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CYTOTOXICITY AND ANTI-MYCOBACTERIAL ACTIVITIES OF *SCLEROCARYA BIRREA* AND *DODONAE VISCOSA ANGUSTIFOLIA* AGAINST *MTB STRAIN*

BY

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DECLARATION

I, Marole Tsireledzo, student number: 14004472, hereby declare that this dissertation submitted for master's degree in microbiology at the University of Venda is my original work and has not been previously submitted to this or any other institution of higher education. I further declare that all sources cited or quoted are indicated and acknowledged by means of a compiled list of references.

03 July 2023

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DEDICATION

This research is dedicated to my mother, my grandmother, and my son.

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ABSTRACT

Background: Tuberculosis is a major public health concern with over 2 billion people currently infected, 8.6 million cases per year and more than 1.3 million deaths annually. Tuberculosis is an infectious disease caused by *Mycobacterium tuberculosis* (MTB) which mainly affects the lungs causing pulmonary TB. In South Africa, the use of the Directly Observed Treatment Short Course (DOTS) strategy is applied where patients have to travel to primary Health care facilities (DOTS clinics) to get their medication. The rising prevalence of multidrug resistant and extensive drug resistant TB throughout the world highlights the critical need for novel anti-tuberculosis compounds/drugs.

Objective: The aim of this study was to determine the cytotoxicity and antimycobacterial activities of Sand Olive and Marula.

Methodology: The leaves (L) and barks (B) of *Dodonaea Viscosa Anguistifolia* (DVA or D) and *S. birrea* (S) plants were collected in the Vhembe district, Limpopo. Separate macerations in methanol (C) and distilled water (H) were done to obtain a total of 8 crude extracts (SBC, SBH, SLC. SLH, DBC, DBH, DLC, DLH). Phytochemical analysis was conducted on all extracts using thin layer chromatography (TLC), the profiling of metabolites was achieved with liquid chromatography-Mass spectrometry (LCMS) method. The antimycobacterial activity against *M. smegmati*s was determined using the microbroth dilution assay. The cytotoxicity of the plant extracts was assessed using the MTT assay.

Results: The percentage yield ranged from 21.07% (DBC) to 4.4% (DBH). The phytochemical screening showed the presence of saponins, cardiac glycosides, steroids, terpenoids and tannins. The BEA solvent system revealed more bands than the CEF solvent system while the EMW solvent system was the least efficient. LCMS method showed sufficient revolving power to separate isomeric forms of several compounds. From the extracts of *DVA and S. birrea*, 508 chemicals were present in all the chromatograms assessed, but only 40 compounds were putatively identified and comprised of flavonoids, phenolics, terpenoids, and saponins. Of the 8 plant extracts that were tested 5 (DLH (50µg/ml (83.5%), (100µg/ml (85.9%)), DBH (50µg/ml (70.9%), (100µg/ml (60.3%)), DBC (50µg/ml (76.6%), 100µg/ml (83.9%) SLH (50µg/ml (66.8%), (100µg/ml (66.1%) and SLC (50µg/ml (79.9%), (100µg/ml (77.1%)) were found to have moderate cytotoxic effects on Vero cells at both treatment concentration while 3 (DLC (50µg/ml (91.5%), (100µg/ml (105%), SBH (50µg/ml (98.7%), (100µg/ml (106.8%) and SBC (50µg/ml (105.3%), (100µg/ml (118.2)) did increase viability of Vero cells. Amongst all the samples screened for antimycobacterial activity, only 3 plant extracts (SBC, DBC and DBH) inhibited the growth of *M. smegmatis*.

Conclusion: The results of the study demonstrate the potential use of *DVA* and *S. birrea* as alternative treatment for tuberculosis. The bark of the plants may contain active compounds with anti-TB activities needing further investigation.

Keywords: Anti-mycobacterial, Cytotoxicity, *Dodonae Viscosa Angustifolia,* LCMS, MTT, *M. smegmatis* and *Sclerocarya Birrea***.**

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LIST OF ABBREVIATIONS

BEA	Benzene: Ethanol: Acetone
CEF	Chloroform: Ethyl acetate: Formic acid
DBC	*D.Viscosa* Bark methanolic extract
DBH	*D.Viscosa* Bark water extract
DLC	*D.Viscosa* Leave methanolic extract
DLH	*D.Viscosa* leave water extract
DPPH	2,2-diphenyl-1-1-picrylhydrazyl
DR-TB	Drug resistant tuberculosis
DVA	*Dodonaea Viscosa Anguistifolia*
EMW	Ethyl acetate: Methanol: Water
FeCl3	Ferric chloride
G	Grams
HCl	Hydrochloric acid
HIV	Human immunodeficiency virus
HPLC	High performance liquid chromatography
IC50	Half maximal inhibitory concentration
LCMS	Liquid chromatography-mass spectrometry
MDR	Multidrug- resistant
M. TB	*Mycobacterium tuberculosis*
Ml	Milliliters
mg/ml	Milligrams per milliliter
Min	Minutes
Mm	Millimeters
NaOH	Sodium hydroxide
RIF	Rifampicin

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Rf	Retention factor
SBC	*S.Birrea bark methanol extract*
SBH	*S.Birrea* Bark water extract
S.birrea	*Sclerocarya birrea*
SLC	*S.Birrea Leave methanolic extract*
SLH	*S.Birrea Leave water extract*
STR	Streptomycin
TB	Tuberculosis
TDR	Totally drug-resistant
TEM	Transmission electron microscopy
TLC	Thin layer chromatography
UV-VIS Spec	Ultraviolet visible spectrophotometry
WHO	World Health Organization
XDR	Extensively drug-resistant
µl	Microliter
µg/ml	Microgram per mililiters

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CHAPTER 1

GENERAL INTRODUCTION

1.1 BACKGROUND

Tuberculosis (TB) is a chronic bacterial illness brought on by the bacterium *Mycobacterium tuberculosis* (*Mtb*) (Obakiro et al., 2020). *Mycobacterium tuberculosis*, an acid-fast bacillus, has rapidly spread over the world, causing major illness and mortality. TB now ranks among the top ten leading causes of mortality in the world caused by a single microbial pathogen (ranking higher than human immunodeficiency virus (HIV) and acquired immune deficiency syndrome (AIDS)) (Swain et al., 2022). Signs and symptoms that can be detected as an early diagnosis of TB is shortness of breath, chest discomfort, decreased appetite, and weight loss as well as chronic cough (Maghfiroh et al., 2021).

TB mostly affects the respiratory system, but it can also affect other organs of the body, leading to pulmonary and extrapulmonary TB. Although tuberculosis affects everyone, those living with HIV/AIDS are more likely to develop active tuberculosis (Zenner, et al., 2015). In 2014, about 9,600,000 persons were infected with tuberculosis, with 5,400,000 men, 3,200,000 women, and one million children afflicted. Approximately twelve percent of these people tested positive for HIV (Sharifi-Rad, 2020). Great strides have been achieved in the treatment of TB during the previous several decades. However, the efficacy of first line antitubercular medicines has been diminished because of the fast appearance and dissemination of multidrug-resistant *Mtb* strains, resulting in treatment failures (Obakiro et al., 2022).

Multi-drug resistant (MDR) TB refers to patients contaminated with the type of TB which is resilient to crucial medications, rifampin (RIF) and isoniazid (INH), whereas XDR-TB occurs when resistance to second-line drugs develop on top of MDR-TB (Sivakumar, 2011). Antibiotics, beginning with penicillin and streptomycin, were widely utilized in the 1940s, revolutionizing medicine by delivering efficient treatments for some common ailments of the day. Resistance to antibiotics shortens antibiotics' life spans thus necessitates an ongoing development of new substances (Nguta, 2015).

Natural medicinal agents are made from naturally existing chemicals wherein plants are being the greatest prevalent sources of natural medicine due to their biological

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and pharmacological diversity (Mathur and Hoskins, 2017). Different medicinal plants have been shown to heal several diseases, and their use is becoming increasingly popular based on the belief that they are harmless and can serve as stimulants of the immune system in the treatment of ailments (Mashamba et al., 2022). Nearly 60% of the world's population, according to the WHO, depends on non-conventional medications for essential health care (Qadir et al., 2007). Historically, natural products have proven to be the most abundant and diverse sources of antibiotics including those used for the treatment of TB (Nguta et al., 2016).

Natural products of plant biodiversity have received considerable attention as potential anti-TB agents since they are a proven template for the development of new molecules against tuberculosis (Anochie et al., 2018). Numerous anti-tubercular substances obtained from traditional medicinal plants show to be promising approaches for tuberculosis therapeutic research (Tuyiringire et al., 2020). The well-known streptomycin (STR) and rifampicin (RIF) are two revolutionizing anti-TB drugs derived from marine resources (Swain et al., 2022). Some of the medicines derived from plants include aspirin (from willow tree bark), digoxin (from the flower of Digitalis lanata) and morphine (from opium); 60% of all medicines are either natural products or secondary metabolites (Mathur and Hoskins, 2017).

Dodonaea viscosa Jacq var. *angustifolia* (L.f) Benth (Gakuubi et al., 2012) and *Sclerocarya birrea* (A.Rich.) Hochst. are South African medicinal plants with wide range of medicinal application (Mcotshana et al., 2022). South African people have historically utilized these herbal plants to treat sore throat, coughing as well as TB symptoms (Nguta et al., 2015).

1.2 RATIONALE

Since 2007, TB has been ranked higher than HIV/AIDS in illnesses with highest mortality rate in the planet and largest cause of death caused by a single infectious agent (Oloya et al., 2022). The rise of drug-resistant and regionally unique TB etiologies has increased the threat in Africa's TB-burdened developing countries, necessitating the hunt for novel therapy protocol which focuses on herbal medicines (Getachew et al., 2022).

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The existing first-line TB medications (isoniazid, rifampicin, pyrazinamide, and ethambutol) were created years back, yet they are losing effectiveness as a result of drug resistance and HIV-related side effects (Anochie et al., 2018). The continued expansion of MDR-TB is currently one of the most important and difficult challenges facing worldwide TB control (Seung et al., 2015), and the recent complexity of tuberculosis chemotherapeutics is due to the rise in the incidence of TB linked with viral diseases like HIV (Nguta et al., 2016).

To achieve global control of this pandemic (Hussain, 2018), modern tuberculosis drugs that can: (1) reduce treatment length; (2) attack MDR or XDR isolates; (3) simplify therapy lessening the number of daily pills required; (4) reduce dosage frequency (for instance, a once-weekly treatment); and (5) be taken with HIV drugs are urgently required (Koul et al., 2011). Rising prevalence of multidrug resistant and extensive drug resistant TB throughout the world highlights the critical need for novel anti-tuberculosis compounds/drugs (Kaur et al., 2015).

There are several indications of remarkable secondary metabolites with antimycobacterial activity in natural sources, indicating that natural products might be a fruitful avenue for the identification of novel anti-TB leads (Sivakumar, 2011). However, traditional medicines can occasionally have negative side effects; hence, further research is necessary to ensure the safety and effectiveness of herbal medicines and procedures used by consultants and clients of folk medicines (Nigussie et al., 2022).

Extraction, isolation, purification, concentration, in-vitro activity, and in-vivo efficacy must all be taken into account when determining phytochemicals from herbal remedies (Mariah et al., 2021). Traditional healers, before using such herbals for healing purposes did not conduct any research analysis of the therapeutic agents in these herbal remedies prior to applying them on clients (Ogu, 2011), necessitating the importance of the research for discovering and establishing the anti-mycobacterial activities of some traditional herbs on MTB together with their toxic effects.

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1.3 OBJECTIVES OF THE STUDY

The main objective of this study was to determine the anti-mycobacterium activities of Sand Olive and Marula against *Mycobacterium Tuberculosis* and their cytotoxicity.

To achieve the main objective of this study the following secondary objectives were followed:

- To obtain the crude extract by maceration
- To profile the components in the plants' extracts using LCMS
- To determine the cytotoxic effects on Vero monkey kidney cells using the MTT assay
- To determine the antimycobacterial activity of the crude extracts using broth-microdilution assay.

1.4 RESEARCH QUESTION

The main question that we need to answer for this study is:

- Do the selected medicinal plants have antimycobacterial activities against mycobacterial tuberculosis as claimed by the traditional healers?

1.5 SIGNIFICANCE OF THE STUDY

The current research will validate the scientific use of these medicinal plants. The results obtained in this study may serve as a guide of therapeutic compounds which can be used in the development of new drugs or supplements. This study might provide additional data towards writing a research article and be a starting point for further investigations. The results obtained might serve as scientific evidence for the community for proper doses and effectiveness of these medicinal plants.

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CHAPTER 2

LITERATURE REVIEW

2.1 TRADITIONAL MEDICINAL HERBS

Traditional herbs were utilized as food and medicinal supplies for centuries. Traditional healing is commonly practiced in African countries, with 80% of the black population claiming to consult traditional therapist (Helwig, 2005). These ancient medicinal plant applications have been tried and proven over the years and passed down from generation to generation (Salmerón et al., 2020). For example, Spilanthes calva or Commelina paludosa, the leaves were boiled, mixed with crushed peppers and taken. The juice of young leaves of Centella asiatica or juice of leaves of Solena amplexicaulis was taken in the raw state and Gymnopetalum cochinchinense fruits were used for prevention of ulcer while Solanum torvum as a preventive measure against leucorrhoea, typhoid and tonsillitis (Anand et al., 2022).

The barks and seeds of Saraca were mashed and taken in the raw state as prevention for irregular menstruation and menorrhagia (Sofowora et al., 2013). Many of these ancient applications and therapies have been developed and marketed as traditional and natural medicines, and they are thought to be safe (Kelmanson, 2000). Medicinal plant constituents serve an important part in therapeutic compounds development efforts (Figure 2.1), acting as both medicines and as framework for the development of novel pharmaceuticals (Anochie et al., 2018).

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Figure 2.1: Schematic diagram of natural products as source of drug discovery (Anochie et al., 2018).

Traditional herbs may be utilized directly as plant crude extracts or indirectly as industrial medications such as pharmaceuticals (Anand et al., 2022). Because they are gentle on the skin and have fewer side effects, the market for herbal medicines is growing rapidly and many herbs are used to treat cardiovascular difficulties, liver illnesses, central nervous system disorders, digestive problems, and metabolic disorders (Sharma et al., 2022). It is reported that traditional healers in India utilizes over 2500 medicinal herbs to treat different conditions such as wounds and fungal infection (Kakkar and Bais, 2014).

Herbal medications or therapeutic plants, as well as their extracts and isolated compounds, have shown a wide range of biological activities (Sharma et al., 2022). Medicinal plants frequently contain a variety of phytoconstituents, many of which have unidentified biological capabilities that might be poisonous or result in medication interactions that are dangerous to human health (Awuchi, 2019). As a result, determining a substance's toxicity is crucial when contemplating its use as a medication, because exposure to poisonous compounds is hazardous to both people and animals (Oloya et al., 2022).

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2.2 MEDICINAL PLANTS USED FOR TB TREATMENT

The World Health Organization defines medicinal herbs as "the sum total of knowledge, skills, and practices based on ideas, beliefs, and experiences unique to many cultures, whether explicable or not, that are used to maintain health and to prevent, diagnose, improve, or cure physical and mental disorders" (WHO, 2002). Utilization of herbal extracts and phytochemicals is extremely important for the prevention of variety illness such as Tuberculosis (Mangwani et al., 2020).

The search for new plant-based drugs is important due to the emergence and widespread of MDR-TB and XDR-TB, that necessitate the development of novel anti-TB drugs. Only few of the plants used by traditional healers to treat respiratory infections have their antimycobacterial activities scientifically tested (Table 2.1).

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Table 2.1: List of medicinal plants used for treating respiratory infections in the Vhembe district.

Name of plants	Family name	Common name	Indigenous names	Parts mostly used	Traditional Usage	References
Dodonaea viscosa angustifolia	Sapindaceae	Gansies kankerbos (Afrikaans)	Mutata-vhana (Venda) Mutepipuma (Shona)	Leaves	Fevers; Colds; Influenza; Sore throat; Stomach trouble; Measles	Cock & Van Vuuren, 2020 Naidoo et al., 2012
Rauvolfia caffra sond	Apocynaeae	Quinine tree	umHlambamanzi/ umKhadluvungu (Zulu) Mundadzi (Venda)	Stem bark	Rheumatism; Pneumonia; Colic; Insomnia; Intestinal worms	Tlhapi et al., 2019
Schinus molle linnaeus	Anacardiaceae	Pepper tree	Xibhaha (Tsonga) Mubibiri (Venda)	Branches with leaves	Ulcers; Respiratory problems; Wounds; Rheumatism; Gout; Diarrhea; Skin diseases; Arthritis	Cock & Van Vuuren, 2020 Salem et al., 2016
Schotia branchypetala sond	Fabaceae	African walnut	Uvovovo (IsiZulu) Mulibi/Mutanswa (Tshivenda)	Bark, Roots and Seeds	Heartburn; Hangovers; Flu symptoms; Purify blood; Treat Nervous system; Heart conditions; Diarrhoea	Sobeh et al., 2016
Tabernaemontana elegans	Apocynaceae	Toad tree	Muhatu **(Venda)** Inomfi/ Umkhahlu **(Zulu)**	Leaves and Roots	Remedy for lung ailments; TB treatment; Remedy for stomachache; Aphrodisiac; Remedy for venereal diseases; Wound wash	Cock & Van Vuuren, 2020 Pallant et al., 2008
Ziziphus mucronata	Rhamnaceae	Buffalo thorn	Isilahla (Zulu) Mutshetshete (Venda)	Leaves; Bark and Roots	Chronic cough; Boils; Toothache; Rheumatism; Swellings; Tuberculosis	Cock & Van Vuuren, 2020 Suliman, 2010 Brunken et al., 2008

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Naturally occurring pure compounds as well as extracts from higher and lower forms of plants, microorganisms and marine organisms have indicated that inhibitory activity against MTB is widespread in nature (Okunade, et al., 2004). Extracts, soaks, suspensions and liquids from traditional herbs components like frond, tuber, trunk, and floret were utilized for years as medicinal therapies against tuberculosis by indigenous folks around the world (Sharifi-Rad, 2020). Many antimycobacterial bioactive chemical compounds have been discovered in the skeletons of natural products, mostly from native vegetation, but also from other creatures like fungi and marine microbes (Anochie et al., 2018).

Plants as well as their natural compounds, which include alkaloids, glycosides, tannins, phenolics, xanthones, quinones, sterols, and triterpenoids, have been proven to show antitubercular action equivalent to currently available antitubercular drugs (Martolia et al., 2020). Saponins, terpenes, anthraquinone, flavones, phlobatannins, and phytochemicals are thought to be accountable for anti-tuberculosis actions (Kumar et al., 2014). *Tabernaemontana elegans* (toad tree) is an alkaloid that is traditionally utilized by African people: they consume tuber extract for pulmonary ailments and chest pains (Anochie et al., 2018). *S.birrea* is a source of anthocyanins, cyanidin-3-*O*-sambubioside (Cy-3-Sa) and cyanidin-3-*O*-glucoside (Cy-3-G) (Manhivi et al., 2022).

2.3 THE SELECTED MEDICINAL PLANTS

Two plant species belonging to two different families were identified for their use in treating respiratory tract infections. Table 2.2 below summarizes the ethnobotanical description of the plants with their common names and their most common usage in traditional medicine. The plants selected belong to the families of Sapindaceae and Anacardiaceae.

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Table 2.2: Biological activities of extracts of the two medicinal plants used for the treatment of respiratory infections in the Vhembe district.

Name of plants	Family name	Phytochemical composition	Scientific studies	References
Dodonaea viscosa angustifolia	Sapindaceae	Tricyclic flavonoids Steroidal compounds Cyclic and acyclic phenolics Pentacyclic saponins Tannins Cardiac glycosides	Antioxidant Anti-inflammatory Cytotoxixity Antibacterial Anti-fungal Anti- diarrheal Anti-mycobacterial	Naidoo et al., 2012 Jeya et al., 2014 Masrullah et al., 2012 Gul et al., 2021
Sclerocarya birrea	Anacardiaceae	Phenolic compounds Tannins and Flavonoids	Anti-oxidant Anti-inflammatory Cytotoxic activity Anti-fungal Anti-parasitic Anti-diabetic	Fotio et al., 2009 Ojewole, 2003 Mai et al., 2019 Russo et al., 2018

2.3.1 *SCLEROCARYA BIRREA*

Sclerocarya birrea (A.Rich.) Hochst *(Anacardiaceae),* also recognized as marula in English and Mufula in Tshivenda, is a medium- to large-sized deciduous tree with an upright trunk and rounded crown (Mabala, 2017). It is one of the plants that was used to feed people in ancient times. Male and female flowers are produced on distinct trees (Figures 2.2 & 2.3), with male flowers providing pollen and female flowers producing the fruit for which the tree is famous. These are green on the tree and yellow when they fall (Feb-June).

Traditional healers combine the bark with other medicinal plants to treat diseases such as syphilis, leprosy, diarrhea, hepatitis, rheumatism, gonorrhea, diabetes, and malaria. Marula has a wide range of medical purposes, the most of which are related to ailments such as dysentery, cold cough, and ulcers (Tapiwa, 2019). Many preparations are made from *S. birrea* leaves and offered as traditional enhanced medicine against diabetes (Do et al., 2020). The powdered bark is used to diagnose the gender of an unborn baby in pregnant women. The bark is an effective treatment

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for hemorrhoids. A drink produced from marula leaves is used to treat gonorrhea (Dossou et al., 2011). It was reported that *S. birrea* has the potential to treat diseases related to *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae* with high efficiency (Mai et al., 2019).

Figure 2.2: The appearance of *Sclerocarya birrea* leaves and its fruits (Image source: South coast Herald).

Figure 2.3: Picture of the *Sclerocarya birrea* tree (Source: Aurum Africa, 2022).

The extract of *S. birrea* roots has been shown to hinder the proliferation of *Candida spp.* and *Cryptococcus neoformans*; it also has significant in vitro antioxidant actions,

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particularly free radical scavenging activity, and shows cytotoxic effects in HepG2 cells (Russo et al., 2018).

2.3.2 DODONAEA VISCOSA ANGUSTIFOLIA (DVA)

Dodonaea Viscosa var. *angustifolia* (L.f) (Family: Sapindaceae). Sand olive (Eng.); sandolien, ysterbos (Afr.); mutata-vhana (Venda); mutepipuma (Shona). DVA is a 5 m high evergreen shrub (Figure 2.4); Its bark is light grey and coarsely fissured. Flowers are produced from April until August (autumn-winter). It is found along a large stretch along the coast from Namaqualand to KwaZulu-Natal, as well as Gauteng, Limpopo, Mpumalanga, and Northwest, and farther north in Mozambique and Zambia. numerous Dodonaea species were frequently and often employed by cultures and health providers for a variety of diseases (Beshah et al., 2020).

Figure 2.4: A picture of *dodonae viscosa leaves* (Source: Gardenia.net)

It is a significant traditional medicinal herb that has been utilized for decades for medical purposes (Kuruppu et al., 2019). According to the literature, *D. viscosa* var. angustifolia is known to have antioxidant, antibacterial, antiviral, and recently

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antimalarial activities (Omosa et al., 2016), and the flavonoids linked to kaempferol are responsible for the plant's antioxidant action (Teffo et al., 2010).

Traditional medicine is made from the leaves, stems, and occasionally the fruits (Figure 2.5) of the plant (Beshah et al., 2020). It is also used to treat a variety of human ailments in Africa and Asia, including diarrhea, gastrointestinal system abnormalities, dermatitis, and rheumatism (Sarainya and Divyabhairathi, 2019; Anodei et al., 2018). DVA leaves are used to treat colds, flu, stomach problems, measles, and skin rashes (Ngabaza et al., 2017).

It was reported that *Dodonaea viscosa* contains tannins, saponins, flavonoids, and terpenoids (Al-Snafi, 2017; Rani et al., 2009). *Dodonaea* species have a high concentration of chemical compounds such as alkaloids, flavonoids, glycosides, terpenoids, anthraquinones, essential oils, and saponin, according to preliminary chemical compound analysis (Beshah et al., 2020). Ngabaza et al (2017) discovered that the confined component flavone-5,6,8-trihydroxy-7-methoxy-2-(4-methoxyphenyl)-4H-chromen-4-one is responsible for its antibacterial activity.

Figure 2.5: Pictures showing leaves and flowers of DVA (Source: Wikimedia Commons)

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2.4 TUBERCULOSIS

2.4.1 GENERALITIES

Tuberculosis (TB) remains a global public health threat. As an aged disease, TB remains the leading cause of human death in 2023 from a single pathogen (Gong et al., 2023). TB is caused by *Mycobacterium tuberculosis* belonging to the family *Mycobacteriaceae*. In developing countries, the human immunodeficiency virus (HIV) pandemic has aggravated the morbidity and mortality by providing high number of susceptible patients which are more likely to be infected by TB (Guinn and Rubin, 2017). TB is extremely infectious through aerosols or droplets from coughs or sneezes (Figure 2.6) and it mostly affects the lungs, while other organs are occasionally affected (Okunade et al., 2004).

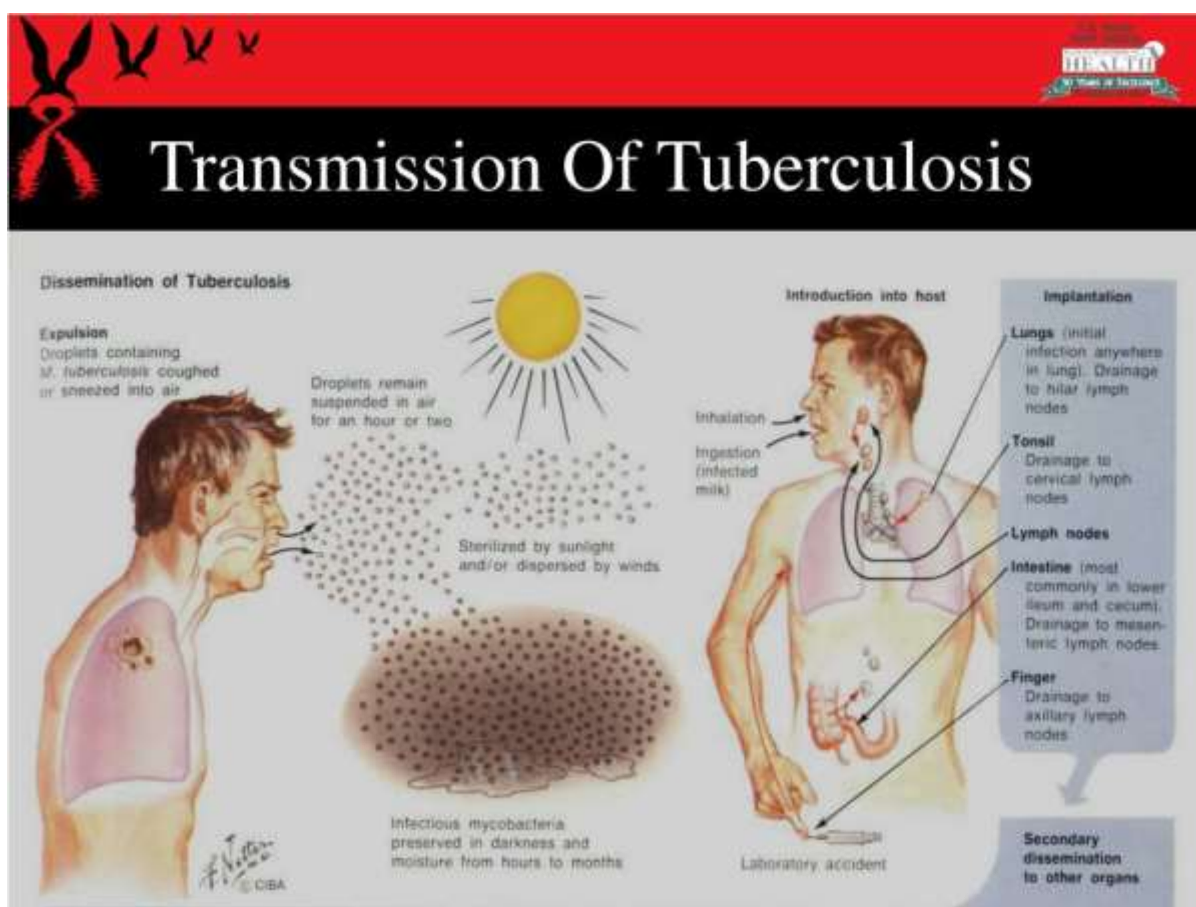

Figure 2.6: Transmission of MTB from one host to another (Source: Marimani et al., 2018)

Recently it was reported that Over 95% tuberculosis cases and death were from countries such as Indonesia, China, Philippines, Pakistan, Nigeria, Uganda, Tanzania

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and South Africa (WHO, 2018). Overwhelming TB burden in African countries is due to many factors including concurrent HIV, public health infrastructure, strategies focused on active disease treatment and less on eradication of latent infection, malnutrition, poverty and other environmental aspects.

In South Africa it was reported that 92% of 236 MDR-TB strains originated in Beijing and was resistant to “four first line: INH, RIF, pyrazinamide, ethambutol and six second line: SM, amikacin, kanamycin, capreomycin, ethionamide and ofloxacin drugs” (Parida et al*.,* 2015). These resistant strains are referred to as “total drug-resistant TB (TDR-TB)” in which to date there have been 30 cases reported in South Africa. TDR-TB poses great challenges for treatment and control strategies.

2.4.2 MYCOBACTERIUM TUBERCULOSIS

With 85 species identified, the genus *Mycobacterium (Mycobacteriaceae)* is quite varied. Some cause illnesses in humans and animals, whereas others are ubiquitous in nature. The 3 leading widespread biological *mtb* human illnesses are *tuberculosis*, *leprosy* (M. leprae), and *buruli* ulcer (M. ulcerans). When common *mycobacteria* are implicated, therapy is very difficult and frequently necessitates administration of clarithromycin probiotic in conjunction with more than one additional antibacterial drugs (Okunade et al., 2004). *TB* bacterial spores are mycobacteria from the *actinobacteria*l collective (MTB complex), that involves *M africanum, M. microtti,* and *M. canetti.* (Sharifi-Rad, 2020).

2.4.3 MYCOBACTERIUM SMEGMATIS

M. smegmatis is used in laboratory experiments for analysing other *Mycobacteria* species. Since it is a "fast-growing" and harmless *mycobacterium*, *M. smegmatis* is frequently employed in *mycobacterium* research (Ward et al., 2008). *Mycobacterium smegmatis* thrives in biofilms, which are communities of cells that are connected to one another. *Mycobacterium smegmatis* is found mostly in soil, water, and plants. They are generally found around vast amounts of water. Unlike certain pathogenic *Mycobacterium*, *M.smegmatis* is categorized like *saprophytes* organisms which seldom leads to illness as it is not reliant in surviving inside a host (Ramaniuk, V.,

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2012.). When *Mycobacterium smegmatis* has been growing for quite some time (generally after 48 hr growth) and is abundant, the color will turn from white to a nonpigmented creamy yellow and it will also be waxy because of the high amount of unique Gram-positive cell wall coated with mycolic acids (Jethva et al., 2016).

2.5 *IN VITRO* ASSAYS OF MEDICINAL PLANTS ON TB

2.5.1 CYTOTOXICITY ASSAY OF MEDICINAL PLANTS

In terms of side effects, medicinal plants are considered safer than modern medicines (Philomena, 2011). Information on the safety of Africans' traditional medicine plants is not usually adequate (Povi etal., 2015).

In vitro cytotoxicity assays represent a well-established model system to assess natural and synthetic substances for potential biological activities and safety (Haudecoeur et al., 2018). The objective of these assays is to monitor the response of cells when exposed to a specific agent or compound. The response is indicated either by a decrease in the number of viable cells (cytotoxicity) or by a decrease in the rate at which the cells proliferate. Moreover, when exposed to a toxic substance, the body cells will initiate physiological processes depending on the level of the injury. The assessment of cell viability is usually performed using several vital dyes such as MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide), INT (Iodonitrotetrazolium chloride), MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-) and XTT ((2,3-Bis(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carbox-anilide)). The principle behind these formazan dyes is that during the cell death, substances that are released (reductase, lactate dehydrogenase) will reduce tetrazolium salts and lead to the formation of chromogenic products showing through a change of coloration (Figure 2.7).

Previous publications show contradictory subchronic toxic effects of marula decoctions, some researchers reported in lowering the number of healthy cells (viable) whereas other reported it to be safe (non-toxic) for use as medicine (Coulidiaty et al., 2021). Major causes of cytotoxicity effects include inaccurate preparations, misidentification or incorrect administration methods; thus, known or documented

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remedies should be administered rather than new and unproven remedies (Philomena, 2011).

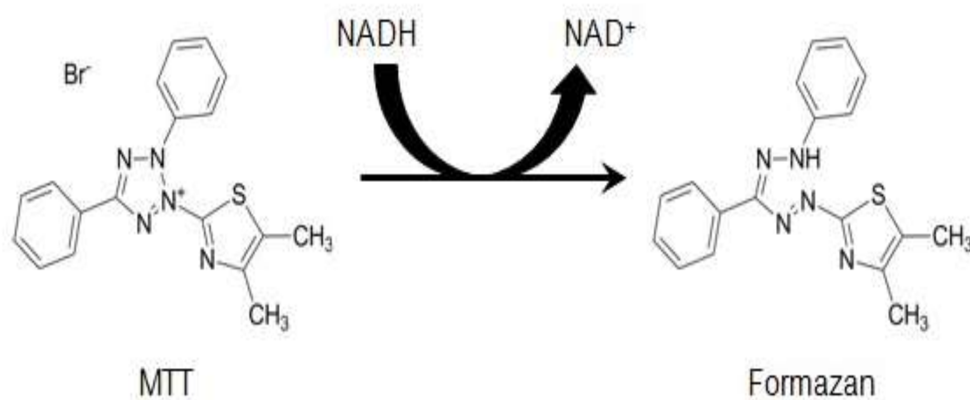

Figure 2.7: Structures of MTT and coloured formazan product after reduction (Riss *et al.*, 2011).

2.5.2 IN VITRO ANTI-TB ASSAYS

M. Smegmatis significance in *in vitro* TB studies

Mycobacterium smegmatis is particularly valuable in cell culture laboratories for research study of other Mycobacteria species. Mycobacterium leprea, Mycobacterium TB, and Mycobacterium bovis are examples of prevalent, hazardous Mycobacterial species. In comparison to these species, Mycobacterium smegmatis is extremely essential since it grows quickly and is non-pathogenic (Ranjitha et al., 2020). Mycobacterium smegmatis grows quickly in most synthetic or complex laboratory media, forming visible colonies in 3-5 days. These characteristics make it an appealing model organism for Mycobacterium tuberculosis and other mycobacterial pathogens. M. smegmatis is also used for mycobacteriophage cultivation (Wu et al., 2018). Mycobacterium smegmatis shares many characteristics with Mycobacteria, which are far more aggressive obligate pathogens. There are several similarities between M. smegmatis and other harmful, infectious Mycobacterium species such as Mycobacterium tuberculosis and the main similarity is the mycothiol biosynthesis to produce basic thiol, which is essential for Mycobacterium spp growth (Ranjitha et al., 2020).

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In vitro Assays for TB Testing

Drug susceptibility testing: Broth method using M. Smeg

The emergence of Antimicrobial Resistance (AMR) is a very worrying phenomenon, which has been identified by influential organizations such as the United Nations and WHO as a major scientific problem in need of urgent, coordinated international action (Pruden et al., 2021). The emergence of resistant pathogen strains around the world requires not only careful monitoring but the adoption of improved behaviors in terms of anti- biotic (AB) selection and consumption (Aiyar and Pingali 2020). Through technological advances which address the problem of AB stewardship it will be possible to achieve substantial reductions in the spread of AMR (Kirchhelle et al., 2020). It is a well-known and established bacterium which over time has developed a number of strategies and resistance mechanisms which allow it to propagate amongst human populations. Antibiotic susceptibility encompasses a number of traditional techniques which include the broth dilution method (also known as Minimal Inhibitory Concentration (MIC)), the disk diffusion test and the gradient diffusion test (Ghorbanpoor et al., 2022).

Alamar Blue Assay: MABA using MTB

Throughput in tuberculosis drug discovery was extremely limited prior to the introduction of microplate-based susceptibility assays (Mathekga et al., 2022.). The 96-well Microplate Alamar Blue Assay (MABA) allows for the quantitative determination of drug susceptibility against any strain of replicating Mycobacterium tuberculosis to be completed within a week at minimal cost (Cho et al., 2015). The Low-Oxygen Recovery Assay (LORA) uses a recombinant M. tuberculosis expressing luciferase and provides results of drug activity against non-replicating M. tuberculosis surviving under hypoxic conditions (Perveen and Sharma, 2022). Determining activity against non-replicating M. tuberculosis is an important factor when developing drug candidates against M. tuberculosis (Cho et al., 2015).

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CHAPTER 3

MATERIALS AND METHODS

3.1 ETHICAL CLEARANCE

This study is part of a larger research effort; ethical permission was acquired from the University of Venda's Health, Safety, and Research Ethics Committee (FSEA/22/MBY/02/1707). The Limpopo Provincial Government's Department of Economic Development, Environment, and Tourism granted permission to harvest the plant.

3.2 SAMPLE COLLECTION AND PLANTS PREPARATION

3.2.1 COLLECTION OF PLANTS

Plant materials (barks and leaves) of *Dodonaea Viscosa Angustifolia* and *Sclerocarya birrea* (A Rich) Hochst were collected from their natural habitat between August and September 2021 from Venda region of Shakadza (South Africa) with the aid of a traditional herbalist to help identify the plants and transported to the Microbiology laboratory at the University of Venda (Figure 3.1). Shakadza is a village in the Musina Local Municipality in the Vhembe District (Limpopo). Shakadza is adjacent to several villages including Muswodi, Mukovhawabale, Tshokotshoko, Gundani, and Tshamutora.

Figure 3.1: Map of Shakadza where the Nwanedi Game Reserve is located (Taken from https://www.viamichelin.ie/web/Maps/Map-Shakadza)

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3.2.2 SAMPLE PREPARATION

Upon arrival at the laboratory the plants materials were cleaned with distilled water to remove soil (sand) and unwanted debris. After washing the barks were cut into small pieces using a clean machete to facilitate drying at room temperature for 2 weeks, and then milled (ground) to powder form using a grinding machine (Bosch grinding mill, Bosch Germany). Ground materials were stored in a tight container to avoid contamination until future use.

3.3 EXTRACTION PROCEDURE

The crude extracts were obtained through maceration of 100 g of barks and leaves powder in separate 1000 ml of solvents (methanol and distilled water) at a ratio of 1:10 in glass bottles (Das et al., 2010). The glass bottles containing the mixture (solution) were left to extract (soak) for 48 hours to mix the plant material and the solvents. Extracts were then strained using filter papers (Sigma; Saint Louis, USA) into a pre-weighed beaker. The methanol extracts were dried under room temperature, and distilled water extracts were dried in a freeze-dryer machine (EPIC Freeze Dryer, Millrock Technology, Kinston, NY, USA). After drying, the extracts were scrubbed and transferred into small pre-weighed bottles to measure the amount of extract obtained.

3.4 PHYTOCHEMICAL ANALYSES

3.4.1 THIN LAYER CHROMATOGTAPHY

Thin layer chromatography (TLC) was used as a confirmatory test for phytochemical screening of the crude extracts to identify the major phytochemical groups contained in the extracts. The principle is based on the components being separated according to their differential migratory velocities (Figure 3.2).

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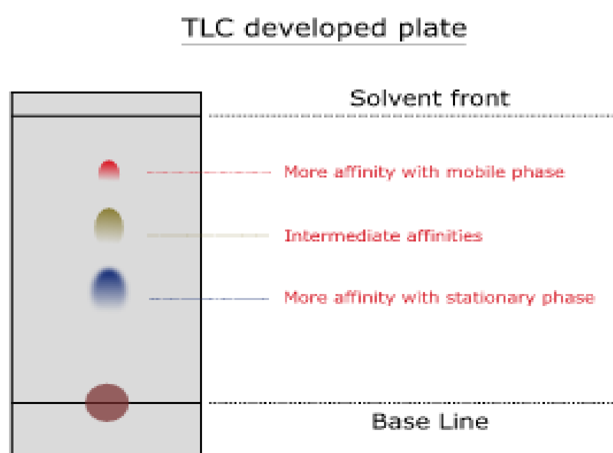

Figure 3.2: Image of a thin layer chromatography (Taken from https://psiberg.com/thin-layer-chromatography).

Briefly, the dried extracts (1 g) were dissolved in 10 ml of the relative extraction solvents to form two working solutions (methanol and water). The solutions were shaken for ten minutes. The solutions was filtered into small conical flask using Whatman filter paper, the filtrate solution was then permitted to dry overnight. The dried extracts were reconstituted with acetone. For each of the extracts, a single drop was dotted onto the aluminium-backed silica TLC plates (Si60 F254; Macherey-Nagel Alugram; Düren, Nordrhein-Westfalen, German) and after drying, they were put in the chamber (glass beaker) containing different solvents (mobile phase) of various polarities, namely benzene/ethanol/ammonia hydroxide (BEA) (non-polar/basic) (18:2:0.2), chloroform/ethyl acetate/formic acid (CEF) (intermediate polarity/acidic) (10:8:2) and ethyl acetate/methanol/water (EMW) (polar/neutral) (10:5.4:4) to run until the mobile phase reached the top line which had been drawn at least 1 cm from the top of the plate. TLC plates were then placed under UV light (254 and 365 nm), to observe the presence of different compounds (band) while under UV lights the spots were outlined with a pencil to mark their locations then sprayed with Vanillin-sulphuric acid reagent and heated at 110℃ for optimal colour development (Kotze and Eloff, 2002). Observed coloured bands on the TLC plates are a positive indication of different phytochemical constituents present in the plant extracts.

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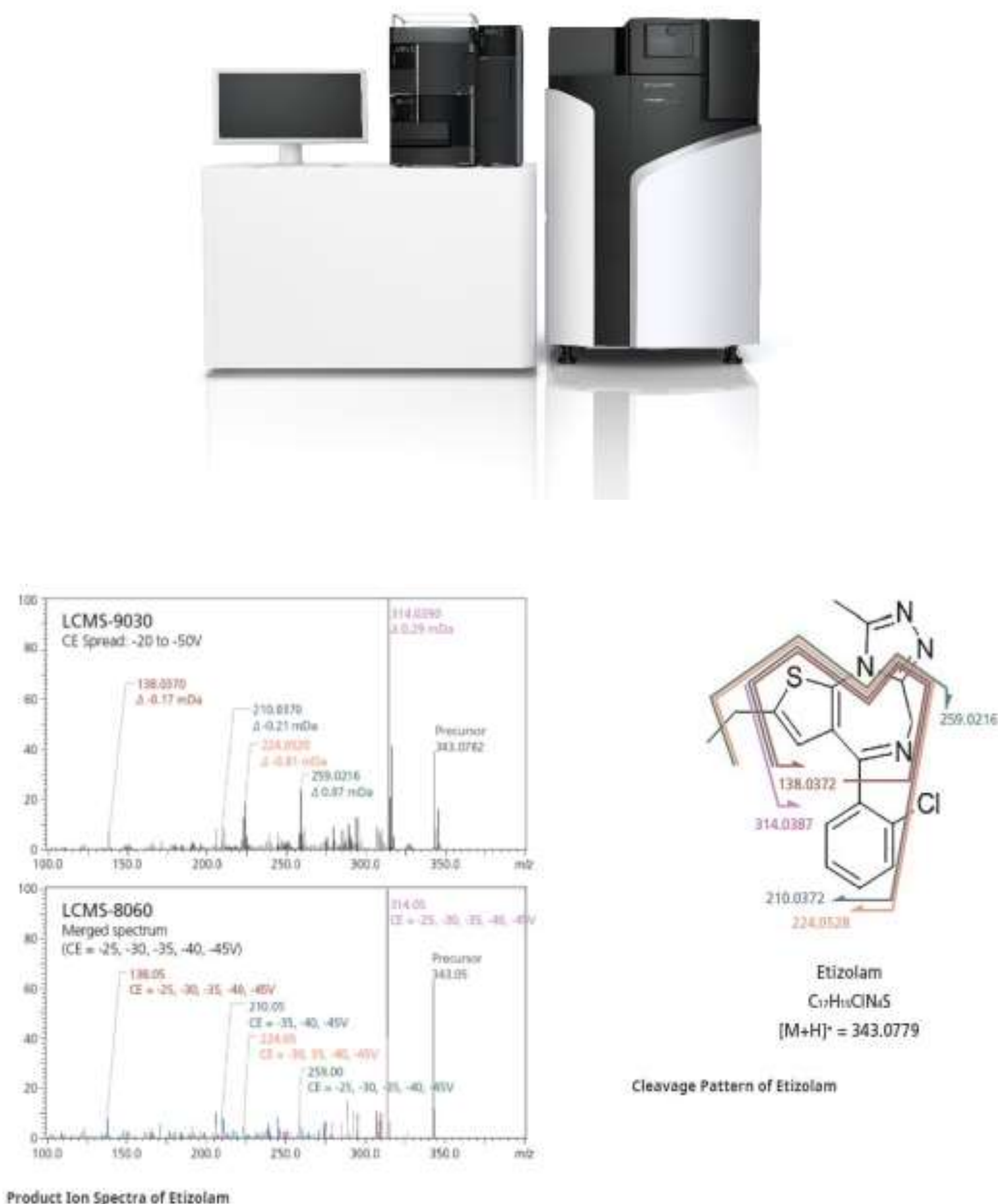
3.4.2 LCMS ANALYSIS

LC-QTOF-MS, model LC-MS 9030 (Shimadzu, Kyoto, Japan), was also used for phytochemical analysis (Figure 3.3). The instrument operates by converting the analyte molecules to a charged (ionised) state, with subsequent analysis of the ions and the produced fragment ions on the basis of their mass to charge ratio (m/z) will be separated as shown in the data acquisition below (Figure 3.3, bottom).



<u>Figure 3.3</u>: Images of LC-MS and data generated (Taken from https://www.antteknik.com/en/products/?p=lcms-9030).

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Briefly, the chemicals were identified in a 4 nm step between 200 and 600 nm. The high-performance liquid chromatography (HPLC) liquid phase contained 0.1% (v/v) formic acid in water (solvent A) and acetonitrile: 0.1% formic acid (1:1, v/v) (solvent B). A 100mm x 2.1mn particle size 2.7m C18 column (Shim Pack Velox, Shimadzu, Kyoto, Japan) was run at 26°C with an injection volume of 5µl and a flow rate of 1ml/min. Based on their absorbance spectrum, retention duration, and mass fragmentation comparison from the literature, the chemicals present in the extracts were recognised using liquid chromatography mass spectrometry (LC-MS).

3.5 CYTOTOXICITY TESTING OF THE PLANT EXTRACTS

The tests were conducted at the Bio-Assay Laboratory in the Biochemistry-Microbiology Department at the Nelson Mandela University (PE, Eastern Cape).

3.5.1 REAGENTS

Low glucose Dulbecco's Modified Eagle Media (DMEM) and PBS with and without Ca^{2+} and Mg^{2+} were purchased from Cytiva (Marlborough, MA, USA). Biowest (Nuaillè, France) supplied the foetal bovine serum (FBS), non-essential amino acids, and penicillin/streptomycin. Sigma-Aldrich provided the MTT and dimethyl sulfoxide (DMSO) (St. Louis, MO, USA).

3.5.2 SAMPLE PREPARATION

Test samples were reconstituted in dimethyl sulfoxide (DMSO) at a stock concentration of 100 mg/mL. Samples were sonicated if insoluble and stored at 4°C until required.

3.5.3 CELL LINE MAINTENANCE

Cellonex in South Africa provided the African green monkey kidney cell line, Vero cells. The cells were maintained using DMEM and 10% FBS at 37°C in a humidified environment with 5% CO_2.

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3.5.4 THE BIO-ASSAY – MTT ASSAY

For the screening experiment, cells were seeded into 96 well microtiter plates at a density of 4 000 cells/well in 100 µl aliquots and left overnight to attach. Treatments were prepared in complete medium and added to cells at 100 µl per well. The concentrations of extracts tested in this screening assay were 50 µg/ml and 100 µg/ml. Melphalan (40 µM) was used as a positive control in all experiments. Treated cells were incubated at 37°C in a humidified 5% CO_2 incubator for 48 hours.

MTT Assay

After incubation, the treatment medium were aspirated and replaced with fresh medium containing MTT at a final concentration of 0.5 mg/ml. Cells were further incubated for 3 hours after which MTT crystals were solubilized using 100 µl DMSO and absorbance measured at 560 nm using a multiplate scanning spectrophotometer (Multiscan MS, Labsystems, San Jose, CA; USA). The pink color in the wells shows the toxicity of the compound (Figure 3.4).

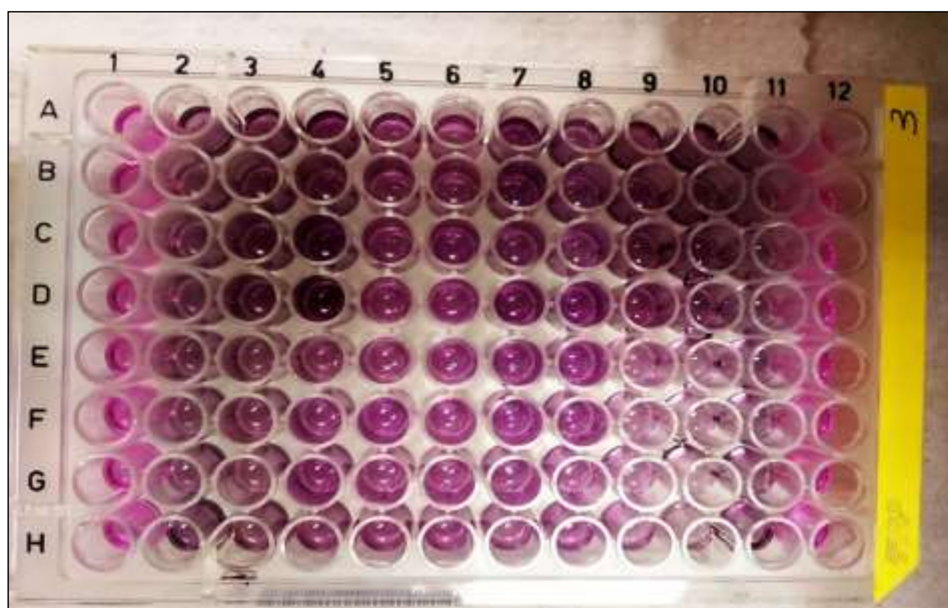

Figure 3.4: Expected plate for MTT reaction (Source: Lab plate).

3.6 ANTIMICROBIAL TESTING

The antimycobacterial activity of the plant extracts was tested against *Mycobacterium smegmatis* mc^{2}155. The culture Mc^{2}155 was maintained in middlebrook 7H9 (Fluka

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M7H9) broth supplemented with glycerol, Tween 80 and middlebrook growth supplemented with OADC (Oleic Albumin Dextrose Catalase) at 37°C. The minimum inhibitory concentration (MIC) to inhibit the growth for M. Smegmatis were analysed following this procedure described by Eloff (1998), with some modifications for mycobacteria (McGaw et al., 2008). Plant crude decoctions were reconditioned in DMSO into desired volume at 5mg/mL which was followed by a two-fold serial dilution in 96 well microliter well plates. DMSO was used as negative control and isoniazid as positive control. M. smegmatis plate was heated in 37ºC for 72h. Addition of 20µL in 0.02% resazurin was done and then incubated for 4 hours. Growth inhibition was indicated by a constant blue resazurin colour (Figure 3.5), while a pink colour indicated viable microorganisms (Farkas et al., 2018). All samples were tested in three technical and biological replicates.

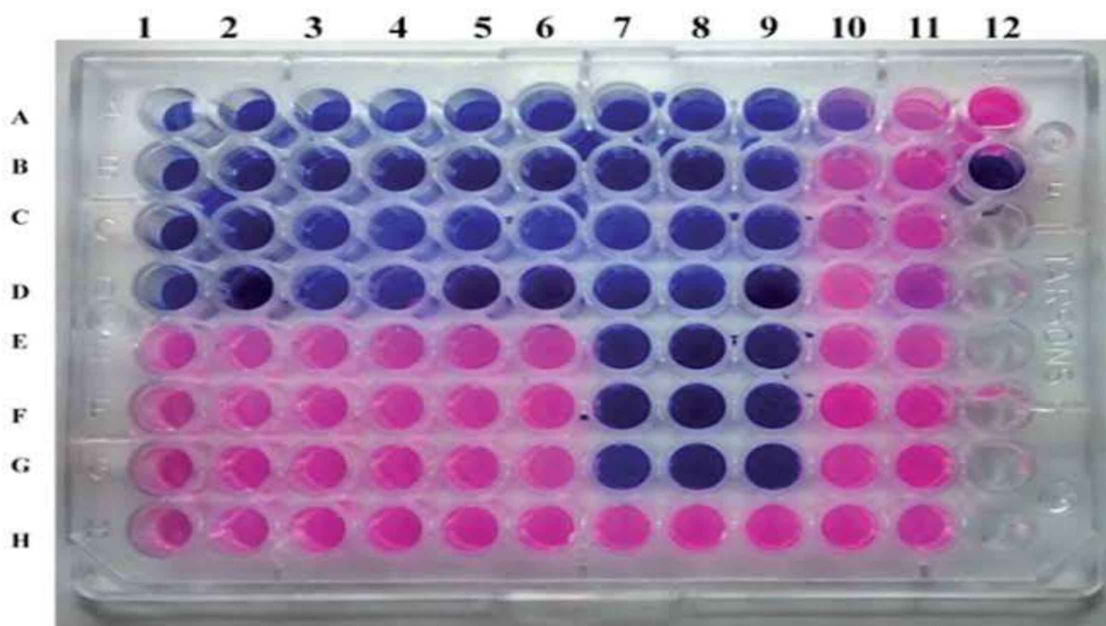

Figure 3.5: An illustration of the MIC microtiter plate after addition of resazurin. Adapted from https://www.researchgate.net/figure/Resazurin-Microtitre-Assay-REMA-for-determining-the-MIC-of-vasicine-against-one-of-the-fig2_282335785/amp on 17 November 22.

3.9 DATA QUANTIFICATION

Quantification of life and dead cells for the screening assay was performed using the ImageXpress Micro XLS Widefield Microscope and acquired images analysed using the MetaXpress software and Multi-Wavelength Cell Scoring Application Module. Each experiment was at least performed in triplicate. All data shown in the tables and

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figures were quantified using Microsoft Excel, ImageJ, and software Origin Pro 9. The mean and standard deviations were calculated.

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CHAPTER 4

RESULTS AND DISCUSSION

This section reports on the results obtained, from the extraction yield to the effects of the extracts on a laboratory strain of *M. tb*.

4.1 EXTRACT YIELDS

Traditional herbs are thought to be phytoconstituent reservoirs with significant role in the plants' characteristics considered to be therapeutic (Belandrin et al., 1985). The filtration part is essential since it is the initial process through extracting the solubilized bioactive components of the plant using solvents.

Most traditional healers use water to extrac active compounds from these plants bec ause it is non-toxic to household pets and people, and it is frequently the only available solvent in use. The type of solvent used in the extraction procedure has a large impact on the successful isolation of compounds from plant materials. The utilization of water only, poses challenges on the extraction of non-polar active compounds (Masoko et al., 2008).

The percentage yield was obtained through calculation by using the formula: Yield = (dried extract/ground weight) * 100. The results are denoted in Table 4.1 below (Where S stand for *S. birrea*, D stand for *D. Viscosa*, L-leaves, B-barks, H-water and C-methanol). Methanol (DBC (21.07%) and SBC (15.77%)) extracts showed higher yield whereas water (DBH (4.4%)) extract showed lowest yield. In this study, water which is a polar solvent; extracted the least amount of compounds as compared to methanol solvent. Water as solvent is known to produce small amount of crude extracts (Maroyi, 2013). In the study done by Adeniran et al (2022) the highest percentage of extract yield was obtained with the polar solvent, (methanol), compared to other solvents that were used and water gave the lowest percentage yield.

Table 4.1: Percentage yield of crude extract after extraction

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s/no	Extracts	Colour of extracts	Yield of extracts (g)	Yield of extracts (%)
1	SLH	Brown	6.73	6.73%
2	SBH	Brownish	7.29	7.29%
3	SLC	Dark brown	7.38	7.38%
4	SBC	Reddish	15.77	15.77%
5	DLH	Brownish	10.82	10.82%
6	DBH	Dark brown	4.4	4.4%
7	DLC	Brown	12.04	12.04%
8	DBC	Dark brown	21.07	21.07%

(S-S. birrea, D- DVA, L-Leaves, B-Bark, C-Methanol and H-Water)

4.2 PHYTOCHEMICAL ANALYSIS OF PLANT EXTRACTS

4.2.1 THIN LAYER CHROMATOGTAPHY (TLC)

Phytochemicals are very important constituents in the plants, especially medicinal plants which can be used to treat the various communicable and non-communicable diseases (Priya et al., 2021). TLC is the most popular technique that can be utilized for identification and quantification of components in mixture (Dwivedi et al., 2020), it is cost effective, time efficient and easy to perform (Kagan and Flythe, 2014). This technique involves the use of different solvent systems that can dissolve compounds that move up the TLC plate, resulting in the development of chromatographs (Tomar et al., 2015). In this study, the phytochemical profiles of the extracts were screened, and the plates were developed in three solvent systems, namely BEA, CEF and EMW. The plates were viewed under UV light and spayed with vanillin sulphuric ($C8H8O3$) reagent for color development. The TLC confirmatory qualitative analysis results are shown below (Figure 4.1).

Some components may have such similar polarity that they appear under one spot after development. After running the chromatogram, more compounds were shown on chromatogram developed inside BEA mobile phase, followed by chromatograms developed inside CEF mobile phase that had fewer compounds in certain extracts

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while TLC plate developed on EMW mobile phase had lowest number of compounds. *Dodnaea viscosa anguistifolia* leaf methanolic (DLC) extract separated more bands (8) as compared to other samples and had the highest Rf value (0.98) on BEA solvent followed by *S.birrea* leaf methanolic (SLC) extract which had five bands.

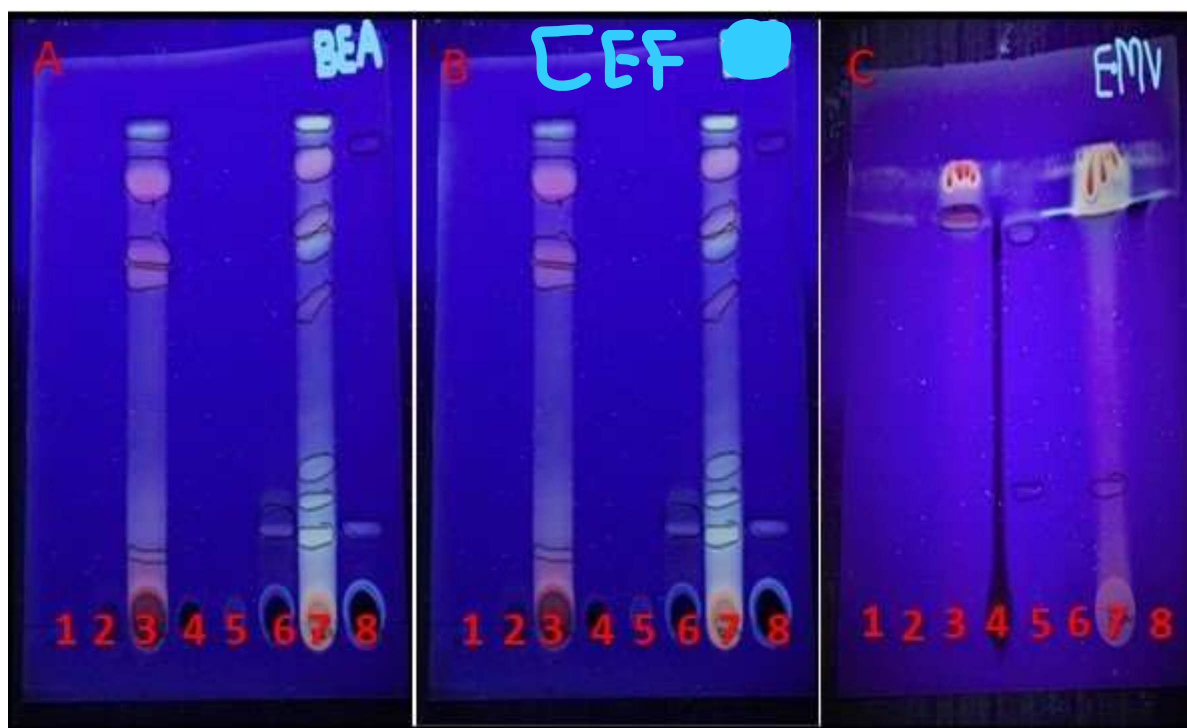

Figure 4.1: TLC- chromatograms showing the separation of S. birrea and DVA leaves and bark extracts developed on **A**-BEA(18:2:02), **B**- CEF(10:8:2), **C**- EMW(10:54:4), with the samples numbered as follow (**1-SLH, 2-SBH, 3-SLC, 4-SBC, 5-DLH, 6-DBH, 7-DLC, 8-DBC**) Different colours (bands) on the plates shows that the extracts have different compounds.

To quantify the movement of the material along the plate, Rf values of the observed bands were calculated (Table 4.2). The Rf values obtained from the samples provide the information about the polarity and separation of these phytochemical in the TLC separation process (Moulishankar et al., 2020). Rf value is the ratio of distance travelled by a substance to distance travelled by a solvent front. The higher the Rf value the lower the polarity of the substance. The lower the Rf value, the higher the polarity of the substance. The highest Rf value was observed for DLC extract (Rf=0.98) that migrated almost at the same speed with the solvent combination (BEA) and the lowest Rf value was observed for SLC (Rf=0.72) that migrated only slightly from the initial point of spotting on the plate on the same solvent system (Njume, et al., 2011).

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We can see here that more phytochemicals compounds were best separated in the BEA solvent system (Table 4.2). Thus, for optimal result, the use of more than one technique for ideal profiling and isolation of active compounds from medicinal plants, is recommended (Ingle et al., 2017; Sharma et al., 2017). For the sample that did not show different compounds, it may be due to the fact that the plant does not have different compounds. Some compounds did not separate because they are moving as the same speed.

Table 4.2: Rf values of the isolated chemical components in the Sand olive and marula barks and leaves extracts

s/no	Extracts	BEA		CEF		EMW	
		Number of spots	Rf value	Number for spots	Rf value	Number for spots	Rf value
1	SLH	-	-	-	-	-	-
2	SBH	-	-	1	0.44	1	0.81
3	SLC	5	0.22 0.72 0.74 0.87 0.94	3	0.27 0.59 0.77	1	0.81
4	SBC	-	-	-	-	-	-
5	DLH	-	-	-	-	3	0.31 0.77 0.80
6	DBH	1	0.27	3	0.43 0.58 0.89	1	0.80
7	DLC	8	0.27 0.36 0.41 0.64 0.73 0.77 0.88 0.98	3	0.73 0.77 0.93	1	0.81
8	DBC	2	0.27 0.92	3	0.44 0.59 0.91	1	0.81

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4.2.2 QUALITATIVE PHYTOCHEMICAL CONSTITUENT OF THE CRUDE EXTRACT USING LC-MS

The determination of phytochemical constituents from *S. birrea* and *D. Viscosa* extracts was done using the LC-MS equipment in the Faculty of Sciences. The LC-MS process demonstrated enough revolving strength in to separate isomeric forms of several compounds. From the analysed extracts (*DVA and S. birrea*), 508 chemicals were observed in all the chromatograms evaluated, but only 46 components were putatively recognized, and they were consisting of flavonoids, phenolics, terpenoids, and saponins, as presented in the tables (Tables 4.3 - 4.6). Although most components were present in every sample, in general *S. birrea* had many polar components that isolated early in the column like (epi)gallocatechin–(epi)gallocatechin dimer (prodephinidin B2) isomers. Quercetins was observed within the stem of *Dodonaea viscosa anguistifolia* as reported by Tong et al (2021). The profiling peaks are reported in the Annexure section.

Gallocatechin showed to have eluted earlier than catechin, this may be due to the fact that the retention time reduces even further as hydroxyl (-OH) group are present (Jiménez-Sánchez et al., 2015). Numerous variations of gallic acid and glucose were detected, including galloyl glucoside, galloyl diglucoside, digalloyl glucoside, gallic acid galloyl glucoside, and digalloyl diglucoside, which were detected at m/z 331, 483, 499 and 577 respectively. Russo et al (2018) revealed that galloyl derivatives of flavonoid glycosides and procyanidins are the popular phenolic components in the *S.birrea* plant.

The compound that showed a deprotonated molecule at m/z 343 was identified as galloyl quinic acid. Compound 1 (Table 4.5) was identified to be ellagic acid based on m/z 301. Previous research found that the ellagic acid are widely distributed on natural plants (Panchal et al., 2013). The far more plentiful polyphenol group observed in marula bark and stem extract were flavonoids (proanthocyanidin).

It has been reported that a level of polymerisation of procyanidins improves the anti-radical activity (effectiveness): dimers and trimers of procyanidins have been shown to be of greater effectiveness as compared to monomeric flavonoids (Russo et al 2018). Cytotoxicity increases with the level of polymerisation. *DVA* has a high concentration of antioxidant compounds flavonoids and phenols, as well as a high

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concentration of fatty acids, primarily oleic acid as previously reported (Tong et al., 2021).

Table 4.3 LCMS profile of *S. birrea* barks

PEAK	RT[1]	MASS	MS/MS[2]	MF[3]	COMP[4] NAME	REF[5]
1	0.76	343.0693	125.0238 169.0151 191.0570	$C_{14}H_{16}O_{10}$	Galloylquinic acid	(Jiménez-Sánchez et al., 2015)
2	1.03	331.0690	125.0241 169.0158	$C_{13}H_{16}O_{10}$	Galloyl glucoside isomer	(Jiménez-Sánchez et al., 2015)
3	1.12	169.0162	124.0169	$C_7H_6O_5$	Gallic acid	(Jiménez-Sánchez et al., 2015)
4	1.39	305.0685	125.0249 167.0358 203.0387	$C_{15}H_{14}O_7$	(Epi)gallocatechin isomer	(Jiménez-Sánchez et al., 2015)
5	1.49	609.1278	165.0197 231.0677 257.0460 289.0733 407.0794 423.0719 485.1217	$C_{23}H_{30}O_{19}$	Proanthocyanidin	Sirius software
6	1.66	761.1407	243.0312 423.0748 591.0965 609.1279 761.1346	$C_{37}H_{30}O_{18}$	(Epi)gallocatechin–(epi)gallocatechin- 3'-O-gallate (prodelphinidin B3-3'-O-gallate)	(Jiménez-Sánchez et al., 2015)
7	1.74	577.1384	125.0247 161.0250 205.0514 289.0733 339.0893 407.0795 425.0904	$C_{30}H_{26}O_{12}$	(Epi)catechin–(epi)catechin (proanthocyanidin B2)	(Jiménez-Sánchez et al., 2015)
8	2.56	745.1457	125.0246 169.0150 217.0518 269.0468 305.0681 331.0457 407.0796 423.0745 467.1007 557.1124 593.1332 745.1451	$C_{15}H_{14}O_6$	(Epi)catechin isomer	(Jiménez-Sánchez et al., 2015)
9	4.29	289.0746	109.0296 159.0458 203.0721	$C_{22}H_{26}O_{13}$	Trimethoxyphenyl glucoside gallate	(Jiménez-Sánchez et al., 2015)
10	4.51	497.1327	125.0248 161.0149 313.0571 497.1293	$C_{22}H_{18}O_{11}$	(Epi)gallocatechin-3-O-gallate isomer	(Jiménez-Sánchez et al., 2015)

[1] RT-Retention time
[2] MS/MS-Tandem mass spectrometry
[3] MF-Molecular Formula
[4] Comp-Compound
[5] REF-References

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11	4.74	457.0804	125.0246 169.0148 193.0150 305. 0684	C37H30O16	(Epi)catechin-3'-O-gallate-–(epi)catechin isomer	(Jiménez-Sánchez et al., 2015)
12	4.83	729.1502	125.0245 169.0147 205.0515 271.0623 289.0731 339.0893 407.0792 451.1059 557.1386 729.1506	C28H28O15	(Epi)catechin-3-O-glucoside-gallate	(Jiménez-Sánchez et al., 2015)
13	5.18	603.1388	125.0249 169.0148 205.0493 245.0838 289.0727 331.0845 465.1004 603.1381	C44H34H21	(Epi)gallocatechin-3-O- gallate–(epi)catechin-3'-O-gallate isomer	(Jiménez-Sánchez et al., 2015)
14	5.49	897.1584	125.0244 169.0148 229.0150 269.0468 287.0577 407.0796 423.0742 467.1013 557.1120 575.1227 619.1121 729.1341 745.1452 897.1568	C28H26O15	Eriodictyol-O-glucoside-O-gallate isomer	Kaigongi et al., 2020
15	6.09	601.1219	125.0252 151.0053 194.9943 313.0564 339.0388 475.0967	C22H18O10	(Epi)catechin-3-O-gallate isomer	(Jiménez-Sánchez et al., 2015)
16	6.26	441.0764	125.0245 169.0147 203.0721	C44H34O20	Digalloylated procyanidin B (P2G2	Russo et al., 2013

Table 4.4: LCMS profile of *S. birrea* leaves

PK	RT	MASS	MS/MS	MF	COMP NAME	REF
1	0.77	331.0692	125.0241 168.0062 211.0254 313.0577	C13H16O10	Galloyl glucoside isomer	(Jiménez-Sánchez et al., 2015)
2	1.02	609.1269	177.0195 243.0293 305.0670 331.0682 423.0727	C30H26O14	(Epi)gallocatechin–(epi)gallocatechin (prodephinidin B2) isomer	Russo et al 2018
3	1.21	593.1323	125.0240 177.0196 219.0664 245.0839 305.0671	C30H26O13	(Epi)catechin–(epi)gallocatechin isomer	Masoko et al., 2018

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			355.0821 423.0731			
4	1.90	523.2215	122.0362 165.0559 205.0716 315.1252 361.1660	$C_{26}H_{36}O_{11}$	Galloyl hexoside derivative	Russo et al., 2013
5	2.00	577.1373	125.0242 161.0243 205.0713 245.0824 289.0723 339.0881 407.0784 425.0892 577.1829	$C_{30}H_{26}O_{12}$	(Epi)catechin–(epi)catechin (proanthocyanidin B2)	Russo et al 2018
6	2.18	745.1432	125.0244 177.0196 273.0428 289.0722 339.0833 407.0797 449.0900 593.1329	$C_{37}H_{30}O_{17}$	(Epi)gallocatechin–(epi)catechin-3'-O-gallate	Masoko et al., 2018
7	2.64	865.2010	125.0242 161.0233 245.0449 287.0570 407.0773 425.0897 525.0820 577.1347 695.1453 739.1662 865.2043	$C_{45}H_{38}O_{18}$	Procyanidin C1	Russo et al 2018
8	4.55	457.0792	125.0243 169.0144 219.0666. 305.0681.	$C_{22}H_{18}O_{11}$	(Epi)gallocatechin-3-O-gallate isomer	Kaigongi et al., 2020
9	4.84	729.1490	125.0244 169.0139 271.0623 289.0723 339.0875 407.0785 451.1045 577.1380 729.1515	$C_{37}H_{30}O_{16}$	(Epi)catechin-3-Ogallate–(epi)catechin isomer	Kaigongi et al., 2020
10	5.32	897.1554	125.0237 169.0145 219.0307 269.0454 287.0564 331.0497 407.0784 423.0737 467.1002 557.1105 575.1218 619.1070 727.1362 745.1417 897.1503	$C_{44}H_{34}O_{21}$	(Epi)catechin-3-O-gallate–(epi) gallocatechin-3-O-gallate isomer	Kaigongi et al., 2020
11	5.64	881.1610	125.0240 169.0141 269.0459	$C_{44}H_{34}O_{20}$	(Epi)catechin-3-Ogallate–(epi)catechin-3'-O-gallate	Russo et al 2018

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			289.0733 407.0779 451.1038 541.1212 559.1245 729.1484 881.1506		(procyanidin B2-3,3' di-O-gallate) isomer	
12	6.22	449.1059	135.0452 151.0037 229.0425 273.0308 302.0352	C21H22O11	Eriodictyol-O-glucoside isomer	Kaigongi et al., 2020
13	6.73	599.1066	137.0233 178.9983 301.0360 599.1074	C28H23O15	Kaempferol 3-O-β-D-(6''-galloyl)glucopyranoside	Russo et al., 2013
14	6.57	431.0992	227.0362 285.0301	C21H20O10	kaempferol 3-O-α-L-rhamnopyranoside	Russo et al., 2013

Table 4.5: LCMS profile of *Dodonaea Viscosa Anguistifolia* leaves

PK	RT	MASS	MS/MS	MF	COMP NAME	REF
1	2.37	301.0910	139.0390 268.5379	C13H18O8	(Iso) tachioside	(Jiménez-Sánchez et al., 2015)
2	4.99	483.0755	125.0232 169.0129 211.0233 271.0441 313.0546 331.0632	C20H20O14	Digalloylglucoside isomer	Kaigongi et al., 2020
3	5.43	457.1895	101.0236 161.0446 233.0688 411.1870	C18H34O3	Oleic acid	Tong et al 2021
4	6.26	337.0908	119.0489 173.0441 191.0546	C16H17O8	p-Coumaric acid	Kaigongi et al., 2020
5	7.20	301.0310	121.0281 151.0021 229.0120 283.9945	C15H1007	Quercetin	(Jiménez-Sánchez et al., 2015)

Both *DVA* and *S.birrea* contains catechins that have been reported to have the potential to treat cancer, particularly breast cancers (Musial et al., 2020 and Kaigongi, et al., 2020).

Triterpenoids found in *DVA* bark and flavanols found in both plants are thought to be accountable for the plants' anticancer, anti-inflammatory, wounds treatment, and antimycobacterial effects (Balingui et al., 2019). The procyanidins is primarily discovered in tuber and trunk of *S.birrea* where they have higher polyphenolic components which are therefore known for their higher anti-oxidant effectiveness and earlier previous studies has shown that the procyanidins also possess antiviral and anticancer properties but limited antimicrobial activity (Russo et al., 2013). According

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to researchers, Oleic acid is known to have anti-cancer, anti-inflammatory, and anti-autoimmune properties, as well as playing an important role in wound healing (Tong et al., 2021).

Table 4.6: LCMS profile of *Dodonaea Viscosa Anguistifolia* bark

PK	RT	MASS	MS/MS	MF	COMP NAME	REF
1	2.54	593.1304	125.0231 177.0178 219.0644 305.0646 355.0801 407.0747 423.0694 467.0956	C30H26O13	(Epi)catechin–(epi)gallocatechin isomer	(Jiménez-Sánchez et al., 2015)
2	3.21	609.1244	125.0231 177.0178 219.0645 273.0403 305.0646 355.0803 423.0695	C30H26O14	(epi)gallocatechin–(epi)gallocatechin (prodephinidin B2) isomer	(Jiménez-Sánchez et al., 2015)
3	6.39	761.1305	125.0220 177.0182 243.0291 305.0650 423.0702 483.0927 609.1234 775.7660	C37H30O18	Epi)gallocatechin–(epi)gallocatechin-3'-O-gallate (prodelphinidin B3-3'-O-gallate) isomer	(Jiménez-Sánchez et al., 2015)
4	4.54	745.1447	125.0231 177.0179 273.0384 289.0696 407.0746 449.0857 467.0941 593.1284 665.2187 745.1759	C37H30O17	(Epi)gallocatechin3-O-gallate–(epi)catechin isomer	Masoko et al., 2018
5	6.98	601.1207	125.0233 169.0128 211.0229 269.0436 313.0545 341.1374 439.0853 491.1895 601.1560	C28H26O15	Eriodictyol-O-glucoside-O-gallate isomer	Russo et al 2018
6	7.28	479.0813	125.0235 178.9972 271.0232 316.0203 341.0636 479.1519	C21H20O13	Myricetin glucoside	(Jiménez-Sánchez et al., 2015)
7	7.19	617.1108	125.0238 177.0175 194.9924 259.0613 313.0536 339.0683	C28H26O16	Dihydroquercetin-O-glucosyl-O-gallate	(Jiménez-Sánchez et al., 2015)

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			465.1030 491.0798			
8	12.70	469.3254	407.3292 425.3398 469.3294	$C_{30}H_{46}O_4$	Oleanonic acid	Masoko et al., 2018

In this research study, subunits up to B-type dimers of procyanidins were identified, that is oligomers and polymers for (epi)catechin monomers; B-type dimers of prodelphinidins, that have been composed of (epi)gallocatechin components; comprised of (epi)afzelechin flavan-3-ol units. The level of galloylation is physiologically significant, as galloyllation have been shown in hindering cellular proliferation.

4.3 CYTOTOXICITY ASSAY

To evaluate the toxicity effects of the traditional plants, the crude extracts were tested against vero cells. The 3-(4,5-dimethylthiazol-2yl)-2,5-diphenyl tetrazolium bromide tests are valued and effective colorimetric test for determining cell viability. MTT, 3-(4,5-dimethylthiazol-2yl)-2,5-diphenyl tetrazolium bromide, is hydrophilic and generates a yellowish colour when prepared in medium or buffers. This test is built on the fact that viable cells' cellular dehydrogenase enzymes can reduce the water-soluble yellow tetrazolium to form a purple non-soluble formazan product. Due to the non permeability of the cell membrane, this purple formazan product cannot escape living cells and thus accumulates inside the cell. As a result, the amount of formazan product produced is proportional to the number of living cells. In general, a plant is considered toxic when the viability of the tested cells is below 35%.

In this study, five of the eight plant extracts tested (DLH, DBH, DBC, SLH and SLC) were found to have significant ($P<0.02$) cytotoxic effects on Vero cells at both treatment concentration and three (DLC, SBH and SBC) did not show any cytotoxic effects on Vero cells ($P<0.001$) at both concentrations as indicated in Figure 4.2 (a & b). From the graphs it is noticeable that total amount of cells with no treatment (negative control) were all at 100% whist treatment of cells with melphalan (positive control) resulted in the reduction of cells to less than 10%. Both + and – controls findings were utilised to determine the level of cytotoxicity of the extracts.

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Amongst all extracts analysed for cytotoxicity, DBH and SLH revealed greater cytotoxicity to the cells (respectively 40% and 34% inhibition), when compared to the negative control (non-treated cells). The cell viability for DBH extract at (100µg/ml) decreased by 40% and at 50µg/ml decreased by 21%, the cell viability for SLH at both concentrations decreased by 33% and 34% as compared to the negative control. Looking at both concentrations DLH, DBC and SLC were less toxic to the cells with the decrease in cell viability ranging from 14% to 29%. SBH and DLC showed less toxicity at 50µg/ml while at 100µg/ml, a stimulation of cellular reproduction was observed (7% and 5% increase respectively). A maximum stimulation of cellular reproduction was observed for SBC samples at 100µg/ml (18.3% increase, $P<0.001$).

The results presented in figure 5b shows a slight dose dependent toxicity of the DBH, DBC, SLH and SLC extracts as the number of cells was decreasing with the increasing concentrations. The toxic effects might be due to presence of secondary metabolites. The concentration of compounds that are known to be toxic such as tannins, cardiac glycosides and terpenoids may explain the toxic nature of these extract as previously reported (Mansoor et al., 2009). Indeed, the toxic effects of procyanidins in various types of monkey cells have been well recorded (Russo et al 2018).

Flavonoids have lower cytotoxicity effects because they are widely distributed in edible plants (Talib et al., 2010). DVA extracts have been reported to have potent activities on breast cancer cell line as shown by Hossain (2019). However, it is not impossible that some of the chemical constituents of the plant extracts would not be toxic to mammals (Ojewole, 2003). In an *in vitro* study looking at the toxic effects of *D. viscosa*, it was reported that the plant displays toxicity towards breast cancer cells and the anticancer activities is done via induction of apoptosis in mammalian breast cancer cell line (Almarfadi et al., 2022). In addition, the high LD50 value found for the water and methanol decoction of S. birrea trunk-bunk indicate that the plant decoctions are generally harmless and/or have no toxic effects on mice.

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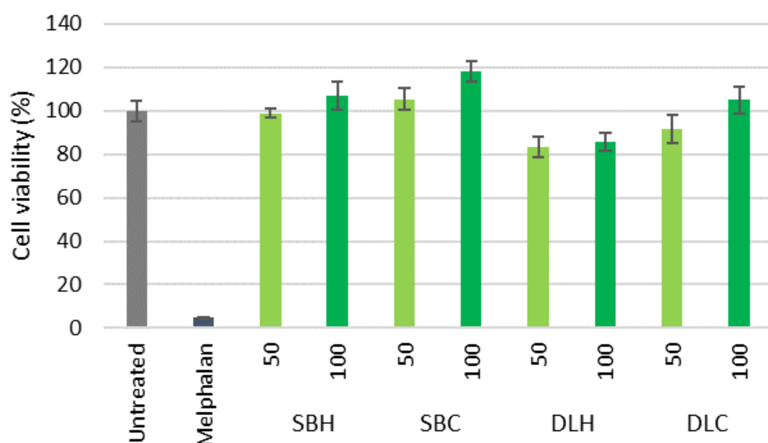
(a)



(b)

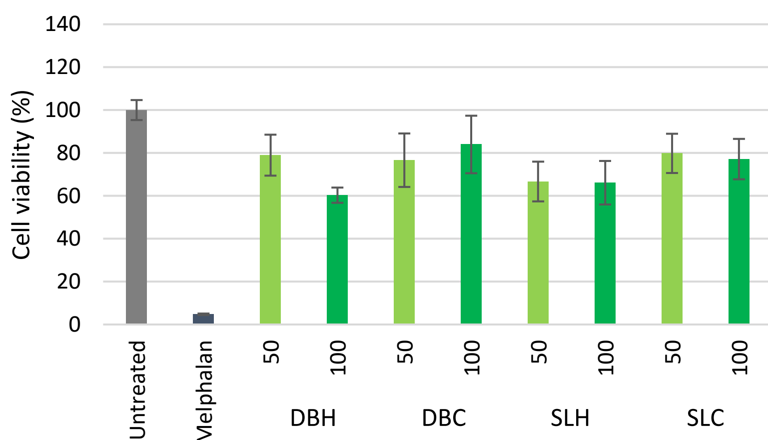


Figure 4.2: Cytotoxicity of eight extract samples in Vero cells after 24 hours of treatment. Melphalan (40 µM) was used as a positive control. Error bars indicate standard deviation of quadruplicate values obtained from this experiment. **(S-S. birrea, D- DVA, L-Leaves, B-Bark, C-Methanol and H-Water**

4.4 ANTIMYCOBACTERIAL TESTING

The antimycobacterial activity of the plant extracts was tested against *Mycobacterium smegmatis* (*M. smegmatis*) mc^{2}155. The extracts were first screened for antimycobacterial activity at the highest concentration (Table 4.7). The extracts that showed inhibition (activity) were evaluated further to determine their lowest inhibitory concentration. The MIC results are shown in Table 4.8. Amongst all the samples

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screened for antimycobacterial activities, only three (3) SBC (0.12 mg/ml), DBC (0.21 mg/ml) and DBH (0.32 mg/ml) showed activities against M. smegmatis, 5 (SBH, SLH, SLC, DLC and DLH) did not show any activity. SBC had the lowest MIC value of 0.12 mg/ml which showed that it was very active against *M.smegmatis*.

Although the *S. birrea* and *D. viscosa* plants extracts have been reported to be used in the treatment of TB and related diseases, most of the extract failed to display any activity against M. smegmatis. The possible explanation could be that the potential active compounds need to be metabolically activated in vivo by specific enzymes or may have a pH dependant biological activity (Singh et al., 2021).

Table 4.7: Antimycobacterial activity screening at 5mg/ml concentration (values)

Samples (mg/ml)	Active	Inactive
SBC	X	
SBH		X
SLC		X
SLH		X
DBC	X	
DBH	X	
DLC		X
DLH		X

(S-S. birrea, D- DVA, L-Leaves, B-Bark, C-Methanol and H-Water)

Table 4.8: Minimum inhibitory concentration (MIC) of extracts against *M. smegmatis.*

	MIC values (mg/ml)		
Plant species	**Water**	**Methanol**	**INH**
SB	>2	0.12	0.63
SL	>2	>2	0.63
DB	0.32	0.21	0.63
DL	>2	>2	0.63

INH- isoniazid (isonicotinic acid hydrazide)

Tong et al (2021) reported that *Dodonaea Viscosa Anguistifolia* has very good activity against Tuberculosis strains. *DVA* also has important bioactivities like antioxidant and anti-tuberculosis. in addition, Hossain (2019) reported that DVA has shown notable activity against both Gram positive and Gram negative pathogenic bacterial strains. Early research shows the leaf material of marula plant to be useful for antibacterial

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uses. The Bark is probably preferred because it contains a larger total quantity of antibacterial activity and because it is easier to transport and store for trading purposes (Eloff, 2001).

Eloff (2001) reported antibacterial activity of *S. birrea* leaves and bark extracts against Gram-positive and - negative bacteria such as *S. aureus*, *P. aeruginosa*, *E. coli* and *E. faecalis* with MIC values ranging from 0.15 to 3 mg/ml. Mai et al (2019) found that the aqueous extract of *S. birrea* bark is effective in inhibiting the growth of bacteria and the zone of inhibition increases with the increase in concentration of the extract.

To determine which plant parts can be used for further testing and isolation, not only the MIC value is important, but also the total activity. The MIC results are shown in Table 4.8. Amongst all the samples screened for antimycobacterial activities, only three (3) SBC (0.12 mg/ml), DBC (0.21 mg/ml) and DBH (0.32 mg/ml) of them showed to have activities against M. smegmatis, 5 (SBH, SLH, SLC, DLC and DLH) did not show any activity. SBC showed to have the lowest MIC value of 0.12 mg/ml which showed that it was very active against *M.smegmatis*.

The fact that most of these extracts did not show any antimicrobial potential does not imply that they will exhibit the same impact *in vivo*; therefore, it must be noted that they only proved poor effectiveness in vitro (Madikizela et al., 2013). Due to the complex lipoglycan calyx on the cell surface, many antibiotics do not work on M. tuberculosis. This could explain the lack of activity shown by some of the plant extracts against MTB in this study. Therefore, the negative results obtained could not preclude the potential antimycobacterial effect of those medicinal plants.

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CHAPTER 5

CONCLUSION AND RECOMMENDATIONS

5. 1 CONCLUSION

This study was aimed to determine anti-mycobacterium activities of the Sand Olive and Marula plants against *Mycobacterium tuberculosis* and their cytotoxicity. To achieve this objective, secondary objectives were followed.

The first secondary objective was to obtain the crude extract by maceration. The crude extracts were obtained and methanolic extracts showed the highest yield as compared to water extracts.

Secondly, profiling of the compounds in the crude extracts using liquid chromatography mass spectroscopy (LC-MS). The plants had abundant flavonoid metabolites that shows to have antioxidative activity and anticancer activity. The flavonoids also play a major role in human health because of various pharmacological activities. Flavonoids are biologically active phytochemicals that are ubiquitous in the plant kingdom which are being used in various herbal medicines.

Thirdly, determination of cytotoxic effects on Vero monkey kidney cells using MTT assay. The *sclerocarya birrea* bark were not toxic on vero cells and the leaves showed toxic effects at both concentrations (50 and 100 µg/mL). From the cytotoxicity assay that was conducted, it can be concluded that *Dodonaea viscosa anguistifolia* exhibited significant cytotoxicity at both concentration except for DLC.

Lastly, the last objective was to determine antimycobacterial activity of the crude extracts on *Mycobacterial smegmatis*. Plant extract SBC, DBC and DBH showed activity against *M.Smegmatis* and SBH, SLC, SLH, DLC and DLH did not show any activity against *M.Smegmatis.*

In addition, the following questions had to be answered:

- From the results obtained it can be concluded that; the selected medicinal plants did not have antimycobacterial activities against *Mycobacterial tuberculosis.*

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5.2 RECOMMENDATIONS

Further research needs to be carried out especially on the formulations to isolate and identify the active compounds responsible for their antimicrobial, antioxidant and low cytotoxicity properties.

In vivo cytotoxicity studies are needed on the formulation to confirm cell proliferation activities found in this study.

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REFERENCES

Adeniran, O.I., Musyoki, A.M., Sethoga, L.S., Mogale, M.A., Gololo, S.S. and Shai, L.J., 2022. Phytochemical profile, anti-glycation effect, and advanced glycation end-products protein cross-link breaking ability of *Sclerocarya birrea* stem-bark crude extracts. *Journal of Herbmed Pharmacology*, *11*(4), pp.529-539

Ahmed, Z. 2001. "Production of natural and rare pentoses using microorganisms and their enzymes." Electronic Journal of Biotechnology. Volume 4. Number 2.

Aiyar, A. and Pingali, P., 2020. Pandemics and food systems-towards a proactive food safety approach to disease prevention & management. *Food Security*, *12*(4), pp.749-756.

Almarfadi, O.M., Siddiqui, N.A., Shahat, A.A., Alqahtani, A.S., Alam, P., Nasr, F.A., Alshahrani, S.S. and Noman, O.M., 2022. Quantification of biomarkers and evaluation of antioxidant, anti-inflammatory, and cytotoxicity properties of Dodonaea viscosa grown in Saudi Arabia using HPTLC technique. *Open Chemistry*, *20*(1), pp.559-569.

Al-Snafi, P.A.E., 2017. A review on *Dodonaea viscosa*: a potential medicinal plant. IOSR J. Pharm. 7, 10–21.

Anochie, P.I., Ndingkokhar, B., Bueno, J., Anyiam, F.E., Ossai-Chidi, L.N., Onyeneke, E.C. and Onyeozirila, A.C., 2018. African medicinal plants that can control or cure tuberculosis. *Int J Pharm Sci Dev Res*, *4*(1), pp.001-008.

Anode, S.O., Abraha, T., Araya, S., 2018. Phytochemical analysis of *Dodonaea angustifolia* plant extracts. Int. J. Herb. Med. 6, 37–42.

Balandrin, M.F., Klocke, J.A., Wurtele, E.S. and Bollinger, W.H., 1985. Natural plant chemicals: sources of industrial and medicinal materials. *Science*, *228*(4704), pp.1154-1160.

Balingui, C.F., Noel, N.J., Emmanuel, T., Benoit, N.M., Gervais, H.M. and Boubakary, A., 2019. LC-MS Analysis, Total Phenolics Content, Phytochemical Study and DPPH Antiradical Scavenging Activity of Two Cameroonian Propolis Samples. *Med Chem (Los Angeles)*, *9*(8), pp.100-106.

© University of Venda

Beshah, F., Hunde, Y., Getachew, M., Bachheti, R.K., Husen, A. and Bachheti, A., 2020. Ethnopharmacological, phytochemistry and other potential applications of Dodonaea genus: A comprehensive review. *Current Research in Biotechnology*, *2*, pp.103-119.

Branca, A., Baglioni, C. 1981. "Evidence that types I and II interferons have different receptors." Nature. Volume 294: p. 768-770.

Brown-Elliott, B., Wallace, R. 2002. "Clinical and Taxonomic Status of Pathogenic Nonpigmented or Late-Pigmenting Rapidly Growing Mycobacteria". Clinical Microbiology Reviews. Volume 15. p. 716–746.

Brunken, U.; Schmidt, M.; Dressler, S.; Janssen, T.; Thiombiano, A.; Zizka, G. 2008. West African plants-A photo guide. Forschungsinstitut Senckenberg.

Cho, S., Lee, H.S. and Franzblau, S., 2015. Microplate alamar blue assay (MABA) and low oxygen recovery assay (LORA) for Mycobacterium tuberculosis. *Mycobacteria protocols*, pp.281-292.

Cock, I.E., Van Vuuren, S.F. 2020. The traditional use of southern African medicinal plants for the treatment of bacterial respiratory diseases: A review of the ethnobotany and scientific evaluations. J Ethnopharmacol., 263, 113204.

Coulidiaty, A.G.V., Youl, E.N.H. and Yam&eogo, T.E.E.M., 2021. Sclerocarya birrea: Review of the pharmacology of its antidiabetic effects and toxicity. *African Journal of Pharmacy and Pharmacology*, *15*(8), pp.164-173.

Das, K., Tiwari, R.K.S. and Shrivastava, D.K., (2010). Techniques for evaluation of medicinal plant products as antimicrobial agent: Current methods and future trends. *Journal of Medicinal Plants Research,* 4(2):104-111

Do, T.K.T., Clark, K., Christen, P. and Reich, E., 2020. Quality assessment of Sclerocarya birrea leaves and leaves products from Burkina Faso based on fingerprinting using HPTLC. *JPC–Journal of Planar Chromatography–Modern TLC*, *33*, pp.439-448.

Dossou, S.S.K., Cosme, K., Pierre, A., Eleonore, Y.L., Brice, S., Mansourou, M., Georges, C.A. and Joeuml; Ile, Q.L., 2011. Chemical composition and seasonal variation of essential oil of Sclerocarya birrea (A. Rich.) Hochst subsp birrea leaves from Benin. *Journal of Medicinal Plants Research*, *5*(18), pp.4640-4646.

© University of Venda

Dwivedi, M.K., Sonter, S., Mishra, S., Patel, D.K. and Singh, P.K., 2020. Antioxidant, antibacterial activity, and phytochemical characterization of Carica papaya flowers. *Beni-Suef University Journal of Basic and Applied Sciences*, *9*, pp.1-11.

Eloff, J.N., 2001. Antibacterial activity of Marula (Sclerocarya birrea (A. rich.) Hochst. subsp. caffra (Sond.) Kokwaro) (Anacardiaceae) bark and leaves. *Journal of ethnopharmacology*, *76*(3), pp.305-308.

Eloff JN (1998) A sensitive and quick microplate method to determine the minimal inhibitory concentration of plant extracts for bacteria. Planta Medica 64: 711–713

Farkas, A., Pap, B., Kondorosi, É. and Maróti, G., 2018. Antimicrobial activity of NCR plant peptides strongly depends on the test assays. *Frontiers in microbiology*, *9*, p.2600.

Fotio, A.L.; Dimo, T.; Nguelefack, T.B.; Dzeufiet, P.D.; Ngo Lemba, E.; Temdie, R.J.; Ngueguim, F.; Olleros, M.L.; Vesin, D.; Dongo, E.; Kamtchouing, P. 2009. Acute and chronic anti-inflammatory properties of the stem bark aqueous and methanol extracts of *Sclerocarya birrea (Anacardiaceae),* Inflammopharmacology, 17, 229-237.

Getachew, S., Medhin, G., Asres, A., Abebe, G. and Ameni, G., 2022. Traditional medicinal plants used in the treatment of tuberculosis in Ethiopia: A systematic review. *Heliyon*, p.e09478.

Ghorbanpoor, H., Dizaji, A.N., Akcakoca, I., Blair, E.O., Ozturk, Y., Hoskisson, P., Kocagoz, T., Avci, H., Corrigan, D.K. and Guzel, F.D., 2022. A fully integrated rapid on-chip antibiotic susceptibility test–A case study for Mycobacterium smegmatis. Sensors and Actuators A: Physical, 339, p.113515.

Glover, R., Kriakov, J., Garforth, S., Baughn, A., Jacobs, W. 2007. "The Two-Component Regulatory System senX3-regX3 Regulates Phosphate-Dependent Gene Expression in Mycobacterium smegmatis." Journal of Bacteriology.

Gong, W., Xie, J., Li, H. and Aspatwar, A., 2023. Research advances of tuberculosis vaccine and its implication on COVID-19. *Frontiers in Immunology*, *14*.

Gul, H.; Awais, M.; Saddick, S.; Khan, F.; Gulfraz, M.; Nazir, K.; Khan, S.; Khan, M. 2021. Determination of in Vivo Biological Activities of Dodonaea Viscosa Flowers

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Against CCL4 Toxicity in Albino Mice With Bioactive Compound Detection. Research square.

Gul, R., Jan, S.U., Faridullah, S., Sherani, S., and Johan, N., 2017. Preliminary phytochemical screening, quantitative analysis of alkaloids, and antioxidants activity of crude plant extracts from Ephedra intermedia indigenous to Balochistan, *The Scientific World Journal, 2017.*

Haudecoeur, R., Peuchmaur, M., Pérès, B., Rome, M., Taïwe, G.S., Boumendjel, A. and Boucherle, B., 2018. Traditional uses, phytochemistry and pharmacological properties of African Nauclea species: A review. *Journal of Ethnopharmacology*, *212*, pp.106-136.

Helwig, D., 2016. Traditional Africa Medicine, Gale Encyclopaedia of Alternative Medicine, ***2*(05).** pp. 20-27.

Herrera-Calderon, O., Rahman, M.H., Pena-Rojas, G. and Andia-Ayme, V., 2020. Dodonaea viscosa Jacq: a medicinal plant with cytotoxic effect on colon cancer cell line (HT-29). *J Pure Appl Microbiol*, *14*(3), pp.1927-1934.

Hossain, M.A., 2019. Biological and phytochemicals review of Omani medicinal plant Dodonaea viscosa. *Journal of King Saud University-Science*, *31*(4), pp.1089-1094.

Huang, Y., Xiao, D., Burton-freeman, B.M., Edirisinghe, I., 2016. Chemical changes of bioactive phytochemicals during thermal processing. Reference Module in Food Science. Elsevier. https://doi.org/10.1016/B978-0-08-100596-5.03055-9

Jethva, K., Bhatt, D. and Zaveri, M., 2016. In-Vitro anti-tuberculosis activity of selected ethnomedicinal plants. *International Journal of Herbal Medicine*, *4*(4), pp.126-128.

Jeya, S.J.; Santhi, V.; Borgia, V.J.F.; Devi, P.S. 2014. In vitro antibacterial activity, phytochemical screening and FT- IR analysis of Dodonaea viscosa and Adhatoda vasica. Asian Journal of Biochemical and Pharmaceutical Research, 2: 289-298.

Jiménez-Sánchez, C., Lozano-Sánchez, J., Gabaldón-Hernández, J.A., Segura-Carretero, A. and Fernández-Gutiérrez, A., 2015. RP-HPLC–ESI–QTOF/MS2 based strategy for the comprehensive metabolite profiling of Sclerocarya Birrea (Marula) bark. *Industrial Crops and Products*, *71*, pp.214-234.

© University of Venda

Joffe, P. 2005. Creative gardening with indigenous plants: A South African guide. Briza Publications, Pretoria.

Kagan, I.A. and Flythe, M.D., (2014). Thin-layer chromatography (TLC) separations and bioassays of plant extracts to identify antimicrobial compounds. Journal of Visualized Experiments, 25(85):51-60

Kaigongi, M.M., Lukhoba, C.W., Ochieng ‘, P.J., Taylor, M., Yenesew, A. and Makunga, N.P., 2020. LC-MS-based metabolomics for the chemosystematics of Kenyan Dodonaea viscosa Jacq (Sapindaceae) populations. *Molecules*, *25*(18), p.4130.

Kakkar, S. and Bais, S., 2014. A review on protocatechuic acid and its pharmacological potential. *International Scholarly Research Notices*, *2014*.

Kaur, R. and Kaur, H., 2015. Antitubercular activity and phytochemical screening of selected medicinal plants. *Orient J Chem*, *31*(1), pp.597-600.

Kirchhelle, C., Atkinson, P., Broom, A., Chuengsatiansup, K., Ferreira, J.P., Fortané, N., Frost, I., Gradmann, C., Hinchliffe, S., Hoffman, S.J. and Lezaun, J., 2020. Setting the standard: multidisciplinary hallmarks for structural, equitable and tracked antibiotic policy. *BMJ Global Health*, *5*(9), p.e003091.

Kotze, M. and Eloff, J.N., (2002). Extraction of antibacterial compounds from Combretum microphyllum (Combretaceae). South African Journal of Botany, 68(1): 62-67

Kumar, J.K., Prasad, A.D. and Chaturvedi, V., 2014. Phytochemical screening of five medicinal legumes and their evaluation for in vitro anti-tubercular activity. *Ayu*, *35*(1), p.98.

Kuruppu, A.I., Paranagama, P. and Goonasekara, C.L., 2019. Medicinal plants commonly used against cancer in traditional medicine formulae in Sri Lanka. *Saudi Pharmaceutical Journal*, *27*(4), pp.565-573.

Maghfiroh, K., Batoro, J., Widyarti, S. and Sumitro, S.B., 2021. Exploration of Medicinal Plants in Pamekasan District: Ethnobotany Study in Herbal Medicine to Prevent Symptoms of Tuberculosis Infection.

Madikizela, B., Ndhlala, A.R., Finnie, J.F. and Staden, J.V., 2013. In vitro antimicrobial activity of extracts from plants used traditionally in South Africa to treat

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tuberculosis and related symptoms. *Evidence-Based Complementary and Alternative Medicine*, *2013*.

Mai, A.J., Emmanuel, M., Ayim, P. and Magaji, M.B., 2019. Evaluation of Antibacterial Activities and Cytotoxicity of Sclerocarya birrea Stem Bark. *Open Access Library Journal*, *6*(9), pp.1-6.

Manhivi, V.E., Slabbert, R.M. and Sivakumar, D., 2022. Co-ingestion of natal plums (Carissa macrocarpa) and Marula Nuts (Sclerocarya birrea) in a snack bar and its effect on phenolic compounds and bioactivities. *Molecules*, *27*(1), p.310.

Mansoor T.A., Barralho P.M., Dewanjee S., Mulhovo S., Rodrigues C.M.P., Ferreria M.U. (2013) Monoterpene bisindole alkaloids, from the African medicinal plants Tabernaemontana elegans, induce apoptosis in HCT116 human colon carcinoma cells. Journal of ethnopharmacology,149:463-470

Mariah, N.M., Mourine, K. and Christine, B., 2021. Qualitative and quantitative phytochemical profiling of crude fractions of Pechuel-Loeschea leubnitziae leaves. *Journal of medicinal plants Research*, *15*(2), pp.64-72.

Marimani, M., Ahmad, A. and Duse, A., 2018. The role of epigenetics, bacterial and host factors in progression of Mycobacterium tuberculosis infection. *Tuberculosis*, *113*, pp.200-214.

Maroyi A.,2013 local knowlwdge and uses of marula (sclerocarya birrea (A.Rich.) Hochst.) in southern central Zimbabwe: Indian Journal of traditional knowledge 12 (3): 398-403

Mashamba, T.G., Adeosun, I.J., Baloyi, I.T., Tshikalange, E.T. and Cosa, S., 2022. Quorum sensing modulation and inhibition in biofilm forming foot ulcer pathogens by selected medicinal plants. *Heliyon*, p.e09303.

Masoko, P., Mmushi, T.J., Mogashoa, M.M., Mokgotho, M.P., Mampuru, L.J. and Howard, R.L., 2008. In vitro evaluation of the antifungal activity of Sclerocarya birrea extracts against pathogenic yeasts. *African Journal of Biotechnology*, *7*(20).

Mathekga, B.S., Nxumalo, Z. and Raj, D.B.T.G., 2022. Micro and nanofluidics for high throughput drug screening. *Progress in Molecular Biology and Translational Science*, *187*(1), pp.93-120.

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Mathur, S. and Hoskins, C., 2017. Drug development: Lessons from nature. *Biomedical reports*, *6*(6), pp.612-614.

McGaw, L. and Eloff, J.N., (2008). Ethnoveterinary use of southern Africa plants and scientific evaluation of their medicinal properties. Journal of ethnopharmacology 119 (3): 550 – 574

Megehee, J., Lundrigan, M. 2007. "Temporal expression of Mycobacterium smegmatis respiratory terminal oxidases" Canadian Journal of Microbiology. Volume 53. p. 459-6.

Miliauskas, G., Venskutonis, P.R. and van Beek, T.A., (2004). Screening radical scavenging activity of some medicinal and aromatic plant extracts. Food Chemistry, 85(2): 231-237

Moulishankar, A., Ganesan, P., Elumalai, M. and Lakshmanan, K., 2020. Significance of TLC and HPTLC in phytochemical screening of herbal drugs. *J. Glob. Pharma Technol*, *13*, pp.30-45.

Musial, C., Kuban-Jankowska, A. and Gorska-Ponikowska, M., 2020. Beneficial properties of green tea catechins. *International journal of molecular sciences*, *21*(5), p.1744.

Naidoo, R.; Patel, M.; Gulube, Z.; Fenyvesi, I. 2012. Inhibitory activity of Dodonaea viscosa var. angustifolia extract against Streptococcus mutans and its biofilm. J Ethnopharmacol, 144, 171-174.

Nasrullah, S.; Rahman, K.; Ikram, M.; Nisar, M.; Khan, I. 2012. Screening of antibacterial activity of medicinal plants. International Journal of Pharmaceutical Science Review and Research, 14, 25-29.

Newton, G., Ta, P., Bzymek, K., Fahey, R. 2006 "Biochemisrty of the Initial Steps of Mycothiol Biosynthesis" Journal of Biological Chemistry. Volume 281. p. 33910-20.

Newton, G., Fahey, R. 2002. "Mycothiol biochemistry." Arch Microbiol. Volume 178. p. 388-94.

Ngabaza, T., Johnson, M.M., Moeno, S. and Patel, M., 2017. Identification of 5, 6, 8-Trihydroxy-7-methoxy-2-(4-methoxyphenyl)-4H-chromen-4-one with antimicrobial activity from Dodonaea viscosa var. angustifolia. *South African Journal of Botany*, *112*, pp.48-53.

© University of Venda

Nguta, J.M., Appiah-Opong, R., Nyarko, A.K., Yeboah-Manu, D., Addo, P.G., Otchere, I. and Kissi-Twum, A., 2016. Antimycobacterial and cytotoxic activity of selected medicinal plant extracts. *Journal of ethnopharmacology*, *182*, pp.10-15.

Nguta, J.M., Appiah-Opong, R., Nyarko, A.K., Yeboah-Manu, D. and Addo, P.G., 2015. Medicinal plants used to treat TB in Ghana. *International Journal of Mycobacteriology*, *4*(2), pp.116-123.

Njume, C., Afolayan, A.J., Green, E. and Ndip, R.N., 2011. Volatile compounds in the stem bark of Sclerocarya birrea (Anacardiaceae) possess antimicrobial activity against drug-resistant strains of Helicobacter pylori. *International Journal of Antimicrobial Agents*, *38*(4), pp.319-324.

Obakiro, S.B., Kiprop, A., K'owino, I., Andima, M., Owor, R.O., Chacha, R. and Kigondu, E., 2022. Phytochemical, Cytotoxicity, and Antimycobacterial Activity Evaluation of Extracts and Compounds from the Stem Bark of Albizia coriaria Welw ex. Oliver. *Evidence-Based Complementary and Alternative Medicine*, *2022*.

Obakiro, S.B., Kiprop, A., Kowino, I., Kigondu, E., Odero, M.P., Omara, T. and Bunalema, L., 2020. Ethnobotany, ethnopharmacology, and phytochemistry of traditional medicinal plants used in the management of symptoms of tuberculosis in East Africa: a systematic review. *Tropical medicine and health*, *48*(1), pp.1-21.

Ogu, A.C., 2011. Anti-tuberculosis activities of medicinal plants used in the treatment of tuberculosis in HIV patients in Nigeria. *African Journal of Microbiology Research*, *5*(10), pp.1126-1130.

Ojewole, J.A., 2003. Evaluation of the anti-inflammatory properties of Sclerocarya birrea (A. Rich.) Hochst.(family: Anacardiaceae) stem-bark extracts in rats. *Journal of ethnopharmacology*, *85*(2-3), pp.217-220.

Oloya, B., Namukobe, J., Ssengooba, W., Afayoa, M. and Byamukama, R., 2022. Phytochemical screening, antimycobacterial activity and acute toxicity of crude extracts of selected medicinal plant species used locally in the treatment of tuberculosis in Uganda. *Tropical medicine and health*, *50*(1), pp.1-13.

Okunade, A.L., Elvin-Lewis, M.P. and Lewis, W.H., 2004. Natural antimycobacterial metabolites: current status. *Phytochemistry*, *65*(8), pp.1017-1032.

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Omosa, L.K., Akala, H., Kenanda, E.O. and Ndunda, B., 2016. Variability of surface exudates of Dodonaea angustifolia Lf, antioxidant and antiplasmodial activities of the compounds. *Journal of Natural Sciences Research*, *6*(10), pp.78-85.

Pallant, C.V.; Steenkamp, V. 2008. In-vitro bioactivity of Venda medicinal plants used in the treatment of respiratory conditions. Hum Exp Toxicol, 27, 859-866.

Perveen, S. and Sharma, R., 2022. Screening approaches and therapeutic targets: The two driving wheels of tuberculosis drug discovery. *Biochemical Pharmacology*, p.114906.

Philomena, G., (2011). Concerns regarding the safety and toxicity of medicinal plants - An overview. Journal of Applied Pharmaceutical Science, 1(6): 40-44

Povi, L.E., Aboudoulatif, D., Batomayena, B., Serge, M., Kwashie, E.G., Kodjo, A., Edmond, C. and Mensanvi, G., 2015. Cytotoxicity of plant extract on Neuro-2a, Caco-2 and Vero cell lines by using MTT assay and Neutral Red uptake test. *Annals of Pharma Research*, *3*(07), pp.129-131.

Priya, V.T., Balasubramanian, N., Shanmugaiah, V., Sathishkumar, P., Kannan, N.D., Karunakaran, C., Alfarhan, A. and Antonisamy, P., 2021. Partially purified lead molecules from Dodonaea viscosa and their antimicrobial efficacy against infectious human pathogens. *Journal of Infection and Public Health*, *14*(12), pp.1822-1830.

Pruden, A., Vikesland, P.J., Davis, B.C. and de Roda Husman, A.M., 2021. Seizing the moment: now is the time for integrated global surveillance of antimicrobial resistance in wastewater environments. *Current Opinion in Microbiology*, *64*, pp.91-99.

Rani, M.S., Pippalla, Rao S., Mohan, P.K., 2009. Dodonaea viscosa Linn.: an overview. J. Pharm. Res. Health Care 1, 97–112.

Ranjitha, J., Rajan, A. and Shankar, V., 2020. Features of the biochemistry of Mycobacterium smegmatis, as a possible model for Mycobacterium tuberculosis. *Journal of Infection and Public Health*, *13*(9), pp.1255-1264.

Riss, T.L., Moravec, R.A. and Niles, A.L., 2011. Cytotoxicity testing: measuring viable cells, dead cells, and detecting mechanism of cell death. *Mammalian Cell Viability: Methods and Protocols*, pp.103-114.

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Riss TL, Moravec RA, Niles AL, 2016. Cell Viability Assays in: Assay Guidance Manual, Bethesda (MD): Eli Lilly & Company and the National Center for Advancing Translational Sciences.

Russo, D., Kenny, O., Smyth, T.J., Milella, L., Hossain, M.B., Diop, M.S., Rai, D.K. and Brunton, N.P., 2013. Profiling of phytochemicals in tissues from Sclerocarya birrea by HPLC-MS and their link with antioxidant activity. *International Scholarly Research Notices*, *2013*.

Russo, D., Miglionico, R., Carmosino, M., Bisaccia, F., Andrade, P.B., Valentão, P., Milella, L. and Armentano, M.F., 2018. A comparative study on phytochemical profiles and biological activities of Sclerocarya birrea (A. Rich.) Hochst leaf and bark extracts. *International journal of molecular sciences*, *19*(1), p.186.

Salem, M.Z., Zayed, M.Z., Ali, H.M., El-Kareem, A. and Mamoun, S.M., 2016. Chemical composition, antioxidant and antibacterial activities of extracts from Schinus molle wood branch growing in Egypt. *Journal of wood science*, *62*(6), pp.548-561.

Saranya, K., Divyabharathi, U., 2019. Phytochemical and histochemical screening of Dodonaea viscosa leaves. Pramana Res. J. 9, 53–59

Schroeder, E., de Souza, N., Santos, D., Blanchard, J., Basso, L. 2002 "Drugs that inhibit mycolic acid biosynthesis in Mycobacterium tuberculosis." Curr Pharm Biotechnol.. Volume 3. p. 197-225.

Sharifi-Rad, J., Salehi, B., Stojanović-Radić, Z.Z., Fokou, P.V.T., Sharifi-Rad, M., Mahady, G.B., Sharifi-Rad, M., Masjedi, M.R., Lawal, T.O., Ayatollahi, S.A. and Masjedi, J., 2020. Medicinal plants used in the treatment of tuberculosis-Ethnobotanical and ethnopharmacological approaches. *Biotechnology advances*, *44*, p.107629.

Sharma, A. and Gupta, S., 2022. Protective manifestation of herbonanoceuticals as antifungals: A possible drug candidate for dermatophytic infection. *Health Science Reports*, *5*(5), p.e775.

Singh, A., Venugopala, K.N., Pillay, M., Shode, F., Coovadia, Y. and Odhav, B., 2021. Antimycobacterial activity of aqueous and methanol extracts of nine plants against Mycobacterium bacteria. *Tropical Journal of Pharmaceutical Research*, *20*(4), pp.849-858.

© University of Venda

Sivakumar, A., 2011. Anti-tuberculosis activity of commonly used medicinal plants of south India. *Journal of Medicinal Plants Research*, *5*(31), pp.6881-6884.

Sobeh, M., ElHawary, E., Peixoto, H., Labib, R.M., Handoussa, H., Swilam, N., El-Khatib, A.H., Sharapov, F., Mohamed, T., Krstin, S. and Linscheid, M.W., 2016. Identification of phenolic secondary metabolites from Schotia brachypetala Sond.(Fabaceae) and demonstration of their antioxidant activities in Caenorhabditis elegans. *PeerJ*, *4*, p.e2404.

Sofowora, A., Ogunbodede, E. and Onayade, A., 2013. The role and place of medicinal plants in the strategies for disease prevention. *African journal of traditional, complementary and alternative medicines*, *10*(5), pp.210-229.

Suliman, A., 2010. *The antimicrobial activity and chemical profile of traditional medicinal plants indigenous to Southern Africa used to treat respiratory tract infections* (Doctoral dissertation, University of the Witwatersrand).

Swain, S.S., Pati, S. and Hussain, T., 2022. Quinoline heterocyclic containing plant and marine candidates against drug-resistant Mycobacterium tuberculosis: A systematic drug-ability investigation. *European Journal of Medicinal Chemistry*, *232*, p.114173.

Sylvester P. W. (2011). Optimization of the tetrazolium dye (MTT) colorimetric assay for cellular growth and viability. *Methods in molecular biology,* 716: 157–168. https://doi.org/10.1007/978-1-61779-012-6_9

Talib, W.H. and Mahasneh, A.M., 2010. Antimicrobial, cytotoxicity and phytochemical screening of Jordanian plants used in traditional medicine. *Molecules*, *15*(3), pp.1811-1824.

Teffo, L.S., Aderogba, M.A., Eloff, J.N. (2010). Antibacterial and antioxidant activities of four kaempferol methyl ethers isolated from Dodonaea viscosa Jacq. var. angustifolia leaf extracts. South African Journal Botany 76(1), 125.

Tlhapi, D.B., Ramaite, I.D., Van Ree, T., Anokwuru, C.P., Orazio, T.S. and Hoppe, H.C., 2018. Isolation, chemical profile and antimalarial activities of bioactive compounds from rauvolfia caffra sond. *Molecules*, *24*(1), p.39.

Tomar, N.S., Sharmar, M. and Agarwal, M.R., (2015). Phytochemical analysis of Jatropha curcas L. during different seasons and developmental stages and seedling

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growth of wheat (Triticum aestivum L) as affected by extracts/leachates of Jatropha curcas L. Physiology and Molecular Biology of Plants, 21(1): 83-92

Tong, Z.W., Gul, H., Awais, M., Saddick, S., Khan, F.S., Gulfraz, M., Afzal, U., Nazir, K., Malik, M.Y., Khan, S.U. and Khan, M.I., 2021. Determination of in vivo biological activities of Dodonaea viscosa flowers against CCL4 toxicity in albino mice with bioactive compound detection. *Scientific Reports*, *11*(1), pp.1-15.

Van Wyk, B.-E., Van Oudtshoorn, B., & Gericke, N. 2009. Medicinal plants of South Africa. Briza Publications, Pretoria.Vlok, J. & Schutte-Vlok, A. 2010. Plants of the Klein Karoo. Umdaus Press, Hatfield, South Africa.

Van Wyk, B.-E. & Gericke, N. 2000. People's plants: A guide to useful plants of southern Africa. Briza Publications, Pretoria.

Van wyk, b-e., van oudtshoorn, b. & gericke, n. 1997. medicinal plants of south africa. briza publications, pretoria.

Van Wyk, Braam [A.E.] & Van Wyk, P. 1997. Field guide to trees of southern Africa. Struik Publishers, Cape Town.

Vega-Avila E, Pugsley MK. (2011). An overview of colorimetric assay methods used to assess survival or proliferation of mammalian cells. Proc West Pharmacol Soc., 54: 10–144. PMID: 22423572.

Venter, F. and Venter, J.A., 1996. *Making the most of indigenous trees*. Briza publications.

World Health Organization, 2022. Rapid communication: key changes to the treatment of drug-resistant tuberculosis (No. WHO/UCN/TB/2022.2). World Health Organization.

WHO, 2014. Global Tuberculosis Report 2014, <http://www.who.int/ tdr/news/2014/global-TB report/en/>.

Wu, K.J., Zhang, J., Baranowski, C., Leung, V., Rego, E.H., Morita, Y.S., Rubin, E.J. and Boutte, C.C., 2018. Characterization of conserved and novel septal factors in Mycobacterium smegmatis. *Journal of Bacteriology*, *200*(6), pp. e00649-17.

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- https://www.researchgate.net/figure/Resazurin-Microtitre-Assay-REMA-for-determining-the-MIC-of-vasicine-against-one-of-the-fig2_282335785/amp on 17 November 22

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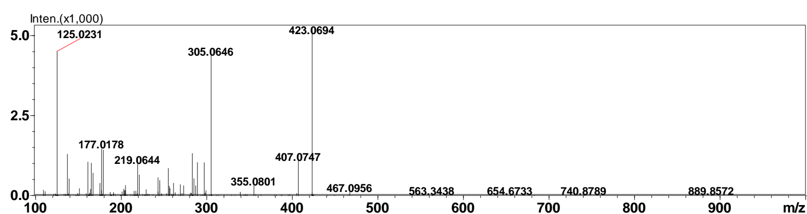
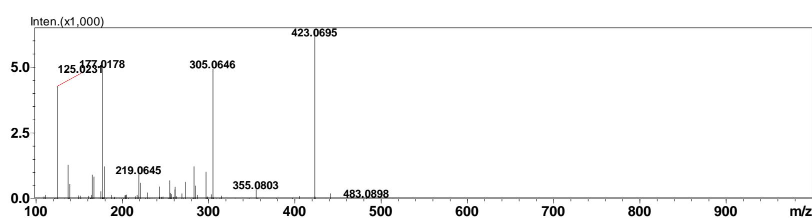
CHROMATOGRAMS

Chromatograms of *Dodonaea Viscosa Anguistifolia* bark extracts

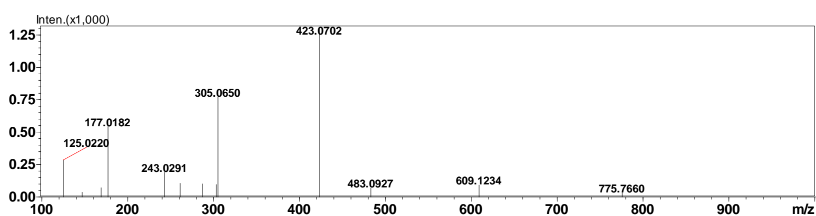
1 (Epi)catechin–(epi)gallocatechin isomer



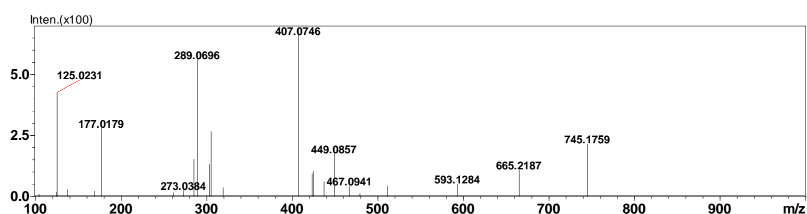
2 (epi)gallocatechin–(epi)gallocatechin (prodephinidin B2) isomer



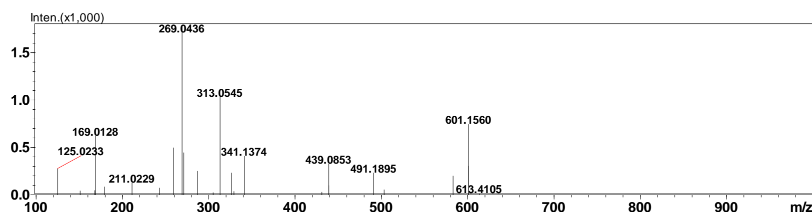
3 (Epi)gallocatechin–(epi)gallocatechin-3'-O-gallate (prodelphinidin B3-3'-O-gallate) isomer



4 (Epi)gallocatechin3-O-gallate–(epi)catechin isomer



5 Eriodictyol-O-glucoside-O-gallate isomer



6 Myricetin glucoside

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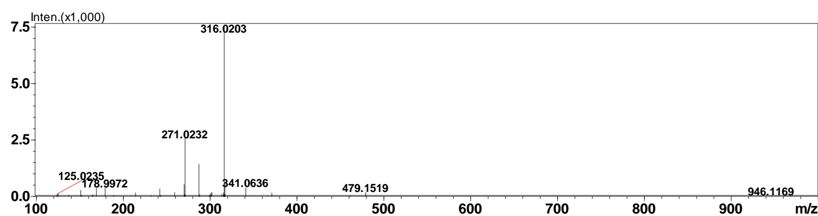
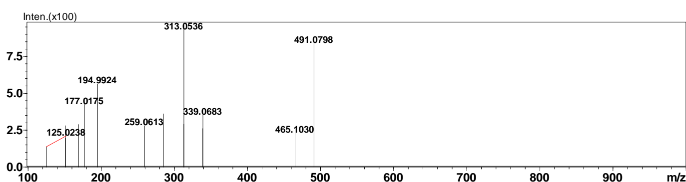
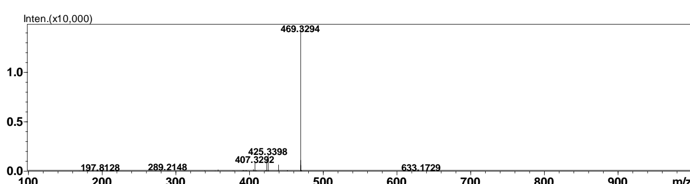


7 Dihydroquercetin-O-glucosyl-O-gallate

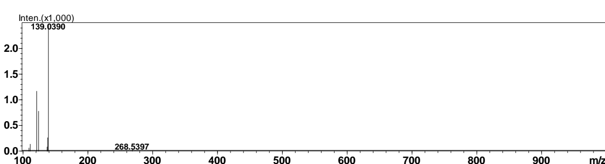


8 Oleanonic acid

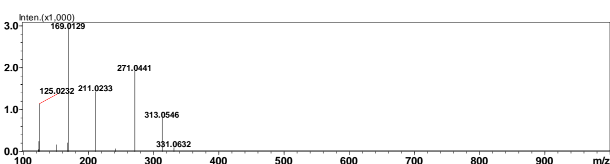


Chromatograms of *Dodonaea Viscosa Anguistifolia* leaves

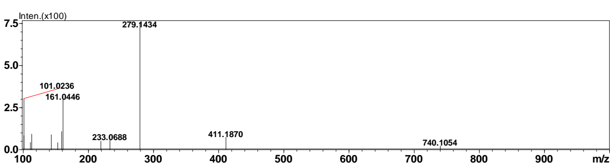
1 (Iso) tachioside



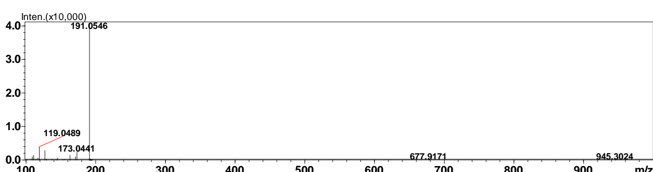
2 Digalloylglucoside isomer



3 Oleic acid



4 p-Coumaric acid



5 Quercetin

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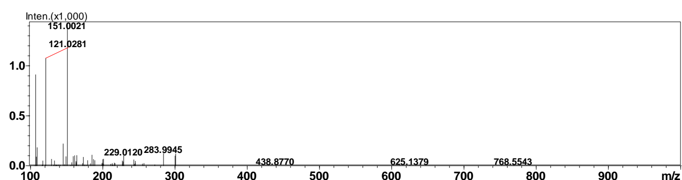
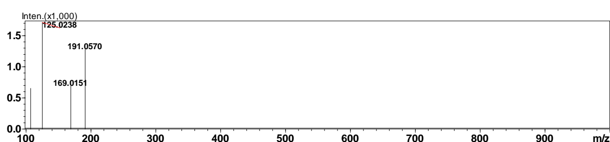
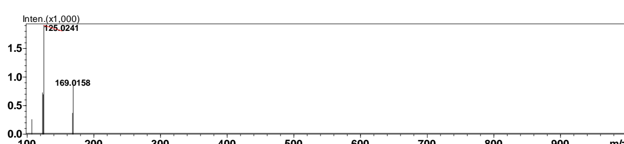


Chromatograms of *S. birrea* barks

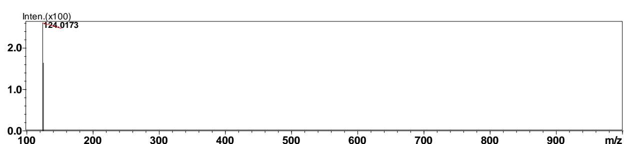
1 Galloylquinic acid



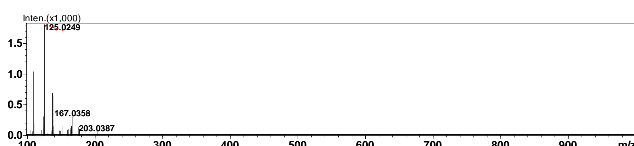
2 Galloyl glucoside isomer



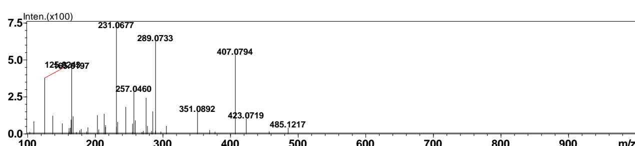
3 Gallic acid



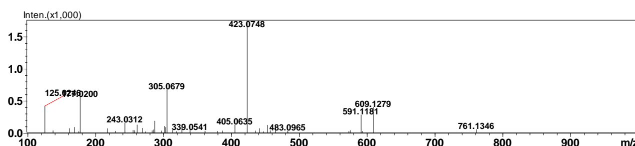
4 (Epi)gallocatechin isomer



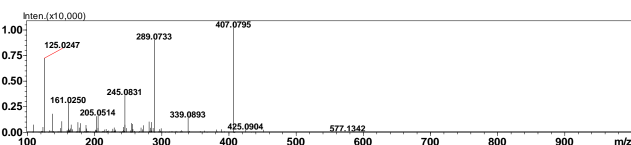
5 (Epi)gallocatechin isomer



6 (Epi)gallocatechin–(epi)gallocatechin- 3’-O-gallate (prodelphinidin B3-3’-O-gallate)



7 (Epi)catechin–(epi)catechin (proanthocyanidin B2)



8 (Epi)catechin isomer

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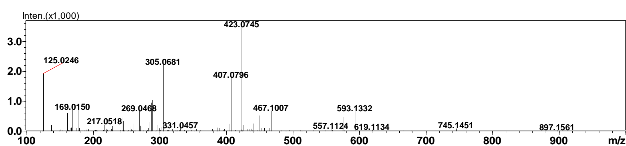
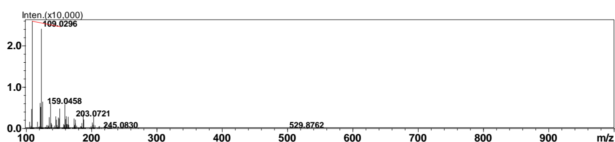
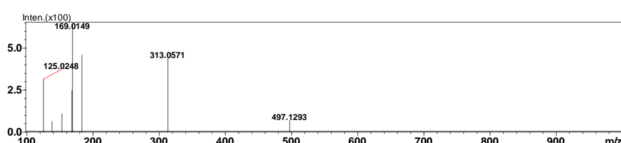


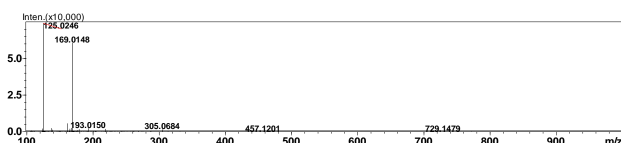
9 Trimethoxyphenyl glucoside gallate



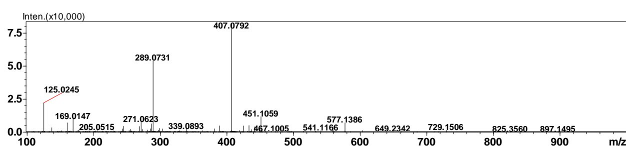
10 (Epi)gallocatechin-3-O-gallate isomer



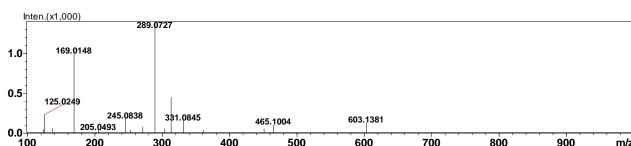
11 Epi)catechin-3’-O- gallate-–(epi)catechin isomer



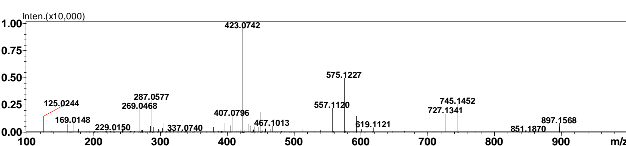
12 (Epi)catechin-3-O-glucoside-gallate



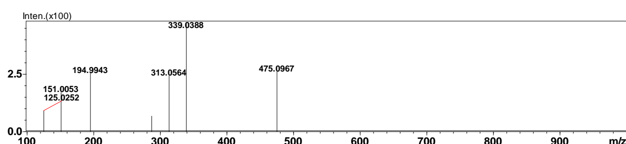
13 Epi)gallocatechin-3-O- gallate–(epi)catechin-3’-O-gallate isomer



14 Eriodictyol-O-glucoside-O-gallate isomer



15 (Epi)catechin-3-O-gallate isomer



16 Digalloylated procyanidin B (P2G2)

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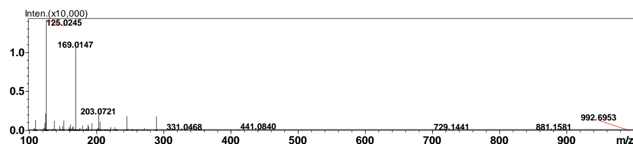
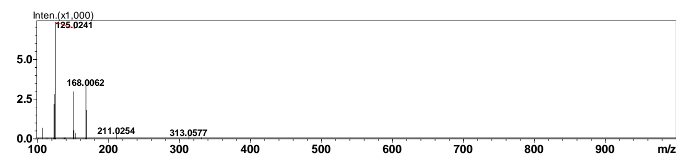
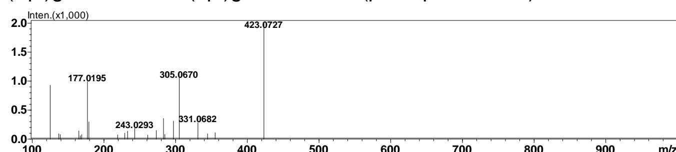


LCMS profile of *S. birrea* leaves

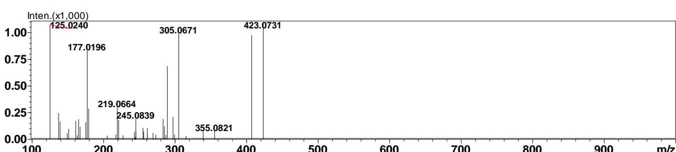
1 Galloyl glucoside isomer



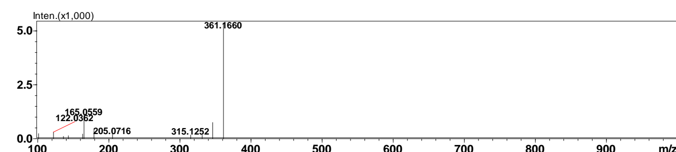
2 (Epi)gallocatechin–(epi)gallocatechin (prodephinidin B2) isomer



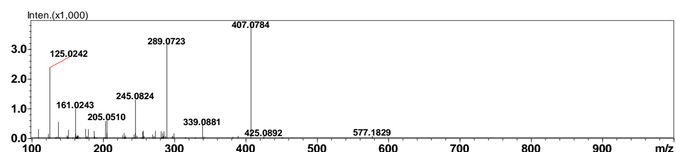
3 (Epi)catechin–(epi)gallocatechin isomer



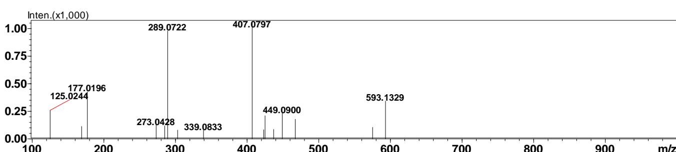
4 Galloyl hexoside derivative



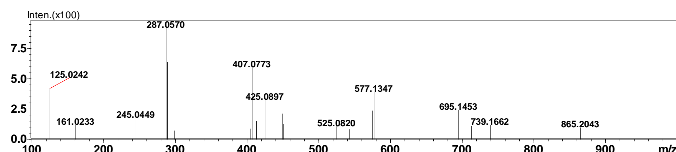
5 (proanthocyanidin B2)



6 (Epi)gallocatechin–(epi)catechin-3'-O- gallate



7 Procyanidin C1



8 (Epi)gallocatechin-3-O-gallate isomer

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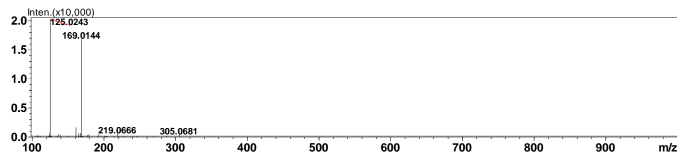
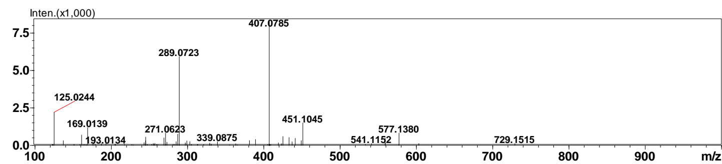
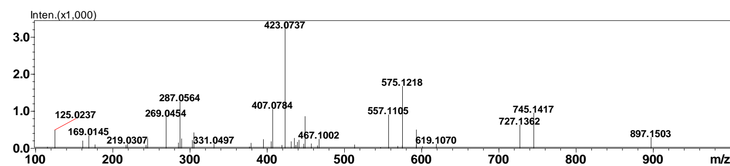


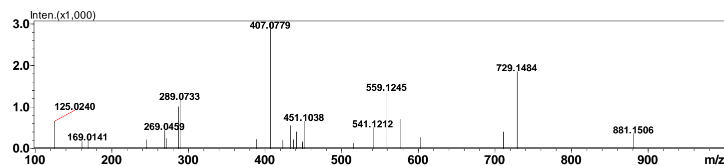
9 (Epi)catechin-3-Ogallate–(epi)catechin isomer



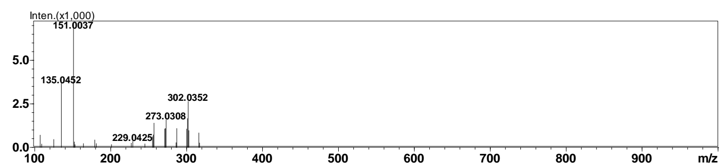
10 (Epi)catechin-3-O-gallate–(epi) gallocatechin-3-O-gallate isomer



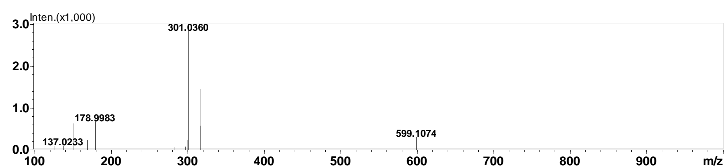
11 (Epi)catechin-3-Ogallate–(epi)catechin-3’-O-gallate (procyanidin B2-3,3’ di-O-gallate) isomer



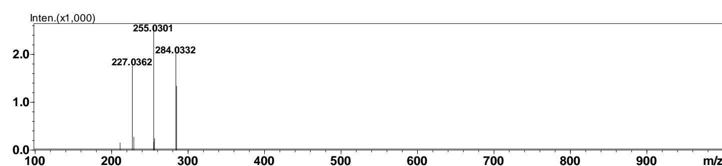
12 Eriodictyol-O-glucoside isomer



13 Kaempferol 3-O-β-D-(6’’- galloyl)glucopyranoside



14 kaempferol 3-O-α-L-rhamnopyranoside



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