ANTIOXIDANT, ANTIMICROBIAL, AND ANTIBIOFILM PROPERTIES OF *OPUNTIA STRICTA* AND *VACHELLIA ERIOLOBA*, AND CHEMICAL CHARACTERIZATION OF VOLATILE COMPOUNDS

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ABSTRACT

Vachellia erioloba, commonly known as *Acacia erioloba* belongs to the family Fabaceae and is native to Namibia, Botswana, and South Africa. The plant is used traditionally to treat ear infections, headaches, and toothaches. *Opuntia stricta* belongs to the family Cactaceae and is found in Namibia. The plant is used in the management of diseases such as diabetes, obesity, and cancer. This study was designed to investigate the antioxidant, antimicrobial, antibiofilm activity, and chemical characterization of *V. erioloba* and *O. stricta*. The radical scavenging capacity of extracts was estimated using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and the reducing power was determined using ferric reducing power assay. Moreover, the antimicrobial activities of the extracts of these medicinal plants were tested against *Staphylococcus aureus*, *Streptococcus mutans*, *Escherichia coli*, and *Candida albicans*. On the other hand, antibiofilm activity was determined against *Staphylococcus aureus*. The phytochemical analysis of *V. erioloba* and *O. stricta* was done using qualitative and quantitative phytochemical analysis. Moreover, the characterization of the volatile constituents was done for the seed extract of *V. erioloba*. Furthermore, chemical characterization of the volatile constituents of the extract was performed using gas chromatography-mass spectrometry (GC–MS). The radical scavenging assay revealed IC_{50} values of 1.973 ± 0.012 and 972.0 ± 22.3 µg/mL for the seed pods and seed of *V. erioloba*, respectively, while the IC_{50} values for the extracts of *O. stricta* were 53.74 ± 0.18, 153.8 ± 0.9, 258.7 ± 0.7, and IC_{50} > 1000 µg/mL for the seeds, flowers, fruits, and leaves of *O. stricta*, respectively. In addition, the reducing power of both *V. erioloba* and *O. stricta* extracts increased as the concentration of the extracts increased, which confirmed the presence of antioxidants in the extracts. The antimicrobial assay revealed that the various extracts of *V. erioloba* and *O. stricta*

exhibited moderate antimicrobial activity with inhibition zones (IZ) ranging from 0 to 12 mm. Moreover, the MIC obtained from this study ranged from ≤ 0.63 to 10 mg/mL. The biofilm inhibition demonstrated BIC_{50} values of *V. erioloba* and *O. stricta* extracts ranged from < 0.08 to 0.498 ± 0.017 mg/mL. Moreover, biofilm eradication revealed BEC_{50} values ranged from < 0.08 to 0.172 ± 0.008 mg/mL. The GC-MS analysis of *V. erioloba* seed extracts revealed the presence of phytosterols, acids, esters, α-tocopherol and α-amyrin, which comprises a number of known bioactive compounds. The results from this study show that *V. erioloba* and *O. stricta* extracts possess antioxidant, antimicrobial, and antibiofilm properties, making these plants good potential sources of antioxidants and antimicrobial agents. The findings of this study support the traditional uses of *V. erioloba* and *O. stricta*.

Keywords: Traditional uses, Biofilm inhibition, biofilm eradication, 2, 2-diphenyl-1-picrylhydrazyl (DPPH), gas chromatography-mass spectrometry (GC–MS)

LIST OF CONFERENCE PROCEEDINGS

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LIST OF ABBREVIATION AND ACRONYMS

DPPH	2,2 – Diphenyl – 1 – picrylhydrazyl
GAE	Gallic acid Equevalents
QE	Quercetin Equivalents
HAT	Hydrogen Atom Transfer
SET	Single Electron Transfer
MIC	Minimum Inhibitory Concentration
MRSA	Methicillin Resistant Staphylococcus aureus
TFC	Total Flavonoid Content
TPC	Total Phenolic Content
WHO	World Health Organisation
ATCC	American Type Culture Collection
DNA	Deoxyribo Ncleic Acids
PIA	Polysaccharide Intercellular Adhesion
GC-MS	Gas Chromatography-Mass Spectroscopy
IC_{50}	Half-Maximal Inhibitory Concentration
NIST	National Institute of Standard and Technology
RI	Retention index
BIC_{50}	Biofilm inhibitory concentration

BEC_{50}	Biofilm eradication concentration
OD	Optical density
iu	Index units

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DEDICATION

This thesis is dedicated to my mother, Ndapewashiwa Nghifiudja, my grandmother, Julia M. Shetuhanga and to my entire family. Thank you so much for seeing the potential and supporting my dreams and for never giving up on me.

DECLARATION

I, **Junias Natangwe Jackson**, hereby declare that this study is my own work and is a true reflection of my research, and that this work, or any part thereof has not been submitted for a degree at any other institution. No part of this thesis/dissertation may be reproduced, stored in any retrieval system, or transmitted in any form, or by means (e.g. electronic, mechanical, photocopying, recording or otherwise) without the prior permission of the author, or The University of Namibia in that behalf. I, **Junias Natangwe Jackson**, grant The University of Namibia the right to reproduce this thesis in whole or in part, in any manner or format, which The University of Namibia may deem fit.

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1. INTRODUCTION

1.1 Background of the study

Medicinal plants have been used traditionally for various diseases over the years because of the presence of bioactive phytochemicals [1]. According to the World Health Organization (WHO), about 80% of the world population use medicinal plants for various purposes which are mostly health related [2]. Namibia has a rich diversity of medicinal plants, many of which are commercially produce such as Devil's claw, Mopane and Marula [3]. There are different local facilities that produce medicinal plant products for commercial purposes, and Farm Vredelus is one of them. Farm Vredelus is located in Mariental (Namibia) and commercializes various medicinal plants mostly based on traditional knowledge. The farm commercializes various plants such as *Hoodia gordonii* [3], *Sceletium tortuosums* [4], and *Bulbine frutescens* [5]. *Vachellia erioloba* and *Opuntia stricta* are some of the plants produced by Farm Vredulus and the farm is considering commercializing the two plants based on traditional knowledge. *Vachellia erioloba* is used traditional to treat ear infection, influenza, gonorrhoea, diarrhoea, and it is also used as a cough remedy, treat toothaches and headaches [6,7]. *Opuntia stricta* is been traditionally used in the treatment of diabetes, obesity and cancer [8,9].

1.2 Statement of the problem

Vachellia erioloba and *Opuntia stricta* are traditionally used in the treatment of various ailments such ear infection, toothaches, diabetes, obesity and cancer. However, no scientific studies have been conducted on the plants in relation to the traditional claims. There is a need for scientific validation to add commercial value to

the two plants. In addition, the chemical composition of both *V. erioloba* and *O. stricta* is not known.

1.3 Objectives of the study

The objectives of this study were to:

a) Determine the antioxidant activity of *V. erioloba* and *O. stricta* extracts using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and ferric reducing antioxidant power (FRAP) assays.
b) Investigate the antimicrobial properties of *V. erioloba* and *O. stricta* extracts against selected microorganisms
c) Investigate the antibiofilm activity of *V. erioloba* and *O. stricta* extracts against *Staphylococcus aureus*.
d) Qualitative Screening for the presence of selected phytochemicals
e) Determine the total phenolic content (TPC) and total flavonoid content (TFC) of *V. erioloba* and *O. stricta*
f) Identification of the volatile and semi-volatile constituents present in the non-polar fraction of the seed extract of *V. erioloba*

1.4 Significance of the study

Research on natural products as antioxidant and antimicrobial agents has become an important aspect in the fight against certain diseases such as cardiovascular diseases and cancers [10]. Moreover, plants have also become the main focus in drug development due to potential antimicrobial activities [11]. Farm Vredelus is considering expanding their range of commercial medicinal plant products. The findings for add study will add commercial value to *V. erioloba* and *O. stricta.*

1.5 Limitation of the study

In a traditional setting most extracts are used in aqueous form, however, analysis in this study could not be conducted on aqueous extracts. Furthermore, various antioxidant assays exist and not all could be conducted in this study. There is no high-pressure liquid chromatography – high resolution mass spectrometry instrument at the University of Namibia, hence the characterization of non-volatile compounds was not possible.

1.6 Delimitation of the study

The study only focused on investigating the Dichloromethane: Methanol extracts of *V. erioloba* and *O. stricta*. Two antioxidant assay were applied in this study; 2,2-diphenyl-1-picrylhydrazyl (DPPH) and ferric reducing antioxidant (FRAP) assays. The antimicrobial activity was determined using selected microorganisms namely *S. aureus, S. mutans, E. coli, and C. albicans*. The antibiofilm activity was only determined using *S. aureus*.

2. LITERATURE REVIEW

2.1 Plant description, and geographical location

2.1.1 Vachellia erioloba

Vachellia erioloba (camelthorn) commonly known as *Acacia erioloba* belongs to the *Vachellia* Genus [7]. It is native to south western Africa and it is found in countries such as Namibia, Angola, Botswana, Mozambique, South Africa and Zimbabwe. *Vachellia erioloba* is considered an important species because many other species and ecosystems depend on it, providing food, shelter, and nesting for many animal species [12]. *Vachellia erioloba* is used traditionally to make coffee-like drink and porridge. The seed pods of *V. erioloba* are spongy on the inside with irregularly placed seeds and are used as nutrients for animals [13]. Figure 1 shows the seed pods and seeds of *V. erioloba* donated by Farm Vredulus. *Vachellia erioloba* is a medium to large spreading tree, and remains green throughout most of the dry season [12]. The ecology of the species suggests that it is adapted to sandy soils that can sometimes be alkaline [13].

Figure 1. *Vachellia erioloba* seed pods and seeds (Source: Junias N. Jackson, Windhoek).

2.1.2 Opuntia stricta

Opuntia stricta is a prickly succulent plant of the Cactaceae family, native to southeast America, eastern Mexico and Caribbean islands. The plant has invaded other regions including, southern and eastern Africa, in countries like Namibia, Botswana and South Africa [9,14]. *Opuntia stricta* is widely spreading succulent plant, usually reaching a height of 50 to 100 cm, but in some cases up to 2 m [15]. The leaves of *O. stricta* are little cone shaped structures and are shed from the cladodes [9]. The flowers of *O. stricta* are bright yellow and bloom throughout the summer [15].The fruits of *O. stricta* are barrel shaped red to purple coloured, usually 46 cm long and 23 cm wide [15]. The seeds of *O. stricta* have a diameter of 45 mm and are embedded in the purple pulp [15].

Figure 2 shows the seeds, flowers, leaves and fruits of *O. stricta* donated by Farm Vredulus.

Figure 2. *Opuntia stricta* seeds (A), flower (B), leaves (C), and fruits (D) (Source: Junias N. Jackson, Windhoek).

2.2 Traditional uses of *Vachellia erioloba* and *Opuntia stricta*

The roots of *V. erioloba* are traditionally used as a cough remedy while the powder from seed pods is used to treat ear infection [16]. No scientific reports exist that support the traditional claims of *V. erioloba*.

Opuntia stricta (prickly pear) is used traditional to treat diabetes, as a cough remedy and treat inflammation [8]. A study done in Tunisia by Koubaa et al [17] reported that fruit peels of *O. stricta* exhibit antioxidant and antibacterial activity. In addition, another study by Kampamba et al [15] reported the in vitro antidiabetic, anti-inflammatory, antihyperglycemic, neuroprotective, and antispermatogenic properties of *O. stricta*.

2.3 Bioactive phytochemicals

Phytochemicals are naturally occurring substances in plants that have been shown to provide health benefits [18]. The most abundant biological storage of diverse phytochemicals are medicinal plants and they are utilized for various diseases and ailments. Primary and secondary metabolites are the two types of phytochemicals. Sugars, amino acids, fatty acids, and nucleotides are examples of primary metabolites, which are chemical molecules required for plant growth, development, and reproduction of plants [19]. Secondary metabolites, on the other hand, are organic substances that are not directly engaged in an organism's regular growth, development, or reproduction but perform protective functions in the plant and the human body. Secondary metabolites, for example, can defend the body against free radicals, affect the immune system, and destroy harmful bacteria [20]. Secondary metabolites include phytochemicals such as phenols, flavonoids, alkaloids, saponins, tannins, steroids, etc.

2.3.1 Phenolic

Phenolic compounds are secondary metabolites of plants with common aromatic rings with one or more hydroxyl groups [21]. Over 8,000 natural phenolic compounds have been discovered to far [22]. Simple phenols, flavonoids, tannins, and coumarins are examples of phenolic chemicals obtained from plant sources [23]. These phenolic compounds have strong anti-cancer effects and are known to fight various diseases associated with oxidative stress [24]. Previous studies have shown that the health-promoting effects of phenolic are due to their ability to exhibit antioxidant, antimicrobial and anti-inflammatory [21–23].

2.3.2 Flavonoids

Flavonoids are an important class of natural products. In particular, they belong to a class of secondary plant metabolites with polyphenolic structures that are widely

distributed in fruits, vegetables, and certain beverages [25]. Flavonoids are associated with a variety of health benefits and are essential ingredients in a variety of dietary supplements, pharmaceuticals, and cosmetics applications. Flavonoids promote antioxidant activity, cell health, normal tissue growth, and whole-body regeneration [26]. It may also work with Vitamin C to reduce oxidative stress on the water-based parts of cells and delay some of the effects of aging [27]. There are over 4,000 unique flavonoids, which are most effective when multiple types are taken together. Flavonoids have several subgroups, which include chalcones, flavones, flavonols, and isoflavones [28]. Flavonoids protect plants from different biotic and abiotic stresses and act as unique UV filters, functioning as signal molecules, allopathic compounds, detoxifying agents, and antimicrobial defensive compounds [29,30].

2.3.3 Alkaloids

Alkaloids are a large, structurally diverse group of microbial, plant, and animal-derived natural products. Alkaloids are characterized by a great deal of structural diversity, with the presence of basic nitrogen atoms being the only unified feature [31]. Despite their structural diversity, alkaloids share many physical and chemical properties. Alkaloids are involved not only in the beneficial effects of traditional medicines, but also in the harmful effects of toxins such as ergot, and are considered a natural blessing and curse [32]. Alkaloids are still the focus of many studies, and their development as antibacterial agents is one of the focal points of drug research [33–35]. They pharmacological properties include analgesics, central nervous system stimulants, central nervous system depressants, and antimalarial drugs activity. These activities are utilized in both traditional and modern medicine [36,37].

2.3.4 Saponins

Saponins are naturally occurring, structurally, and functionally diverse phytochemicals that are widespread in plants. They are a complex and chemically diverse group of compounds composed of triterpenoids or steroid aglycones bound to oligosaccharide units [38]. The combination of a hydrophobic aglycone skeleton and hydrophilic sugar molecules makes saponins highly amphipathic, having both foaming and emulsifying properties [38]. These molecules play an important role in plant ecology and are also used in a wide range of commercial applications in the fields of food, cosmetics, and pharmaceuticals. Saponins are also hypocholesterolemia, anti-carcinogenic, anti-inflammatory, antibacterial, and antigenic and have antioxidant properties [39]. Due to their antibacterial, antifungal, insecticidal, insecticidal, and feeding inhibitory properties, it is widely recognized that they play an important role in plant defense against pathogens, pests, and herbivore [40,41].

2.3.5 Tannins

Tannins are plant phenolic secondary metabolites used in the treatment of animal skins to avoid their putrefaction. Due to the interaction between tannins and the collagen from the skins, the collagen can be stabilized and the animal skins tanned and transformed into leather [23]. Tannins are molecules able to interact and precipitate proteins, among other molecules, such as polysaccharides and polyphenols [24]. Hydrolyzable tannins, condensed tannins and phlorotannins are the main groups of tannins that are found in various parts of the higher plants. As natural products in plants, they act as a natural barrier against insects, pathogens and animals, because of their ability to react with proteins and their antioxidant properties [21,42,43]. Therefore, tannins contribute to the reduction of cardiovascular diseases and of some cancer risks.

2.3.6 Steroids

Steroids are a subgroup of compounds that belongs to terpenoid class. They have a wide range of biological activities, including anti-plasmodium activity [39]. Due to their chemical structure, they show medicinal properties, including some antimalarial activity. Examples of these steroids include lupeol, cardiac glycosides and phytosterols [31].

2.4 Natural antioxidants

Natural antioxidants of plant origin can be divided into three major classes: phenolic compounds, vitamins and carotenoids [44]. Some phenolic compounds are not only the most important plant compounds with antioxidant activity, but also exhibit antibacterial, antibiofilm and antifungal activity [44,45]. Phytochemicals, in particular flavonoids, phenolic and tannins have shown antioxidant properties that could be used in the treatment of oxidative stress induced neurological disorders [44].

2.4.1 Free radicals and reactive oxygen species (ROS)

Oxidative stress is caused by an imbalance between the production and accumulation of oxygen reactive species (ROS) in cells and tissues and the biological system to detoxify these reactive products [46]. Reactive oxygen species are highly reactive chemicals formed from O_2 that include superoxide radicals (O_2), hydrogen peroxide (H_2O_2), hydroxyl radical (OH), and singlet oxygen ($^1O_2$) they are produced as by-products of metabolism in biological systems and they cause oxidative stress [45,47,48]. Oxidative stress is associated with several disease such as cancer, cardiovascular disease, neural disorder, Alzhimer, Parkinson, atherosclerosis, diabetes and metabolic disorder [48]. Reactive oxygen species can be neutralized by antioxidant compounds such a vitamin C, E, reduced thiols and carecholamines.

2.4.2 The role of antioxidants in diseases prevention

Antioxidants are compounds that has the ability to donate an electron to oxidants in order to stop the chain reaction of oxidation [49,50]. Studies shows that antioxidants neutralize free radical and maintain the intracellular balance of the body [49,50]. They are associated will the prevention of many diseases such as cancer, atherosclerosis, and cardiovascular disease. Cancer is thought to be caused by damage to DNA which is an oxidative process [51]. Furthermore, antioxidants can interfere with the metabolic activation of chemical carcinogens and may then inhibit the development of cancer [49,51]. Antioxidants such as vitamin C, Vitamin E and beta-carotene are known to lower the rates of cancer. Atherosclerosis is a chronic inflammatory disease and the major cause of coronary heart disease and stroke in humans. antioxidants facilitate atherosclerosis treatment through a variety of mechanisms, including the inhibition of LDL oxidation, the reduction of generated reactive oxygen species, the inhibition of cytokine secretion, the prevention of atherosclerotic plaque formation and platelet aggregation [47].

2.5 Antioxidant assays

2.5.1 Diphenyl-1-PicrylHydrazyl (DPPH) Radical

DPPH is a stable free radical, which has a violent colour with an absorbance maximum between 515 - 520 nm. Antioxidant and other radical species are able to react with this stable radical (DPPH) by providing an electron or hydrogen atom, hence reducing it to 2,2 – diphenyl-1-hydrazine (DPPH-H) this is characterized by a colourless or pale yellow colour (fig. 3) which could be easily measure with a spectrophotometer [52]. The system is sensitive for radical reaction/H-transfer (HAT) or reduction/single e^--transfer (SET). DPPH scavenging capacity is usually evaluated in organic media such as ethanol or methanol by monitoring the absorbance decrease at 515–528 nm until

the absorbance remains constant. Light, oxygen and pH have an influence on DPPH absorbance [53,54]. Test compounds with absorption at 520 nm (e.g. carotenoids) interfere with the absorption maximum of DPPH. Small molecules react more easily with DPPH than polymers (thus polymeric antioxidants in plant extracts are underestimated) [53–55]. Generally, the results are reported as half-maximal inhibitory concentration (IC_{50}). The IC_{50} value is the amount of antioxidant necessary to decrease by 50% the initial DPPH concentration. Percentage inhibition and IC_{50} are used very frequently as parameters characterizing the radical scavenging activity. The lower IC_{50} indicates the higher radical scavenging potential [53].

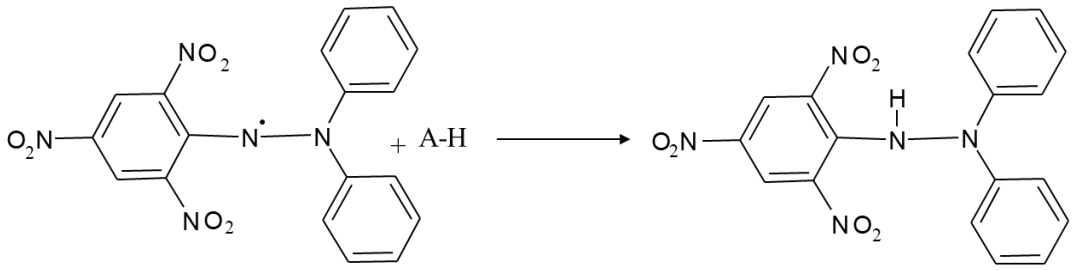

Figure 3. Principle of DPPH free radical scavenging assay.

2.5.2 Reducing Power Assay

The reducing power assay measures the ability of antioxidants to bind to the ferric 2,4,6-tripyridyl-s-triazine complex $[Fe^{3+} (TPTZ)_2]^{3+}$ to produce an intense blue iron complex $[Fe^{3+} (TPTZ)_2]^{2+}$ in acidic medium [53,54]. The reducing power assay measure the reducing power in plasma, but has been adopted and used as the assay for antioxidants found in various foods and beverages. Figure 4 illustrates the reduction reaction of ferric 2,4,6-tripyridylstriazine (TPTZ) to a coloured product [53]. The reducing assay is conducted at acidic pH 3.6 to maintain iron solubility. Reaction at low pH decreases the ionization potential that drives electron transfer and increases the redox potential, causing a shift in the dominant reaction mechanism [53,54]. The

oxidation or reduction of radicals to ions the radical chain, and the reduced power reflects the compound's ability to regulate the redox tone of plasma and tissues. The reducing value is calculated by measuring the increase in absorbance at 593 nm and referring to a ferrous ion standard solution or an antioxidant standard solution. The change in absorbance is proportional to the sum of the FRAP values of the antioxidants in the sample.

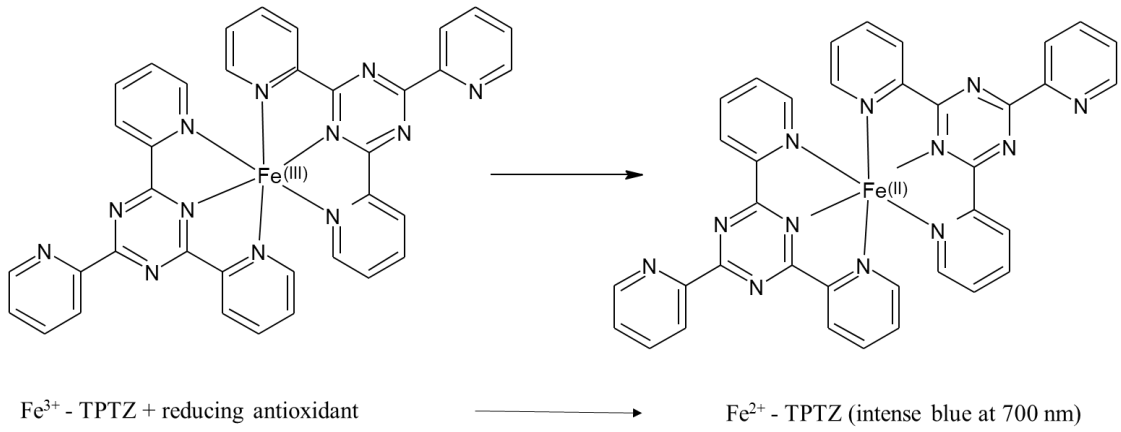

Figure 4. $[Fe^{3+}\text{-}(TPTZ)_2]^{3+}$- $[Fe^{3+}\text{-}(TPTZ)_2]^{2+}$ reduction reaction for reducing power assay

2.6 Natural products as antimicrobial agents

Many phytochemicals and secondary metabolites from plants such as, flavonoids, tannins, and saponins have been shown to have in vitro antimicrobial activities with less toxicity and side effects [56]. Antibacterial resistance is the resistance of bacteria to treatment with antibiotic drugs that was originally effective for the treatment of infection caused by that microorganism. Antimicrobial substances derived from natural products, such as phytochemicals, has gained increasing importance alongside the discovery of new synthetic chemical compounds with antibiotic and bacteriophage properties [57].

2.6.1 Test microorganisms

The antimicrobial activity of the extracts of *V. erioloba* and *O. stricta* was evaluated against strains of *Staphylococcus aureus* (ATCC 25923), *Streptococcus mutans* (ATCC 25175), *Escherichia coli* (ATCC 33849), and *Candida albicans* (ATCC 90029).

2.6.1.1 *Staphylococcus aureus*

Staphylococcus aureus (ATCC 25923) is a gram positive round-shaped bacterium that belongs to the firmicutes family [58]. *Staphylococcus aureus* is part of the normal human flora and found in the mucous membranes and skin of healthy individuals. It causes respiratory infection, food poisoning and other life threatening disease such as toxic syndrome, pneumonia, meningitis and endocarditis [59]. Methicillin-resistant *Staphylococcus aureus* (MRSA) is a strain of *S. aureus* with an altered penicillin-binding protein it is a multi-resistant strain [60]. Biofilms formed by *S. aureus* may resist antibiotic treatments and survive in hostile environments through impaired penetration and by evading host immune responses, subsequently leading to the establishment of chronic infections or medical device-related infections [61,62]. *S. aureus* can adhere to and develop biofilms on food contact surfaces, affecting the quality and safety of food products [63].

2.6.1.2 *Streptococcus mutans*

Streptococcus mutans (ATCC 25175) is a gram positive facultative anaerobic bacterium that belongs to a group of Streptococci such as *S. sobrinus* [64]. *S. mutans* is commonly found in the human oral cavity. Oral streptococci, like *Streptococcus mutans*, are associated with pyogenic and other infections in various sites including the mouth, heart, joints, skin, muscle, and central nervous system [64].

2.6.1.3 *Escherichia coli*

Escherichia coli (ATCC 33849) are a rod-shaped gram-negative bacterium that is found in the lower intestines and is part of the normal flora of the human body [65]. Most *E. coli* are harmless and helps keep the digestive tract healthy. *E. coli* is one of the most common causes of bacterial infections including Cholecystitis, bacteraemia, urinary tract infections, neonatal meningitis and pneumonia [65].

2.6.1.4 *Candida albicans*

Candida albicans (ATCC 90029) is a commensal fungal species commonly colonizing human mucosal surfaces [66]. However, under conditions of immune dysfunction such as HIV infection, *C. albicans* can become opportunistic pathogens causing mucosal and disseminated infections [66] and Osteomyelitis [67]. As a microorganism commonly found in the intestine, *Candida albicans* (*C. albicans*) can invade the gut epithelium barrier through microfold cell and enter the bloodstream [68].

2.7 Biofilm formation and development

Biofilm is a complex structure of microbiome having different bacterial colonies or single type of cells in a group this biofilm can adhere to surfaces. These cells are embedded in extracellular polymeric substances, a matrix which is generally composed of DNA, proteins and polysaccharides that shows high resistance to antibiotics [69]. Biofilm can tolerate antimicrobial agents needed to kill planktonic bacteria and resistant to phagocytosis making films extremely difficult to eradicate from living host [69–71]. Moreover, biofilm cause many bacterial diseases including native valve endocarditis, osteomyelitis, dental caries, ear infection, medical device-related infections, ocular implant infections and chronic lung infections in cystic fibrosis patients [72]. Figure 5 shows the formation and development of biofilm that occurs in five stages.

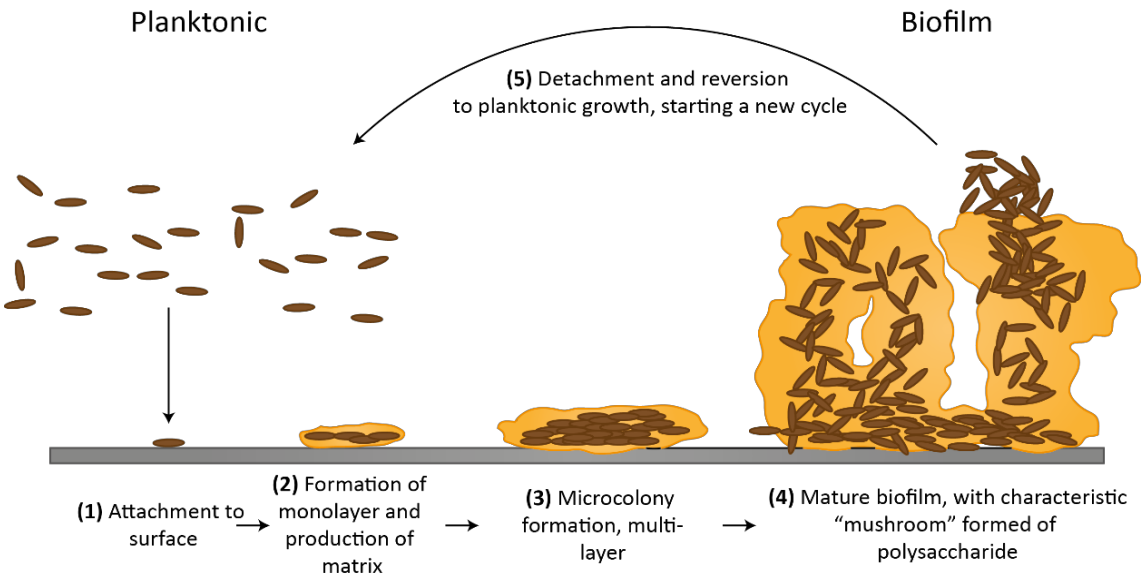

Figure 5. Schematic representation for single bacterial species biofilm formation on a solid surface. The schematic depicts the five main steps for the formation and spreading of biofilms [73].

2.7.1 Biofilm formation: Initial stage of Attachment

Biofilm formation begins with the adhesion of single cells to material surfaces that are exposed to an aqueous medium and formation of a conditioning layer [74]. Adhesion is known to be a critical step in biofilm formation; once the bacteria attach to the surface, the chances of further transport of other free-floating microbes increases resulting in coaggregation and the creation of multiple layers [75]. The conditioning layer is an organic monolayer which forms on surfaces and acts as a docking place for the first reversibly attached cells; however, the strength of the biofilm is dependent upon the cohesiveness of the conditioning film [76]. The conditioning film plays a significant role in cellular or microbial adhesion and can be dependent on the concentration of organic molecules in the medium that is in contact with the surface [76]. After the establishment of the conditioning layer, planktonic microorganisms in the aqueous medium attach themselves to this layer [74]. The attachment depends on the motility of microbes or the moving of the planktonic (free-floating) cells through

gravity, diffusion or the forces of the fluid dynamic forces from the surrounding liquid phase [77]. Adhesion is affected by the availability of nutrients in the surrounding medium.

2.7.2 Biofilm formation: Irreversible Attachment

In this stage, microorganisms are irreversibly attached to the surface and synthesize EPS [77]. Secretion of EPS by bacteria reaches a certain level, forming a strong interaction between the microbe and the surface [78]. During this stage, planktonic microorganisms can stick to each other or different species of surface-bound organisms, forming aggregates on the substratum and the adhesion becomes irreversible in the absence of physical or chemical intervention, thus the bacterial cells become attached firmly to the surface [74]. The irreversibly attached cells start growing and dividing using the nutrients in the conditioning film and in the surrounding fluid to form micro colonies and produce the further polymer (EPS) which helps anchor the cells to the surface and stabilize the colony from environmental fluctuations [74]. In this stage, both physical and chemical processes contribute to the initial adhesion ends, biological processes start to dominate; production of polysaccharide intercellular adhesion (PIA) polymers and the presence of divalent cations interact to form stable bonding [70].

2.7.3 Biofilm formation: Maturation

During this stage, the attached small colonies grow into a mature biofilm, with the characteristic three-dimensional biofilm structure, through reproduction and by accumulating debris and new planktonic bacteria from the surrounding environment [78]. In this stage, cell to cell and cell to substratum attachment depends on the EPS [78]. At high cell density, cell signalling mechanisms that use a range of different signal types known as quorum sensing are used by the biofilm; however, QS is

important for biofilm maturation processes since bacteria monitor cell density and regulate collective behaviour [76,78]. Although quorum sensing is typically thought to mediate interspecies communication, there is evidence that interspecies interaction also occurs [60]. Once mature, the biofilm has three layers: a joining film binding the biofilm to the surface; a base film composed of a dense layer of bacteria; and a surface film from which free-floating bacteria can arise and spread [79].

2.7.4 Biofilm formation: Dispersal

The final stage of the biofilm process is dispersal where attached cells detach and disperse to colonize a new niche [80]. Biofilm cells can be dispersed either by shedding of daughter cells from actively growing cells or detachment can arise due to various factors, such as nutrient limitation, fluid dynamics and shear effects of the bulk fluid, secretory proteins and catabolite repression [78,80]. The detachment stage consists of sloughing, erosion and abrasion [74]. Erosion refers to the continuous removal of single cells or small biofilm fragments [74]. Sloughing is the loss of large particles of biofilm biomass [81]. This loss is due to nutrient and dissolved oxygen depletion at the base of the biofilm or to a sudden increase in nutrient concentration in the bulk liquid [70]. Abrasion is a loss of biofilm by suspended particles [74]. Any released cells may be transported to new locations and then restart the process of biofilm formation [80]. Detachment from biofilms is thought to be a key reason for the spread of pathogens [78].

2.8 Gas chromatography - mass spectroscopy

Gas chromatography – mass spectroscopy is the method of choice for the analyses of volatile and semi-volatile compounds present in plant extracts [82,83]. Volatile and semi-volatile compounds have boiling points ranging from 50 to 400˚C and can easily be separated using the GC-MS. GC-MS is used for the analysis of esters, fatty acids,

alcohols, aldehydes, alkaloids, terpenes, etc [84]. Some of these compounds reportedly display antioxidant, antimicrobial and antibiofilm activities [85,86].

3. RESEARCH METHODS

3.1 Research design

Powdered plant materials of *V. erioloba* and *O. stricta* were extracted using a mixture of dichloromethane and methanol (1:1). Antioxidant activity was determined using the DPPH radical scavenging and reducing power methods. Followed by the antimicrobial screening which was determined using the disc diffusion. Antibiofilm activity of the extracts was determined through biofilm inhibition and eradication of *Staphylococcus aureus.* Qualitative and quantitative phytochemical screening was performed on the plant extracts and the volatile constituents were identified using GC-MS.

3.2 Procedures

3.2.1 Plant materials

Plant materials of *V. erioloba* (seed pods and seeds) and *O. stricta* (seeds, fruits, flowers and leaves) were donated by Farm Vredelus (2020 and 2021).

3.2.2 Plant Extraction

Extraction of dried *V. erioloba* (seed pods and seeds) and *O. stricta* (seeds, fruits, flower and leaves) was performed using a method described by Njateng et al [87]. The samples were blended and dissolved in a 1:1 mixture of dichloromethane and methanol (DCM: MeOH) and was then filtered using a vacuum filter. The filtrate evaporated and dried at 40°C under reduced pressure using rotatory vacuum evaporator. The extracts were then allowed to dry at room temperatures, weighed, and stored in vials in the fridge at 4°C. The yield percentages were calculated using the following formula: Extract percentage yield (%) = **R**/**S** ×100 (where **R** - weight of extracted plants residues and **S** - weight of plant raw sample).

3.2.3 Phytochemical screening

3.2.3.1 Qualitative phytochemical screening

Phytochemical screening was conducted to determine the presence of flavonoids, phenols, tannins, alkaloids, saponins and steroids [88].

3.2.3.2 Alkaloids

Ten mg of extract was treated with Dragendroff's reagent and the formation of a red precipitate indicated the presence of alkaloids.

3.2.3.3 Flavonoids

Shinoda Test: Ten mg of extract was added to a pinch of magnesium turnings followed by 2 drops of concentrated hydrochloric acid. The formation of a pink colour indicated the presence of flavonoids.

3.2.3.4 Tannins

Ferric chloride test: Five percent of ferric chloride (0.5 mL) was added to 5 mg of extract. The development of a dark bluish black colour indicated the presence of tannins.

3.2.3.5 Phenols

Sodium hydroxide test: Five mg of extract was dissolved in 0.5 mL of 20% sulphuric acid solution, followed by the addition of a few drops of aqueous 1M sodium hydroxide solution. The formation of a blue colour indicated the presence of phenols.

3.2.3.6 Saponins

Foam Test: The extract stock solution (1 mL) was diluted with 20 mL of distilled water and then was shaken by hand for 15 min. The formation of a foam layer indicated the presence of saponins.

3.2.3.7 Steroids

The crude plant extract (1 mg) was placed into a test tube dissolved with chloroform (10 mL), and then equal volume of concentrated sulphuric acid was added to the test tube. The formation of a red colour in the upper layer and yellow colour in the bottom layer indicated the presence of steroids.

3.2.4 Quantitative phytochemical screening

Total phenolic and total flavonoid contents were determined using procedures by Saeed et al [89] and Josipovic et al [90] with minor modifications.

3.2.4.1 Analysis of total phenolic content

The total phenolic content was determined using the spectrophotometric method described by Saeed et al [89] with minor modification. In brief, 1 mL of sample (1 mg/ml) was mixed with 1 mL of Folin-Ciocalteu's phenol reagent. After 5 min of incubation at room temperature, 10 ml of a 7% Na_2CO_3 solution was added to the mixture followed by the addition of 13 mL of deionized distilled water and mixed thoroughly. The mixture was incubated in the dark for 90 min at room temperature, after which the absorbance was read at 750 nm. The TPC was determined from extrapolation of calibration curve, which was made by preparing Gallic acid solution (31.125-500 µg/mL). The estimation of the phenolic compounds was carried out in triplicate. The TPC was expressed as milligrams of Gallic acid equivalents (GAE) per g of dried sample.

3.2.4.2 Analysis of total flavonoids content

Total flavonoid content was determined spectrophotometrically using the method outlined by Josipovic et al [90] with minor modification. One ml of the extract was mixed with 4 mL of distilled water and 0.3 mL of 5% $NaNO_2$ (w/v). The mixtures

were vortexed and left at room temperature for 5 min. After that, 0.3 mL of 10% aluminium chloride (w/v) was added, vortexed and left for 6 min. Two ml of 1 M sodium hydroxide and 2.4 mL distilled water were added. Absorbance was immediately measured at 510 nm. Total flavonoids were calculated from a Quercetin standard curve and reported as mg Quercetin equivalents (QE)/g of dried weight.

3.2.5 Antioxidant assays

3.2.5.1 DPPH free radical scavenging assay

The free radical scavenging activity of the extracts was evaluated using a modified method outlined by Kapewangolo et al [91]. The extracts dissolved in ethanol were mixed with a 90 µM 2,2-diphenyl-1-picrylhydrazyl (DPPH) ethanol solution to give final extract concentrations of 6–1000 µg/mL. Incubation of extract with DPPH was done in the dark at room temperature for 30 min. The absorbance values were measured at 520 nm with a SpectraMax M2 spectrophotometer (Molecular devices, USA). The absorbance were converted into percentage of antioxidant activity. A known antioxidant, ascorbic acid, was used as a standard control. Half-maximal inhibitory concentration (IC_{50}) values were calculated using Graphpad Prism (Graphpad Software Inc. California, USA).

3.2.5.2 Reducing power assay

The reducing power of the extracts was determined using a method outlined by Jayanthi and Lalitha [92]. Extracts at various concentrations (0.5 - 1000 µg/mL) were mixed with phosphate buffer (0.2 M, pH 6.6) and 1% potassium ferricyanide. The mixtures were incubated at 50°C for 20 min, 10% trichloroacetic acid was added to the mixture and centrifuged at 3000 rpm for 10 min. The upper layer of the resultant solution was mixed with distilled water and 0.1% ferric chloride. The absorbance was

measured at 700 nm with a SpectraMax M2 spectrophotometer (Molecular devices, USA). Control samples included a blank and ascorbic acid.

3.2.6 Antimicrobial Activity

3.2.6.1 Test microorgasms and growth conditions

The microorganisms used for this study included *Staphylococcus aureus* ATCC 25923, *Streptococcus mutans* ATCC 25175, *Escherichia coli* ATCC 33849 and *Candida albicans* ATCC 90029. Stock cultures were prepared and kept at -4ºC until needed. The strains were grown in nutrient broth at 37ºC.

3.2.6.2 Antimicrobial screening

Antimicrobial screening was done using the disc diffusion method by Kaaria et al [49]. Ten microliters of each microbial strain was placed on different nutrient agar plates; spread evenly using a glass spreader then allowed to stand for 10 min before the extract was introduced. About 10 µL of plant extracts (10 mg/mL) was added to a paper disc and placed on to the agar plate these were allowed to stand for 30 min then the plates were incubated for 24h at 37ºC. The plant extract that showed activity against bacterial strains through clear inhibition zones after 24h were recorded and were investigated further. Ampicillin (10 µg) was used as the positive control and sterile distilled water as negative control.

3.2.6.3 Minimum Inhibitory Concentration

The determination of the MIC also was done following the disc diffusion method as described above. The filter paper discs were loaded with extracts at different concentrations (0.63 – 10 mg/mL). The least concentration that showed inhibition on the different strains was recorded as the minimum inhibitory concentration (MIC).

3.2.7 Antibiofilm activity

The inhibition or eradication of biofilm formation by the different plant extracts was done against a well-known biofilm producer; *Staphylococcus aureus*.

3.2.7.1 Biofilm inhibition

A modified crystal violet assay was employed to test the effect of plant extracts on biofilm formation [69]. Serial dilutions of plant extracts (0.078 up to 10 mg/mL) were made in sterile 96 flat wells microtiter plates containing 150 µL of nutrient broth per well. Fifty microliters (50 µL) of fresh bacterial suspension was added to each well and the plates incubated at 37 °C for 24 h. Positive control (bacterial suspension in broth) and negative control (broth) were included. Following incubation, the content of each well was gently removed by tapping the plates. The wells were washed with 200 µL of sterile distilled water to remove free floating bacteria. Biofilms formed by adherent cells in plate stained with 0.1% crystal violet and incubated at the room temperature for 30 min. Excess stain was rinsed off thorough washing with distilled water and plates were fixed with 200 µL of ethanol 70%. Optical densities (OD_{630}) of stained adherent bacteria were measured using the SpectraMax M2 spectrophotometer (Molecular devices, USA).

$$\textbf{Percentage inhibition} = \frac{\mathbf{C} - \mathbf{T}}{\mathbf{C}} \times \mathbf{100}\%$$

Where **C** = absorbance of control and **T** = absorbance of test (biofilm and treatment)

3.2.7.2 Biofilm eradication

The potential of the plant extracts to remove pre-formed biofilm was evaluated using modified method by Abidi [81]. Briefly, microtitre plate containing 150 µL broth and 50 µL strain were incubated at 37˚C for 24 h. After incubation, plates were washed

with sterile distilled water to remove planktonic cells and 200 µL of each plant extract (0.078195 up to 10 mg/mL) was inoculated in each well. Plates were then incubated for a period of 15 min. After incubation, planktonic bacteria were removed from the microtiter plates by washing the plates with distilled water. Remaining biofilm stained with 200 µL of 0.1% crystal violet solution and incubated for 10 min at room temperature. Following incubation, the plates emptied and washed again with sterile distilled water. Finally, the dye was solubilized by adding 200 µL of 70% ethanol to plate, and repeated pipetting mixed the contents of each well. The Optical densities (OD) of the extracts were measured at a wavelength 630 nm.

Measurement of anti-biofilm efficacy called Percentage eradication was calculated from blank, control and test OD, using equation:

$$\textbf{Percentage eradication} = \frac{[(\boldsymbol{C} - \boldsymbol{B}) - (\mathbf{T} - \mathbf{B})]}{[\mathbf{C} - \mathbf{B}]} \times 100\%$$

Where **B** = absorbance of blank (no biofilm, no treatment),

C = absorbance of control (biofilm, no treatment) and

T = absorbance of test (biofilm and treatment)

3.2.8 Identification and quantification of volatile compounds

3.2.8.1 Sample preparation

The seed sample of *V. erioloba* were donated by Farm Vredulus and extracted using DCM: MeOH (1:1). Ten grams (10 g) of the DCM: MeOH extract was further separated into polar and no-polar fractions using a mixture of DCM and water (2:1). The DCM layer was dried using anhydrous sodium sulfate and the solvent evaporated

using the rotatory evaporator which yielded 16.75% of the non-polar extract. Only the non-polar fraction was analysed using the GC-MS.

3.2.8.2 GC-MS analyses

The GC-MS analyses was performed as described by Sheehama et al [93], using a ThermoFisher Scientific Focus GC coupled to an ITQ 700 MS with helium as the carrier gas at a flow rate of 1.0 mL min⁻1 (constant flow) and a split ratio of 10. The temperature of the injector was maintained at 220°C, and the source and transfer line temperatures were 200°C and 250°C, respectively. An SGE Analytical Science BP5MS capillary GC column (30 m × 0.25 mm) with a 5% diphenyl, 95% polysilphenylene-siloxane stationary phase (0.25 μm film thickness) was used for the separations. The oven was programmed at a rate of 2°C.min^{-1} from 40 to 300°C to ensure optimal separation of the volatile constituents. The electron ionization (EI) mass spectra were recorded at ionization energy of 70 eV and a mass range of m/z 25–625 was scanned. A mixture of alkanes (C_{10}-C_{40}) was analysed under the same conditions as the sample solutions and the linear Kővats retention indices of the compounds were calculated using the following equation [94]:

$$I = 100[n + (N - n)\frac{\log t'_r\ (unknown) - \log t'_r\ (n)}{\log t'_r\ (N) - \log t'_r\ (n)}$$

Where: I is the retention index. N and n are the number of carbon atoms in the larger alkane and the number of carbon atoms in the smaller alkane, respectively. $t'_r(unknown)$ is the adjusted retention time of the unknown. $t'_r(n)$, and $t'_r(N)$ are the adjusted retention time of the small alkane and the adjusted retention times of the bigger alkane, respectively. t'_r is the adjusted retention time = t_r - t_m, where t_r is the actual retention time and t_m is the time that it takes for an un-retained compound to move through the column.

4. RESULTS AND DISCUSSION

4.1 Extraction

Table 1 shows the percentage yield extracted for different plant parts of *O. stricta* and *V. erioloba*. The highest percentage yield was obtained for the leaves of *O. stricta* (13.72%) followed by the flower of *O. stricta* (11.97%).

Table 1: The percentage yield of crude extracts of *V. erioloba* and *O. stricta*.

Plant sample	Starting material (g)	Extract yield (g)	Percentage yield (%)
Vachellia erioloba			
Seed pods	208.3863	22.2630	10.68
Seeds	520.2591	42.2672	8.12
Opuntia stricta			
Seeds	113.0148	9.3058	8.23
Fruit	97.2186	2.8871	2.97
Flower	56.6788	6.3784	11.97
Leaves	34.6600	4.7570	13.72

4.2 Phytochemical screening

4.2.1 Qualitative phytochemical screening

The phytochemical screenings of *V. erioloba* and *O. stricta* extracts revealed the presence of different phytochemicals such as phenols, flavonoids, tannins, steroids, alkaloids and saponins. Flavonoids and steroids were found to be present in all the extracts of *V. erioloba* screened. However, phenols, tannins and saponins were not detected in the seed extract of *V. erioloba* (Table 2).

Table 2: Phytochemical screening of the DCM: MeOH extracts of *V. erioloba* and *O. stricta*.

Plant sample	Phytochemicals					
	Phenol	**Tannins**	**Flavonoids**	**Steroids**	**Alkaloids**	**Saponin**
Vachellia erioloba						
Seed pods	+	+	+	+	+	+
Seeds	-	-	+	+	-	-
Opuntia stricta						
Seeds	-	-	+	+	-	+
Fruit	+	+	+	+	+	+
Flower	+	+	+	-	-	+
Leaves	+	+	+	-	-	+

The phytochemical screenings done on the extracts of *O. stricta* found that all extracts contained flavonoids and saponins. However, phenols, alkaloids and tannins were not detected in the seed extract of *O. stricta.* Steroids and alkaloids were also not detected in the flower and leave extracts of *O. stricta* (Table 2). The findings of this study confirms those done on *V. erioloba* aqueous leaves extract, which revealed the presence of phenol, saponins, tannins, and flavonoid [95]. Previous studies done on related species of *V. erioloba* also confirms the presence of these phytochemicals [96,97]. No study has been reported on the phytochemicals presence in *O.* stricta. However, reports on phytochemical screening of related species of *O. stricta* confirms the presence of phenols, flavonoid and tannins [98]. The phytochemicals screened in this study are known to be biologically active compounds and they are responsible for

different activities such as antimicrobial, antioxidant, antifungal, anticancer and antibiofilm activities [22,26,56,57].

4.2.2 Quantitative phytochemical analysis

The total phenolic contents of the different plant parts of *V. erioloba* and *O. stricta* ranged from 18.8 ± 0.0 and 290.9 ± 2.1 mg GAE/g dry sample mass (Table 3). The seed pod extract of *V. erioloba* showed the highest TPC value of 290.9 ± 2.1 mg GAE/g dry sample mass and the fruit extract of *O. stricta* showed the lowest TPC value of 18.8 ± 0.1 mg GAE/g dry sample mass. The total flavonoid content of *V. erioloba* and *O. stricta* varied from 6.0 ± 0.9 to 595.5 ± 1.5 mg QE/g dry sample mass. The seed pod extract of *V. erioloba* showed the highest TFC value of 595.5 ± 1.5 mg QE/g dry sample mass and the seed extract of *O. stricta* showed the lowest TFC value of 6.0 ± 0.9 mg QE/g dry sample mass.

Table 3. Total phenolic and flavonoid content of the DCM: MeOH extracts of *V. erioloba* and *O. stricta***.**

Plant sample	**TPC (mg GAE/g dry sample mass)**	**TFC (mg QE/g dry sample mass)**
Vachellia erioloba		
Seed pods	290.9 ± 2.1	595.5 ± 1.5
Seeds	-	22.6 ± 0.4
Opuntia stricta		
Flowers	94.8 ± 0.1	114.8 ± 1.9
Fruit	18.8 ± 0.1	17.2 ± 3.4
Leaves	60.8 ± 0.0	12.8 ± 1.1
Seeds	-	6.0 ± 0.9

A literature search revealed that there are no study done on the TPC and TFC of both *V. erioloba* and *O. stricta*. However, studies on the TPC and TFC of related species of *V. erioloba* [99] and *O. stricta* [100] revealed that both species have high TPC and TFC. The high phenolic compounds in *Vachellia* species could be the reason for their high antioxidants activities, especially in the seed pod extract of *V. erioloba* [96,99,101].

4.3 Antioxidant activity

4.3.1 DPPH radical scavenging activity

The DPPH free radical scavenging activity of *V. erioloba* (seeds and seed pods) and *O. stricta* (seeds, fruits, flower and leaves) is summarized in Figures 6 and 7.

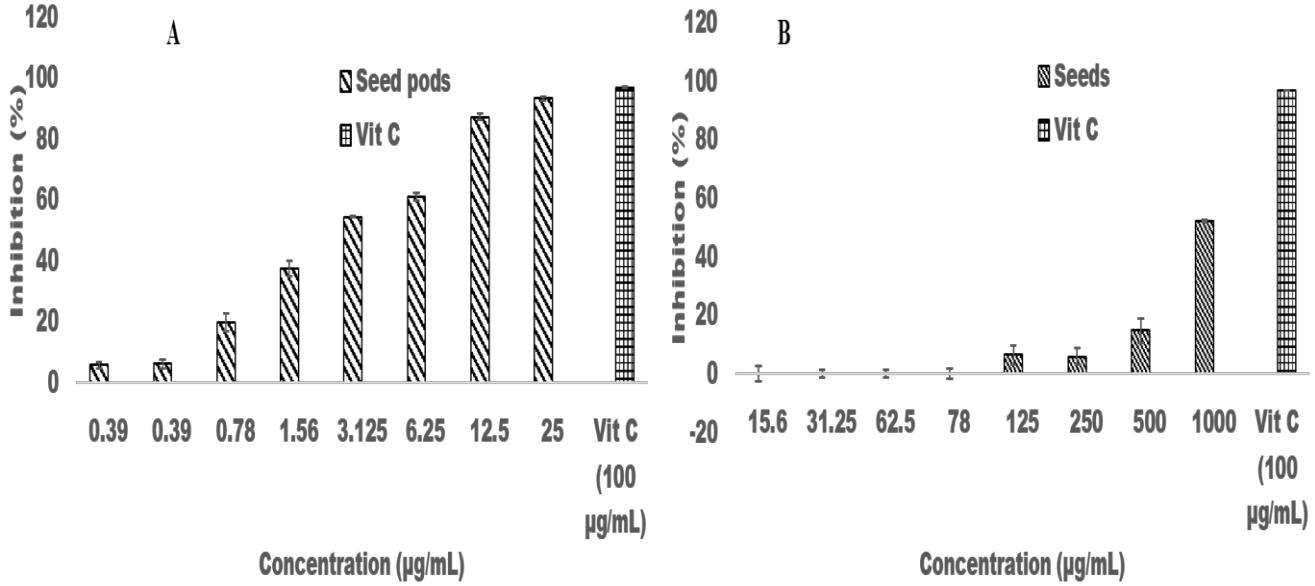

Figure 6. DPPH free radical scavenging activity of *V. erioloba* seed pods (A) and seeds (B) extracts.

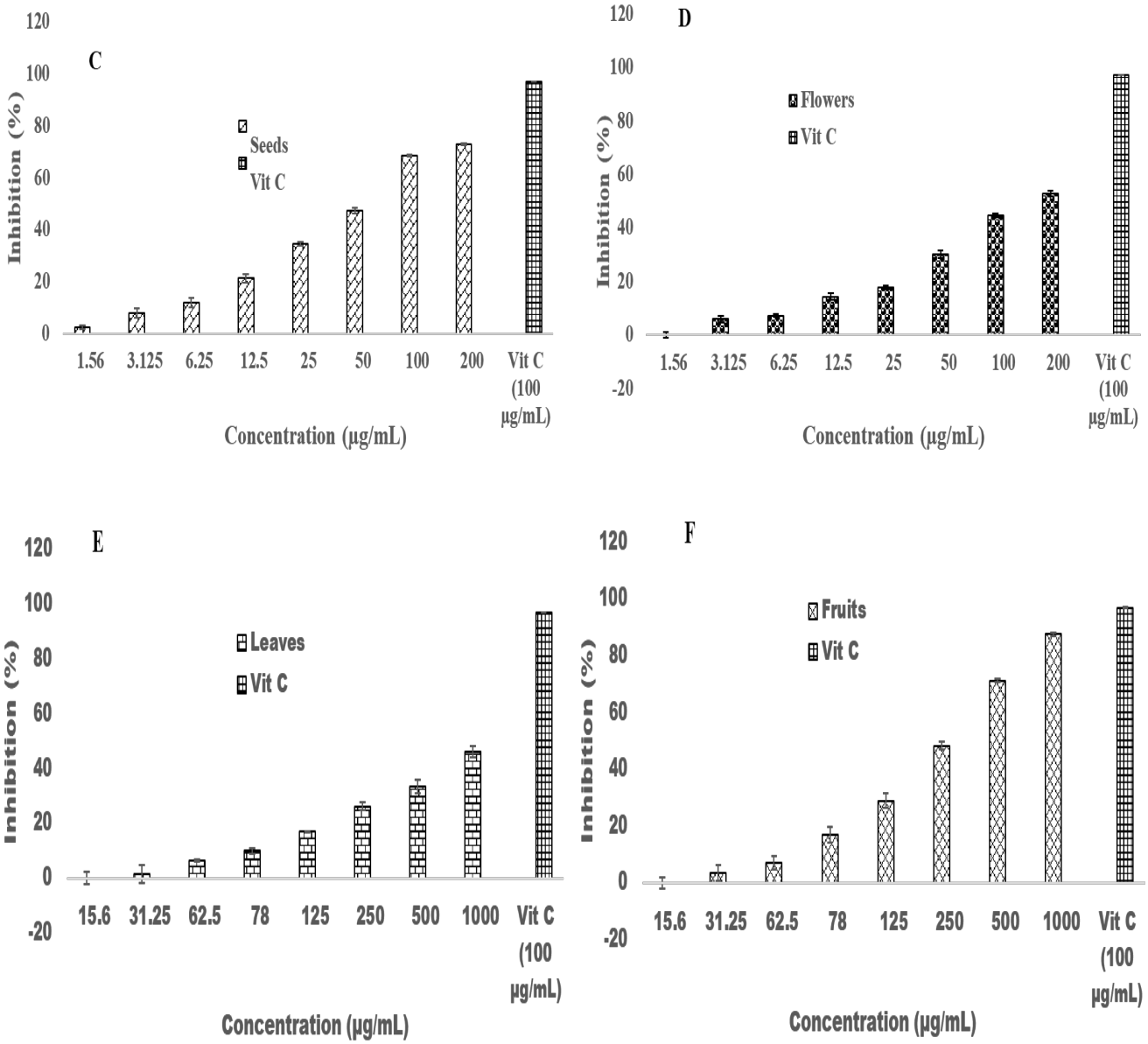

Figure 7. DPPH free radical scavenging activity of *O. stricta* seeds (C), flowers (D), leaves (E) and fruits (F) extracts.

Table 4 shows the half maximal inhibitory concentration (IC_{50}) value of *V. erioloba* seed pods and seeds extracts which are 1.973 ± 0.0012 and 972.0 ± 22.3 µg/mL, respectively. The IC_{50} of *O. stricta* extracts ranged from 53.74 ± 0.18 to > 1000 µg/mL.

Table 4. DPPH free scavenging activity IC_{50} values of the *V. erioloba* and *O. stricta* extracts

Plant sample	IC_{50} value (µg/mL)
Vachellia erioloba	
Seed pods	1.973 ± 0.012
Seeds	972.0 ± 22.3
Opuntia stricta	
Seeds	53.74 ± 0.18
Fruits	258.7 ± 0.7
Flowers	153.8 ± 0.9
Leaves	IC_{50} > 1000
Ascorbic acid	3.097 ± 0.395

The seed pods of *V. erioloba* had the best IC_{50} value of 1.973 0.012 µg/mL, which was lower than the control (3.097 0.395 µg/mL) ascorbic acid. The leaves extract of *O. stricta*, on the other hand, had the lowest IC_{50} value (IC_{50} > 1000 g/mL). The difference in the DPPH radical scavenging activities between the extracts of *V. erioloba* and *O. stricta* can be attributed to the difference in the quantities of phenolic compounds present in these extracts. Furthermore, studies suggest that phenolic compounds and flavonoid found in plant extracts may be responsible for their antioxidant activity [3,22,50,102].

The free radical scavenging of *V. erioloba* and *O. stricta* has not been reported before. However, reports on the antioxidant properties of related species of *V. erioloba* [99,101,103] and *O. stricta* [17,104,105] are similar to those of the present study,

further confirming that these plants are potential antioxidant agents. Antioxidant compounds have the potential to donate an electron or hydrogen atom to DPPH radicals [52]. The ability of *V. erioloba* and *O. stricta* extracts to scavenge DPPH radicals makes them promising antioxidant agents.

4.3.2 Reducing power

The reducing power of the extract of *V. erioloba* (seeds and seed pods) and *O. stricta* (seeds, flower, leaves, and fruits) are summarized in figures 8 and 9 respectively.

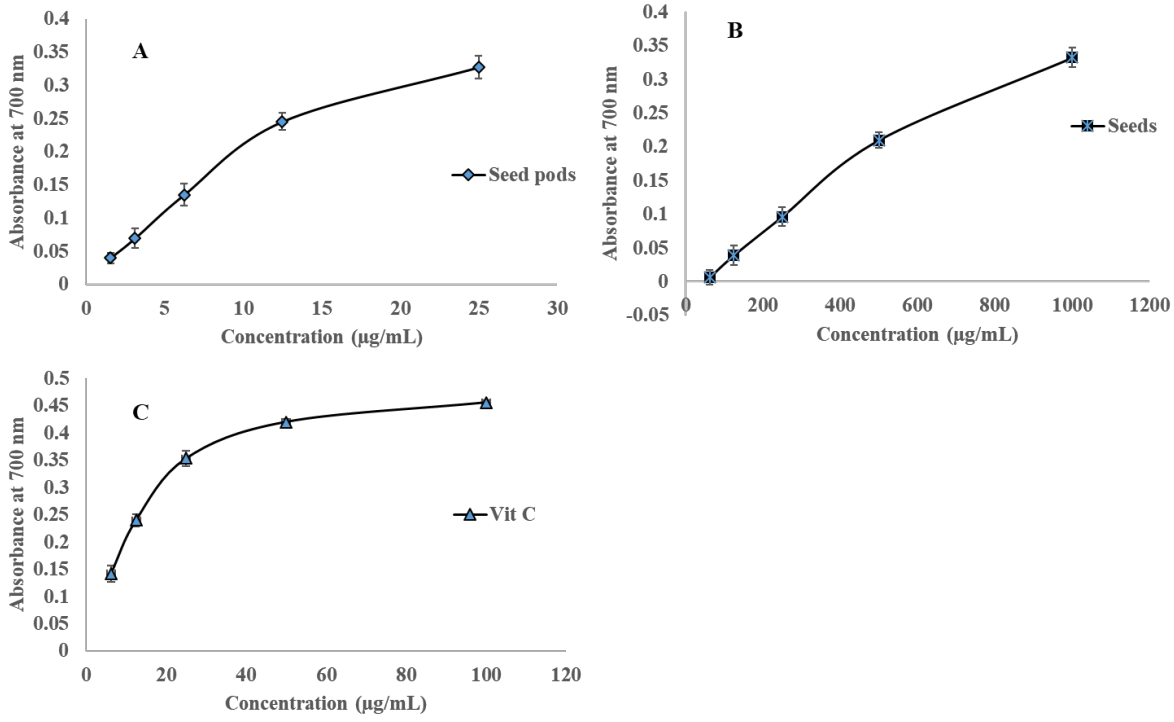

Figure 8. Reducing power activity of *V. erioloba* seed pods (A), seeds (B), and Vit C (F).

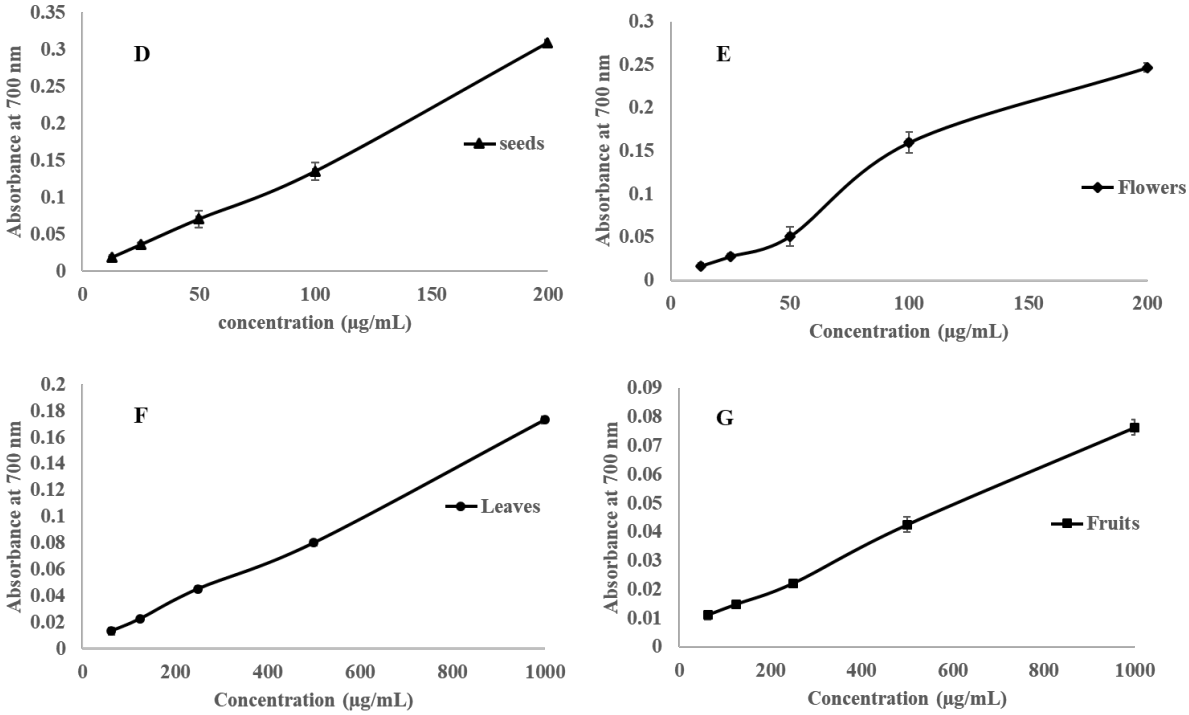

Figure 9. Reducing power activity of O. stricta seeds (D), flower (E). Leaves (F) and fruits (G) extracts.

This study shows the ability of the extracts from *V. erioloba* and *O. stricta* to reduce the ferricyanide (Fe^{3+}) complex to the ferrous (Fe^{2+}) form. The ferric reducing power of *V. erioloba* and *O. stricta* has been shown to be concentration-dependent, as the concentration increase so does the reducing power of the extracts.

The difference in the ferric reducing capacity found among the extracts of *V. erioloba* and *O. stricta* could be attributed to the difference in their total phenolic and total flavonoid contents. The seed pod extract of *V. erioloba*, for example, exhibited a high phenolic and total flavonoid content, indicating a high capacity to act as electron donors, demonstrating their ability to react with free radicals and therefore increasing antioxidant activity. Studies shows that quantity of phenolic compounds has a direct link with the reducing power of plant extracts [103,106]. High phenolic

contents exhibited by extracts of *V. erioloba* and *O. stricta* in this study suggest that they are potential sources of antioxidant.

4.4 Antimicrobial activity

4.4.1 Antimicrobial screening

The various extracts of *V. erioloba* and *O. stricta* exhibited moderate antimicrobial activity with inhibition zones (IZ) ranging from 0 to 12 mm as shown in table 5 below. Not all extracts inhibited the tested microbes; however, *V. erioloba* seed pods had the best inhibition of 10. 4 ± 0.5, 11.2 ± 0.7 and 12.0 ± 0.8 against *S. aureus, S. mutans* and *E. coli* respectively. The best inhibition against *C. albicans* was recorded for the seeds extracts of *V. erioloba*.

Table 5. The antimicrobial screening of the seeds and seed pods of *V. erioloba* and seed, fruits, flowers and leaves of *O. stricta.*

Plant sample	**Inhibition Zones (mm)**			
	Gram (+ve) bacteria		**Gram (-ve) bacteria**	**Fungi**
	S. aureus	*S. mutans*	*E. coli*	*C. albicans*
Vachellia erioloba				
Seed pods	10.4 ± 0.5	11.2 ± 0.7	12.0 ± 0.8	9.8 ± 0.6
Seeds	0.0 ± 00	0.0 ± 00	9.9 ± 0.3	10.2 ± 0.5
Opuntia stricta				
Seeds	0.0 ± 00	8.7 ± 0.5	0.0 ± 00	0.0 ± 0.0
Fruits	8.6 ± 0.5	8.5 ± 0.6	10.0 ± 0.0	9.6 ± 0.3
Flowers	10.2 ± 0.7	9.8 ± 0.4	10.3 ± 0.6	9.9 ± 0.2
Leaves	9.2 ± 0.7	8.7 ± 0.5	9.9 ± 0.1	10.0 ± 0.1
Ampicillin	19.2 ± 0.3	21.0 ± 0.0	20.3 ± 0.6	20.0 ± 0.0

4.4.2 Minimum inhibitory concentration

The MIC values recorded for the various extracts of *V. erioloba* and *O. stricta* are shown in table 5. The MIC obtained from this study ranged between < 0.63 and 10 mg/mL depending on the microorganism and the various extracts. All the extract of *V. erioloba* and *O. stricta* shows MIC (< 0.63 mg/mL) value against *C. albicans*. The highest MIC value of 10 mg/mL was recorded for the seed extract of *V. erioloba* and the fruit extract of *O. stricta*.

Table 6. Minimum inhibitory concentration (MIC) of the extracts of *V. erioloba* and *O. stricta* against four microbial strains.

Plant sample	**MIC (mg/mL)**			
	Gram (+ve) bacteria		**Gram (-ve) bacteria**	**Fungi**
	S. aureus	*S. mutans*	*E. coli*	*C. albicans*
Vachellia erioloba				
Seed pods	1.25	≤ 0.63	1.25	≤ 0.63
Seeds	5.0	-	10	≤ 0.63
Opuntia stricta				
Seeds	-	1.25	-	-
Fruits	2.5	1.25	10.0	≤ 0.63
Flowers	2.5	1.25	2.5	≤ 0.63
Leaves	5.0	1.25	5.0	≤ 0.63
Ampicillin	≤ 0.63	1.25	1.25	≤ 0.63

The antimicrobial activity exhibited by *V. erioloba* and *O. stricta* were concentration-dependent, as the concentration decreases so does the inhibition zones. The antimicrobial screening results revealed that most extracts were active against all microbes, except for the seeds of *V. erioloba*, which were not active against *S. aureus* and *S. mutans*. The seeds of *O. stricta* were not active against *S. aureus, E. coli,* and *C. albicans*. The highest activity was determined for the seed pods of *V. erioloba* against most of the strains. This could be due to the high contents of phenolic compounds as well as high content of flavonoids compounds present in the seed pod extracts. Furthermore, the seed extract of *V. erioloba* and fruit extract of *O. stricta* were less active, exhibiting MIC of 10 mg/mL.

The antimicrobial activity of *V. erioloba* and *O. stricta* have not been reported before. However, a report on the essential oils extracts of *O. stricta* shows that the plant has antimicrobial activity against *E. coli* and *C. albicans*, but not against *S. aureus* [15]. These could be due to the facts that the current study was done on a Dichloromethane and methanol (DCM: MeOH) seed extract and those in literature were carried out on an aqueous extract. Moreover, the antimicrobial activities done on other species of *V. erioloba* [101,107] and *O. stricta* [108] revealed the species are active against *S. aureus, E. coli, S. mutans,* and *C. albicans*. The moderate antimicrobial activities of these plants could be attributed to the presence of compounds such as flavonoids, tannins and alkaloids which are reportedly good antimicrobial agent [109].

4.5 Antibiofilm activity

4.5.1 Biofilm inhibition

The biofilm inhibition of *V. erioloba* (seedpods and seeds) and *O. stricta* (seeds, flowers, leaves, and fruits) are summarized in figures 10, and 11. The results illustrate a concentration-dependent activity, all the extract inhibited the formation of biofilm produced by *S. aureus*. The seedpod extract of *V. erioloba* showed the best inhibition against *S. aureus* (95% inhibition), whereas the leave extract of *O. stricta* was the least effective (78% inhibition)

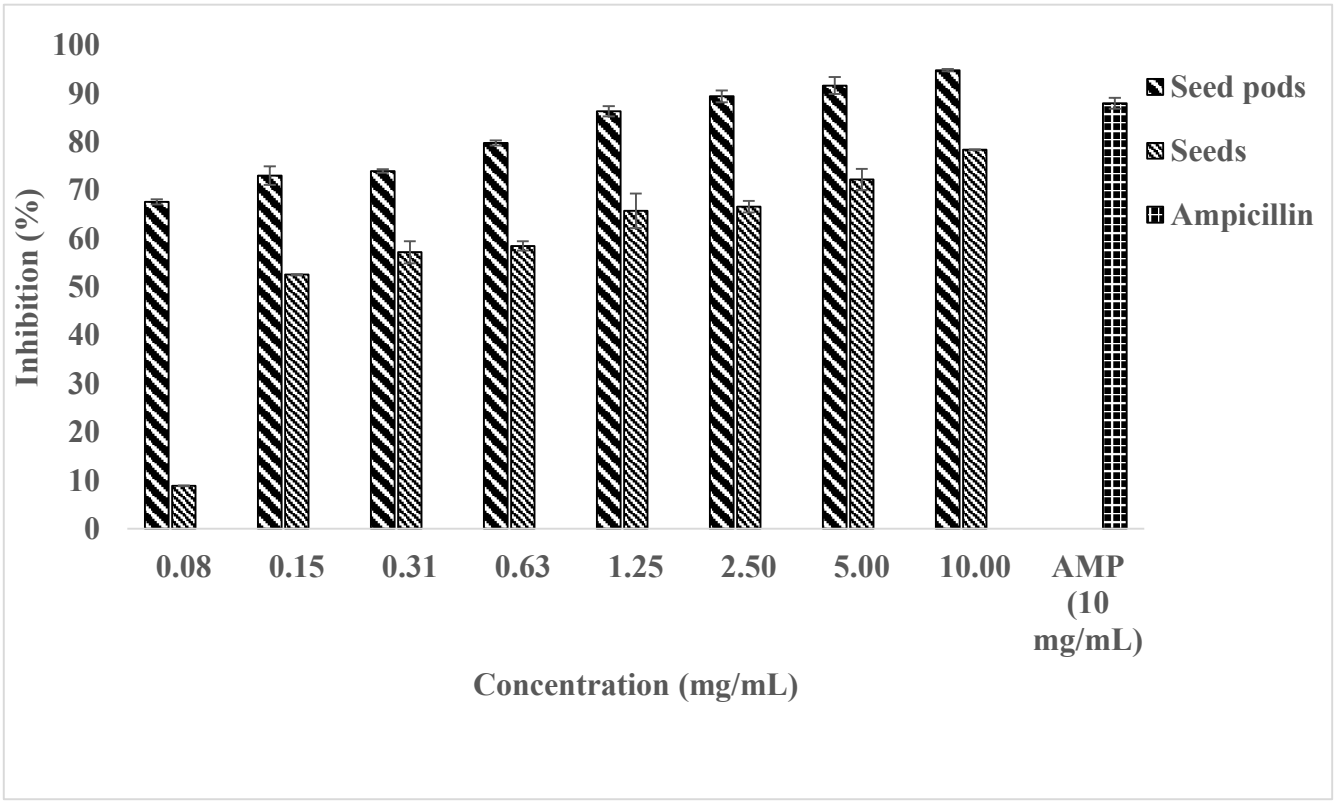

Figure 10. Biofilm inhibition of the extracts of *V. erioloba* seed pods and seeds, against *Staphylococcus aureus*.

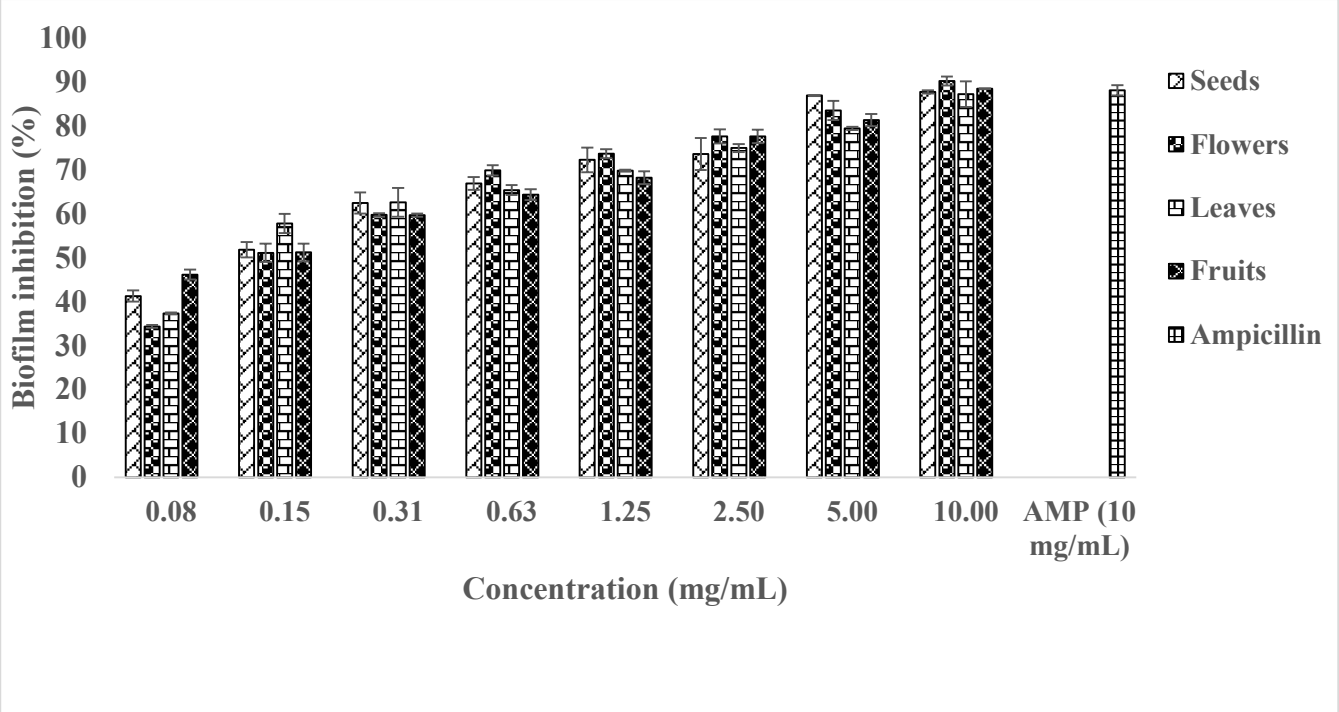

Figure 11. Biofilm inhibition of the extracts of *O. stricta* seeds, flowers, leaves, and fruits against *Staphylococcus aureus*.

4.5.2 Biofilm eradication

The biofilm eradication of *V. erioloba* (seed pods and seeds) and *O. stricta* (seeds, flowers, leaves, and fruits) are also summarized in figures 12 and 13. The results showed that the biofilm eradication of the extracts is concentration-dependent; as the concentration increases, the biofilm eradication potential of the extracts increases as well. The seed pod extract of *V. erioloba* showed the best inhibition against *S. aureus* (98% biofilm eradication), whereas the flowers of *O. stricta* was the least effective (86% biofilm eradication).

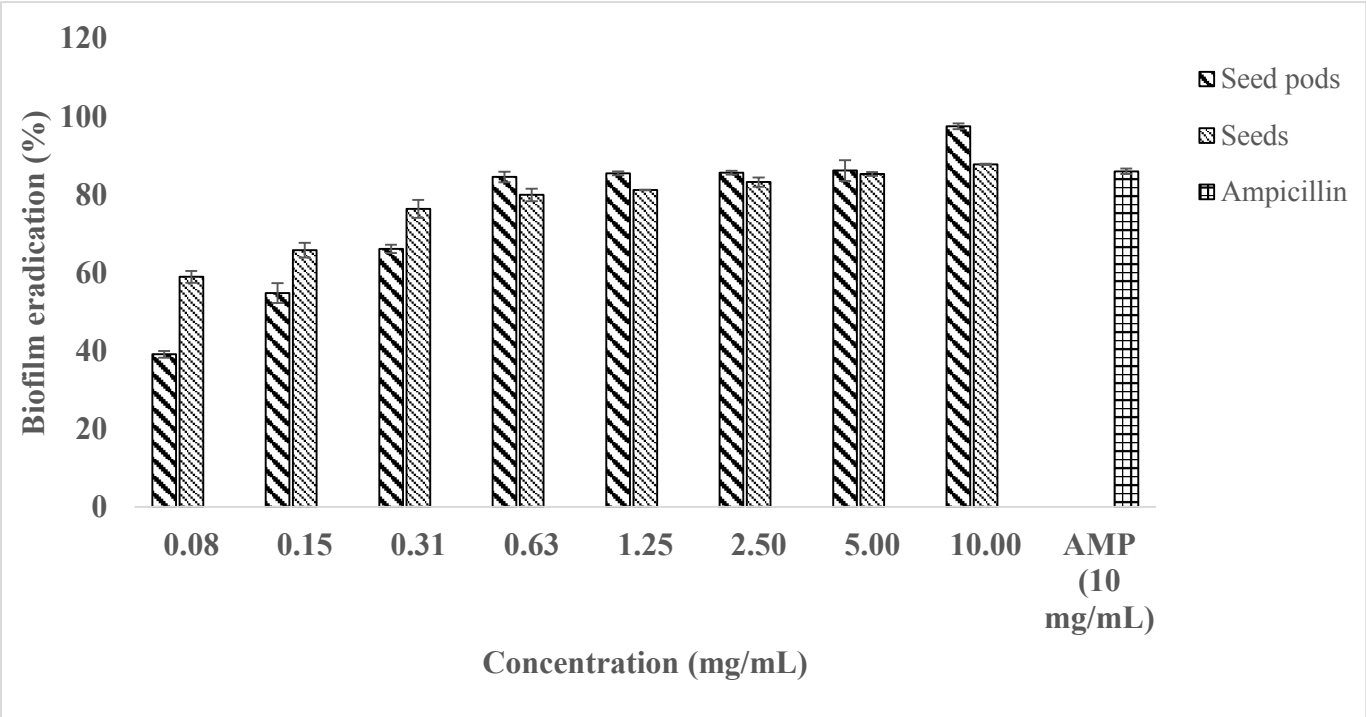

Figure 12. Biofilm eradication of *V. erioloba* seed pods and seeds, against *Staphylococcus aureus*.

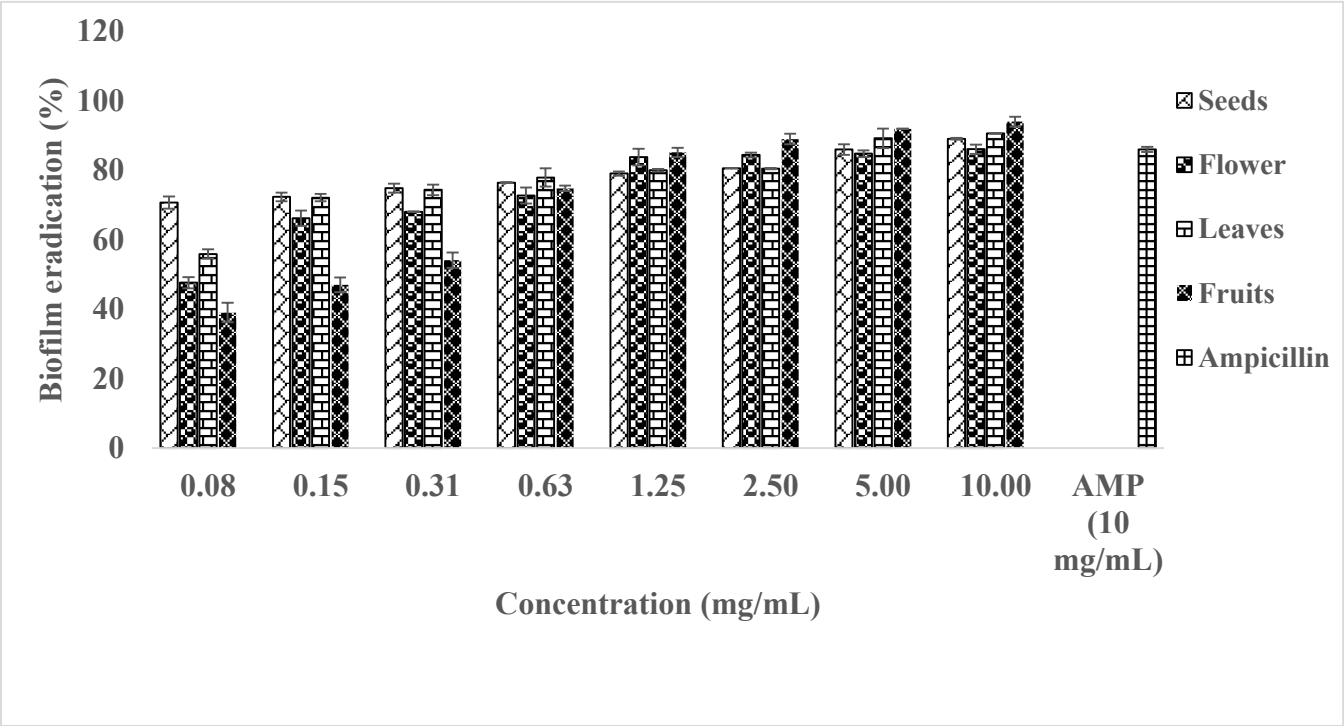

Figure 13. Biofilm eradication of *O. stricta* seeds, flower, leaves and fruits, against *Staphylococcus aureus.*

4.5.3 Biofilm inhibitory concentration (BIC_{50}) and Biofilm eradication concentration (BEC_{50})

Table 7 shows the biofilm inhibitory concentration (BIC_{50}) and biofilm eradication concentration (BEC_{50}) value for the extracts of *V. erioloba* and *O. stricta*. The BIC_{50} value of *V. erioloba* and *O. stricta* ranged from < 0.08 to 0.498 ± 0.017 mg/mL. The BEC_{50} value of *V. erioloba* and *O. stricta* ranged from < 0.08 to 0.172 ± 0.008 mg/mL.

Table 7. The biofilm inhibitory concentration (BIC_{50}) and biofilm eradication concentration (BEC_{50}) value of the extract of *V. erioloba* and *O. stricta* against S. aureus.

Plant sample	BIC_{50} (mg/mL)	BEC_{50} (mg/mL)
Vachellia erioloba		
Seed pods	BIC_{50} < 0.08	0.121 ± 0.006
Seeds	BIC_{50} < 0.08	BEC_{50} < 0.08
Opuntia stricta		
Seeds	BIC_{50} < 0.08	BEC_{50} < 0.08
Fruits	0.498 ± 0.017	0.172 ± 0.008
Flowers	0.1646 ± 0.0139	0.1289 ± 0.0580
Leaves	BIC_{50} < 0.08	BEC_{50} < 0.08

The best BIC_{50} (0.121 ± 0.006 mg/mL) was obtained for the seeds of *V. erioloba* and the least BIC_{50} (0.498 ± 0.017 mg/mL) was obtained for the fruits of *O. stricta.* On the other hand, the best BEC_{50} (0.1289 ± 0.0580 mg/mL) was obtained for the flowers of *O. stricta* and the least BEC_{50} (0.172 ± 0.008 mg/mL) was obtained for the fruits of *O. stricta* in this study.

The effects of the extracts to inhibit or eradicate biofilm formed by *S. aureus* (a strong biofilm producer) was investigated in this study. The findings of this study demonstrated that all extracts could inhibit and eradicate the biofilm that had formed, with higher concentrations being more effective. The extracts of *V. erioloba* and *O. stricta* were found to inhibit and eradicate the production of biofilms against *S. aureus*. The high activities illustrated in this study could be attributed to the high TFC demonstrated by the plant extracts. Furthermore, secondary metabolites, such as flavonoids, and alkaloids, have been reported to exhibit antibiofilm activities against *S. aureus*, which could also explain the antibiofilm activities in these plant extracts [110]. There are no studies on the antibiofilm activity of *V. erioloba* and *O. stricta*. However, previous studies on related species of *O. stricta* revealed that the species is a good biofilm inhibitor against *S. aureus* [111].

Antibiofilm activity of plant extracts has also been linked to antioxidants presence in these plants [101,112]. Previous studies, reported that oxidative stress can be one of the mechanisms for the change of microorganisms from their planktonic stage to their biofilm stage [113]. Antioxidants are capable of inhibiting against ROS, these may prevent the formation of biofilm as antioxidants interferes with ROS [113]. In the present study, the antioxidants potential of the extracts could also be linked to their ability to inhibit or eradicate the formation of biofilms against *S. aureus* (Table 5 and 7).

4.6 Chemical characterization of *V. erioloba* seed extract

The non-polar fraction of the seed extract of *V. erioloba* were analysed using GC-MS (Figure 15). A total of 12 compounds (Table 8) were identified in these extracts, including phytosterols, acids and ester, α-tocopherol and α-amyrin. The volatile and semi-volatile constituents of the extract were identified by comparison of their EI mass

spectra with those reported in the literature and those in the National Institute of Standards and Technology (NIST) MS and RI libraries. Compounds are regarded as being positively identified if their RI values are within 10 index units of the corresponding literature value. In addition, the literature value must be one that was experimentally determined by the reporting authors, using an authentic standard [114].

4.6.1 Phytosterols

Comparison of the mass spectrum of compound **9** (Figure 17), the major compound in the seed extract of *V. erioloba*, to those in the NIST MS database revealed that it might be a phytosterol, β-sitosterol. The diagnostic ions used to identify phytosterols are, (1) $[M - H_2O]^+$, (2) $[M – (CH_3 + H_2O)]^+$, (3) $[M – (\text{side chain} + H_2O)]^+$ and (4) $[M - (\text{D-ring cleavage} + CH_3 + H_2O)]^+$ [115]. The ions observed at m/z 414, and m/z 381 in the mass spectrum of compound **9** corresponds to the molecular ion and diagnostic ion (2), respectively. When the experimentally determined RI of compound **9**, 3304 iu, was compared to the literature values, it was found that none of the RI values reported for β-sitosterol matched that of compound **9**. However, one RI value reported for γ-sitosterol, the 24*S* enantiomer of β-sitosterol, is only 14 iu lower than that of compound **9**. Therefore, compound 9 was tentatively identified as γ-sitosterol (Figure 14).

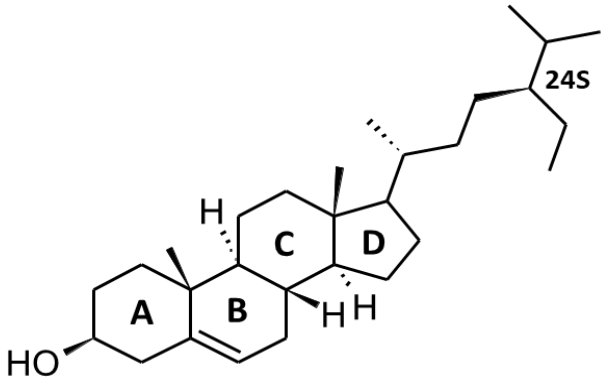

Figure 14. Possible structure of compound 9, tentatively identified as of γ-sitosterol.

The ions observed at m/z 412 and m/z 225 and 213 in the mass spectrum of compound **8** (Figure 19) corresponds to the molecular ion and diagnostic ion (3) and (4), respectively. Based on the MS data, comparison of the RI values and the presence of the characteristic fragment ions, compounds **7** and **8** were tentatively identified as an unknown steroid and stigmasterol, respectively. Since the experimentally determined RI value of compound **8** is within 10 index units of the one reported in the literature (Table 8), compound **8** was positively identified as stigmasterol.

Phytosterols represent a family of lipids present in plant cells and are structurally and biosynthetically similar to cholesterol [93]. The most common phytosterols are sitosterol, campesterol, and stigmasterol. Phytosterols have properties and biological effects, such as hypocholesterolaemia, anti-inflammatory, anti-oxidative, and antitumor. They are widely used in pharmaceuticals, nutritional supplements, and cosmetics [94]. β-Sitosterol is a bioactive Phytosterol, which is found in the cell membranes of plants. This compound reportedly displays antioxidant, anticancer and antimicrobial activities [95]. Stigmasterol is one of the important plant sterols that can inhibit the development of various cancerous cells by inhibiting the promotion and growth of apoptosis of cancer cells. Moreover, studies show that it displays antiosteoarthritic, anti-hypercholesteraemic, cytotoxicity, antitumor, hypoglycaemic, antimutagenic, antioxidant, anti-inflammatory and central nervous system effects [116]**.**

4.6.2 Fatty acids and esters

After comparison of the mass spectra of compounds **2**, **3**, **4**, and **5** (Figures 20-23) to those published, it was determined that compounds **2** – **5** are fatty acids (FAs) and esters. Based on the MS data, compound **4** was tentatively identified as methyl linoleate. Furthermore, since the experimentally determined RI value of compound **4**

is within 10 index units of the one reported in the literature (Table 8), compound **4** was positively identified as methyl linoleate. Similarly, compounds **2**, **3** and **5** were identified as methyl palmitate, palmitic acid, and unidentified octadecenoic acid, respectively, based on their MS and RI data. On the other hand, compounds **11** and **12** (Figures 24 and 25) were tentatively identified as long chain esters, since no RI matches could be found.

FAs are generally derived from triglycerides and phospholipids and are the main components of dietary fats. Most naturally occurring FAs have an unbranched chain of even number (4-28) carbons [117]. The major roles of unsaturated FAs in the body are to store energy, provide energy, and provide stable cellular membranes; however, there have been found to be bioactive molecules. Previous studies have shown many fatty acids and esters display antifungal, antioxidant, hypocholesterolemia, insecticidal, haemolytic, and potent antimicrobial activities [86,118–120]. Unsaturated FAs such as oleic acid are known for their antibacterial, and antifungal properties [121–123]. It has also been reported that the saturated FA palmitic acid, one of the most common FAs has antifungal, antioxidant, antimalarial, and antimicrobial activities [86,119,120].

4.6.3 α-Tocopherol

A comparison of the mass spectrum of compound **6** (Figure 26) to those in the NIST MS database showed that this compound might be α-tocopherol. Hence, it is assumed that the peak that appears at *m/z* 430 in its mass spectrum corresponds to the molecular ion. The base peak $[M\text{-}C_{19}H_{37}]^+$ ion is observed at *m/z* 165, and the peak at *m/z* 205, corresponds to the $[M–\text{side chain}]^+$ ion. Based on the MS data, RI data and the presence of the characteristic fragment ions, compound **6** was identified as α-tocopherol.

α-Tocopherol (vitamin E) is a one of the most abundant biologically active compounds present in plants and it is essential in the maintenance of the cell membrane integrity [124]. Studies of the antioxidant activity of α-tocopherol revealed that it can scavenge many lipid soluble and reactive oxygen species (ROS) compounds produced during oxidative stress [125]. α-Tocopherol also has antiaging, analgesic, antidiabetic anti-inflammatory, and antioxidant, antitumor, and anticancer properties [126–128].

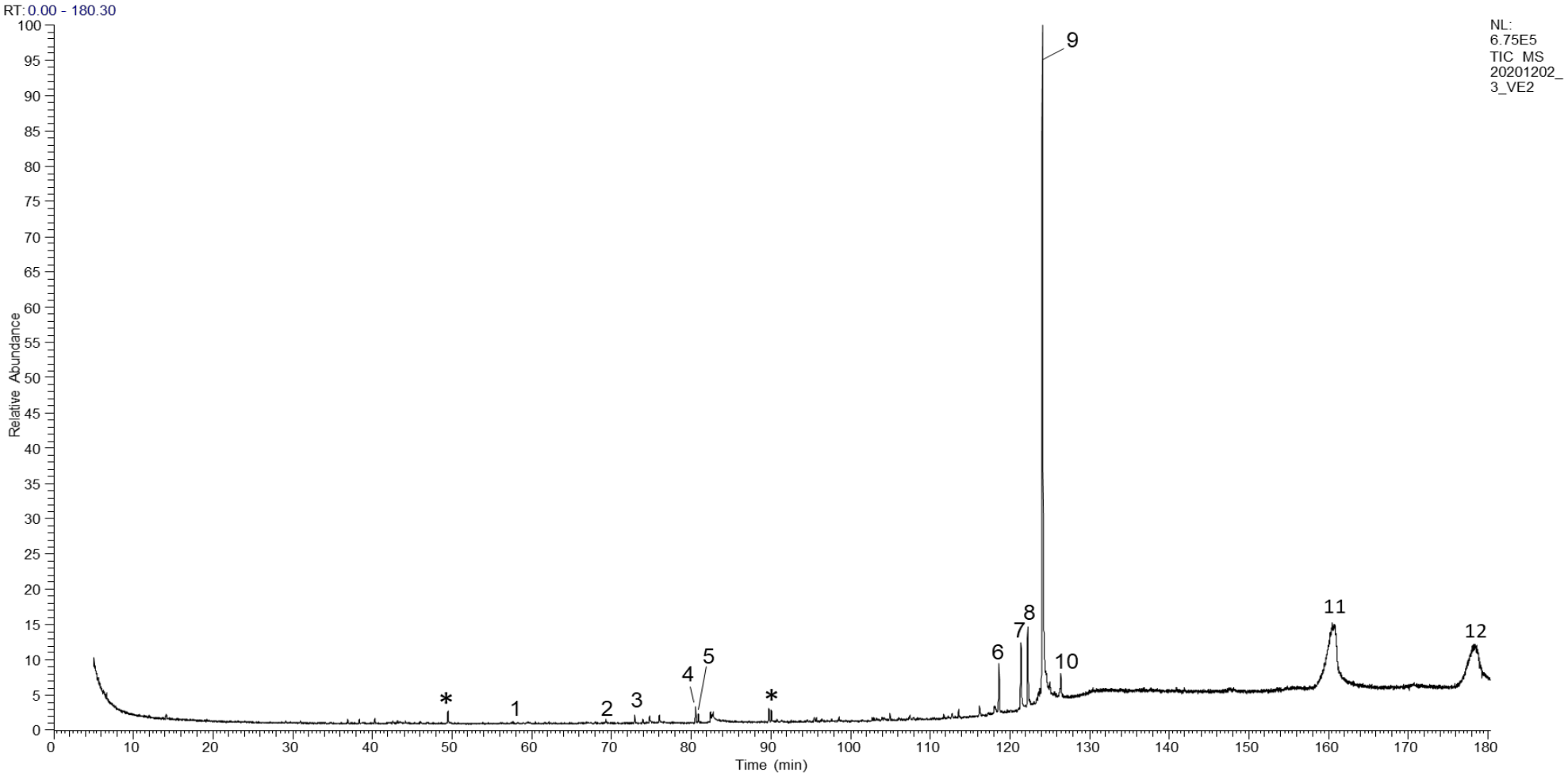

Figure 15. Total ion chromatogram of the non-polar fraction of the seed extract of *V. erioloba*. The identified compounds are numbered in order of elution from the GC column. The numbers correspond to those in Table 8. *contaminant

Table 8. Compounds identified in the non-polar fraction of the seed extracts of *Vachellia erioloba.*

COMPOUND NUMBER [A]	T_R (MIN)	RI (EXP.)[B]	RI (LIT.)	COMPOUND NAME	IDENTIFICATION METHODS[D]
1	59.62	1671	-	Unknown	A
2	72.87	1928	1921[c]	Methyl palmitate	A, B
3	74.76	1965	1963[e]	Palmitic acid	A, B
4	80.5	2091	2085[c]	Methyl linoleate	A, B
5	80.85	2098	-	Unidentified octadecenoic acid	A, C
6	118.58	3120	3111[f]	α-Tocopherol	A, B
7	121.37	3210	-	Unidentified steroid	A, C
8	122.24	3241	3249[f]	Stigmasterol	A
9	124.06	3304	3290[f]	γ-Sitosterol	A
10	126.36	3383	3376[f]	α-Amyrin	A
11	160.90	-	-	Unidentified long chain ester	A, C
12	178.63	-	-	Unidentified long chain ester	A, C

[a] Compounds are numbered in order of elution from the GC column.

[b] RI values determined experimentally on an HP-5 equivalent column.

[c] Kovátz RI values reported for authentic reference standards on an HP-5 equivalent column by Adams [129], except where other publications are cited.

[d] A: Comparison of mass spectrum with NIST MS database and published data, B: RI matches published value (within 10 RI units), C: mass spectrum interpretation (tentative identification).

[e] RI value reported for authentic reference standard on an HP-5 equivalent column [130].

[f] RI value reported in the NIST RI and PubChem database

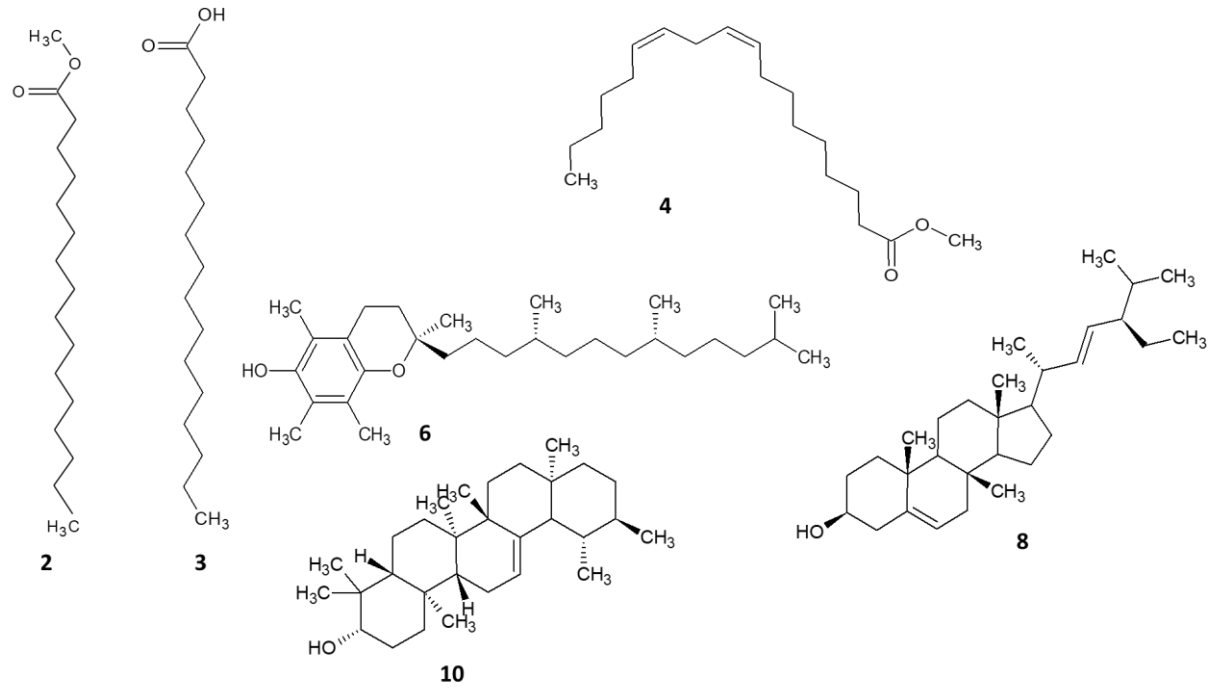

Figure 16. Structures of the minor compounds identified in the non-polar fraction of the seed extracts of *Vachellia erioloba*

4.6.4 α-Amyrin

A comparison of the mass spectrum of compounds **10** (Figure 27) to those in the NIST MS database showed that this compound might be α-amyrin. Hence, it is assumed that the peak that appears at *m/z* 426 in the mass spectrum of compound **10** corresponds to the molecular ion. The base peak ion, formed by the retro-Diels-Alder rearrangement is observed at *m/z* 218, and the peak at *m/z* 203, corresponds to the $[218 - CH_3]^+$ ion [131,132]. Based on the MS data, RI data and the presence of the characteristic fragment ions, compound **10** was identified as α-amyrin.

α-Amyrin is a plant-derived triterpenoid, and reportedly has physiological and pharmacological activities. Previous studies showed that it displays antioxidant [133], antimicrobial [134] and antitumor, & anti-inflammatory [135] activities.

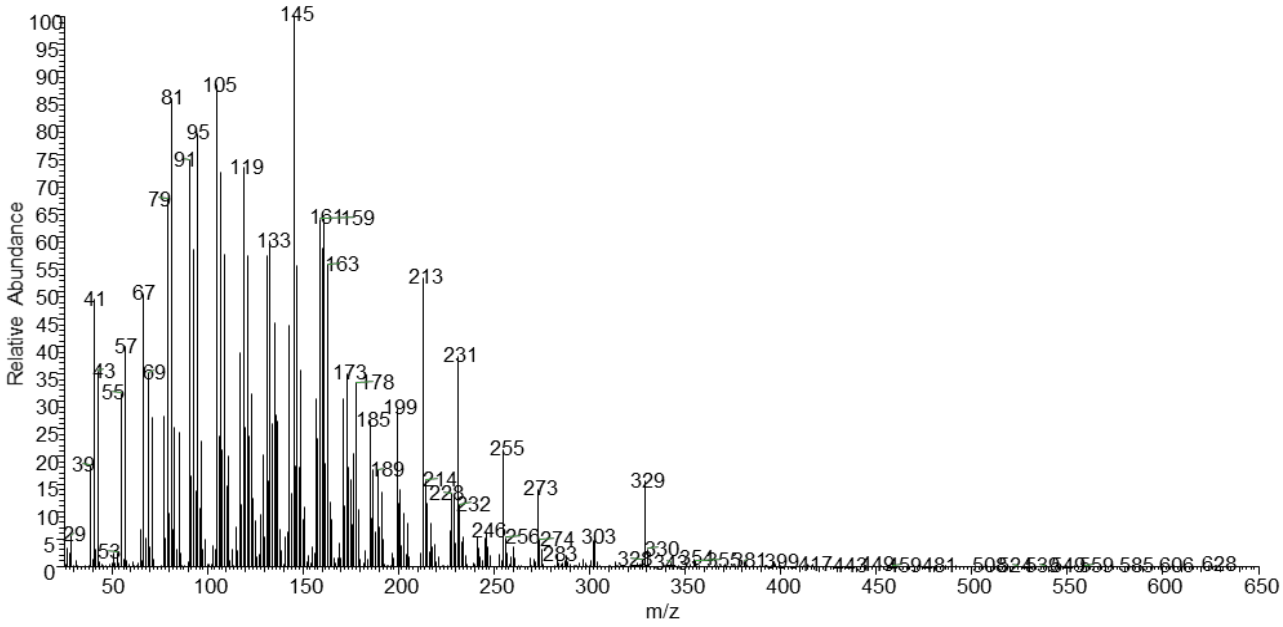

Figure 17. Mass spectrum for compound 9, tentatively identified as γ-sitosterol in the non-polar fraction of the seed extract of *V. erioloba.*

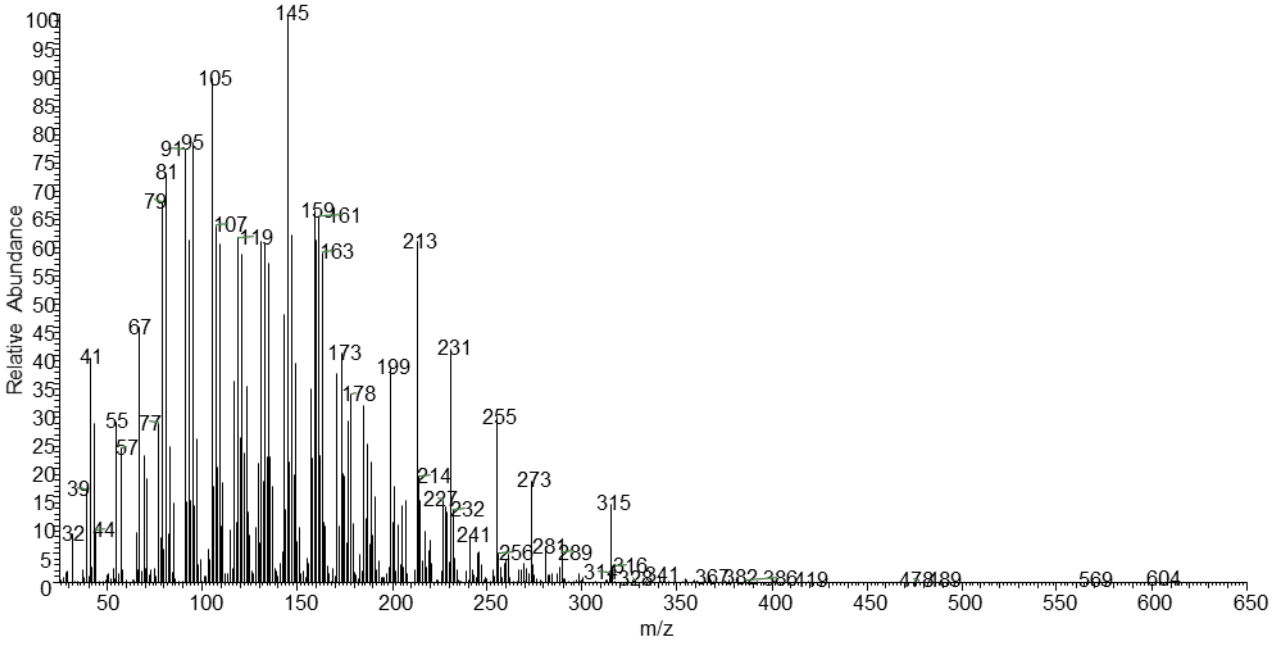

Figure 18 Mass spectrum for compound 7, tentatively identified as an unidentified steroid in the non-polar fraction of the seed extract of *V. erioloba.*

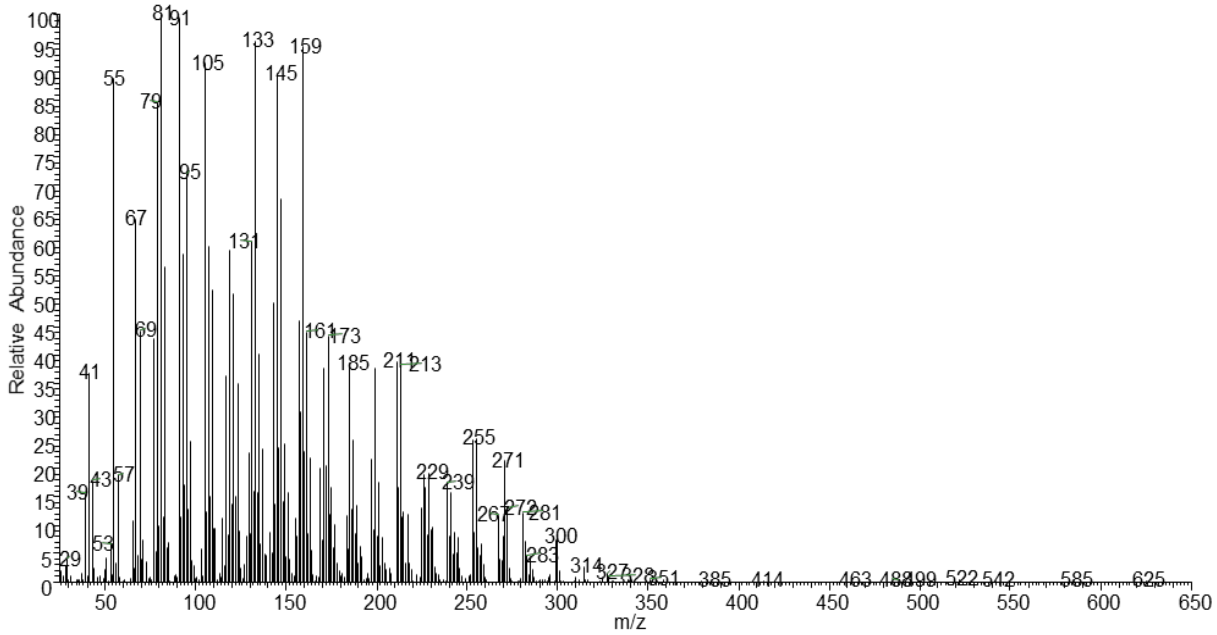

Figure 19. Mass spectrum for compound 8, tentatively identified as stigmasterol in the non-polar fraction of the seed extract of *V. erioloba.*

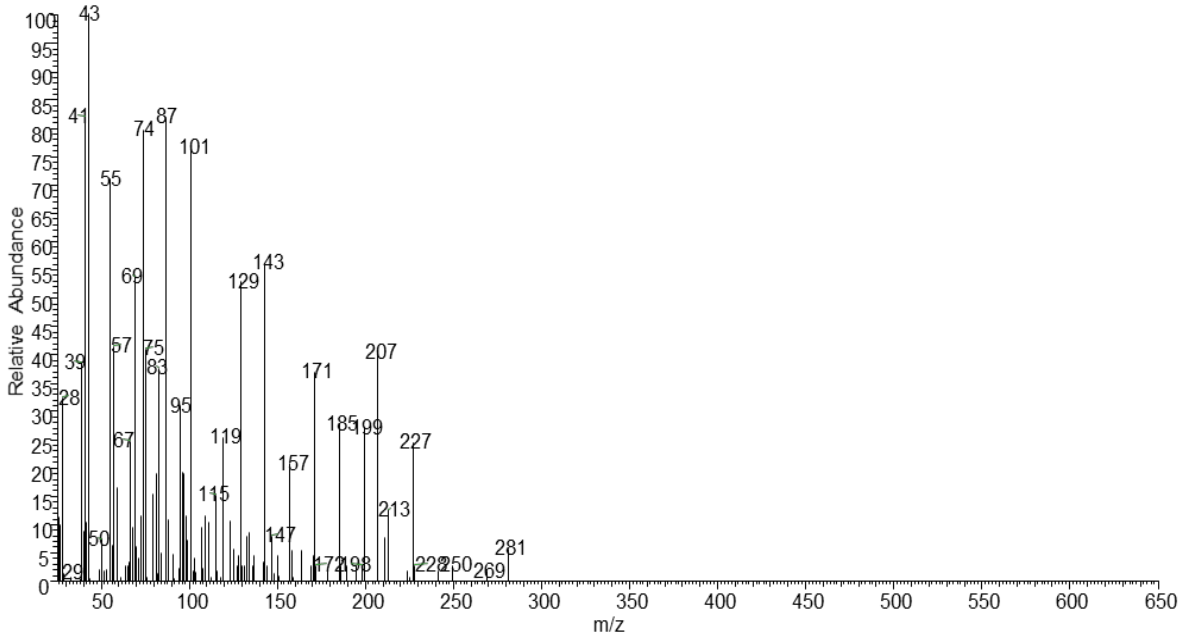

Figure 20. Mass spectrum for compound 2, identified as methyl palmitate in the non-polar fraction of the seed extract of *V. erioloba*

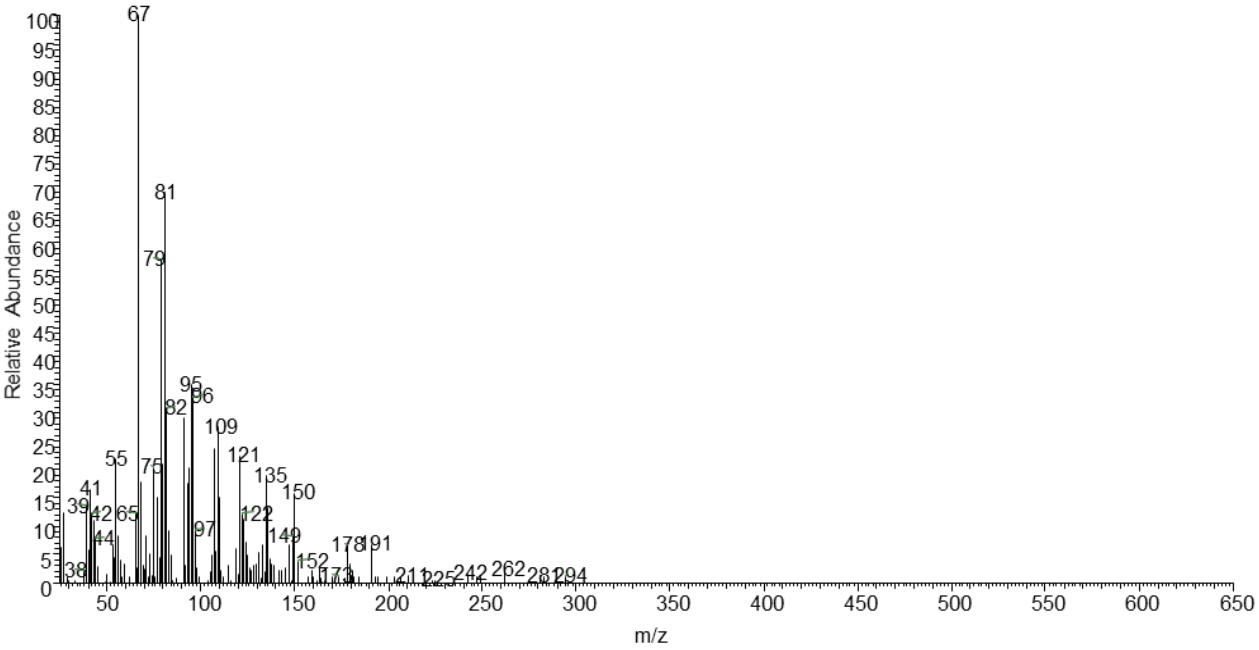

Figure 21. Mass spectrum for compound 4, identified as methyl linoleate in the non-polar fraction of the seed extract of *V. erioloba*

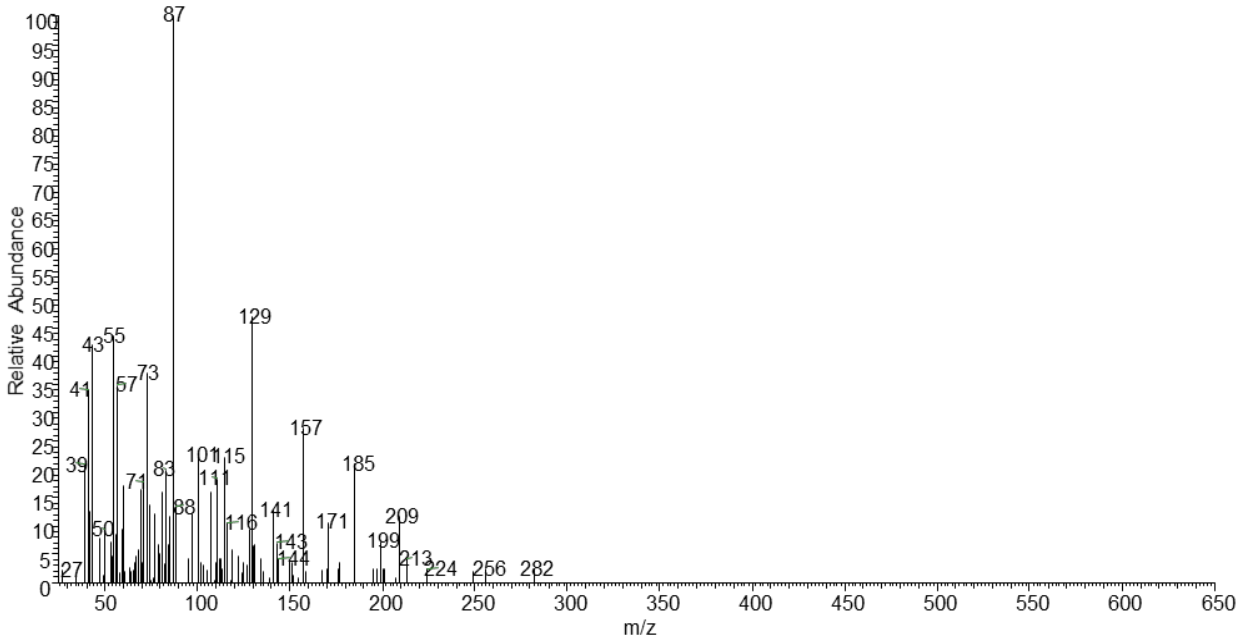

Figure 22. Mass spectrum for compound 3, identified as palmitic acid in the non-polar fraction of the seed extract of *V. erioloba*

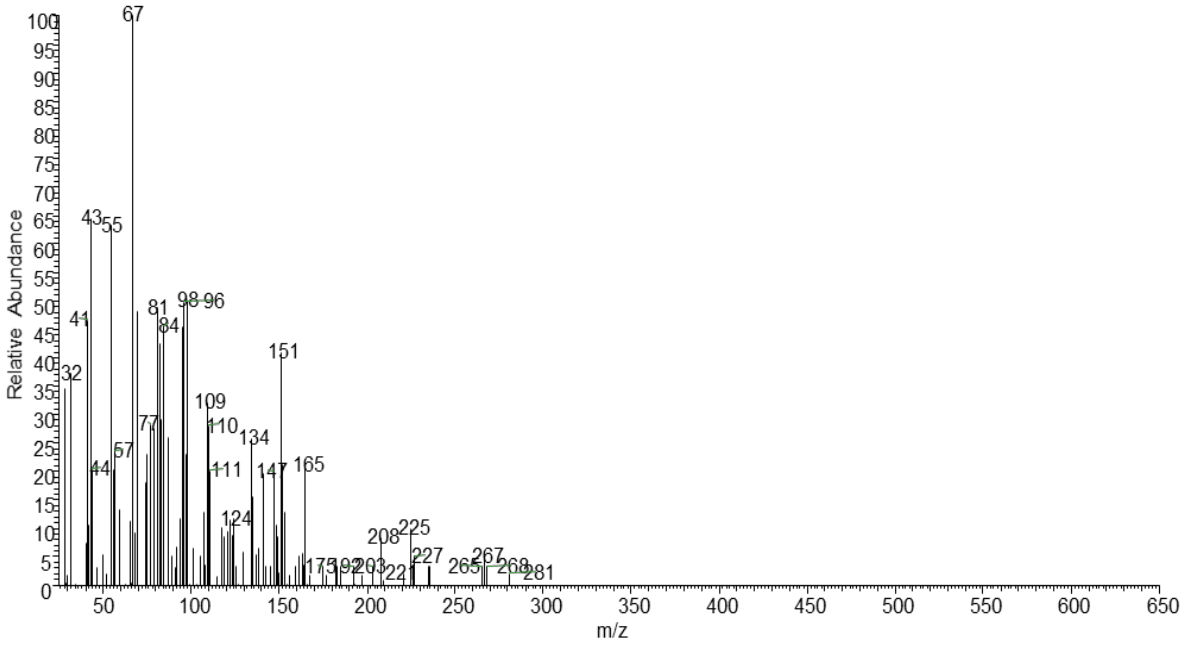

Figure 23. Mass spectrum for compound 5, tentatively identified as an unidentified octadecenoic acid in the non-polar fraction of the seed extract of *V. erioloba*

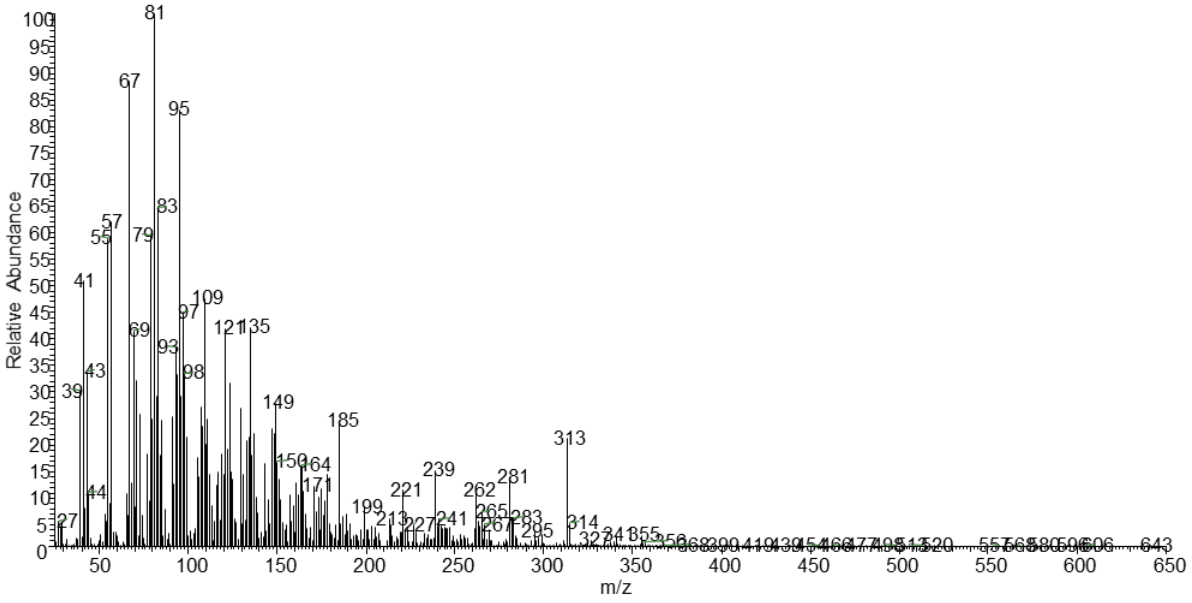

Figure 24. Mass spectrum for compound 11, tentatively unidentified long chain ester in the non-polar fraction of the seed extract of *V. erioloba*

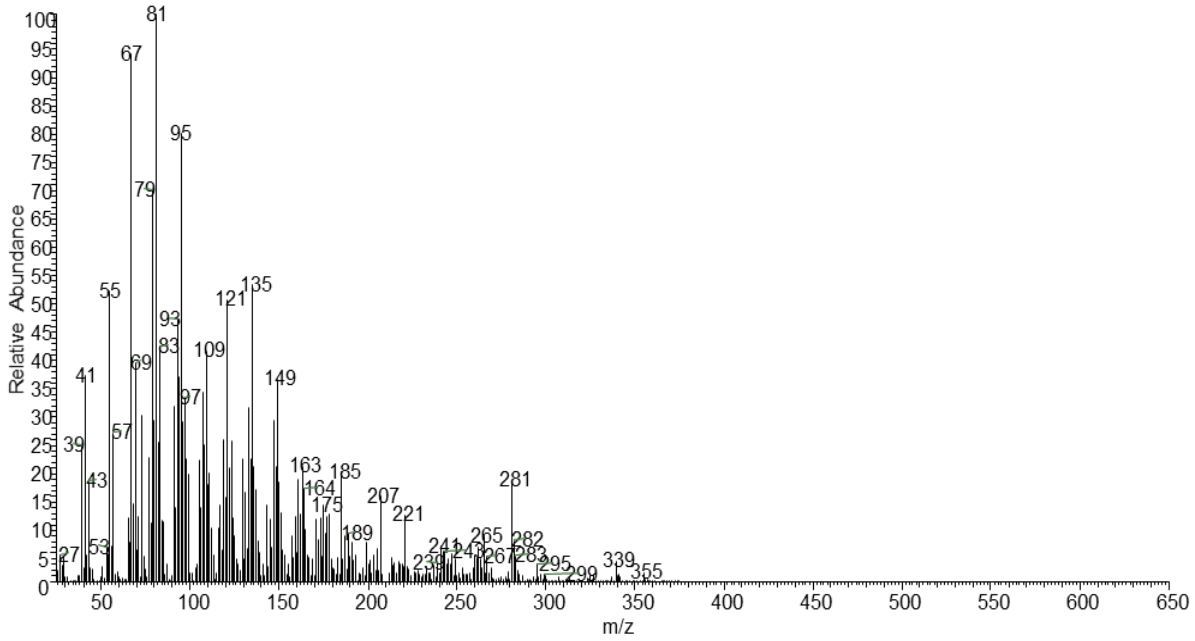

Figure 25. Mass spectrum for compound 12, tentatively unidentified long chain ester in the non-polar fraction of the seed extract of *V. erioloba*

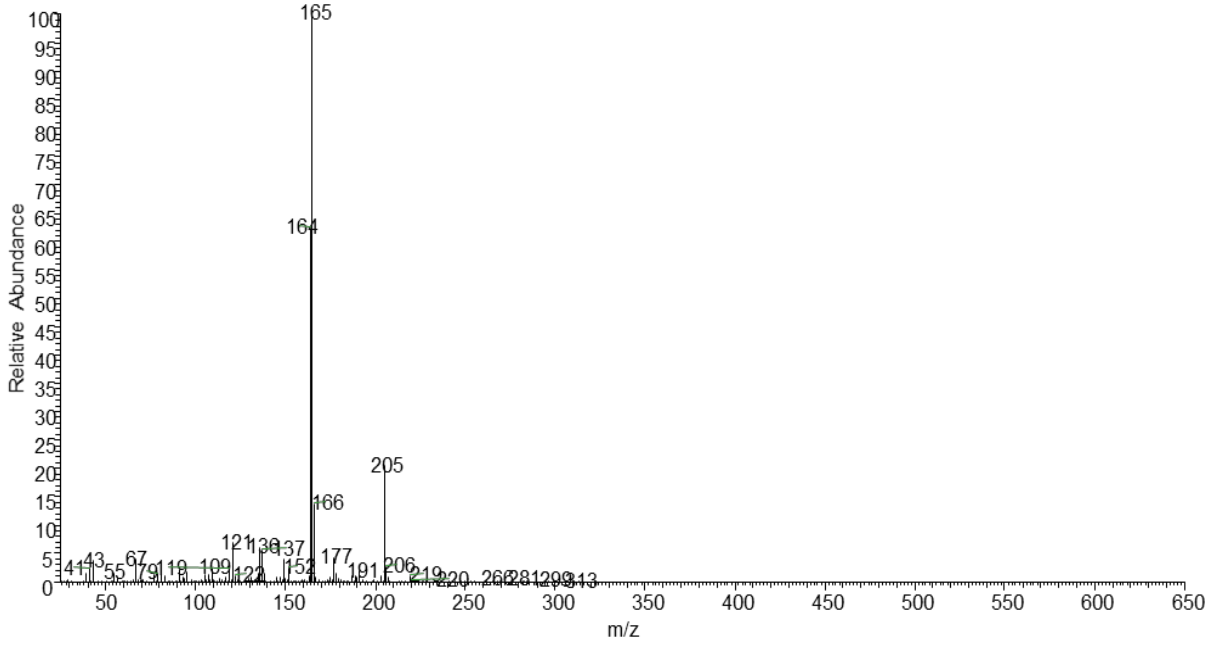

Figure 26. Mass spectrum for compound 6, identified as a-tocopherol (vitamin E) in the non-polar fraction of the seed extract of *V. erioloba*

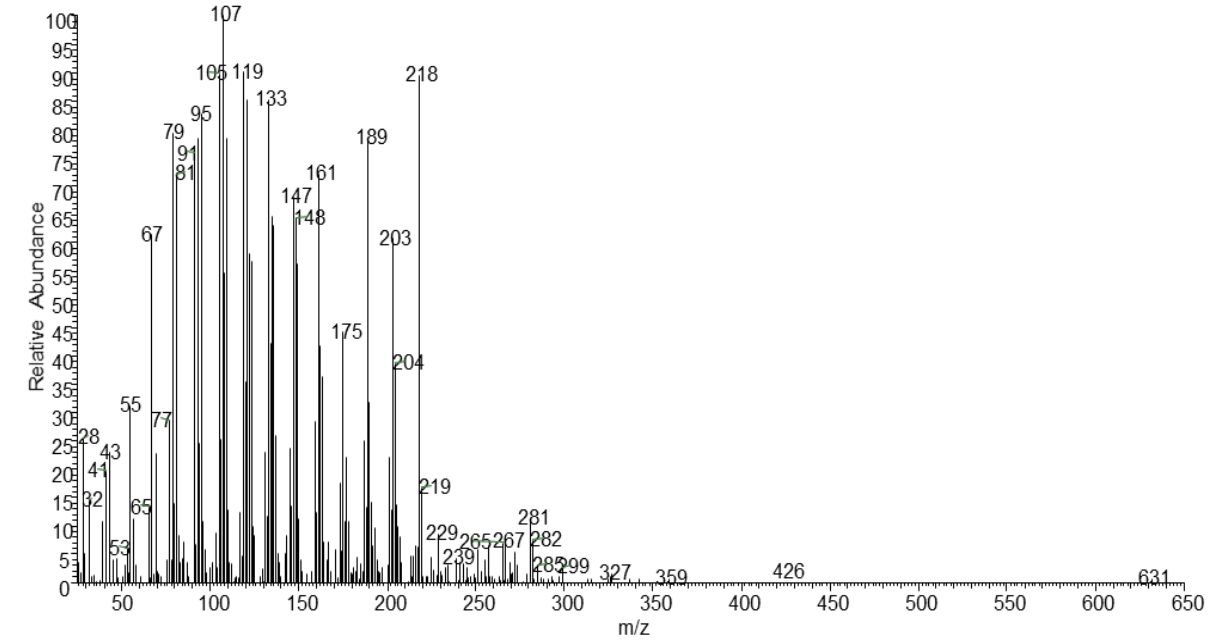

Figure 27. Mass spectrum for compound **10**, tentatively identified as α-amyrin in the non-polar fraction of the seed extract from *V. erioloba*

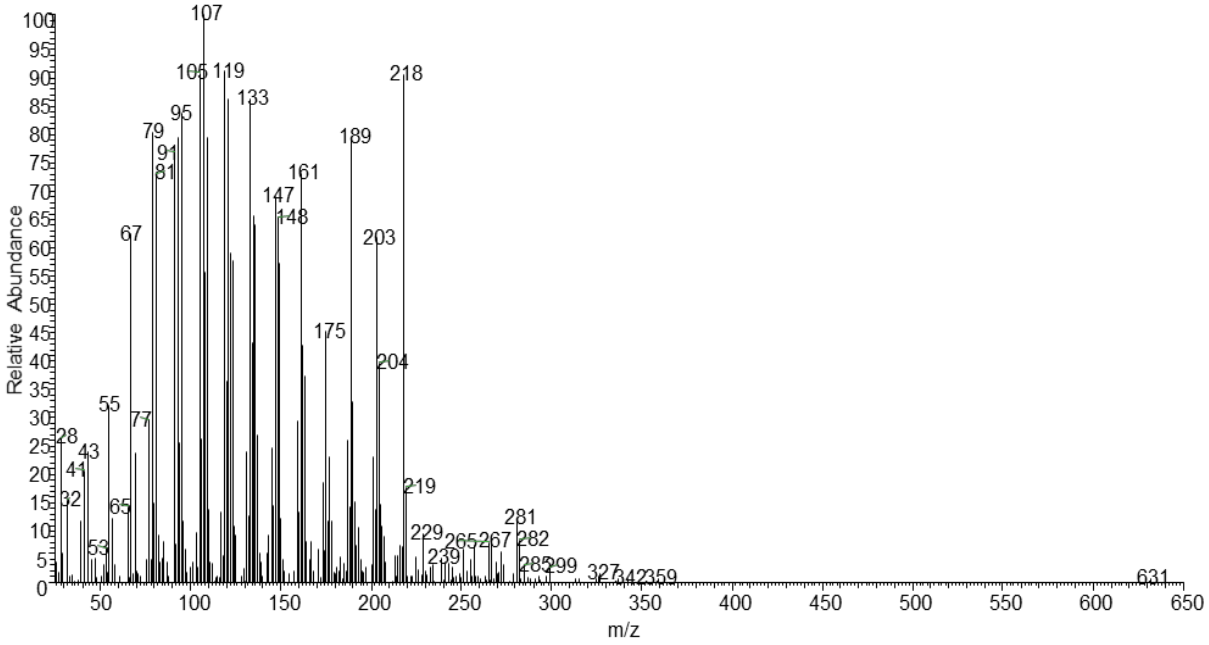

Figure 28. Mass spectrum for compound 1, tentatively identified as an unknown in the non-polar fraction of the seed extract of *V. erioloba*

5. CONCLUSION

The extracts obtained from *V.* erioloba (seed pods and seeds), and *O. stricta* (seeds, flower, leaves and fruits) showed good antioxidant. In addition, the seed pods extracts of *V. erioloba* (IC_{50} = 1.973 ± 0.012 µg/mL) showed excellent activity as compared to the control ascorbic acid (IC_{50} = 3.097 ± 0.395 µg/mL). The excellent antioxidant activity of this extract could be attributed to the high phenolic content and high flavonoid contents observed for this extract. Furthermore, the good antioxidant activity of the other extracts of *V. erioloba* and *O. stricta* could as be attributed to the high TPC and TPC in these extracts.

The antimicrobial and antibiofilm activity of *V. erioloba* and *O. stricta* were investigated for the first time in this study. The antimicrobial activity of *V. erioloba* and *O. stricta* in this study showed moderate activity against the tested strains. In addition, the moderate activity can be attributed to the amount of phenolic and flavonoid contents in these plants. The biofilm inhibition and eradication of *V. erioloba* and *O. stricta* was found to be very good and can be attributed to the phytochemicals such flavonoids, saponin and alkaloids presents in these extracts.

The chemical characterisation of volatile and semi volatile compounds of *V. erioloba* seeds were also investigated for the first time in this study. Twelve compounds (Table 8) were identified in these extracts, including phytosterols, acids and ester, α-tocopherol and α-amyrin. These compounds have been reported to display biological activities.

The findings of this study support the use of both *V. erioloba* and *O. stricta* as traditional remedies that are used for the treatment of various ailment. The data from

this study adds value to the plants as potential antioxidant, antimicrobial and antibiofilm agent.

6. RECOMMENDATION

6.1 Antioxidant activity

The antioxidant activity of *V. erioloba* and *O. stricta* in this study was determined for the DCM: MeOH extracts. Further studies can look into the antioxidant activity of the aqueous extract of *V. erioloba* and *O. stricta.* Traditionally the plants are preparing in aqueous media hence it is important to study the water extracts. In this study two antioxidant assays were used namely 2,2-diphenyl-1-PicrylHydrazyl (DPPH) radical and ferric reducing antioxidant power (FRAP). Various antioxidant assays exist such as Nitric oxide, Hydrogen peroxide, 2,2-Azino-Bis-3-Ethylbenzothiazoline 6-sulfonic acid (ABTS) etc. These assays can be used to obtain different results as compare to DPPH and FRAP assays used. Further studies should there for use other antioxidant assays to studies

6.2 Antimicrobial and antibiofilm activity

Future studies on the antimicrobial and antibiofilm activity of *V. erioloba* and *O. stricta* should focus on the aqueous extracts of the plants since traditionally water extracts are used. Four microbial strains were used to determine the antimicrobial activity in these studies namely, *S. aureus, S. mutans, and E. coli* and *C. albicans*. Other studies should look at other strains such as *Streptococcus pneumoniae, Bordetella pertussis,* and *Neisseria gonorrhoeae*, these microbial are known to cause ear infection and inflammation. Since most of the extract displayed MIC values below the lowest concentration, (0.63 mg/mL) tested. This studies only looked at the antibiofilm activity of *V. erioloba* and *O. stricta* against *S. aureus* a strong biofilm producer, however, further studies can look at other biofilm producers such as *Escherichia coli, Candida albicans, Klebsiella pneumonia* etc. I recommended further studies look at testing the extracts at lower concentrations.

6.3 Chemical characterization of volatile and semi-volatile compounds

In the present study, only the volatile and semi-volatile compounds of the non-polar fraction of the seed extract of *V. erioloba* were investigated. Future research could look into identifying compounds present in all the extracts of *V. erioloba* and *O. stricta.* In addition, there is no high-pressure liquid chromatography – high-resolution mass spectrometry instrument at the University of Namibia, hence the characterization of non-volatile compounds was not possible. Further studies could therefore also look into the identification of non-volatile compound present in these plants.

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8. APPENDIX

Appendix 1. Ethical clearance certificate

UNAM

ETHICAL CLEARANCE CERTIFICATE

Ethical Clearance Reference Number: SOS-0004 Date: 25 October 2021

This Ethical Clearance Certificate is issued by the University of Namibia Ethics Committee (REC) in accordance with the University of Namibia's Research Ethics Policy and Guidelines. Ethical approval is given in respect of undertakings contained in the Research Project outlined below. This Certificate is issued on the recommendations of the ethical evaluation done by the ethics committee.

Title of Project: ANTI OXIDANT, ANTIMICROBIAL, AND CYTOTOXIC PROPERTIES OF OPUNTIA STRICTA AND VACHELLIA ERIOLOBA, AND CHEMICAL CHARACTERISATION OF VOLATILE COMPOUNDS

Student: JUNIAS JACKSON

Student Number: 201612026

Supervisor(s): PROF PETRINA KAPEWANGOLO (UNIVERSITY OF NAMIBIA)

Centre for Research Services

Take note of the following:

1. Any significant changes in the conditions or undertakings outlined in the approved Proposal must be communicated to the ethics committee. An application to make amendments may be necessary.
2. Any breaches of ethical undertakings or practices that have an impact on ethical conduct of the research must be reported to the ethics committee
3. The Principal Researcher must report issues of ethical compliance to the ethics committee (through the Chairperson) at the end of the Project or as may be requested by the ethics committee
4. The ethics committee retains the right to:

i) Withdraw or amend this Ethical Clearance if any unethical practices (as outlined in the Research Ethics Policy) have been detected or suspected,

ii) Request for an ethical compliance report at any point during the course of the research.

The ethics committee wishes you the best in your research.

Dr. Zivayi Chiguvare (Chairperson Ethics Committee)

Prof. Davis Mumbengegwi (Head, Multidisciplinary Research)

Appendix 2: Authorization of research projects

NCRST

AUTHORIZATION OF RESEARCH PROJECTS

Authorization is hereby granted in terms of section 21 of the RST Act No. 23 of 2004, to:

Name: Dr Stefan Louw

Address: University of Namibia
Private Bag 13301
Windhoek

Coworkers: Prof Petrina Kapewangolo, Dr Celine Mukakalisa and Mr Junias Jackson.

Certificate Number (if applicable): RCIV00022018 **Authorization No:** AN202101016

Type of research

Non-commercial research and the use of the resources be limited to what is specified in the proposal.

Title of Research authorized:

Bio-evaluation of Vachellia eriloba (Camelthorn) seeds and pods as potential nutritices for animals and humans.

Locality:

Windhoek

Duration: 26 March 2021 - 31 March 2022

Research/Sample collection conditions:

N/A

Yours sincerely,

National Commission on Research Science & Technology
21 MAR 2021
P/Bag 13253
Windhoek, Namibia

Ms. A. Ngurare
Acting Chief Executive Officer