga)	Small to medium tree	Yes/No	
Diospyros mespiliformis - African ebony, jackal-berry (English) and Mgula (Tsonga)	Tree	Yes/No	
Indigofera daleoides - Indigo (Tsonga)	Shrub	Yes/No	

Other disease(s)/symptoms you used the plant against……

Do you mix it with other plants? Yes ( ) No ( ) If yes specify:……

How a plant is used: Fresh ( ) Dried ( ) Powdered ( )

Method of preparation for use: Extraction with water, cold ( ) Hot ( ) Boiled ( )

Extraction with local gin ( ) other ( ) If other please specify:……

Mode of administration:……

Dosage:…… Duration of treatment:……..

Status of plant: Wild ( ) or Cultivated ( )

Any other comments:……

Participant signature……

Thank you for participating in the Study.