ANTIMICROBIAL SCREENING AND CHARACTERISATION OF PHYTOCHEMICAL COMPOUNDS FROM THE STEM BARK OF *Butyrospermum paradoxum*

BY

ERUUT DAVID

19/U/18946/GMCH/PD

A DISSERTATION SUBMITTED TO THE DIRECTORATE OF RESEARCH AND GRADUATE TRAINING IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF MASTERS OF SCIENCE IN CHEMISTRY OF KYAMBOGO UNIVERSITY

OCTOBER, 2024

DECLARATION

This research thesis is entirely original and has never before been submitted for degree award in any higher institution of learning or University. In all instances where someone else's work has been used, it has been properly acknowledged with citations to supporting sources.

Name: ERUUT DAVID

Reg No.: 19/U/18946/GMCH/PD

Sign……...

Date: ……

APPROVAL

This Dissertation has been submitted to the Department of Chemistry for examination with the approval of the following university supervisors:

Sign…….. Date…….

Dr Kodi Phillip
Department of Chemistry
Kyambogo University

Sign…….. Date…….

Dr. Hannington Twinomuhwezi
Department of chemistry
Kyambogo University

DEDICATION

THIS DISSERTATION IS DEDICATED

TO

MY DEAR PARENTS;

ERUUT JOHN ADAKUN

AND

ANYAIT JOYCE

ACKNOWLEDGEMENT

I would like to take this opportunity to express my gratitude to my supervisors; Dr. Kodi Phillip and Dr. Twinomuhwezi Hannington for all the advise and assistance rendered to me throughout the duration of my Msc Chemistry degree programme.

My sincere thanks also go to Dr Gumula Ivan, Dr. Nanyonga Sarah and Prof. Kwetegyeka Justus for the continued advice and encouragement given to me during my period of study.

In the same vein I wish to thank all the academic staff in the Chemistry Department of Kyambogo University for the guidance they offered in different ways that helped me go through this programme.

In a special way I wish to convey my gratitude to the members of the Chemistry laboratory at Kyambogo University led by Mr Opedun John Chrysostom and Mr Olado Simon Peter for the technical advice rendered to me in the course of this research work. Special thanks also go to the technicians at the Biology laboratory of Kyambogo University for the technical assistance rendered during this research.

I also want to acknowledge my parents and the entire family members for the different efforts they put to see me through this journey. Their continued encouragement and advice was of great importance.

Special thanks also go to Ms Nekesa Sarah, Mr Kabala Hakim, Mr Makamba Ronald, Mr Okiring Isaac and Mr Niringiyimana Eric for the different kinds of support that they rendered to me at different levels of this programme.

I also wish to acknowledge all my course mates and friends for the assistance and encouragement given to me.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABREVIATIONS

$CDCl_3$	Deuterated trichloromethane
DCM	Dichloromethane
DMSO	Dimethylsulfoxide
HMBC	Heteronuclear Multiple Bond Correlation
HSQC	Heteronuclear Single Quantum Coherence
NMR	Nuclear Magnetic Resonance
TLC	Thin Layer Chromatography
TMS	Tetramethylsilane
UV	Ultra violet
WHO	World Health Organisation
STD's	Sexually Transmitted Diseases
UTI's	Urinary Tract Infections
MIC	Minimum Inhibitory Concentration
MBC	Minimum Bactericidal Concentration
MFC	Minimum Fungicidal Concentration
COSY	Correlation Spectroscopy
ID	Inhibitory Dose

ABSTRACT

There is a growing risk in bacterial and fungal infections as a result of increased resistance of the disease causing bacteria and fungi to the existing medicines. A lot of scientific research has therefore been directed to finding reliable alternatives to the synthetic antimicrobial agents and this has led to studies on natural products.

In this study, the antimicrobial activity of the stem bark crude extracts and the pure compounds of *Butyrospermum paradoxum* was investigated. Extraction from the air dried powdered plant material was done sequentially using n-hexane, DCM, ethyl acetate and methanol respectively to obtain four crude extracts.

The crude extracts obtained were subjected to silica gel column chromatography and further purification of different fractions was done using sephadex LH-20 to obtain pure compounds. Using various NMR techniques like ^{1}HNMR, ^{13}CNMR, COSY, HSQC and HMBC, the isolated bioactive compounds were characterized i.e. α-amyrin cinnamate (**48**), butyrospermol cinnamate (**49**), lupeol acetate (**50**) and lupeol cinnamate (**51**).

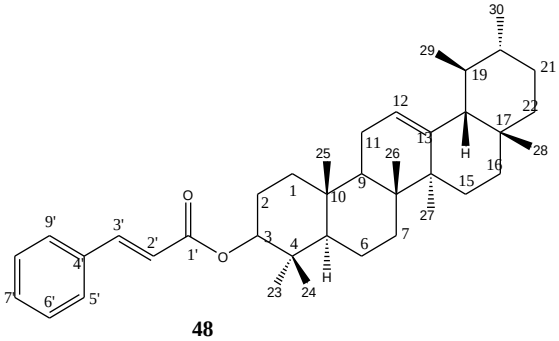
The antimicrobial activity of the crude extracts against *Salmonella typhi*, *Escherichia coli*, *Candida albicans, Pseudomonas aeruginosa, Klebsiella pneumoniae* and *Staphylococcus aureus* was determined using the agar well diffusion assay procedure while the MIC and MBC was determined by using the broth dilution method.

All the four crude extracts were found to be active against most of the selected microbial organisms. The hexane extract was most active against *C. albicans* with average diameter of zone of inhibition of 14.3 mm and least active against *P. aeruginosa* giving a diameter of zone of inhibition of 11 mm. The DCM extract was most active against *S. aureus* with average diameter of zone of inhibition of 16.3 mm and was also least active against *P. aeruginosa* giving a diameter of zone of inhibition of 13.7 mm. . Meanwhile the ethyl acetate and methanol extracts were most active against *S. typhi* with average diameter of zone of inhibition of 18.0 mm and 22.0 mm respectively. The MIC and MBC values of all the crude extracts ranged between 12.5 and 50 mg/mL. The methanol extract gave the least antibacterial activity with an average diameter of zone of inhibition of 9.7 mm against *K. pneumoniae*.

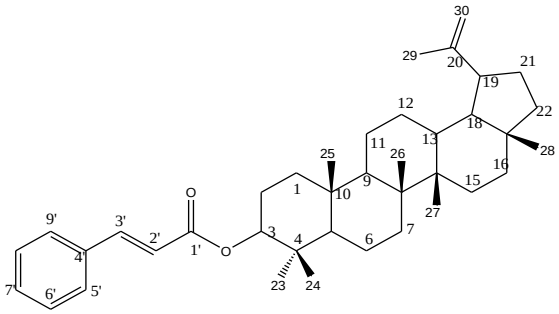
The antimicrobial activity of isolated compounds **48** and **51** against *S. typhi*, *E. coli*, *C. albicans*, and *S. aureus* was also investigated. Compound **51** gave the highest activity against

C. albicans with average diameter of zone of inhibition of 22 mm and with MIC and MBC values of 0.125 and 0.50 mg/ mL respectively. Meanwhile the least activity was noted on compound **48** against *S. aureus* with an average diameter of zone of inhibition of 17.0 mm and with similar MIC and MBC values of 0.25 and 0.50 mg/ mL respectively.

The study showed that both crude extracts and isolated pure compounds from the stem bark of *B. paradoxum* have significant antimicrobial activity and therefore may be used as alternative antimicrobial agents in place of the synthetic agents.



48



51

CHAPTER ONE: INTRODUCTION

1.1 Background of the study

1.1.1 Brief history of medicinal plants

Mankind in history all over the world has used several medicines obtained from plants. Societies world over have often resorted to nature in form of plant herbs, trees and bushes to treat an unending list of ailments (Petrovska, 2012). For centuries, even before microbes were discovered, humans used plant materials to treat diseases without knowledge of the causative agents (Rios and Recio, 2005). For example opium, one of the most ancient remedies, obtained from poppies was used as a hypnotic and also as an analgesic in several countries across Europe and Asia while the bark of the cinchona tree was used for hundreds of years to treat fever and malaria (Patrick, 2001).

1.1.2 The Global burden of microbes

One of the major public health challenges in developing countries like Uganda and all across the world is the high prevalence of infectious diseases caused by pathogens like bacteria and fungi. The common bacterial strains including *Staphylococcus aureus, Eschericia coli, Pseudomonas aeruginosa, Salmonella species, Klebsiella pneumonia, Enterococcus species, Mycobacterium tuberculosis*, and *Neisseria gonorrhea* are all known to cause a series of highly risky infections (Al-judaibi, 2014).

A growing list of these common bacteria especially those that are Gram-negative have been listed as being antibiotic resistant and hence their infections have proved to be difficult to treat. *E. coli* for example has been found resistant to most commonly used antibiotics such as ampicillin and trimethoprim (sulfamethoxazole) where a susceptibility of as low as 24% has been reported (Maina *et al*., 2016). A report by the World Health Organization shows that a number of common infections such as pneumonia, tuberculosis, blood poisoning, gonorrhea and food borne diseases are becoming hard to treat because the antibiotics have become less effective against the causing bacteria (Talebi and Abadi, 2019).

Antibiotic resistance is a very old health problem and originates way back from the beginning of the synthetic antibiotics era. For example in 1945, just a couple of years after one of the first antibiotics, penicillin was massively produced, 20% of strains of *S. aureus* that were isolated from hospitalized patients were found to be resistant against the drug. Latterly other antibiotics such as cephalothin, streptomycin, methicillin and gentamicin have followed the

same trend against strains like *Staphylococcus aureus*, *Enterococcus spp*, *Pseudomonas aeruginosa*, *Acinetobacter* and *Enterobacteriaceae* (Rossolini *et al*., 2014).

New alternatives to the synthetic antibiotics need to be looked for and extracts from medicinal plants such as *B. paradoxum* could provide a good option. In traditional medicine, decoctions from the leaves of the plant are used in treatment of malaria, oil from the seeds is applied on the skin of scabies patients while extracts from stem barks have been used as a remedy for gastric ulcers (Ogunwande *et al*., 2000).

1.2. Statement of the problem

The misuse and/or overuse of synthetic antimicrobials among other causes have led to escalation of cases of antibiotic resistance. Several of these cases have been reported throughout the world. Many infections are becoming resistant and are slowly responding to the synthetic antibiotics and at times showing no response at all, making them impossible to treat. The ability of man to treat common infections is decreasing due to emergence of resistance mechanisms like decreased uptake of drug, inactivation of the drug, alteration of drug target and drug efflux pumps activation. As a result a number of infections like pneumonia, gonorrhea and tuberculosis are becoming increasingly more difficult to treat. Therefore, there is need to explore new alternatives and extracts from plants could provide an excellent option for synthetic therapeutic agents. *Butyrospermum paradoxum* is a plant under the family *Sapotaceae* and extracts from several plants under this family are known to have good antimicrobial activity, hence in this study the stem bark of *B. paradoxum* was investigated for its antimicrobial activity.

1.3 Objectives

1.3.1 General objective

To assess the antimicrobial activity of the stem bark crude extracts and pure compounds and to characterize pure compounds from *B. paradoxum.*

1.3.2 Specific objectives

i. To determine the *in vitro* antibacterial and antifungal activity of crude extracts from stem bark of *B. paradoxum* against selected bacteria and fungi.

ii. To isolate, purify and characterize structures of the compounds isolated from the potent crude extracts of the stem bark of *B. paradoxum.*

iii. To evaluate the *in vitro* antifungal and antibacterial activities of isolated compounds from stem bark of *B. paradoxum*.

1.4 Justification of the study

There is a rapid rise in cases of bacterial and fungal infections among the population in Uganda and all over the world. Several studies have revealed that a growing number of these bacteria and fungi are becoming multi-drug resistant and hence difficult to treat. It is therefore necessary to look for new alternatives to synthetic antibiotics in order to achieve good health and wellbeing of the populace which is a key Sustainable Development Goal (SDG). Medicinal plants are well documented to be having several phytochemicals, many of which offer therapeutic importance. The purpose of the study is thus to determine the antimicrobial activity, then characterize and identify the active phytochemicals from *B. paradoxum.*

1.5 Significance of the study

The proposed study will generate information about the potential antimicrobial activity of *B. paradoxum* stem bark extract against selected bacteria and fungi. The study will generate information about the phytochemical composition of the stem bark extract of *B. paradoxum*. The active phytochemicals will be characterized and there structures can be used to develop new effective antimicrobials. The phytochemical compounds identified can be used to make antimicrobial formulations for use in areas like cosmetics, foods and detergents.

1.6 Scope of the study

i. Isolate pure phytochemical compounds from the crude extract.
ii. To characterize the active phytochemical compounds isolated.
iii. Test for the antimicrobial activity of crude extracts and pure compounds obtained from the stem bark of *B. paradoxum* against *Klebsiella pneumonia*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella typhi* using the agar well diffusion method.
iv. Determine the MIC and MBC of crude extracts and pure compounds against the test microbial organisms.

CHAPTER TWO: LITERATURE REVIEW

2.1 The extent of microbe infection globally

In the United States of America, a study was conducted on the use of 50 antibacterial drugs among adults who were treated in 130 hospitals spread across different states, for the period between 1st- Aug-2002 and 31st-July-2003. The findings showed that 1,074,174 out of 1,795,504 patients had received at least a single dose of antibacterial drugs representing 59.8% of the study population (Polk *et al*., 2007). This shows that there is a very high prevalence of bacterial infections among the adults in the said country.

In Africa, studies have shown that the extent of microbial infections is quite high especially in Sub-Saharan Africa. Bacterial infections actually compete with malaria as a common cause of illness and mortality. A study was conducted by Duke University researchers on the extent of bacterial blood stream infection among 58,296 patients spread across major regions in Africa. It was found that 13.4% of patients with a fever history and 7.4% of the patients without a fever history had a bacterial or fungal blood infection (Duke Global Health Institute, 2010).

In East Africa the burden of microbial infections is indeed high. In Kenya for instance, a study found out that approximately 7% of the country's populace suffered from a fungus related infection at any given time, with recurrent vaginitis and tinea capitis responsible for more than three quarters of the cases (Guto *et al*., 2016). In another study between 1997 and 2003, the bacterial causes of diarrhea were examined among selected cases in parts of Western Kenya. Stool specimens from a total of 1092 (32%) out of 3445 persons yielded at least one bacterial pathogen with *Shigella species* being the most commonly isolated. *Campylobacter species* and diarrheagenic *Escherichia coli* were dominant among children below 5 years and were progressively replaced by *Shigella species* with increasing age (Brooks *et al*., 2006).

In Uganda, the biggest burden on health arises from communicable diseases which are caused by microbial organisms such as bacteria, viruses, parasites and fungi. The microbial infections are responsible for over 50% of illness and death in the country (WHO, 2018b). A study was carried out in Uganda to investigate the occurrence of neonatal sepsis which is an entire blood stream bacterial infection in newly born infants who are a month old or younger. The results showed that 32.5% of infant blood samples collected was infected with

unidentified bacterial strains while 2.5% of the cerebral fluid cultures was infected with bacteria (Kiwanuka *et al*., 2013).

2.2 A selection of common antibiotic drugs on the market

World over, there are synthetic antibiotics used as remedy for common microbial infections such as acne, Sexually Transmitted Diseases (STD's), dermal infection, diarrhea, Upper Respiratory Tract Infection and infections of the urinary tract.

The common classes of antibiotic drugs in the market are discussed below.

2.2.1 Penicillins

They are also known as the beta-lactam antibiotics due to the fact that they contain beta-lactam ring in their structure. Penicillins work by preventing synthesis of bacterial cell walls thereby allowing water to enter into the cell by osmosis, the growing cell swells and this causes lysis (Prescott, 2013). Under this class are five groups of antibiotics which include aminopenicillins, antipseudomonal penicillins, beta-lactamase inhibitors, natural penicillins, and the penicillinase resistant penicillins (Ann, 2019). Examples of penicilins incude amoxicillin, ampicillin and oxacillin all of which are commonly used antibiotics. Resistance to penicillin drugs by some bacteria has been widely reported e.g. the methicillin resistant *S. aureus* causes problems world over as a cause of noscomial infections (Rivera and Boucher, 2011).

2.2.2 Tetracyclines

Tetracyclines are used to treat a number of common infections caused by either Gram-positive or Gram-negative bacteria like skin acne, STDs and infections of the eye, gum, intestinal tract and urinary tract (Ann, 2019). Examples of drugs in this class include demeclocycline, doxycycline and eravacycline. However several species of bacteria such as *Shiggela*, *Staphylococcus* and *Enterobacteriaceae* have been reported as being resistant against many forms of tetracycline drugs (Roberts, 2003).

2.2.3 Cephalosporins

The cephalosporins belong to the β-lactam subgroup of antibiotics that are often used to treat a range of simple bacterial infections like skin infections, strep throat and UTIs. They kill bacteria using a mechanism very related to that of penicillin. Cases of resistance to cephalosporins have been documented in species like *Serratia* and *Enterobacter* that are known to have inducible β-lactamase enzymes (Roberts, 2009).

2.2.4 Quinolones

The quinolones represent a class of antibiotics that share a bicyclic core structure that is strongly related to 4-quinolone. A number of reports show that fluoroquinolones, like ciprofloxacin and ofloxacin, are effective against both against both Gram-negative and Gram-positive bacteria, including *Mycobacterium tuberculosis,* the causative agent of tuberculosis (Pham *et al*., 2019). The quinolones are often used to treat resistant UTIs, hospital-acquired pneumonia and bacterial prostatis, however resistance has been reported by some microbial organisms like *Bacillus anthracis*, *Escherichia coli* and *Streptococcus pneumonia* (Hooper and Jacoby, 2015).

2.2.5 Lincomycins

The lincomycins are primarily used to combat both aerobic and anaerobic Gram-positive bacteria and in a few cases on certain strains of Gram-negative anaerobic bacteria. Drugs derived from lincomycin are occasionally used to treat serious infections like those of the lower respiratory tract while some are applied topically on the skin to treat acne (Ann, 2019). Clindamycin and lincomycin are some of the common lincomycin drugs. Resistance to this type of drugs has also been reported e.g. some coagulase-positive *Staphylococci* render both clindamycin and lincomycin inactive (Vishovan *et al*., 2020).

2.2.6 Sulfonamides

Sulfonamides are among the first antibiotic drugs discovered and act on certain Gram-positive and several Gram-negative bacteria. With their wide range of activity, sulfonamides are effective against several species of bacteria such as intestinal bacteria like *Escherichia coli*, *Klebsiella*, *Salmonella*, *Shigella* and *Enterobacter* species. However widespread resistance has been documented by species such as *Enterobacter aerogenes*, *Pseudomonas aeruginosa*, and other *Proteus species* (Connor, 1998). In addition, resistance against several sulfonamides by *Serratia* species and *Pseudomonas aeruginosa* is well documented (Tacic *et al*., 2017).

2.3 Plant under study

Butyrospermum paradoxum is a plant species under the family *Sapotaceae*. The tree originates from many countries in Western Africa and in Uganda it is found in eastern and northern parts of the country. It is a small tree with a trunk diameter of up to 2 m and an average height of 7 to 15 m at maturity. The tree typically has a rounded hemispherical crown

and is highly branched. Its bark is distinctively corky, thick, and is deeply fissured longitudinally and horizontally (**Figure 2.1**). The slash pale pink bark secrets latex just like all plants under this family. The leaves have a thick bark showing several leaf scars and are 4 - 7 cm wide, grouped in clusters and arranged spirally at the end of sturdy twigs.

Figure 2.1: A bark of a fully grown *Butyrospermum paradoxum*

2.3.1 Classification of plant under study

The scientific classification of *Butyrospermum paradoxum* is outlined below (**Table 2.1)**.

Table 2.1: Classification of *Butyrospermum paradoxum*

Kingdom	Plantae
Class	Dicotyledonae
Order	Ericales
Family	*Sapotaceae*
Subfamily	*Sapotoideae*
Genus	*Vitellaria*
Species	*Vitellaria paradoxa*

2.3.2 Traditional uses of *Butyrospermum paradoxum*

Butyrospermum paradoxum is a widely used medicinal plant across many West African countries and also in parts of East Africa due to its known medicinal properties. Aqueous extracts from *B. paradoxum* stem bark and seeds are used to stop diarrhea and hemorrhoids in Nigeria (Sharaibi and Osuntogun, 2017) while the oil extracts from seed kernels treat

tuberculosis and cough (Ogbole *et al*., 2010). Also, rash and several skin infections are treated using decoctions from aerial parts (Abubakar *et al*., 2017).

Similarly, extracts of *B. paradoxum* act as a remedy for various conditions in other parts of West Africa. In Ghana, extracts from the bark or leaves help reduce symptoms of diabetes, stroke and fractures (Imoro *et al,* 2013) while in Guinea, malaria is treated using decoctions from the bark (Traore *et al*., 2013).

In addition, the people of Burkina Faso use extracts from the leaves to lower symptoms of malaria (Nadembega *et al*., 2011) and also neuropsychiatric disorders (Kinda *et al*., 2017). The Togolese on the other hand treat high blood pressure using herbal medicines from the root bark of this plant (Karou *et al*., 2011) while several liver illnesses are also treated using extracts from the plant (Kpodar *et al*., 2016).

In parts of Eastern and Northern Uganda, an aqueous root extract of *B. paradoxum* is used to cure several ailments like stomach ache and skin infections. In addition, decoctions from the roots are also used for treating symptoms of HIV/AIDS (Anywar *et al*., 2020).

2.4 Antimicrobial properties of *Sapotaceae* family

The family *sapotaceae* comprises of over 800 species of plants distributed in more than 35 genera. Several studies have revealed presence of many bioactive compounds from plants under this family and include flavonoids, tannins, triterpenes and saponins. A number of these compounds are said to have antibacterial and antifungal activity (Fayek *et al*., 2012).

The antimicrobial activity of extracts from several plants under the indigenous *Sapotaceae* family has been investigated. For example in a study, the leaf and stem methanol extracts of the genus *Labourdonnaisia* species (*Labourdonnaisia glauca*, *Labourdonnaisia revolute*, and *Labourdonnaisia calophylloides*), *Sideroxylon* species (*Sideroxylon cinereum, Sideroxylon puberulum* and *Sideroxylon grandiflorum*) and *Mimusops* species (*Mimusops maxima, Mimusops erythroxylum* and *Mimusops petiolaris*) were all found to have inhibitory effect on all of the tested bacteria with the MIC values varying from 0.5 to 8 mg/mL (Gurib-Fakim *et al*., 2005).

Another study investigated the activity of the leaves and stem bark extracts of *Chrysophyllum boivinianum*, a popular medicinal plant in Madagascar. Both the stem and leaf extracts were found to have a high inhibitory effect against *S. typhi*, *E. coli* and *C. albicans*. For all the

three micro-organisms, the zone of inhibition diameter exceeded 7 mm with the leaf extract generally showing stronger activity than the stem bark extract (Rakotoniaina *et al*., 2020).

2.5 Plant secondary metabolites from the family *Sapotaceae*

There are several classes of compounds which have been found to be present in extracts from medicinal plants under the family *Sapotacea* and include alkaloids, terpenoids, saponins, steroids, flavonoids and phenolics. Several compounds from each class have been isolated and have proven to have *in vitro* antimicrobial activity.

2.5.1 Terpenes and Terpenoids

Terpenes form a broad class of phytochemicals generated from the five carbon units of 2-methyl-1,3-butadiene (isoprene), assembled and often transformed in several ways. They are categorized as hemiterpenes, monoterpenes, sesquiterpenes, diterpenes, sesterpenes, and triterpenes if the carbon skeleton has five, ten, fifteen, twenty, twenty five and thrity atoms respectively (Roberts *et al*., 2018). Terpenoids represent a refined category of terpenes with distinct functional groups and varying positions of an oxidized methyl group. Esessential oils for example contain monoterpenes and sesquiterpenes with monoterpenes being the most common molecules. In addition medicinal plant parts like roots, leaves, barks, stems and flowers usually contain terpenoids like linalool and citral. All these are now known to have a significant antimicrobial activity (Jain and Sharma, 2020).

Several studies have resulted in isolation of terpenoids from plants in the family *Sapotaceae.* In a study the antimicrobial activity of the DCM – methanol extract from the leaves and twigs of *Donella welwitshii*, a Cameroonian medicinal plant was investigated. It led to isolation of several triterpenoids including: diospyric acid (**1**), 28-hydroxy-β-amyrin (**2**), and oleanolic acid (**3**). Diospyric acid was found to signifcantly inhibit *E. coli*, *Enterobacter aerogenes*, *K. pneumoniae*, *Providencia stuartii* and *S. aureus* with MIC values of < 10 µg/mL in all cases. Meanwhile, 28-hydroxy-β-amyrin greatly inhibited *E. aerogenes*, *K. pneumoniae* and *S. aureus* with MIC values of 8, 4 and 8 µg/mL respectively while oleanolic acid strongly inhibited *E. coli*, *E. aerogenes* and *P. stuartii* with an MIC value of 8 µg/mL in each case (Guefack *et al*., 2022).

A study to investigate the phytochemical composition of the fruit pericarp DCM – methanol extract of *Omphalocarpum procerum* yielded a new fatty acid triterpenoid; 3β-hexadecanoyloxy-28-hydroxyolean-12-en-11-one (**4**), a white powdered solid. The isolated compound was tested for its antiprotozoal property and along with five other isolated

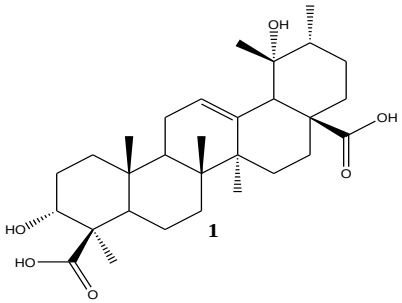
compounds gave moderate inhibition with IC_{50} values ranging from 9 to 80 µg/mL (Fotsing *et al*., 2014).

A study was carried out on the antimicrobial activity of the leaves of *Gambeya lacourtiana*, a medicinal plant widely found across several West African countries. It led to isolation of a number of pentacyclic triterpenoids including; lupenone (**5**), lupeol (**6**) and taraxerol (**7**) from the DCM – methanol extract. In addition corosolic acid (**8)** and tormentic acid (**9)** were also isolated (Mangoua *et al*., 2021).

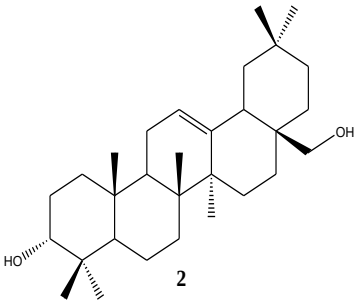
The phytochemical composition of the *Manilkara zapota*, stem bark was investigated in yet another study. It led to isolation of two previously unknown pentacyclic triterpenoids; 3-acetyltaraxer-14-en-12-one (**10**) and 3-hydroxy-7- oxolup-20(29)-en-28-oic acid (**11**), and 11 already known compounds from the methanol extract (Aristide *et al.*, 2015). Several other studies have led to isolation of many other terpenoids (**Table 2.2**) from various parts of plants under the *Sapotaceae* family.

Table 2.2: Selected triterpenoids reported from the family *Sapotaceae*

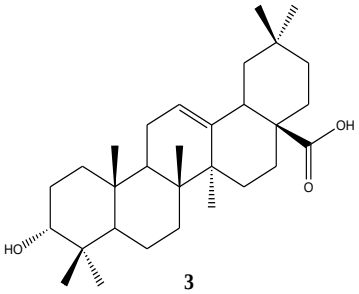
Compound	Plant	Plant part	Reference
α-amyrin acetate (**12**)	*Madhuca longifolia*	Bark	(Aanchal and Rajesh, 2023)
β-amyrin acetate (**13**)	*Madhuca longifolia*	Bark	(Aanchal and Rajesh, 2023)
α-terpeneol (**14)**	*Madhuca longifolia*	Bark	(Aanchal and Rajesh, 2023)
Lupeol acetate (**15**)	*Chrysophyllum albidum*	Bark	(Olanudun *et* al., 2018)
Betulin (**16**)	*Monotheca buxifolia*	Aerial parts	(Nightingale *et al*., 2018)
β-amyrin (**17**)	*Monotheca buxifolia*	Aerial parts	(Nightingale *et al*., 2018)
ursolic acid (**18**)	*Pouteria venosa*	Aerial parts	(Montenegro *et al*., 2006)
betulinic acid (**19**)	*Pouteria torta*	Twigs	(Che *et al*., 1980)



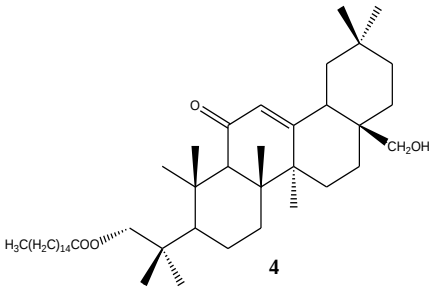
1



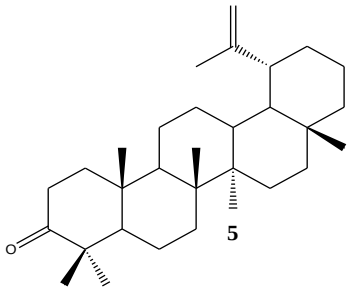
2



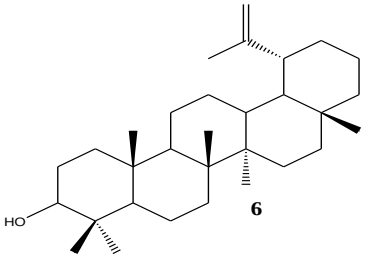
3



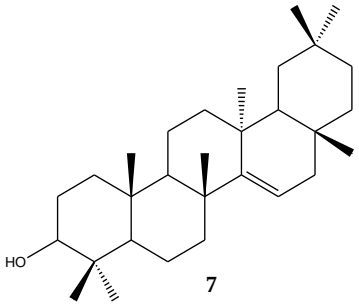
4



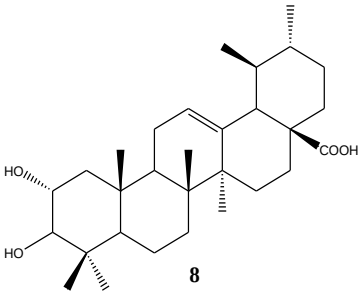
5



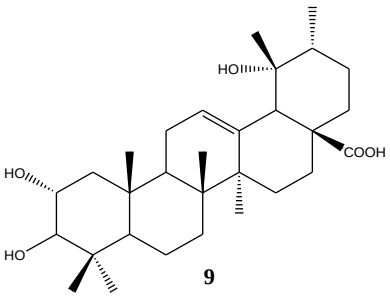
6



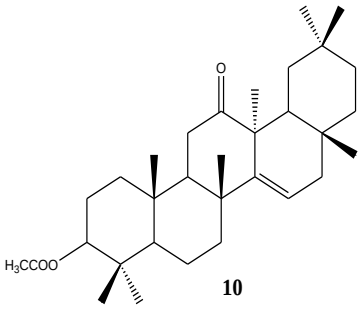
7



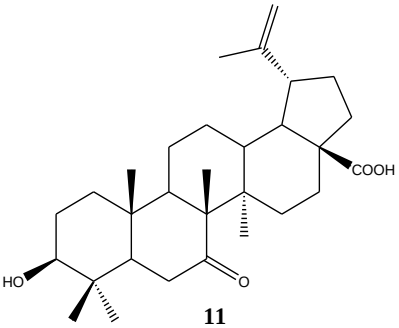
8



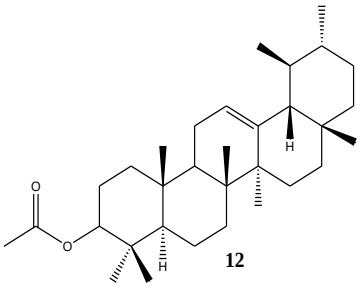
9



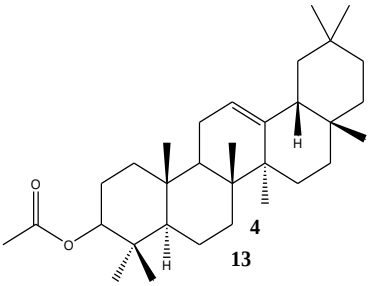
10



11

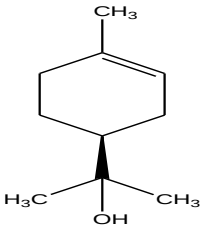


12

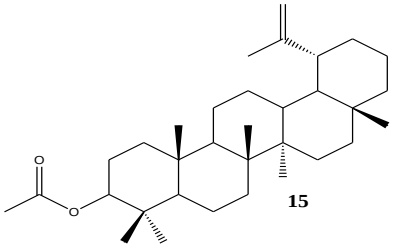


4

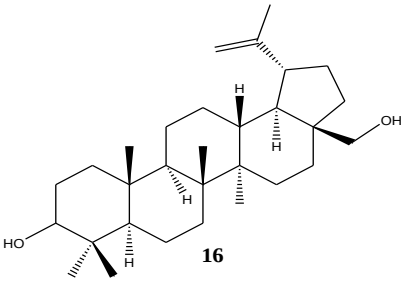
13



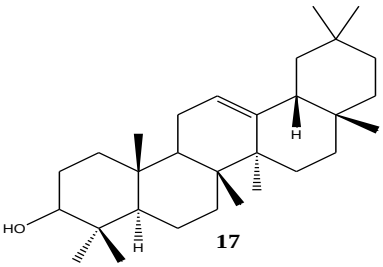
14



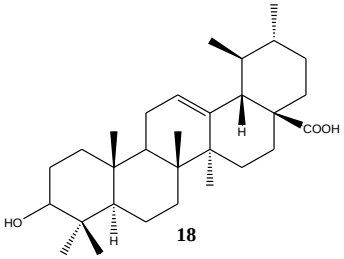
15



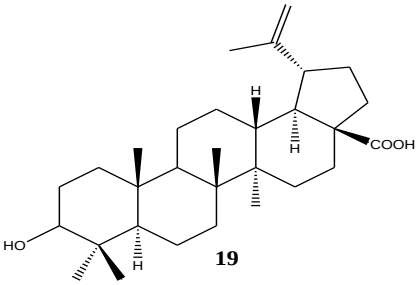
16



17



18



19

2.5.2 Flavonoids

Flavonoids form a very diverse class of plant secondary metabolites and are distributed in all plant parts (Panche *et al*., 2016). Several flavonoids have been isolated from plants under *Sapotaceae* (**Table 2.3**) as discussed by (Baky *et al*., 2016). Many of these compounds have been proved to have *in vitro* antimicrobial activity.

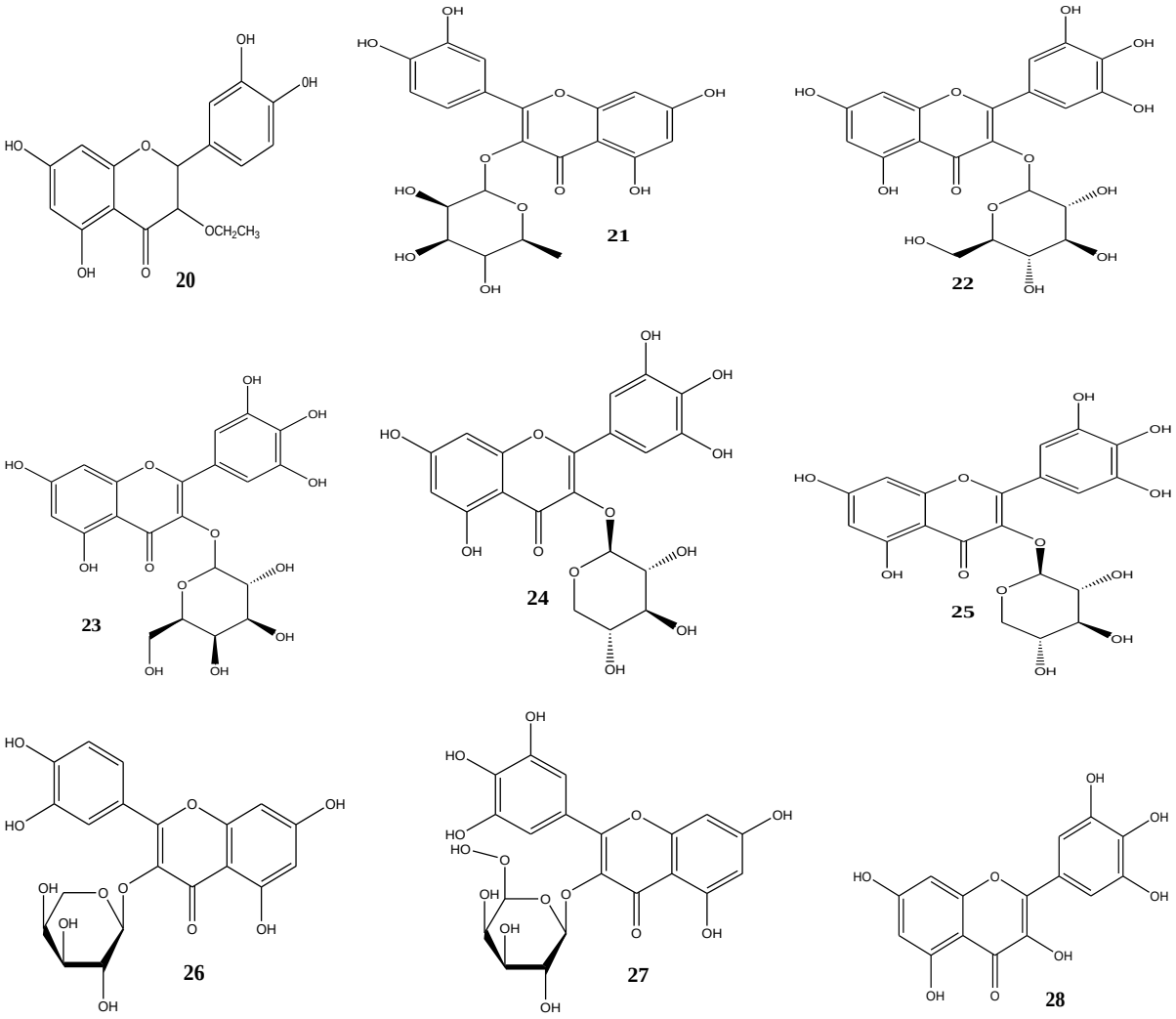
A study on the flavonoid composition of the leaves and fruit of *Manilkara hexandra* led to identification of two new flavonoid compounds; "myricetin3-*O* (3″-*O*-methyl) *α*-L-rhamnopyranoside and 3-*O*-ethyl-dihydroquercetin (3-*O*-ethyltaxifolin)" (**20**). In addition five other known flavonoid compounds i.e. " *β*-4′-methoxy-myricetin-3-*O*-*α*-L-rhamnoside, 4′-methoxy-quercetin-3-*O*-*α*-L-rhamnoside**,** quercetin-3-*O*-*α*-L-rhamnoside (**21**) and myricetin-3-*O*-*α*-L-rhamnoside" were also isolated. The two new compounds had strong activity against selected fungi and bacteria, the highest being against the Gram-negative *Pseudomonas aeruginosa* with MIC values 3.9 µg/mL in each case (Baky *et al*., 2016).

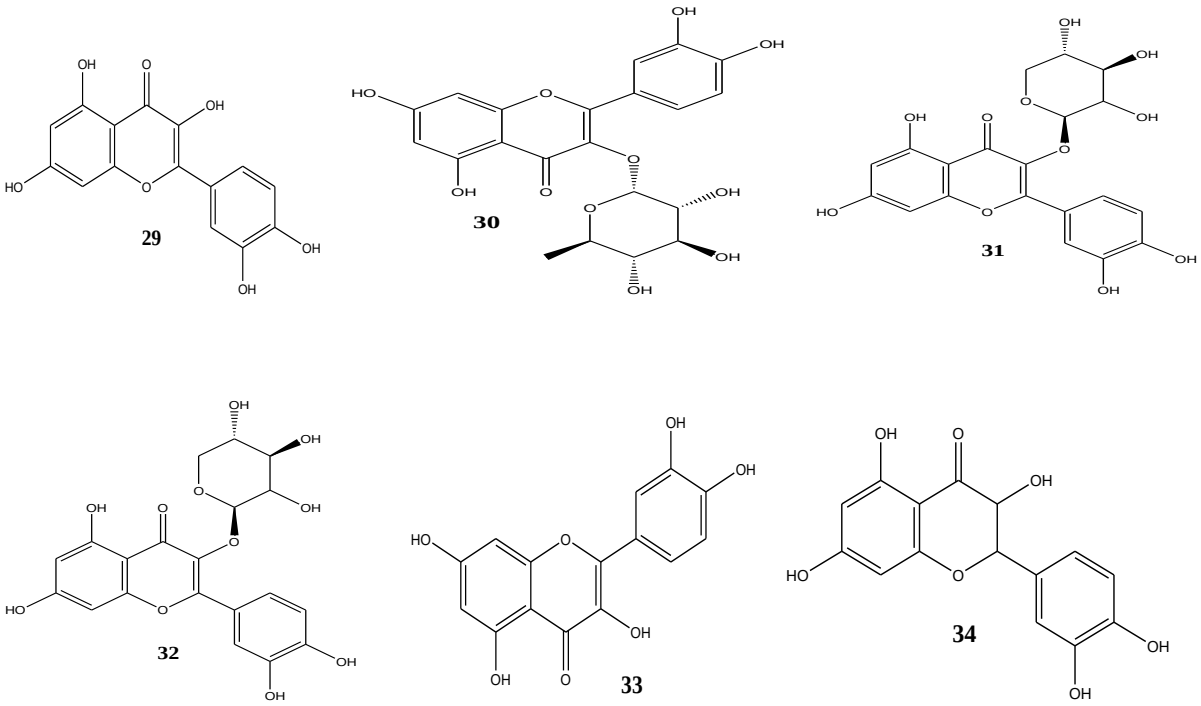
Another study investigated the antimicrobial activity of flavonoids from the leaves of *Manilkara zapota* and lead to isolation of three flavonoids (Flavon-3-ol glycosides). One of the isolated compounds, myricetin-3-glucoside (**22**) was found to be potentially active against *S. aureus* (MIC of 8 µg/mL) and also showed strong inhibition of *T. rubrum* (MIC of 16 µg/mL) (Layany *et al.*, 2015).

In yet another study, the phenolic composition and antimicrobial activity of *Argania spinosa* leaves was investigated. The results revealed abundance of flavonoids in the leaf extract (66.86 ± 3.36 µg CAEq/1 g). Profiling by "ultra-high performance liquid chromatography-electrospray ionization-quadrupole time-of-flight-mass spectrometry (UPLC-ESIQTOF-MS)" led to identification of "myrecitin-3-galactoside (**23**), myrecitin-3-xyloside (**24**), quercetin-3-galactoside (**25**), quercetin-3-glucoside, quercetin-3-arabinofuranoside, and quercetin-3-rhamnoside". The *A. spinosa* leaf extract was found to inhibit both Gram-positive and Gram-negative bacteria, with the highest activity noted against *B. subtilis* (diameter of zone of inhibition of 16 mm) (Dakiche *et al*., 2016).

Table 2.3: Selected flavonoids reported from the family *Sapotaceae*

Flavonoid	Source	Plant part	Reference
Myricetin-3-O-α-L-arabinopyranoside (**26**)	*Pouteria torta*	leaves	(Baky *et al*., 2016)
Myricetin-3-O-β-D-galactopyranoside (**27**)	*Pouteria torta*	leaves	(Baky *et al*., 2016)
Myricetin-3-O-α-L-rhaminopyranoside	*Pouteria torta*	leaves	(Baky *et al*., 2016)
Myricitin (**28**)	*Argania Spinosa*	Seed oil	(Baky *et al*., 2016)
Quercetin (**29**)	*Argania Spinosa*	Seed oil	(Baky *et al*., 2016)
Quercetin-3-O-α-L-rhamnopyranoside (**30**)	*Pouteria campechiana*	leaves	(Baky *et al*., 2016)
Quercetin-3-O-β-arabinopyranoside (**31**)	*Pouteria campechiana*	leaves	(Baky *et al*., 2016)
Rutin (**32**)	*Argania Spinosa*	Seed oil	(Baky *et al*., 2016)
Luteolin (**33**)	*Argania Spinosa*	Seed oil	(Baky *et al*., 2016)
Dihydroquercitin (**34**)	*Madhuca longifolia*	Nut shell	(Baky *et al*., 2016)





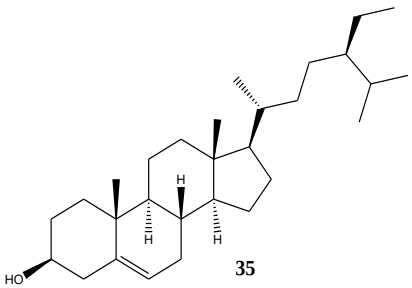
2.5.3 Steroids

Initial studies have indicated that a number of plants from the family *Sapotaceae* contain considerable amounts of steroids and cardiac glycosides. For example extracts from the seeds of *Mimusops elengi* were found to be very rich in steroids and cardiac glycosides (Gopalkrishnan and Shimpi, 2011). In addition, the results of a histochemical study showed the presence of steroids in the leaf blades of *Manilkara zapota*, a plant whose aqueous extracts are used in folk medicine to manage many ailments like inflammation, pain, fevers, coughs, dysentery, and diarrhea (Moura *et al.*, 2019).

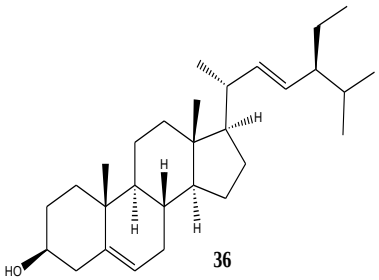
A number of steroids have been obtained from plants under the family *Sapotaceae*, many of which are said to have antimicrobial activity. For example , a number of steroids have been isolated from the leaves of *Synsepalum dulcificum* including β-sitosterol (**35**) and stigmasterol (**36**) (Chen *et al*., 2010).

In another study, the antimicrobial activity of the petroleum ether, DCM, ethyl acetate and methanol extracts of *Pachystela brevipes* was investigated against organisms including *S.* aureus, *E. coli, S. typhi, S. dysenterea, P. aeruginosa, K pneumonia, C. stellatoidea, Candida tropicalis, Candida krusei* and *Proteus mirabilis*". The most active extract was that of ethyl acetate giving diameters of zones of inhibition in the range of 20 - 27 mm in all the test organisms. The MIC value was found to be 2.5 mg/mL in all cases while the MBC/MFC

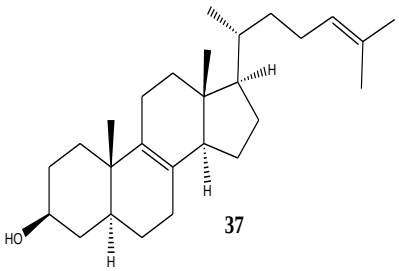
values ranged between 5 - 10 mg/mL. Using column chromatography, a compound zymosterol (**37**) was isolated and was linked to the significant antimicrobial activity of the extract (Ndukwe and Ezuruike, 2015). Other steroids have been isolated from plants under *Sapotaceae* e.g. α-Spinasterol (**38**) and α-Spinasterol fatty acid ester were isolated from *Mimusops species* (Kadam *et al*., 2012).



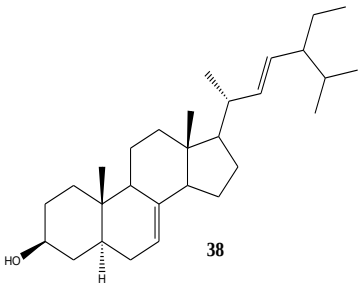
35



36



37

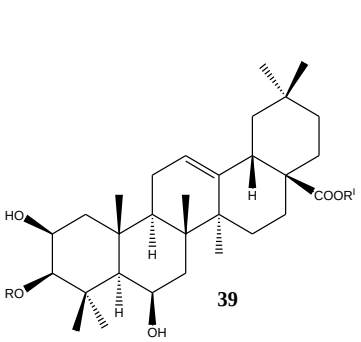


38

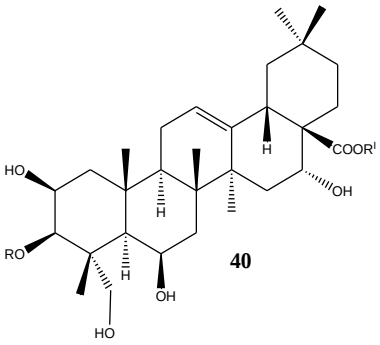
2.5.4 Saponins and Sapogenols

Saponins are a category of plant secondary metabolites containing at least one glycosidic linkage at C-3 between a sugar chain and aglycone. Saponins are often classified as monodesmosidic or bidesmosidic if they contain one or two sugar moieties respectively. The saponins are reported to have several medicinal properties like anti-inflammatory, antibacterial and antifungal (Ashour *et al*., 2019).

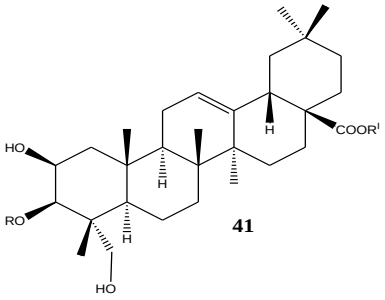
The triterpenoid saponins and sapogenols isolated from the family *Sapoaceae* contain a hydroxylated oleanane skeleton as the aglycone. Saponins isolated from this family are therefore derivatives of protobassic acid (**39**), 16α-hydroxyprotobassic acid (**40**), 6-deoxyprotobassic acid (**41**), 2-oxouncargenin (**42**), bassic acid (**43**) and oleanolic acid (**44**). In all cases, the aglycone is attached to a sugar moiety such as β-D-apiofuranosyl, α-L-arabinopyranosyl, β-D-glucopyranosyl, β-D-glucuronopyranosyl, 6-O-methyl-β-D-glucuronopyranosyl, α-L-rhamnopyranosyl and β-D-xylopyranosyl. Meanwhile two sapogenols have been isolated from the *Mimusops species* and contain a rearranged-oleanane skeleton i.e mimusopic acid (**45**) and mimusopsic acid (**46**) (Akihisa *et al*., 2018).



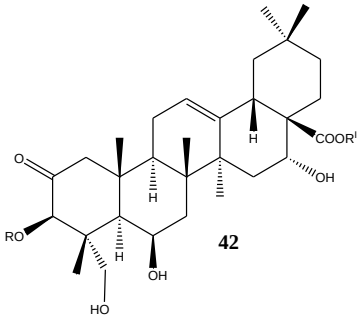
39



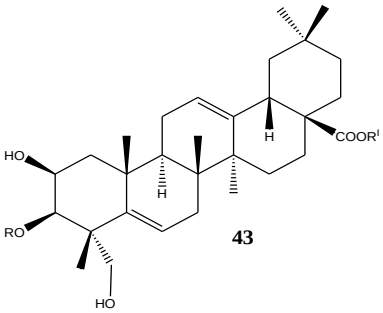
40



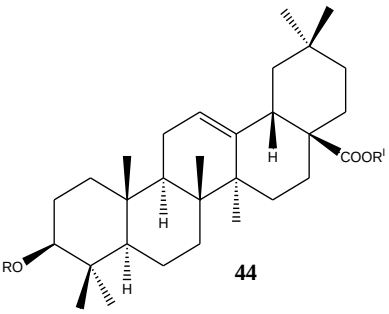
41



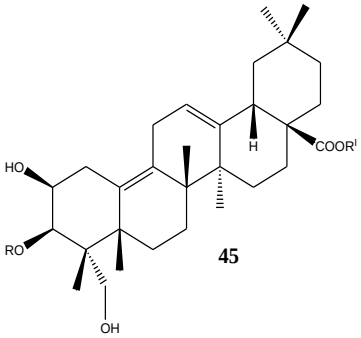
42



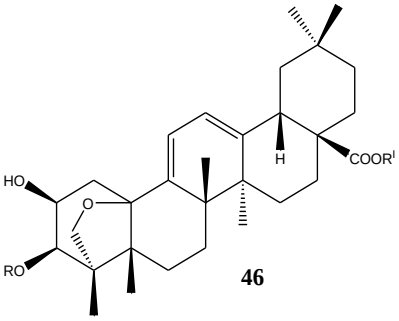
43



44



45



46

2.5.5 Alkaloids

Alkaloids form a very diverse class of plant secondary metabolites and are mainly produced by flowering plants (Wansi *et al*., 2013). They contain a nitrogen atom located in a ring or cyclic system. Alkaloids are divided into groups such as indoles, quinolones and others by basing on their structures. Indole alkaloids for example posses a nitrogen atom in the indole ring system (Aniszewski, 2007).

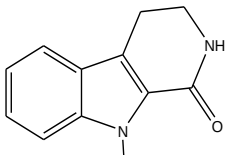
Many alkaloid monomers from classes such as indole, aaptamine, and quinolone have been found to have significant antibacterial potential with MIC values of $\leq 10x10^{-6}$ g/mL. In addition, a number of alkaloid dimers from classes such as hapalindole have been found to have comparable antibacterial activity. The antibacterial activity of alkaloids is based on efflux pump inhibition except squalamine which enhances antibacterial activity by making cells permeable (Cushnie *et al*., 2014).

In a study, fractionation of a methanol extract of *Mimusops elengi* led to isolation of an alkaloid fraction. The alkaloid fraction (50 µg/mL) displayed activity against several test fungi with a generally high percentage mycelial inhibition (**Table 2.4**) in all cases (Satish *et al*., 2008).

Table 2.4: Percentage inhibition of fungi by alkaloid fraction of *Mimusops elengi*

Fungi	Percentage mycelial inhibition
Alternaria alternata	95.48 ± 0.75
Drechslera halodes	98.45 ± 0.25
D. tetramera	96.43 ± 0.56
Fusarium equiseti	82.34 ± 0.35
F. lateritium	68.45 ± 0.67
F. oxysporum	68.18 ± 0.78
Aspergillus candidus	95.45 ± 0.56
A. ochraceus	96.85 ± 0.56
A. tamari	88.79 ± 0.56
A. versicolor	85.86 ± 0.45
Penicillium chrysogenum	95.56 ± 0.34
P. oxalicum	86.67 ± 0.67

Relatedly, a study to investigate the bioactive compounds in the stem bark of *Tridesmostemon omphalocarpoides* led to isolation of alkaloid 1,2,3,4-tetrahydronorharman-1- one (**47**) from the DCM – methanol extract. The isolated compound was active against several of the tested bacteria including the methicillin resistant *E. coli* where an MIC value of 16 µg/mL was recorded (Fru *et al.*, 2013).



47

2.6 A review of analytical techniques commonly used in natural product research

In natural products research, the reliability of findings from both qualitative and quantitative studies depends on selection of proper methods. The common methods employed in natural products research are briefly discussed below.

2.6.1 Extraction

Extraction is a process of separating compounds that have medicinal properties from plant samples through a chosen procedure and by using solvents that are selective in order to leave out the insoluble compounds. After extraction, the solvent is removed and the extract remains either as a liquid or a dry solid containing a number of phytochemicals. Generally, extraction is achieved by washing out the phytochemicals from the plant sample into the extraction solvent (Odii *et al*., 2022). Three conventional methods are used in extraction of medicinally active compounds from natural products: soxhlet extraction, maceration and hydro distillation (Azmir *et al*, 2013). Other methods of extraction used include: percolation, digestion and decoction.

Soxhlet extraction involves placing the plant material into a thimble holder of soxhlet which is gradually filled with a condensed fresh solvent from a distillation flask during its operation. A siphon removes all the liquid from the thimble holder and returns it to the distillation flask that has the extracted analyte when the liquid reaches the overflow level. The process is repeated several times to ensure that the target phytochemicals are fully extracted (Luque and Garcia, 2000).

Maceration is an extraction procedure where the plant material is ground into powder which is then carefully transferred into a container, usually a bottle; followed by addition of a suitable extraction solvent to entirely submerge the powdered plant sample. The container is then tightly closed and kept for at least three days with occasional shaking to ensure complete extraction. After the extraction, the crude extract is obtained by either decantation or filtration (Mainul and Haque, 2020).

Hydro distillation is a traditional method used for extracting compounds from plant materials without using any organic solvents. During hydro distillation, the plant sample is packed in a stationary compartment like a flask and an adequate amount of water is added which is then heated for it to boil. The other option is to inject steam directly into the plant material in a flask to free up bioactive compounds. The vapour obtained from hydro distillation is condensed to obtain a mixture of water and oil (Rasul, 2018a).

Decoction is a method that uses water to extract bioactive phytochemicals from different parts of medicinal plants. Compounds extracted by this method are usually those that are water soluble and at the same time heat resistant. The method involves cutting plant parts into small pieces which are then added into a suitable apparatus containing water from where the mixture will be heated at a relatively low heat until a concentrated extract is formed. The concentrated extract is then cooled then filtered or decanted into another clean apparatus (Rasul, 2018a).

Percolation is procedure that is commonly used to extract active compounds which are thermolabile. This method is based on a solvent continuously flowing through a stationary bed containing the plant material, so as to wash out the soluble phytochemicals. The procedure involves wetting the plant material with a suitable quantity of solvent then allowing it stand for about 4 hours in a tightly closed container. The mixture is then left to macerate in a percolator for up to 24 hours after which the apparatus is opened from the bottom to allow the extract to drip slowly (Mihai G. and Maria H., 2019). More solvent is periodically added from the top until the extraction process is complete, observed when no residue remains after evaporation upon dropping a solvent from the percolator on a white piece of paper (Rasul, 2018b)**.**

Digestion is a form of a maceration procedure that involves use of gentle heat during the extraction stage. By warming gently, the extraction efficiency of the solvent increases and this leads to increase in the extract quality and yield (Mihai and Maria, 2019).

2.6.2 Column chromatography

It is a technique that is often used to isolate pure compounds from crude extracts of different plant parts. In column chromatography, a solid adsorbent to act as the stationary phase, usually silica gel, is parked in a standing glass column. An organic solvent which acts as the mobile phase is added into the column from the top. The crude extract is carefully placed on top of the silica gel in the column and the solvent is continuously added to elute different fractions. The different compounds in the extract interact differently with the eluting solvent and this effects separation. Compounds that weakly interact with the stationary phase are eluted first while those with a strong interaction are eluted last (Dongare *et al*., 2023).

2.6.3 Thin Layer Chromatography (TLC)

This technique is often used to compare different fractions obtained from column chromatography in order to ascertain which fractions can be pulled together without affecting purity. It also helps confirm the purity of a given isolated compound. A typical TLC plate is made up of a sheet coated with a uniform layer of silica or alumina to act as the stationary phase through which the mobile phase flows by capillarity. The separation of compounds by TLC is based on the difference in the extent of absorption of compounds on the mobile and stationary phase. Compounds which are highly absorbed travel a shorter distance on the TLC plate than those which weakly interact with the stationary phase (Tiwari and Talreja, 2022). After separation, the TLC plate is first stained using agents like iodine, bromocresol green and anisaldehyde before the compounds can be visualized as spots using a U.V lamp at both short and long wavelength (Cai, 2014).

2.6.4 Nuclear magnetic resonance (NMR)

It is a popular method employed for structure elucidation in studies involving natural products. It is an analytical technique that depends on the absorption of energy by the nucleus of some atoms that get excited and transition from the lowest spin state to the next state of higher energy. Atoms of elements with a non zero magnetic moment like C-13 and H-1 are known to be NMR active (Gerothanassis *et al*., 2002). Organic compounds primarily consist of carbons and hydrogens whose interactions give spectra that can be used effectively in structure determination. Different NMR techniques have been employed, some of which are 1D and others are 2D. The 1D methods used are proton (^{1}H) NMR and carbon-13 (^{13}C) NMR while the 2D experiments include COSY, HMBC and HSQC. The COSY spectrum is used to detect correlations between protons while HSQC is used to detect carbons and protons that are directly bonded (^{1}J). Meanwhile HMBC is used to detect hydrogens and carbons which are two or three bonds apart (^{2}J or ^{3}J) (Keeler, 2002).

2.7 A review of methods used to assess the antimicrobial activity of medicinal plants

2.7.1 Agar well diffusion method

It is a commonly used technique when assessing the antimicrobial activity of extracts of medicinal plants. In this procedure, inoculation of the agar surface is achieved by spreading a suitable amount of the microbial inoculums over the whole surface. A hole of diameter 6 to 8 mm is made by punching aseptically using a sterilized cork borer or a tip, and a volume of 20 to 100 mL of the plant extract solution at a suitable concentration is added into the well. The

agar plates are then incubated under suitable conditions depending on the test microorganism. The bioactive plant extract diffuses in the agar medium and inhibits the growth of bacteria under study. The diameter of zone of inhibition is then measured in millimeters (Balouiri *et al*., 2016).

A conclusion on the level of activity of both the crude extracts and isolated compounds against the selected bacteria and fungi can be made basing on the summary (Table 3.1) given below (Ekhuemelo *et al.*, 2021).

Table 2.5: Summary of diameter of zone of inhibition range and a conclusion

Diameter of inhibition	Conclusion
< 10.0 mm	Not active
10 -13 mm	Partly active
14 -19 mm	Active
> 19 mm	Very active

2.7.2 Broth dilution method

It is among the primary methods often used to gauge the susceptibility of microbial organisms to medicinal plant extracts. The procedure involves preparing several solutions of plant extracts of dilution factor 2 in tubes containing at least 2 mL of the liquid growth medium. Every tube is then inoculated with microbial inoculums which have been prepared in the same medium after dilution of the standardized microbial suspension adjusted to 0.5 McFarland scale. This is followed by incubation of the inoculated tubes at appropriate conditions that depend on the bacteria or fungi being tested. The MIC is the least concentration of the extract that visibly inhibits the growth of microbial organisms. Meanwhile the MBC or MFC is the least concentration which inhibits 99.9% of the activity of the tested bacteria and fungi respectively (Balouiri *et al*., 2016).

CHAPTER THREE: MATERIALS AND METHODS

3.1 Sample collection

Mature fresh stem bark from a healthy plant of *B. paradoxum* was harvested from Kokorio parish, Kapujan sub-county in Katakwi District, Teso sub-region, North Eastern Uganda on 3rd January 2022. The plant material was authenticated by a plant taxonomist at Makerere University Herbarium and a voucher specimen No. 1 with plant Accession No. 51246 was generated.

The stem bark was then thoroughly washed using borehole water to remove all the dirt particles and latex attached, and then allowed to drain in order to remove excess water. The stem bark was then carefully packed in a clean polythene bag from where it was transported to the Chemistry laboratory at Kyambogo University. It was then dried under a shade to prevent decomposition of any phytochemicals as a result of sunlight. The drying was intended to help remove water from the plant material so as to increase its duration of storage without getting spoilt.

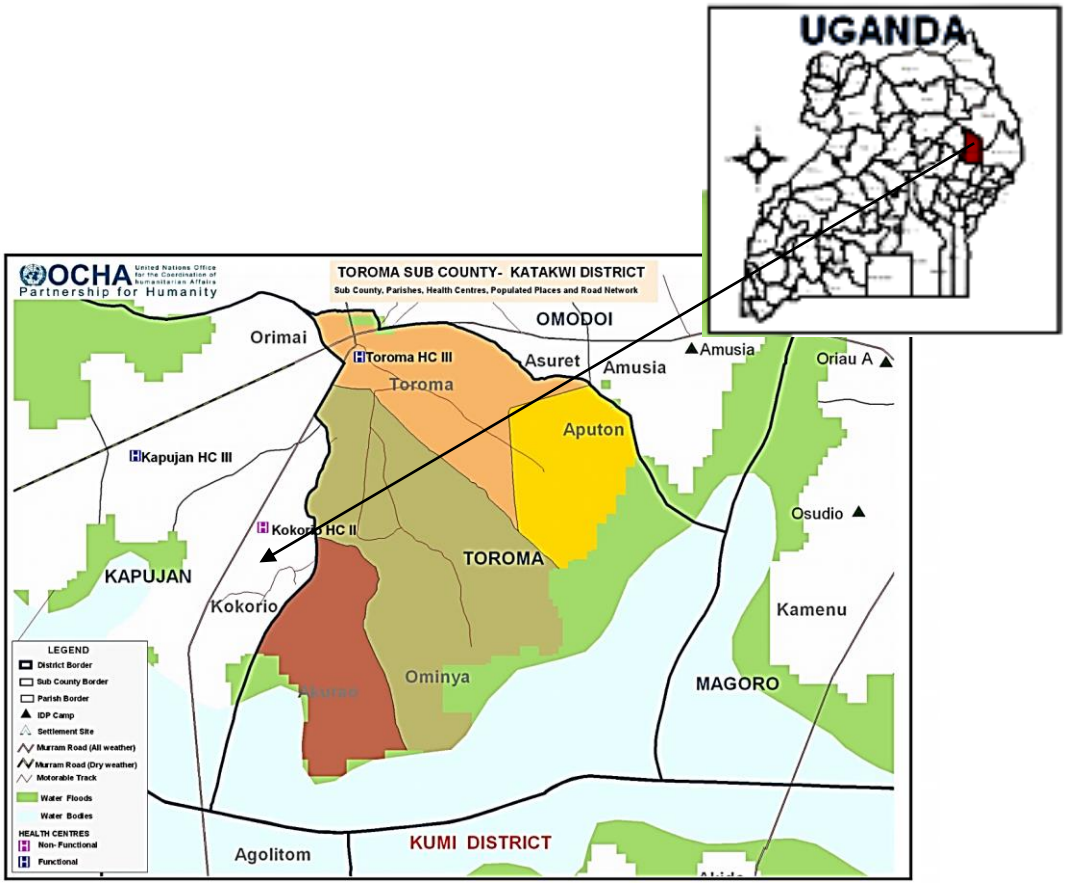


Figure 3.1: Location of Kokorio, Kapujan Subcounty in Katakwi District

3.2 Preparation of the collected sample

The stem bark which had been shade dried was then pounded using a clean wooden mortar and pestle and then ground into a fine powder using a clean water free electric grinder. Grinding helped reduce the particle size of the plant material, thereby creating a homogenous substance which provided a large surface area of contact between itself and the extraction solvents. The clean dry powder obtained was then kept carefully tied in a clean dry polythene bag ready for the extraction process.

3.3 Extraction and isolation

The concentrated plant extracts were prepared by maceration using four solvents: hexane, dichloromethane, ethyl acetate and methanol.

3.3.1 Summary of Experimental design of the extraction process

The schematic flow diagram (**Figure 3.2**) below shows a summary of the extractions that were carried out on the powdered plant material. The extractions were done sequentially with solvents arranged in order of increasing polarity. This helped to minimize the amount of analogous compounds in the different extract.

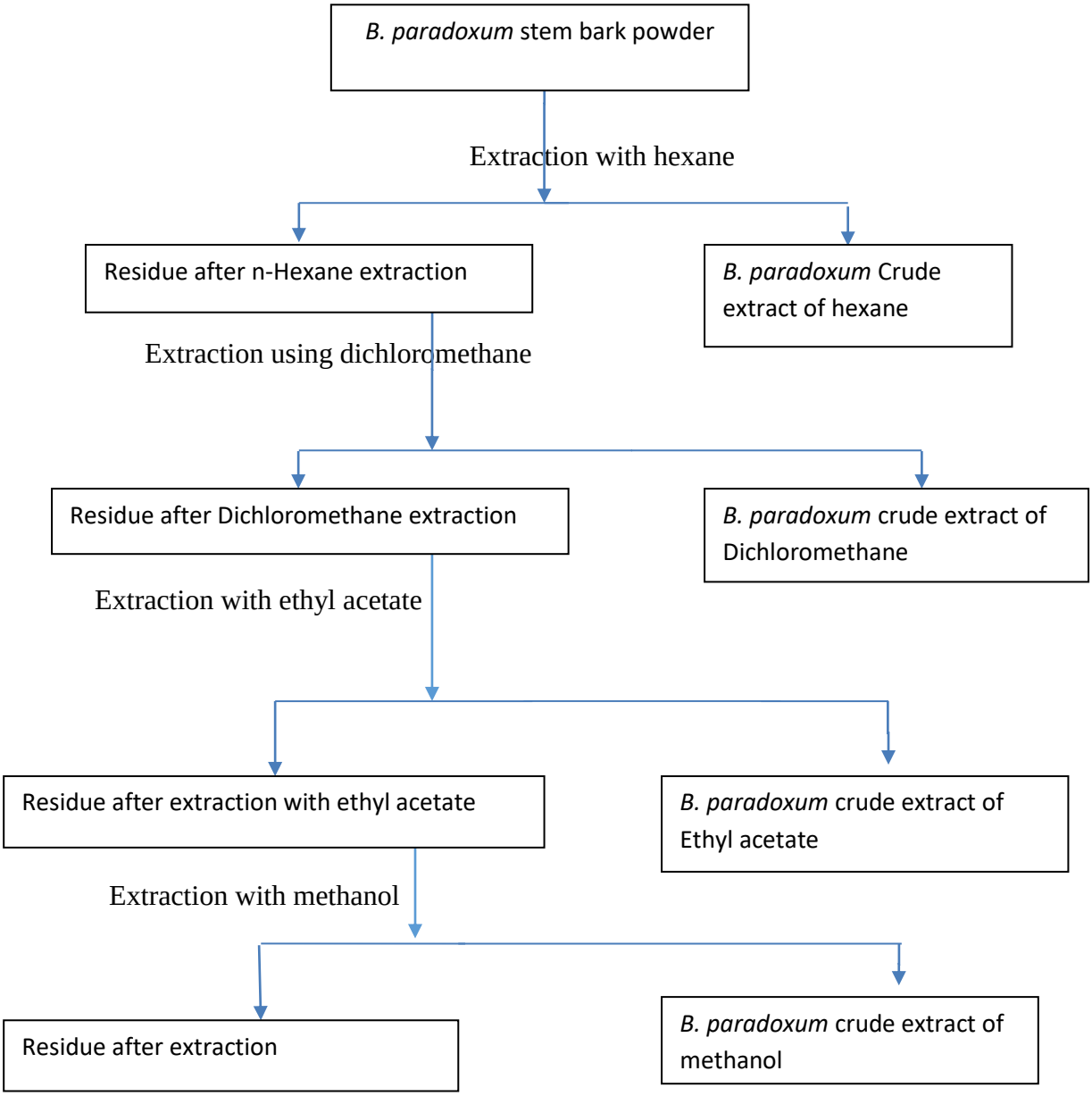


Figure 2.2: Flow chart showing the summary of the extraction procedure

3.3.2 General preparation of the crude extracts

The powdered plant material (1.8 kg) was added to Winchester glass bottles followed by enough n-hexane with the aid of a funnel. The bottles were tightly sealed and then shaken thoroughly to ensure uniform mixing. The mixture was left to stand for 72 hours at room temperature in a cupboard in the laboratory accompanied with occasional shaking.

After 72 hours, the mixture was filtered; first using cotton wool plunged in a filter funnel, then using a Whatmann filter paper. The crude n-hexane extract was kept in a clean glass

bottle at room temperature. The residue was then put back into the Winchester bottles and again soaked in a sufficient quantity of n-hexane in each bottle for 72 hours, filtered and the filtrate (extract) obtained was added to the one in the first extraction. The residue obtained from the second extraction with n-hexane was once again put back in the Winchester bottles and soaked in more n-hexane in each case for third extraction. The mixture was again filtered and the solution was added to that obtained in the first two extractions with n-hexane.

The stem bark residue after extraction with n-Hexane was once again dried in a shade before it was extracted three times with dichloromethane as was the case with n-hexane to obtain dichloromethane crude extract. The procedure was repeated using ethyl acetate and methanol to yield crude ethyl acetate and methanol extracts respectively. All the four crude extracts were then concentrated using a water bath operating at about 40^{o}C under a reduced pressure on a rotary evaporator machine (Neo-Tech SA). The concentrated crude extracts were dried in a fume cupboard to produce solid crude extracts which were placed in sterile glass bottles and kept in a refrigerator at 4^{o}C. The extraction yield was calculated by expressing the masses of the extracts as a percentage of the mass of original plant material using the equation below.

$$\text{Extract yield (\%)} = \frac{M1}{M2} \times 100$$

M_1= Net weight of dry crude extract measured in grams

M_2 = Total weight of the medicinal plant powder

3.3.3 Isolation of pure compounds from the hexane crude extracts of *B. paradoxum*

The following steps were followed to isolate pure compounds **48** and **49** from the crude hexane extract of the stem bark of *B. paradoxum* (**Figure 3.3**).

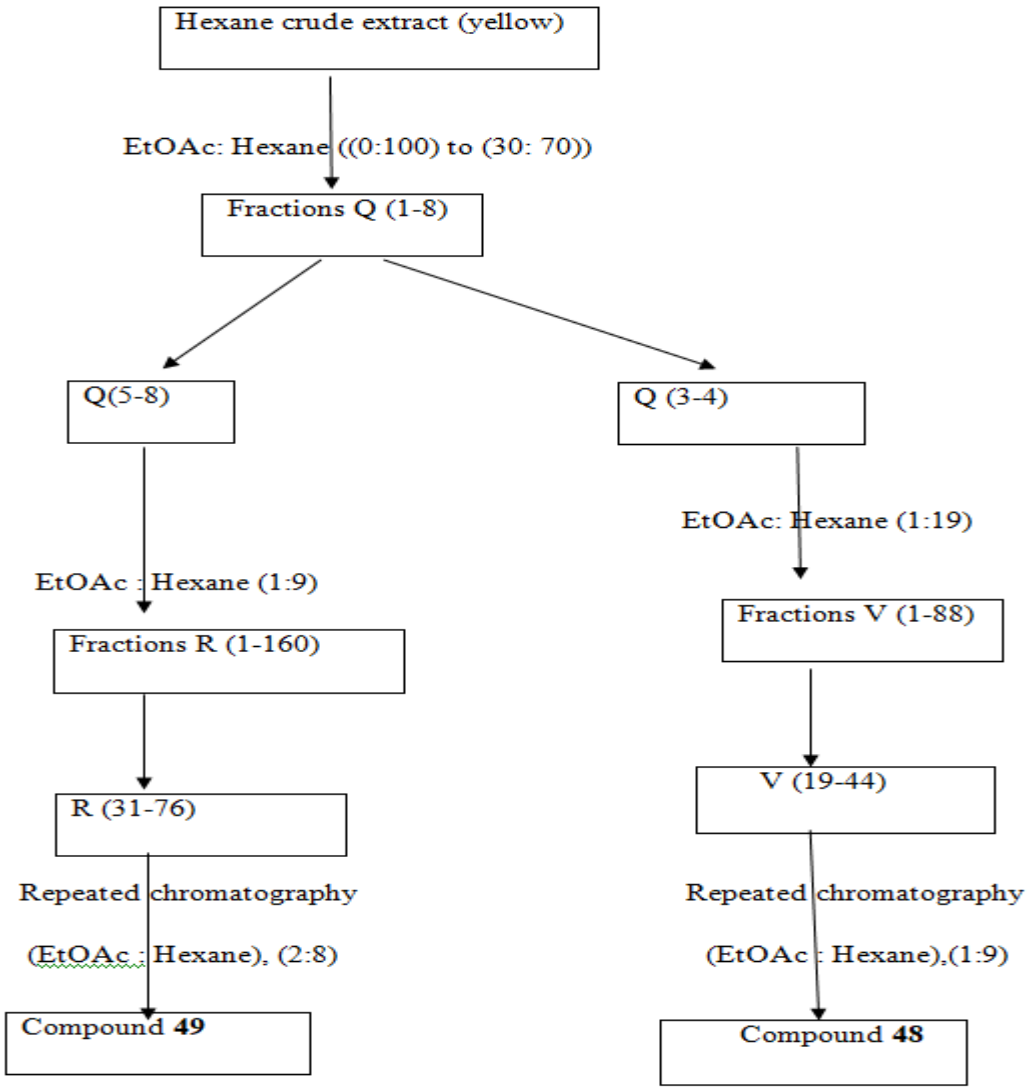


Figure 3.3: Flowchart showing procedure of isolation of compounds from the hexane extract

The yellow hexane crude extract (40.0 g) was eluted with increasing polarity of ethyl acetate: hexane i.e. (0:100), (2:98), (5:95), (10:90), (15:85), (20:80), (25:75), (30:70) to obtain eight broad fractions Q (1-8) respectively. Fractions Q (3-4) were pulled together as guided by thin layer chromatography, TLC and was then eluted with ethyl acetate: hexane system (1:19) to obtain fractions V (1-88). The fractions V (19-44) were pulled together and then subjected to repeated column chromatography on ethyl acetate: hexane system (1:9) to obtain compound

(**48**) which was U.V active on both short and long wavelength. The isolated compound (**48**) was further purified by a sephadex column using a DCM: methanol system (1:1) to obtain 150 mg of the white needle like pure compound.

Meanwhile fractions Q (5-8) were pulled together as guided by TLC and the pulled fractions were eluted with ethyl acetate: hexane system (1:9) to obtain 160 fractions R (1-160) of which fractions, R(31-76) were further pulled together. The combined fractions were further subjected to repeated column chromatography using ethyl acetate: hexane system (1:9) to obtain compound (**49**) which was also U.V active on both short and long wavelength. The isolated compound (**49**) was further purified with the help of a sephadex column using DCM: methanol system (1:1) which gave 40 mg of the white amorphous solid.

3.3.4. Isolation of pure compounds from the DCM crude extract of *B. paradoxum*

The DCM crude extract was subjected to a series of steps (**Figure 3.4**) leading to the isolation of pure compounds **50** and **51**.

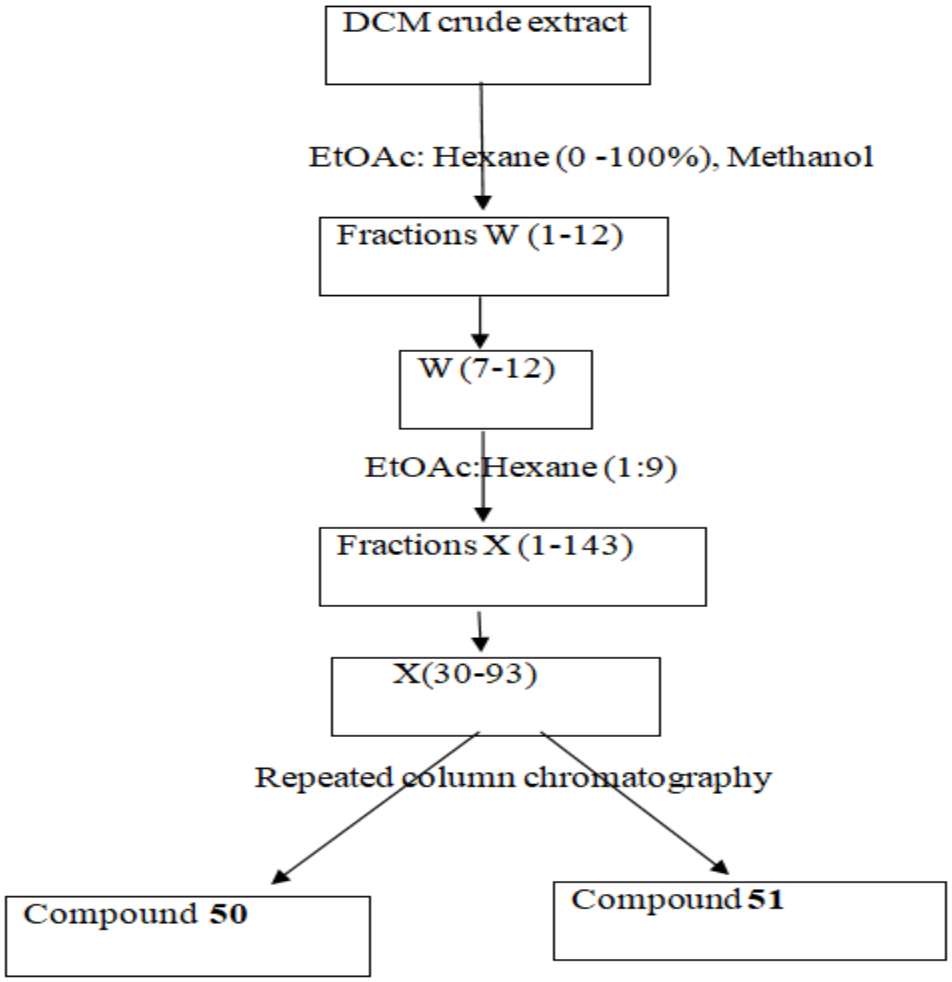


Figure 3.4: Flowchart showing procedure of isolation of compounds from DCM extract

The light-green DCM crude extract (15.0 g) was eluted stepwise with increasing polarity of ethyl acetate: hexane i.e. (0:100), (1:9), (2:8), (3:7), (4:6), (5:5), (6:4), (7:3), (8:2), (9:1), (0:100) followed by 100% methanol to obtain 12 broad fractions W (1-12) respectively. Fractions W (7-11) were pulled together as guided by TLC and was then eluted with ethyl acetate: hexane system (1:9) to obtain fractions X (1-143). The fractions X (30-93) were further pulled together and then subjected to repeated column chromatography on ethyl acetate: hexane system (1:9) then (2:8) to obtain compound (**50**) and compound (**51**) which were also U.V active on both short and long wavelength. The isolated compounds were also

further purified by a sephadex column using a DCM: methanol system (1:1) to obtain 28 mg and 125 mg of the white amorphous solids of pure compound **50** and **51** respectively.

3.3.5 Isolation of compounds from ethyl acetate and methanol extracts

The ethyl acetate crude extract was also fractionated by silica gel column chromatography assisted by TLC and it led to isolation of 60 mg of a white amorphous compound which on NMR analysis, formed spectra very similar to those of compound **51** isolated from the DCM extract. Analysis of the spectra led to a conclusion that the isolated compound was indeed lupeol cinnamate (**51**). Meanwhile the crude methanol extract was not fractionated due to its highly polar nature.

3.3.6 Structure determination of bioactive compounds

The bioactive compounds were characterized using data obtained from NMR spectroscopy carried out in Germany. In all cases, the samples of isolated compounds were dissolved in deuterated trichloromethane ($CDCl_3$) and then further prepared for NMR analysis. Both 1D and 2D experiments were done in order to characterize the isolated compounds. The 1D methods used were proton (^1H) NMR and carbon-13 (^{13}C) NMR while the 2D experiments included COSY, HMBC and HSQC. The raw data (FID format) obtained in different experiments was processed with the help of MestreNova version 8.1.1. The chemical shifts of peaks were recorded in (ppm), measured relative to the TMS signal. Meanwhile the multiplicities and coupling constants (*J*) of selected peaks were also recorded. The results obtained were compared with the information available from the already published literature in order to deduce the exact structure of the compound.

3.4 Determination of antimicrobial activity of plant extracts

The antibacterial activity of the four crude extracts from the stem bark of *B. paradoxum* was determined by the agar well diffusion assay and was presented as average diameter of the zone of growth inhibition measured in millimeters. The bacteria used in the study include: *Klebsiella pneumoniae*, *Salmonella typhi*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*, the first four of which are Gram-negative and the last being Gram-positive. *Candida albicans* was the only fungal strain used in addition to the said bacteria.

3.4:1 Collection of microbial strains and preparation of microbial cultures

The stock cultures of the different bacterial strains and fungi were obtained from the Biology laboratory, Faculty of Science Kyambogo University. Inoculums of all the test micro-organisms were prepared in a way that their turbidity matches the 0.5 McFarland standards.

3.4.2 Determination of zones of inhibition

The antimicrobial activity of the four crude extracts of the stem bark of *B. paradoxum* was determined by the Agar well diffusion assay described in (Ramalivhana *et al*., 2014).

About 100 µL of microbial organisms was spread over the surface of an agar plate and the same procedure was repeated for all the test microbial organisms. Five holes were punched in each of the culture petri dishes with the help of a glass pipette sterilized using ethanol. One of the holes was punched in the center of the plate and three drops of 12 mg/mL solution of tetracycline was added as a positive control. Three drops of dimethylsulfoxide (DMSO) was then added to the second hole which acted as a negative control. The remaining three holes were each added an equal volume of the crude plant extract solutions obtained by dissolving the dry extract in DMSO. Thereafter, the culture petri dishes were incubated for 24 hours at 37°C. The diameter of the clear zone of inhibition around the crude plant extract solution was then measured in millimeters.

3.4.3 Determination of Minimum Inhibitory Concentration (MIC) by Broth dilution method

The antimicrobial activity of the four crude extracts of stem bark of *B. paradoxum* was further investigated to determine the least concentration of the extracts that could inhibit bacterial growth. This was done by employing a micro dilution technique. The dry extract (1.0g) for hexane, DCM and methanol extracts and 0.8g for ethyl acetate extract was added to 10 mL of dimethylsulfoxide to dissolve in a sterilized test tube. The solution formed was then subsequently diluted in 6 dilution blanks, each containing 1 mL of solvent to obtain solutions with dilution factor 2, 4, 8, 16 and 32. The solutions obtained by serial dilution were tested separately with each of the six test microbial organisms. In each case, bacterial inoculums was pipetted into the respective test tubes and then gently shaken to ensure uniform mixing. The test tubes were then carefully sealed using aluminium foil and then incubated in an oven operating at 37 ^{o}C for 24 hours. Growth of bacteria in a given test tube was implied by presence of turbidity or by formation of froth as compared with the un-inoculated control. The MIC value was obtained from the least concentration test tube where growth of bacteria was not physically visible and was recorded in milligrams per milliliter.

3.4.4 Determination of Minimum Bactericidal concentration (MBC)

The MBC was determined using a solution at a concentration representing the MIC and at least two more solutions of the crude extract or isolated compounds at lower dilution factors. Subculture was then carried out on Mueller Hinton agar medium and incubated for 48 hours

at 37 ^{0} C. The lowest media concentration that contained no bacterial colonies was taken as the MBC (Hendiani *et al*., 2020).

3.4.5 Antifungal activity of the crude extracts and pure compounds 48 and 51

The fungal stock culture was obtained from Biology laboratory, Faculty of Science at Kyambogo University where they were stored at about 4^{o}C. A loop full of cultures was transferred into a sabouraud dextrose broth, and then incubated for 72 hours at 25^{o}C to generate fresh fungal cultures. Using the prepared sabouraud dextrose agar, the diameter of zone of inhibition was determined by the well diffusion method in a procedure similar to that of bacteria.

The Minimum inhibitory concentration and Minimum fungicidal concentration of the crude extracts and isolated compounds against *Candida albicans* were also determined using the two fold dilution method. The stock solutions of both crude extracts and pure compounds in DMSO were subjected to a serial dilution to obtain solutions with dilution factor 2, 4, 8, 16 and 32 in different tubes. About 100 µL of the suspended *C. albicans* was transferred to each tube which were then incubated at 28^{o}C for 72 hours. The least concentration that showed no growth of fungi after incubation was taken as the MIC. To determine the MFC, all the tubes used in MIC that showed no fungal growth were first diluted in a sabouraud dextrose broth and then re-incubated at 28^{o}C for 72 hours. The MFC was recorded as the least concentration of extract or compound that didn't have any fungal colony after re-incubation (Chandrasekaran and Venkatesalu, 2004).

CHAPTER FOUR: RESULTS AND DISCUSSION

4.1 Extraction yield

The extraction yield for different solvents on the same powdered plant material is shown below (**Table 4.1**). It can be seen that the *B. paradoxum* stem bark is rich in fats and oils as evidenced by the high extract yield of hexane solvent, which extracts mostly the less polar compounds. In addition, the extract is also rich in sugars and this is shown by the high extract yield of the methanol solvent which mostly extracts highly polar compounds including sugars (Xu *et al*., 2018). Ethyl acetate had the lowest yield of all the four solvents.

Table 4.1: Extract yields from different solvents

Extraction solvent	Appearance	Mass of dry extract (g)	Percentage yield
Hexane	Yellow sticky	45.37	2.52
Dichloromethane	Light green	28.08	1.56
Ethyl acetate	Dark brown	11.80	0.66
Methanol	Black	53.65	2.98

4.2 Characterization of isolated bioactive compounds

4.2.1 Introduction

Different chromatographic techniques were used to isolate bioactive compounds from crude extracts, including TLC analysis which showed various spots indicating presence of compounds. These spots were identified using U.V (254 and 366 nm) and iodine tank. The isolated compounds were characterized and their structures elucidated as discussed below.

4.2.2 Identification of compound 48

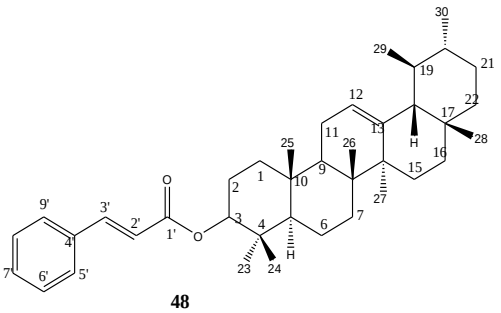
Compound **48** was isolated from the hexane crude extract as a white needle like solid. The ^{1}HNMR spectrum of the compound showed eight methyl signals at δ_H 0.91, 0.92, 0.99, 0.85, 1.12, 0.83, 0.85 and 0.96 and the compound was identified as a triterpene cinnamate. The cinnamyl group was identified due to the presence of signals at δ_H 7.40, 7.41, 7.53, and 7.43 which confirm presence of an aromatic ring while the signal δ_H 7.69 and 6.43 confirm the presence of a carbon to carbon double bond. The presence of the methyne signal (δ_H 5.16, on H-12) represents α-amyrin skeleton.

The ^{13}CNMR spectrum showed peaks similar to that of a cinnamate derivative of a triterpene alcohol. Two signals at δ_C 124.2 and 145.2 represent carbon atoms characterized by double

bonds. The signal at δ_C 80.5 was for carbon 3 which was attached to an oxygen atom of the ester group. In addition, the signals at δ_C 134.6, 128.1, 128.9, and 130.1 represent carbon atoms of the benzene ring while the signals at δ_C 144.3 and 118.9 represent carbon 2^1 and 3^1 of the cinnamyl group. The signal at δ_C 166.3 represents the carbon of the ester group in the cinnamyl. Using the NMR data given and in comparison with the available literature (**Table 4.2)**, the compound was identified as α-amyrin cinnamate **(48).**

In the HMBC spectrum protons resonating at δ_H 4.66 (H-3) showed correlations with carbons at δ_C 38.0 (C-1), 23.5 (C-2), 28.1 (C-23) and 16.3 (C-24) while the protons resonating at δ_H 0.90 (H-5) showed correlation with carbons at δ_C 37.1 (C-4), 47.9 (C-9) and 28.1 (C-23). More cross peaks correlations were observed and include; protons resonating at δ_H 5.16 (H-12) with carbons at δ_C 23.2 (C-11), 42.0 (C-14) and 59.0 (C-18), protons resonating at δ_H 0.95 (H-24) with carbons at δ_C 80.5 (C-3), 37.1 (C-4), 53.8 (C-5) and 28.1 (C-23), protons resonating at δ_H 1.03 (H-26) with carbons at δ_C 39.5 (C-8) and 42.0 (C-14), protons resonating at δ_H 1.08 (H-27) with carbons at δ_C 39.5 (C-8), 139.6 (C-13), 42.0 (C-14) and 26.7 (C-15), protons resonating at δ_H 7.69 (C-2^1) with carbons at δ_C 166.3 (C-1^1), 118.9 (C-3^1), 128.1 (C-5^1) and 134.6 (C-4^1), protons resonating at δ_H 7.55 (H-5^1) with carbons at δ_C 134.6 (C-4^1) and 144.3 (C-2^1) and the protons resonating at δ_H 7.37 (H-8^1) with carbons at δ_C 128.9 (C-6^1) and 134.4 (C-4^1).

Some of the major correlations in the HSQC spectrum are those of the olefinic protons resonating at δ_H 5.16 which correlated with carbons at δ_C 124.2 (C-12) and olefinic protons resonating at δ_H 6.43 with carbons at δ_C 118.9 (C-3^1).There were other major correlations observed in the HSQC spectrum (**Table 4.2**). In the COSY spectrum, the protons resonating at δ_H 4.60 (H-3) correlated with those at δ_H 1.68 (H-2) while those resonating at δ_H 5.16 (H-12) correlated with those at δ_H 1.86 (H-11).



48

Table 4.2: ^{1}HNMR and ^{13}CNMR data for compound **48**

Position	δ_H (H, m, J in Hz) Expe.	δ_H (Literature) (Ragasa *et al*., 2015)	δ_C Expe.	δ_C Literature (Akihisa *et al*., 2010)
1	1.08, 1.64		38.0	38.5
2	1.68 (s, *J*=12.4)	1.70	23.5	23.6
3	4.66 (m, *J*=8.53)	4.65 (dd,*J* = 6.0, 10.1)	80.5	81.0
4			37.1	37.9
5	0.90(m, *J*=8.86)	0.89 (d, *J* = 6.0)	53.8	55.2
6	1.57, 1.41 (s, J=11.5)		18.1	18.2
7	1.31 (d, *J*=9.3)		32.2	32.9
8			39.5	40.0
9	1.61(d, *J*=10.11Hz)		47.9	47.6
10			36.8	36.8
11	1.86 (d, *J*=7.50)		23.2	23.4
12	5.16 (t, *J* = 3.7Hz)	5.13 (t, *J* = 3.8)	124.2	124.3
13			139.6	139.6
14			42.0	42.0
15	1.72 (d, *J*= 4.79)		26.7	26.7
16	1.44, 2.04		27.1	28.2
17			33.7	33.7
18	1.32		59.0	59.0
19	0.91		39.7	39.6
20	1.31		39.7	39.7
21	1.25, 1.39		31.2	31.2
22	1.27, 1.42		41.5	41.5
23	0.92 (s)	0.92 (s)	28.1	28.1
24	0.95 (s)	0.95 (s)	16.3	16.9
25	1.01(s)	1.01 (s)	15.7	15.7
26	1.03(s)	1.03 (s)	16.8	16.9
27	1.08(s)	1.08 (s)	23.2	23.2
28	0.80(s)	0.80 (s)	28.8	28.8
29	0.80(s)	0.80(d, *J = 5.9*)	17.5	17.5
30	0.93(s)	0.92 (d, *J = 5.9*)	21.4	21.4
1^1			166.3	166.8
2^1	7.69 (d, *J* = 14.90)	7.67 (d, *J* = 16.0)	144.3	144.2
3^1	6.43 (d, *J* = 16.0)	6.44 (d, *J* = 16.0)	118.9	118.8
4^1			134.6	134.5
5^1	7.55	7.53	128.1	128.0
6^1	7.37 (m, *J* = 3.74)	7.38	128.9	128.8
7^1	7.37	7.38	130.1	130.1
8^1	7.37	7.38	128.9	128.8
9^1	7.55	7.53	128.1	128.0

A survey through the literature shows that α-amyrin cinnamate has been isolated from a number of plants across different families. In one study, α-amyrin cinnamate was isolated from a dichloromethane extract of the fruits of *Sonneratia alba* (Ragasa *et al*., 2015). Another study also shows that α-amyrin cinnamate was isolated from the DCM extract of the leaves of *Hoya melifua* (Ng *et al*., 2019).

4.2.3 Identification of compound 49

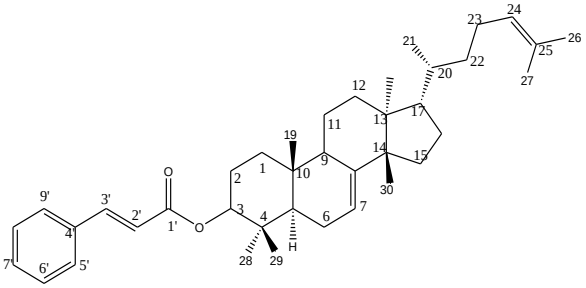
Compound **49** was isolated from the hexane crude extract as a white amorphous solid. The ^{1}HNMR spectrum of the compound showed eight methyl signals at δ_H 0.80, 0.77, 0.83, 1.64, 1.57, 0.88, 0.95 and 1.00, and the compound was identified as a triterpene cinnamate. The cinnamyl group was identified due to the presence of signals at δ_H 7.50, 7.41, 7.52, and 7.40 which confirm presence of an aromatic ring while the signal at δ_H 7.69 and 6.45 confirm the presence of a proton on a carbon to carbon double bond. The presence of a signal at δ_H 5.13 (H-24) represents a hydrogen in the methine group of the butyrospermol skeleton.

The ^{13}CNMR spectrum showed peaks similar to that of a cinnamate derivative of a triterpene alcohol. Two signals at δ_C 124.5 and 130.9 represent carbon atoms characterized by double bonds. The signal at δ_C 80.9 was for carbon 3 which was attached to the oxygen atom of the ester group. In addition, the signals at δ_C 134.6, 128.1, 128.7, 128.2, 128.8 and 130.1 represent carbon atoms of the benzene ring while the signal at δ_C 144.5 and 118.3 represent carbon 2^1 and 3^1 of the cinnamyl group. Meanwhile the signal at δ_C 167.0 represents the carbon of the ester group of cinnamyl. Using the NMR data given (**Table 4.3**) and in comparison with the available literature, the compound was identified as butyrospermol cinnamate (**49**).

In the HMBC spectrum protons resonating at δ_H 4.69 (H-3) showed correlation with carbons at δ_C 16.1 (C-29). More cross peak correlations were observed and include; protons resonating at δ_H 5.27 (H-7) with carbons at δ_C 48.8 (C-9), protons resonating at δ_H 2.26 (H-9) with carbons at δ_C 117.3 (C-7) and 33.9 (C-12), protons resonating at δ_H 0.83 (H-21) with carbons at δ_C 53.3 (C-17), 34.7 (C-20) and 34.2 (C-22), protons resonating at δ_H 1.05 (H-22) with carbons at δ_C 53.3 (C-17) and 34.7 (C-20), protons resonating at δ_H 5.13 (H-24) with carbons at δ_C 130.9 (C-25), protons resonating at δ_H 0.88 (C-28) with carbons at δ_C 80.9 (C-3), 38.2 (C-4) and 16.1 (C-29), protons resonating at δ_H 1.00 (H-29) with carbons at δ_C 80.9 (C-3), 38.2 (C-4) and 50.3 (C-5), and the protons resonating at δ_H 7.69 (H-2^1) with carbons at δ_C 128.1 (C-5^1) and 167.0 (C-1^1). Meanwhile the protons resonating at δ_H 6.45 (H-3^1) showed

correlation with carbon at δ_C 134.6 (C-4[1]) and those resonating at δ_H 7.40 (H-7[1]) showed correlation with carbons at δ_C 128.1 (C-5[1]) and 128.2 (C-9[1]).

Some of the major correlations in the HSQC spectrum are that of the olefinic protons resonating at δ_H 5.13 which correlated with carbons at δ_C 124.5 (C-24) and olefinic protons resonating at δ_H 6.45 with carbons at δ_C 118.9 (C-3[1]). There were other major correlations observed in the HSQC spectrum (**Table 4.3**). In the COSY spectrum, the protons resonating at δ_H 7.69 (H-2[1]) showed correlation with those resonating at δ_H 6.45 (H-3[1]) while the protons resonating at δ_H 5.13 (H-24) correlated with those at δ_H 2.07 (H-23). In addition the protons resonating at δ_H 4.69 (H-3) correlated with those at δ_H 1.64 (H-2).



49

Table 4.3: ^{1}HNMR and ^{13}CNMR data for compound **49**

Position	δ_H (H, m, *J* in Hz) Expe.	δ_H (Literature) (Akihisa *et al*., 2010)	δ_C Expe.	δ_C (Literature) (Akihisa *et al*., 2010)
1	1.28 (d, s, *J* = 4.83, 9.38)	1.25, 1.69	36.8	36.9
2	1.64 (s, *J* = 4.34)	1.67	21.3	24.4
3	4.69 (d,d *J* = 4.30)	4.67 (dd, *J* = 4.2,10.5)	80.9	81.3
4			38.2	38.2
5	1.46 (s)	1.44	50.3	50.9
6	1.94 (d, *J* = 3.27))	1.95, 2.16	23.8	23.8
7	5.27 (*J* = 3.1,6.9)	5.26 (dd, *J* = 3.0, 6.6)	117.3	117.6
8			151.1	146.1
9	2.26 (m, *J* = 7.88)	2.22	48.8	48.8
10			34.2	34.9
11	1.56 (m, *J* = 6.38)	1.49	18.7	18.7
12	1.80 (d, *J* = 3.94)	1.65, 1.80	33.9	33.8
13			43.0	43.6
14			51.5	51.8
15	1.45 (s, *J* = 5.67)	1.44	34.3	34.0
16	1.27 (dd, *J* = 3.76)	1.27, 1.93	29.5	28.5
17	1.52 (d, *J* = 2.80)	1.47	53.3	53.3
18	0.80 (s, *J* = 5.73)	0.79 (s)	22.1	22.2
19	0.77 (s, *J* = 3.04))	0.77 (s)	13.5	13.3
20	1.41	1.41	34.7	35.9
21	0.83 (d, *J* = 3.76)	0.83 (d, *J* = 6.4)	18.3	18.8
22	1.05	0.95, 1.06	34.2	35.2
23	2.07 (t, *J* = 5.07)	1.91, 2.08	25.3	25.4
24	5.13 (m, *J* = 6.33)	5.10 (tt, *J* = 1.1, 6.9)	124.5	125.2
25			130.9	131.1
26	1.64 (s)	1.66 (s)	25.8	25.9
27	1.57 (s, *J* = 4.60)	1.58 (s)	17.5	17.8
28	0.88 (s, *J* = 3.23)	0.88 (s)	27.5	27.7
29	1.00 (s, *J* = 5.22)	1.00 (s)	16.1	16.1
30	0.95 (s, *J* = 2.55)	0.93 (s)	27.7	27.4
1^1			167.0	166.9
2^1	7.69 (d, *J* = 14.50)	7.65 (d, *J* = 16.0)	144.5	144.4
3^1	6.45 (d, *J* = 16.00)	6.43 (d, *J* = 16.0)	118.9	118.9
4^1			134.6	134.6
5^1	7.52	7.51 (d, *J* = 16.0)	128.1	128.1
6^1	7.40 (m)	7.36	128.7	128.9
7^1	7.40 (m)	7.36	130.1	130.1
8^1	7.41	7.36	128.8	128.9
9^1	7.52	7.51	128.2	128.1

A survey through the literature shows that butyrospermol cinnamate has been previously isolated from parts of two plants. In a study, the compound was isolated from air dried twigs of *Ficus ampelas*; a plant used to treat several ailments in parts of Asia (Ragasa *et al*., 2014).

In another study, butyrospermol cinnamate was also isolated as fine needles from defatted seed kernels of *Vitellaria paradoxum* along with three other triterpenoid esters after fractionation of the n-hexane extract using preparative TLC (Akihisa *et al*., 2010).

4.2.4 Identification of compound 50

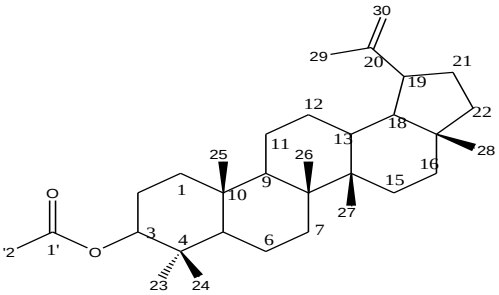
Compound **50** was isolated from the DCM crude extract as a white amorphous solid. The proton spectrum of the compound revealed eight methyl signals; six at δ_H 0.84, 0.87, 0.88, 0.91, 0.76 and 1.03. The compound was identified as a triterpene acetate due to the presence of an acetate methyl signal at δ_H 2.06 as a singlet. The presence of two exo-methylene (δ_H 4.70 on H-30 and 4.51 on H-3) and one methyl signal at δ_H 1.69 (H-29) represents lupane skeletons.

The ^{13}CNMR spectrum showed peaks which are in agreement with an acetate derivative of a triterpene alcohol. Two signals at δ_C 109.3 and 151.0 represent olefinic carbon atoms characterized by double bonds. The signal at δ_C 81.0 was for carbon 3 which was attached to an oxygen atom of the ester group. In addition, the signal at δ_C 171.4 is due to the carbon of the ester group while that at δ_C 21.4 is associated with the carbon in the methyl group of acetate. By comparing all the NMR data of the isolated compound with that in the literature (**Table 4.4**), it was identified as lupeol acetate (**50**).

In the HMBC spectrum protons resonating at δ_H 1.32 (H-9) showed correlations with carbons resonating at δ_C 37.2 (C-10). More cross peak correlations were observed and include; protons resonating at δ_H 1.38 (H-18) with carbons at δ_C 30.0 (C-21) and 18.2 (C-28), protons resonating at δ_H 1.04 (H-25) with carbons at δ_C 38.9 (C-1), 55.4 (C-5) and 50.4 (C-9), protons resonating at δ_H 0.96 (H-27) with carbons at δ_C 40.9 (C-8), 37.9 (C-13), 42.6 (C-14) and 14.6 (C-27) , protons resonating at δ_H 1.69 (H-29) with carbons at δ_C 150.8 (C-20), 109.9 (C-30) and 48.5 (C-19), protons resonating at δ_H 4.70 (H-30) with carbons at δ_C 48.5 (C-19), 150.8 (C-20) and 19.4 (C-29), and the protons resonating at δ_H 2.06 (C-2^1) with carbon at δ_C 171.4 (C-1^1).

Some of the major correlations in the HSQC spectrum are those of the olefinic protons resonating at δ_H 4.70 with carbons at δ_H 109.9 (C-30) and protons resonating at δ_H 4.51 with

carbons at δ_C 81.0 (C-3). There were other major correlations in HSQC spectrum (**Table 4.4**). In the COSY spectrum, the protons resonating at δ_H 1.43 (H-11) correlated with those at δ_H 1.92 (H-12) while the protons resonating at δ_H 4.51 (H-3) correlated with those at δ_H 1.65 (H-2).



50

Table 4.4: ^{1}HNMR and ^{13}CNMR data for compound **50**

Position	δ_H (H, m, *J* in Hz) Expe.	δ_H (Literature) (Akihisa *et al*., 2010)	δ_C Expe.	δ_C Literature (Akihisa *et al*., 2010)
1	0.95(m)	0.99 (m)	38.9	37.7
2	1.65 (m)	1.62 (m)	24.3	21.3
3	4.51 (dd, *J*=6.0, 12.0)	4.47(*dd*, *J*=5.3, 11.3)	81.0	81.0
4			38.1	38.0
5	0.86 (m)	0.80 (m)	55.4	55.4
6	1.42 (m), 1.54 (m)	1.52 (m), 1.42 (m)	18.1	18.2
7	1.39	1.49 (m), 1.38 (m)	34.2	34.2
8			40.9	40.8
9	1.32 (m)	1.30 (m)	50.4	50.3
10			37.2	37.1
11	1.43 (m)	1.42 (m), 1.23 (m)	20.9	20.9
12	1.92 (m)	1.91 (m), 1.63 (m)	23.8	23.7
13	1.66	1.66 (m)	37.9	37.1
14			42.6	42.8
15	1.69	1.69 (m), 1.01 (m)	27.3	27.4
16	1.50	1.49 (m), 1.36 (m)	35.5	35.6
17			43.1	43.0
18	1.38	1.37 (m)	48.4	48.3
19	2.39 (dt, *J*=5.4)	2.39 (m)	48.5	48.0
20			150.8	151.0
21	1.94	1.93 (m), 1.34 (m)	30.0	29.8
22	1.22	1.39 (m), 1.20 (m)	40.2	40.0
23	0.87(s)	0.88(,s)	28.2	28.0
24	0.85(s)	0.86(,s)	16.6	16.5
25	1.04(s)	1.05 (,s)	16.3	16.5
26	0.82(s)	0.85 (,s)	16.1	16.0
27	0.96(s)	0.95 (,s)	14.6	14.5
28	0.80(s)	0.80 (,s)	18.2	18.0
29	1.69 (s)	1.70 (,s)	19.4	19.3
30	4.70 (dd, *J*=14.3, 2.6)	4.70 (*s*)	109.9	109.3
1^{1}			171.4	171.3
2^{1}	2.06	2.06 (*s*)	21.4	21.3

Several investigations have resulted in isolation of lupeol acetate and in all cases, the compound has registered considerable antimicrobial activity. In a study, the crude hexane extract of the leaves of *Ficus sanasibaraca*; a popular medicinal plant in the tropics, was subjected to chromatographic fractionation leading to isolation of lupeol acetate. The

compound was found to be active against *S. aureus* with a reported average diameter of zone of inhibition of 13.5 mm (Awolola *et al*., 2014).

Another study revealed presence of lupeol acetate in the butanol root bark extract of *Ficus sycomorus*. The findings showed that at 100 µg/mL concentration, the compound inhibited *S. aureus, B. subtilis* and *S. typhi* with inhibition diameter of 18, 16 and 16 mm respectively. In all the three cases, the MBC and MIC values were found to be 25 µg/mL and 12.5 µg/mL respectively (Muktar *et al*., 2018).

The antifungal potential of lupeol acetate against *Macrophomina phaseolina* mold was investigated in another study. The compound was isolated from *Monotheca buxifolia* methanolic extract and the results showed that at low concentrations in the range of 0.104 to 6.66 µg/mL, the biomass of the test fungi was reduced by approximately 80% (Javed et al., 2021). Lupeol acetate was also isolated from other plants e.g. stem bark of *Hoya pubicalyx*, a plant with medicinal properties from the Phillipines (Didibhuku and Thwala, 2010).

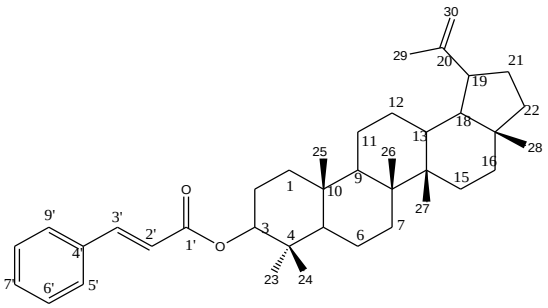
4.2.5 Identification of compound 51

Compound **51** was isolated from the DCM crude extract as a white amorphous solid. The ^{1}HNMR spectrum of the compound showed seven methyl signals at chemical shift δ_H 0.84, 0.87, 0.88, 0.91, 0.76, 1.03 and 1.69 and it was identified as a triterpene cinnamate. The cinnamyl group was identified due to the presence of signals at δ_H 7.37, 7.51, 7.36 and 7.37 which confirm presence of an aromatic ring while the signals at δ_H 7.65 and 6.43 confirm presence of a hydrogen on a carbon to carbon double bond. The presence of two exo-methylene (δ_H 4.68, 4.48 on H-30) and one methyl signal at 1.69 (H-29) represents lupane skeletons.

The ^{13}CNMR spectrum showed peaks which are in agreement with a cinnamate derivative of a triterpene alcohol. Two signals at δ_C 109.3 and 151.0 represent olefinic carbon atoms characterized by double bonds. The signal at δ_C 81.0 was for carbon 3 which was attached to an oxygen atom of the ester group. In addition, the signals at δ_C 134.6, 128.3, 128.9, 129.9, 128.9 and 128.1 represent the carbon atoms of benzene while the signal at δ_C 144.3 and 119.1 represent carbon 2^1 and 3^1 of the cinnamyl group. The signal at δ_C 167.2 represents the carbon of the ester group in cinnamyl. By comparing the NMR data obtained and that available literature (**Table 4.5**), this compound was characterized as lupeol cinnamate (**51**). The findings agree with literature reported in (Wood *et al*., 2001).

In the HMBC spectrum the protons resonating at δ_H 1.32 (H-9) showed correlations with carbons resonating at δ_C 37.2 (C-10). More cross peak correlations were observed and include; protons resonating at δ_H 1.38 (H-18) with carbons at δ_C 30.0 (C-21) and 18.2 (C-28), protons resonating at δ_H 1.04 (H-25) with carbons at δ_C 38.9 (C-1), 55.4 (C-5) and 50.4 (C-9), protons resonating at δ_H 0.96 (H-27) with carbons at δ_C 40.9 (C-8), 37.9 (C-13), 42.6 (C-14) and 14.6 (C-27), protons resonating at δ_H 1.69 (H-29) with carbons at δ_C 150.8 (C-20), 109.9 (C-30) and 48.5 (C-19), protons resonating at δ_H 4.70 (H-30) with carbons at δ_C 48.5 (C-19), 150.8 (C-20) and 19.4 (C-29), protons resonating at δ_H 7.71 (C-2^1) with carbons at δ_C 128.3 (C-5^1) and 167.2 (C-1^1), protons resonating at δ_H 6.43 (H-3^1) with carbons at δ_C 134.4 (C-4^1) and the protons resonating at δ_H 7.51 (H-5^1) with carbons at δ_C 129.9 (C-7^1).

Some of the major correlations in the HSQC spectrum are those of the olefinic protons resonating at δ_H 4.70 which correlated with carbons at δ_H 109.9 (C-30), protons at δ_H 6.43 with carbons at δ_C 119.1 (C-3^1) and protons resonating at δ_H 4.51 with carbons at δ_C 81.0 (C-3). There were other major correlations in HSQC spectrum (**Table 4.5**). In the COSY spectrum, the protons resonating at δ_H 6.43 (H-3^1) showed correlation with those at δ_H 7.71 (H-2^1) while the proton at δ_H 4.51 (H-3) correlated with those at δ_H 1.65 (H-2). In addition the protons resonating at δ_H 1.43 (H-11) correlated with those at δ_H 1.92 (H-12).



51

Table 4.5: ^{1}HNMR and ^{13}CNMR data for compound **51**

Position	δ_H (H, m, *J* in Hz) Expe.	δ_H (Literature) (Akihisa *et al*., 2010)	δ_C Expe.	δ_C Literature (Wood *et al*., 2001)
1	0.95	1.02, 1.68	38.9	38.43
2	1.65	1.69	24.3	23.82
3	4.5 1(dd, *J*=6.0)	4.6 1(dd, *J*=6.4,11.8)	81.0	81.06
4			38.1	37.84
5	0.86, 0.88	0.84	55.4	55.44
6	1.42, 1.54	1.39. 1.53	18.1	18.23
7	1.39	1.41	34.2	34.23
8			40.9	40.87
9	1.32	1.32	50.4	50.37
10			37.2	37.113
11	1.43	1.41, 1.21	20.9	20.97
12	1.08, 1.67	1.07, 1.66	23.8	25,12
13	1.66	1.64	37.9	38.06
14			42.6	42.85
15	1.70, 1.06	1.67, 1.09	27.3	27.45
16	1.50	1.33, 1.47	35.5	35.58
17			43.1	43.00
18	1.38	1.35	48.4	48.31
19	2.39 (dt, *J*=5.4)	2.38 (dt, *J*=5.4, 11.0)	48.5	48.02
20			150.8	150.98
21	1.94	1.91, 1.34	30.0	29.85
22	1.22	1.19, 1.38	40.2	40.01
23	0.87 (s)	0.89 (s)	28.2	28.02
24	0.85 (s)	0.92 (s)	16.6	16.67
25	1.04 (s)	0.88 (s)	16.3	16.21
26	1.05 (s)	1.04 (s)	16.1	16.09
27	0.96 (s)	0.94 (s)	14.6	14.54
28	0.80 (s)	0.79 (s)	18.2	18.01
29	1.69 (s)	1.69 (s)	19.4	19.30
30	4.70 (dd, *J*= 14.2, 2.4)	4.58 (dd, *J*=14.2, 2.4)	109.9	109.35
1^1			167.2	166.83
2^1	7.71	7.66(d, *J*=16.0)	144.3	144.23
3^1	6.43	6.44(d, *J*=16.0)	119.1	118.90
4^1			134.4	134.56
5^1	7.51	7.52	128.3	128.03
6^1	7.36	7.37	128.3	128.83
7^1	7.37	7.37	129.9	130.11
8^1	7.36	7.37	128.9	128.83
9^1	7.51	7.52	128.3	128.03

A few investigations have resulted in isolation of lupeol cinnamate from parts of medicinal plants for example the compound was isolated from n-hexane extract of defatted seed kernels of *B. paradoxum.* (Akihisa *et al*., 2010). However there is limited information about the antibacterial and antifungal activity of this compound.

4.3 Antimicrobial activity tests

The results for the antimicrobial activity of the crude extracts of stem bark of *B. paradoxum* are presented as average diameter of zone of inhibition in mm ± SEM.

4.3.1 Antimicrobial activity of hexane extract

The results for antimicrobial activity of the hexane extract (**Table 4.6**) show that it was either weakly or moderately active against all the six test microbial organisms. The extract gave the highest activity against *C. albicans* and the gram-negative *S. typhi* with average diameters of zone of inhibition of 14.3 ± 0.3 and 14.0 ± 1.0 mm respectively. However the hexane extract was least active against *P. aeruginosa* and *K. pneumoniae* with an average clearance of 11.0 ± 0.0 and 11.3 ± 0.3mm respectively.

Table 4.6: Antimicrobial activity for hexane extract

Microbial organism	Diameter of zone of inhibition of Tetracycline (mm)	Diameter of zone of inhibition of DMSO (mm)	Average diameter of zone of inhibition of the plant extract (mm)
K. pneumoniae	20.0	0.0	11.3 ± 0.3
S. typhi	20.0	0.0	14.0 ± 1.0
E. coli	20.0	0.0	12.0 ± 0.0
P. aeruginosa	16.0	0.0	11.0 ± 0.0
S. aureus	25.0	0.0	13.3 ± 0.3
C. albicans	24.0	0.0	14.3 ± 0.3

4.3.2 Antimicrobial activity of Dichloromethane extract

The results for antimicrobial activity of the DCM extract (**Table 4.7**) show that it was active against all the six test microbial organisms. The extract gave the highest activity against the Gram-negative *S. typhi* and the Gram-positive *S. aureus* with average diameters of zone of inhibition of 16.0 ± 0.6 and 16.3 ± 0.7 mm respectively. The DCM extract was found to be least active against *P. aeruginosa* with an average diameter of zone of inhibition of 13.7 ± 0.3 mm.

Table 4.7: Antimicrobial activity for Dichloromethane extract

Microbial organism	Diameter of zone of inhibition of Tetracycline (mm)	Diameter of zone of inhibition of DMSO (mm)	Average diameter of zone of inhibition of the plant extract (mm)
K. pneumoniae	20.0	0.0	15.7 ± 0.3
S. typhi	20.0	0.0	16.0 ± 0.6
E. coli	19.3	0.0	14.7 ± 0.3
P. aeruginosa	16.0	0.0	13.7 ± 0.3
S. aureus	25.0	0.0	16.3 ± 0.7
C. albicans	23.0	0.0	15.0 ± 0.6

4.3.3 Antimicrobial activity of Ethyl acetate extract

The results for antimicrobial activity of the ethyl acetate extract (**Table 4.8**) also show that it was active against all the six tested microbial organisms. The extract gave the highest activity against the gram-negative *S. typhi* with average diameter of zone of inhibition of 18.0 ± 0.0 against the typhoid causing bacteria. The ethyl acetate extract was least active against *E. coli* and *K. pneumoniae* with an average diameter of zone of inhibition of 12.0 ± 0.6 and 12.7 0 ± 0.3 respectively.

Table 4.8: Antimicrobial activity for ethyl acetate extract

Microbial organism	Diameter of zone of inhibition of Tetracycline (mm)	Diameter of zone of inhibition of DMSO (mm)	Average diameter of zone of inhibition of the plant extract (mm)
K. pneumoniae	20.0	0.0	12.7 ± 0.3
S. typhi	20.0	0.0	18.0 ± 0.0
E. coli	18.0	0.0	12.0 ± 0.6
P. aeruginosa	18.0	0.0	15.0 ± 0.6
S. aureus	25.0	0.0	16.0 ± 0.6
C. albicans	24.7	0.0	16.0 ± 0.6

4.3.4 Antimicrobial activity of methanol extract

The results for antimicrobial activity of the methanol extract (**Table 4.9**) also show that it was active against four of the six test microbial organisms. The extract was very active against the Gram-negative *S. typhi* with average diameter of zone of inhibition of 22.0 ± 0.0 mm which was actually matching that of the tetracycline positive control. The methanol extract can however be considered inactive against *E. coli* and *K. pneumoniae* with an average diameter of zone of inhibition of 9.3 ± 1.2 and 9.7 0 ± 0.3 respectively.

Table 4.9: Antimicrobial activity for methanol extract

Microbial organism	Diameter of zone of inhibition of Tetracycline (mm)	Diameter of zone of inhibition of DMSO (mm)	Average diameter of zone of inhibition of the plant extract (mm)
K. pneumoniae	18.0	0.0	9.7 ± 0.3
S. typhi	22.0	0.0	22.0 ± 0.0
E. coli	18.0	0.0	9.3 ± 1.2
P. aeruginosa	18.0	0.0	14.7 ± 0.7
S. aureus	25.0	0.0	14.3 ± 0.0
C. albicans	25.0	0.0	15.0 ± 0.3

The results generally show that the hexane, DCM, ethyl acetate and methanol extracts all have compounds that are active against most of the tested microbial organisms. The activity of the less polar hexane and DCM extract can be attributed to presence of lipids since they extract mostly lipophilic compounds. The lipids are well known to kill bacteria by disrupting the cell membrane while they also have antifungal activity because they can penetrate the peptidoglycan in cell wall without causing significant changes and this enables them reach the cell membrane causing it to disintegrate (Bergsson, 2005). In addition, the DCM extract usually contains terpenoids which are commonly known to have antifungal activity and these extracts being lipophilic helps them interact with the cell membrane constituents causing toxicity (Yao *et al*., 2004).

Extracts from the more polar solvents like ethyl acetate and methanol generally showed better antimicrobial activity than the less polar ones like hexane. This could be attributed to the higher solubility of most bioactive compounds in polar solvents than in the non polar ones. In addition, the polarity of a compound may help enhance its inhibitory effect (Parekh and Chanda, 2006).

All the four extracts were active against *S. aureus* and this is in agreement with common knowledge that Gram-positive bacteria show a higher susceptibility to antibiotic agents than the Gram-negative ones (Chanda *et al*., 2005). The Gram-negative bacteria have an outer membrane made of phospholipids that contains structural lipopolysaccharide components, which prevents lipophilic solutes from penetrating the cell wall. Because Gram-positive bacteria, such as *S. aureus*, only have an exterior peptidoglycan layer, which is ineffective as a permeability barrier, they are more vulnerable (Nostro *et al*., 2000). The findings show that

the crude extracts from *B. paradoxum* give antimicrobial activity which is comparable with that of other medicinal plants in the family *Sapotaceae*, being slightly higher in comparison with extracts of some plants and slightly lower in some cases. E.g. The methanol extract of leaves of *Manilkara zapota* was noted to be active against *S. aureus*, *K. pneumoniae, E. coli* and *P. aeruginosa* with average diameter of zone of inhibition of 10.0, 11.0, 5.0 and 3.0 mm respectively (Chanda, 2008) while the ethyl acetate extract of *Mimusops elengi* was active against both *K. pneumonia* and *E. coli* giving average zone of inhibition diameters of 17.23 and 13.16 mm respectively (Kannadhasan *et al*., 2016). By comparison, these findings show that the extracts from the stem bark of *B. paradoxum* are generally more active against the said microbial organisms.

4.3.5 Minimum Inhibitory Concentration and Minimum Bactericidal / Fungicidal Concentration

The results of MIC and MBC / MFC (**Table 4.10**) show that all the four crude extracts have inhibitory effect against the test organisms. The MBC values are generally higher than the MIC values because to completely kill the microbial organism, a more concentrated solution of the extract is needed. The ethyl acetate extract was most effective for inhibition of *E. coli*, *P. aeruginosa* and *S. aureus* with an MIC and MBC value of 10.0 and 20.0 mg/mL respectively in each case. Meanwhile *S. typhi* and *K. pneumoniae* were most susceptible to the crude dichloromethane extract giving an MIC value of 6.25 mg/mL in both cases. However, the extract of methanol was also highly inhibiting against *K. pneumonia* with a low MIC value of 6.25 mg/mL.

Table 4.10: MIC and MBC values of crude extracts in mg /mL

	Klebsiella pneumoniae		*Salmonella typhi*		*Escherichia coli*		*Pseudomonas aeruginosa*		*Staphylococcus aureus*		*Candida albicans*	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MFC
Hexane extract	12.5	25.0	25.0	50.0	12.5	25.0	25.0	50.0	25.0	50.0	25.0	50.0
DCM extract	6.25	12.5	6.25	25.0	12.5	25.0	25.0	50.0	25.0	50.0	6.25	25.0
Ethyl acetate extract	20.0	40.0	20.0	40.0	10.0	20.0	10.0	20.0	10.0	20.0	10.0	20.0
Methanol extract	6.25	12.5	12.5	25.0	12.5	25.0	12.5	25.0	12.5	25.0	12.5	25.0

The results of MIC and MBC / MFC are generally high implying that a high concentration of the stem bark extract is required to inhibit or kill the different microbial organisms under study. This justifies the frequent administration of the extract to patients in traditional medicine which is often done two or three times a day.

Extracts from different parts of plants in the family *Sapotaceae* have been previously investigated for antimicrobial activity and their MIC and MBC values have been compared with those obtained in this study. The methanol extract of the fruit peel of *Pouteria caimito* significantly inhibit *S. aureus, E. coli* and *K. pneumonia* with MIC values of 1.2 and 0.6 mg/mL respectively (Abreu *et al*., 2019), showing stronger inhibition at lower concentrations than the methanol extract of *B. paradoxum*. Relatedly, sthe methanol extract of *Tridesmostemon omphalocarpoides* stem bark strongly inhibited the growth of *E. coli*, *K. pneumoniae*, *S. typhi* and *C. albicans* giving MIC values of 78.12, 156.25, 156.25 and 312.5 mg/mL respectively and MBC values of 156.25, 625, 312.5 and 625 mg/mL respectively (Kuete *et al*., 2006). A general comparison in this case shows that the *B. paradoxum* extracts are more inhibiting at lower concentrations.

4.3.6 Antimicrobial activity of isolated compounds

The results for antimicrobial activity (**Table 4.11**), MIC and MBC / MFC (**Table 4.12**) of the isolated pure compounds **48** and **51** against selected bacteria and fungi are given. The antimicrobial activity of compounds **49** and **50** was not determined because a very small amount of each compound was isolated. The results for antimicrobial activity are presented as the average diameter of zones of inhibition in mm while the MIC and MBC/MFC are presented in mg/mL.

The two isolated compounds showed significant antibacterial and antifungal activity against selected organisms. Compound **51** was most active on *S. typhi* and *C. albicans* and gave the least MIC and MBC values of 0.125 and 0.25 mg/mL respectively in both cases. Meanwhile compound **48** showed similar activity in all the four test organisms with MIC and MBC of 0.25 and 0.50 mg/mL respectively in all cases.

Table 4.11: Antimicrobial activity of compounds **48** and **51**

Bacteria	Average diameter of zone of inhibition (mm) ± SEM	
Salmonella typhi	Compound **48**	18.0 ± 0.0
	Compound **51**	20.0 ± 0..3
	Tetracycline	21.0 ± 0.0
Escherichia coli	Compound **48**	21.0 ± 1.2
	Compound **51**	21.0 ± 0.3
	Tetracycline	26.0 ± 0.0
Staphylococcus aureus	Compound **48**	17.0 ± 0.3
	Comound **51**	18.0 ± 0.6
	Tetracycline	25.0 ± 0.0
Candida albicans	Compound **48**	20.0 ± 0.3
	Compound **51**	22.0 ± 0.0
	Tetracycline	25.0 ± 0.0

Table 4.12: MIC and MBC of compounds **48** and **51**

	Salmonella typhi		*Escherichia coli*		*Staphylococcus aureus*		*Candida albicans*	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MFC
Compound **48**	0.25	0.50	0.25	0.50	0.25	0.50	0.25	0.50
Compound **51**	0.125	0.25	0.25	0.50	0.25	0.50	0.125	0.50

The two tested compounds: lupeol cinnamate and α-amyrin cinnamate belong to the same class of secondary plant metabolites known as terpenoids. All the four tested microbial organisms were found to be susceptible to the isolated compounds as the diameter of zones of inhibition were within the range of standard antibiotics like tetracycline (17-25 mm) according to the Clinical and Laboratory Standard Institute CSLI (2007).

The terpenoids are well documented to have antimicrobial activity and this could be as a result of their functional group and considerable water solubility. In addition, terpenoids readily dissolve in lipids and this is of great importance as it enables them dissolve in the phospholipid bilayer of microbial membranes thereby enabling them exert their antimicrobial activity (Griffin, 2000). The generally high activity of the isolated compounds against the tested bacteria and fungi justifies the use of extracts of the stem bark of *B. paradoxum* in traditional medicine.

CHAPTER FIVE: CONCLUSION AND RECOMMENDATION

5.1 Conclusion

The study found that the hexane extract of *B. paradoxum* was active against *S. typhi*, *C. albicans,* , and *S. aureus* but only partially active against *E. coli*, *K. pneumoniae* and *P. aeruginosa* while the DCM extract was active against all the six microbial organisms. The ethyl acetate and methanol extracts were active against all the test organisms apart from *E. coli* and *K. pneumonia* where they were only partially active.

The study led to isolation of four compounds: α-amyrin cinnamate, butyrospermol cinnamate, lupeol acetate and lupeol cinnamate. Two compounds: α-amyrin cinnamate and lupeol cinnamate were both active against *S. typhi, C. albicans, S. aureus* and *E. coli*.

The findings of this study justify the use of stem bark extracts of *B. paradoxum* in traditional medicine. In addition the herbal extracts from the stem bark of *B. paradoxum* can be standardized with the help of the isolated and characterized compounds.

5.2 Recommendations

i. Clinical trials on the antimicrobial activity of the isolated compounds should be done so as to determine the antimicrobial activity *in vivo* and also ascertain the extent of safety.
ii. The antimicrobial activity of joint isolated compounds should be investigated and compared with the activity of individual compounds.
iii. More studies should be directed to modification of the isolated compounds to obtain derivatives with even better antimicrobial activity.

REFERENCES

A., A. R., & Haque, M. (2020). *Preparation of Medicinal Plants: Basic Extraction and Fractionation Procedures for Experimental Purposes*. 1–10. https://doi.org/10.4103/jpbs.JPBS

Aanchal Mittal, R. S. (2023). *World Journal of Pharmaceutical Research*. *12*(9), 1031–1057. https://doi.org/10.20959/wjpr20239-28291

Abreu, M. M., De Nobrega, P. A., Sales, P. F., De Oliveira, F. R., & Nascimento, A. A. (2019). Antimicrobial and antidiarrheal activities of methanolic fruit peel extract of pouteria caimito. *Pharmacognosy Journal*, *11*(5), 944–950. https://doi.org/10.5530/pj.2019.11.150

Abubakar, U., Yusuf, K., Abdu, G., Saidu, S., Jamila, G., & Fatima, A. (2017). Ethnopharmacological survey of medicinal plants used for the management of pediatric ailments in Kano State, Nigeria. *Research Journal of Pharmacognosy (RJP)*, *4*(3), 29–39. http://rjpharmacognosy.ir

Akihisa, T., Kojima, N., Kikuchi, T., Yasukawa, K., Tokuda, H., Masters, E. T., Manosroi, A., & Manosroi, J. (2010). *Anti-Infl ammatory and Chemopreventive Effects of Triterpene Cinnamates and Acetates from Shea Fat*. *280*(6), 273–280.

Al-judaibi, E. (2014). Infection and Antibiotic Resistant Bacteria in Developing Countries : A Genetic Review. *Journal of Microbiology Research*, *4*(11), 10–17. https://doi.org/10.5923/s.microbiology.201401.02

Aniszewski, T. (2007). *Alkaloids-Secrets of Life:: Aklaloid Chemistry, Biological Significance, Applications and Ecological Role*. Elsevier.

Ann Anderson, L. (2019). Antibiotics: List of Common Antibiotics & Types - Drugs.com. In *Drugs.com*. https://www.drugs.com/article/antibiotics.html

Anywar, G., Kakudidi, E., Byamukama, R., Mukonzo, J., Schubert, A., & Oryem-Origa, H. (2020). Indigenous traditional knowledge of medicinal plants used by herbalists in treating opportunistic infections among people living with HIV/AIDS in Uganda. *Journal of Ethnopharmacology*, *246*, 112205. https://doi.org/10.1016/j.jep.2019.112205

Aristide, F., Toze, A., Fomani, M., Nouga, A. B., Chouna, J. R., François, A., Waffo, K., &

Wansi, J. D. (2015). *Taraxastane and Lupane Triterpenoids from the Bark of Manilkara zapota*. *7*(4), 157–164. https://doi.org/10.9734/IRJPAC/2015/17436

Ashour, A. S., El Aziz, M. M. A., & Gomha Melad, A. S. (2019). A review on saponins from medicinal plants: chemistry, isolation, and determination. *Journal of Nanomedicine Research*, *7*(4), 282–288. https://doi.org/10.15406/jnmr.2019.07.00199

Awolola, G. V., Koorbanally, N. A., Chenia, H., Shode, F. O., & Baijnath, H. (2014). Antibacterial and anti-biofilm activity of flavonoids and triterpenes isolated from the extracts of Ficus sansibarica Warb. subsp. sansibarica (Moraceae) extracts. *African Journal of Traditional, Complementary, and Alternative Medicines : AJTCAM / African Networks on Ethnomedicines*, *11*(3), 124–131. https://doi.org/10.4314/ajtcam.v11i3.19

Baky, M. H., Kamal, A. M., Elgindi, M. R., Haggag, E. G., & Mohamed Elgindi, C. R. (2016). A Review on Phenolic Compounds from Family Sapotaceae. *~ 280 ~ Journal of Pharmacognosy and Phytochemistry*, *5*(2), 280–287.

Balouiri, M., Sadiki, M., & Ibnsouda, S. K. (2016). Methods for in vitro evaluating antimicrobial activity: A review. *Journal of Pharmaceutical Analysis*, *6*(2), 71–79. https://doi.org/10.1016/j.jpha.2015.11.005

Bergsson, G. (2005). *Antimicrobial polypeptides and lipids as a part of innate defense mechanism of fish and human fetus*.

Brooks, J. T., Ochieng, J. B., Kumar, L., Okoth, G., Shapiro, R. L., Wells, J. G., Bird, M., Bopp, C., Chege, W., Beatty, M. E., Chiller, T., Vulule, J. M., Mintz, E., & Slutsker, L. (2006). Surveillance for bacterial diarrhea and antimicrobial resistance in rural western Kenya, 1997-2003. *Clinical Infectious Diseases*, *43*(4), 393–401. https://doi.org/10.1086/505866

Cai, L. (2014). Thin layer chromatography. *Current Protocols in Essential Laboratory Techniques*, *2014*(October), 6.3.1-6.3.18. https://doi.org/10.1002/9780470089941.et0603s08

Chanda, S. (2008). *IJPhS-70-390*. *June*.

Chanda, S., Parekh, J., Jadeja, D., & Chanda, S. (2005). *Efficacy of Aqueous and Methanol Extracts of Some Medicinal Plants for Potential Antibacterial Activity Efficacy of*

Aqueous and Methanol Extracts of Some Medicinal Plants for Potential Antibacterial Activity. *29*(4).

Chandrasekaran, M., & Venkatesalu, V. (2004). *Antibacterial and antifungal activity of Syzygium jambolanum seeds*. *91*(August 2002), 105–108. https://doi.org/10.1016/j.jep.2003.12.012

Che, C. T., Koike, K., Cordell, G. A., Fong, H. H. S., & Dobberstein, R. H. (1980). Triterpenes of pouteria torta (sapotaceae). *Journal of Natural Products*, *43*(3), 420–421. https://doi.org/10.1021/np50009a016

Chem, J. (2022). *1 2 1 1*. *47*(4), 807–817.

Chen, C. Y., Wang, Y. D., & Wang, H. M. (2010). Chemical constituents from the roots of Synsepalum dulcificum. *Chemistry of Natural Compounds*, *46*(3), 448–449. https://doi.org/10.1007/s10600-010-9639-9

Connor, E. E. (1998). Honorable Mention Paper Sulfonamide Antibiotics ! T. *Primary Care Update Ob/Gyn*, *5*(97), 32–35.

Cushnie, T. P. T., Cushnie, B., & Lamb, A. J. (2014). Alkaloids: An overview of their antibacterial, antibiotic-enhancing and antivirulence activities. *International Journal of Antimicrobial Agents*, *44*(5), 377–386. https://doi.org/10.1016/j.ijantimicag.2014.06.001

Dakiche, H., Khali, M., Abu-el-Haija, A. K., Al-Maaytah, A., & Al-Balas, Q. A. (2016). Biological activities and phenolic contents of Argania spinosa L (Sapotaceae) leaf extract. *Tropical Journal of Pharmaceutical Research*, *15*(12), 2563–2570. https://doi.org/10.4314/tjpr.v15i12.5

Didibhuku, W., & Thwala, W. D. (2010). The land question and sustainable development in South Africa: Issues and challenges. In *African Journal of Agricultural Research* (Vol. 5, Issue 25, pp. 3553–3560). https://doi.org/10.5897/AJAR10.013

Dongare, V. S., Kohale, N. B., & Rathod, S. B. (2023). A Review of Chromatograph: Principal, Classification, Applicatio. *International Journal of Humanities Social Science and Management (IJHSSM)*, *3*(2), 367–373. www.ijhssm.org

Ekhuemelo, D., Anyam, J. and Ekhuemelo, C. (2021). *Antimicrobial Efficacy of Vitellaria paradoxa fractions and compounds on some wood Fungi and Bacteria*. *38*(Ju), 1–13.

https://doi.org/DOI: https://dx.doi.org/10.4314/njb.v38i1.1 Antimicrobial

Fayek, N. M., Abdel Monem, A. R., Mossa, M. Y., Meselhy, M. R., & Shazly, A. H. (2012). Chemical and biological study of Manilkara zapota (L.) Van Royen leaves (Sapotaceae) cultivated in Egypt. *Pharmacognosy Research*, *4*(2), 85–91. https://doi.org/10.4103/0974-8490.94723

Fotsing, R., Yankam, R., Rodolphe, J., Lanz, C., & Furrer, J. (2014). *Procerenone : a Fatty Acid Triterpenoid from the Fruit Pericarp of Omphalocarpum procerum (Sapotaceae).* *13*(September 2013), 1425–1430.

Fru, C. G., Sandjo, L. P., Kuete, V., Liermann, J. C., Schollmeyer, D., Yeboah, S. O., Mapitse, R., Abegaz, B. M., Ngadjui, B. T., & Opatz, T. (2013). Omphalocarpoidone , a new lanostane-type furano-spiro- g -lactone from the wood of Tridesmostemon omphalocarpoides Engl . (Sapotaceae). *Phytochemistry Letters*, *6*(4), 676–680. https://doi.org/10.1016/j.phytol.2013.08.017

GEROTHANASSIS, I. P., TROGANIS, A., EXARCHOU, V., & BARBAROSSOU, K. (2002). Nuclear Magnetic Resonance (Nmr) Spectroscopy: Basic Principles and Phenomena, and Their Applications To Chemistry, Biology and Medicine. *Chem. Educ. Res. Pract.*, *3*(2), 229–252. https://doi.org/10.1039/b2rp90018a

Gopalkrishnan, B., & Shimpi, S. N. (2011). Seeds of Mimusops elengi Linn. Pharmacognosy and phytochemical studies. *International Journal of Pharmacognosy and Phytochemical Research*, *3*(1), 13–17.

Griffin, S. (2000). *view.pdf*.

Guefack, M. G. F., Ngangoue, M. O., Mbaveng, A. T., Nayim, P., Kuete, J. R. N., Ngaffo, C. M. N., Chi, G. F., Ngameni, B., Ngadjui, B. T., & Kuete, V. (2022). Antibacterial and antibiotic - potentiation activity of the constituents from aerial part of Donella welwitshii (Sapotaceae) against multidrug resistant phenotypes. *BMC Complementary Medicine and Therapies*, *3*, 1–14. https://doi.org/10.1186/s12906-022-03673-3

Gurib-Fakim, A., Subratty, H., Narod, F., Govinden-Soulange, J., & Mahomoodally, F. (2005). Biological activity from indigenous medicinal plants of Mauritius. *Pure and Applied Chemistry*, *77*(1), 41–51. https://doi.org/10.1351/pac200577010041

Guto, J. A., Bii, C. C., & Denning, D. W. (2016). Estimated burden of fungal infections in Kenya. *Journal of Infection in Developing Countries*, *10*(8), 777–784. https://doi.org/10.3855/jidc.7614

Hendiani, I., Susanto, A., Carolina, D. N., Ibrahim, R., & Balatif, F. F. (2020). Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of mangosteen (Garcinia mangostana Linn.) rind extract against Aggregatibacter actinomycetemcomitans. *Padjadjaran Journal of Dentistry*, *32*(2), 131–135. https://doi.org/10.24198/pjd.vol32no2.27366

Hooper, D. C., & Jacoby, G. A. (2015). Mechanisms of drug resistance: Quinolone resistance. *Annals of the New York Academy of Sciences*, *1354*(1), 12–31. https://doi.org/10.1111/nyas.12830

Imoro, A. Z., Khan, A. T., & Eledi, J. D. A. (2013). Exploitation and use of medicinal plants, Northern Region, Ghana. *Journal of Medicinal Plants Research*, *7*(27), 1984–1993. https://doi.org/10.5897/jmpr12.489

Institute, D. G. H. (2010). *Bacterial Infections a Common Cause of Illness, Death in Africa*. https://globalhealth.duke.edu/news/bacterial-infections-common-cause-illness-death-africa

J. Azmir, I.S.M. Zaidul, M.M. Rahman, K.M. Sharif, A. Mohamed, F. Sahena, M.H.A. Jahurul, K. Ghafoor, N.A.N. Norulaini, A. K. M. O. (n.d.). *Author ' s personal copy Techniques for extraction of bioactive compounds from plant materials : A review*.

Jain, N., & Sharma, M. (2020). Inhibitory effect of some selected essential oil terpenes on fungi causing superficial infection in human beings. *Journal of Essential Oil Bearing Plants*, *23*(4), 862–869.

Javed, S., Mahmood, Z., Khan, K. M., Sarker, S. D., Javaid, A., Khan, I. H., & Shoaib, A. (2021). Lupeol acetate as a potent antifungal compound against opportunistic human and phytopathogenic mold Macrophomina phaseolina. *Scientific Reports*, *11*(1), 1–11. https://doi.org/10.1038/s41598-021-87725-7

Kadam, P. V., Deoda, R. S., Shivatare, R. S., Yadav, K. N., & Patil, M. J. (2012). Pharmacognostic, phytochemical and physiochemical studies of Mimusops Elengi Linn stem bark (Sapotaceae). *Der Pharmacia Lettre*, *4*(2), 607–613.

Kannadhasan, M., Valarmathi, S., Kadirvelmurugan, V., Karthik, V., Priya, G., Rajesh, E., Amarasuriyan, C., & Raju, K. (2016). The Medicinal Plant of Mimusops Elengi (Sapotaceae) in Antimicrobial Activities. *Journal of Engineering Research and Application*, *6*(7), 26–31. www.ijera.com

Karou, S. D., Tchacondo, T., Djikpo Tchibozo, M. A., Abdoul-Rahaman, S., Anani, K., Koudouvo, K., Batawila, K., Agbonon, A., Simpore, J., & De Souza, C. (2011). Ethnobotanical study of medicinal plants used in the management of diabetes mellitus and hypertension in the Central Region of Togo. *Pharmaceutical Biology*, *49*(12), 1286–1297. https://doi.org/10.3109/13880209.2011.621959

Keeler, J. (2002). *Understanding NMR Spectroscopy*.

Kinda, P., Zerbo, P., Guenné, S., Compaoré, M., Ciobica, A., & Kiendrebeogo, M. (2017). Medicinal Plants Used for Neuropsychiatric Disorders Treatment in the Hauts Bassins Region of Burkina Faso. *Medicines*, *4*(2), 32. https://doi.org/10.3390/medicines4020032

Kiwanuka, J., Bazira, J., Mwanga, J., Tumusiime, D., Nyesigire, E., Lwanga, N., Warf, B. C., Kapur, V., Poss, M., & Schiff, S. J. (2013). The Microbial Spectrum of Neonatal Sepsis in Uganda: Recovery of Culturable Bacteria in Mother-Infant Pairs. *PLoS ONE*, *8*(8), 1–6. https://doi.org/10.1371/journal.pone.0072775

Kpodar, M. S., Karou, S. D., Katawa, G., Anani, K., Gbekley, H. E., Adjrah, Y., Tchacondo, T., Batawila, K., & Simpore, J. (2016). An ethnobotanical study of plants used to treat liver diseases in the Maritime region of Togo. *Journal of Ethnopharmacology*, *181*, 263–273. https://doi.org/10.1016/j.jep.2015.12.051

Kuete, V., Tangmouo, J. G., Penlap Beng, V., Ngounou, F. N., & Lontsi, D. (2006). Antimicrobial activity of the methanolic extract from the stem bark of tridesmostemon omphalocarpoides (Sapotaceae). *Journal of Ethnopharmacology*, *104*(1–2), 5–11. https://doi.org/10.1016/j.jep.2005.08.002

Layany, C., Mourão, S., Stien, D., & Garcia, V. L. (2015). *ANTIMICROBIAL ACTIVITY OF FLAVONOIDS FROM Manilkara zapota (L .) LEAVES*. 5355.

Maina, D., Omuse, G., Revathi, G., & Adam, R. D. (2016). Spectrum of microbial diseases and resistance patterns at a private teaching hospital in Kenya: Implications for clinical practice. *PLoS ONE*, *11*(1), 1–12. https://doi.org/10.1371/journal.pone.0147659

Mangoua, R., Jouda, J., Deccaux, G., Fotso, W., Ndjakou, B., Sewald, N., & Wandji, J. (2021). *Pentacyclic triterpenoids and other constituents isolated from the leaves of Gambeya lacourtiana and their antibacterial activity*. *98*(July). https://doi.org/10.1016/j.bse.2021.104322

Mihai Grumezescu, A., & Maria Holban, A. (2019). *Functional and Medicinal Beverages: Volume 11: The Science of Beverages*.

Montenegro, L. H. M., Oliveira, P. E. S., Conserva, L. M., Rocha, E. M. M., Brito, A. C., Araújo, R. M., Trevisan, M. T. S., & Lemos, R. P. L. (2006). Terpenóides e avaliação do potencial antimalárico, larvicida, anti-radicalar e anticolinesterásico de Pouteria venosa (Sapotaceae). *Revista Brasileira de Farmacognosia*, *16*, 611–617. https://doi.org/10.1590/s0102-695x2006000500005

Moura, B. I. de V., de Araújo, B. P. L., Sá, R. D., & Randau, K. P. (2019). Pharmacobotanical study of Manilkara zapota (L.) p.royen (sapotaceae). *Brazilian Journal of Pharmaceutical Sciences*, *55*, 1–10. https://doi.org/10.1590/s2175-97902019000117227

Muktar, B., Bello, I., & Sallau, M. (2018). Isolation, characterization and antimicrobial study of lupeol acetate from the root bark of Fig-Mulberry Sycamore (*Ficus sycomorus* LINN). *Journal of Applied Sciences and Environmental Management*, *22*(7), 1129. https://doi.org/10.4314/jasem.v22i7.21

Nadembega, P., Boussim, J. I., Nikiema, J. B., Poli, F., & Antognoni, F. (2011). Medicinal plants in Baskoure, Kourittenga Province, Burkina Faso: An ethnobotanical study. *Journal of Ethnopharmacology*, *133*(2), 378–395. https://doi.org/10.1016/j.jep.2010.10.010

Ndukwe, G. I., & Ezuruike, I. T. (2015). *Isolation , characterization and antibacterial evaluation of Zymosterol from the Root of Pachystela Brevipes (Sapotaceae)*. *4*(1), 35–41.

Ng, V. A. S., Malabed, R. S., Aurigue, F. B., & Ragasa, C. Y. (2019). Triterpenes and sterols from leaves of hoya meliflua merr. *Pharmacognosy Journal*, *11*(1), 48–52. https://doi.org/10.5530/pj.2019.1.9

Nightingale, S., Spiby, H., Sheen, K., & Slade, P. (2018). LJMU Research Online m. *Tourism Recreation Research*, 19. http://researchonline.ljmu.ac.uk/id/eprint/8705/

Nostro, A., Germano, M. P., Angelo, V. D., Marino, A., & Cannatelli, M. A. (2000). *Extraction methods and bioautography for evaluation of medicinal plant antimicrobial activity*. *Atcc 9027*, 379–384.

Ogbole, O. O., Gbolade, A. A., & Ajaiyeoba, E. O. (2010). Ethnobotanical survey of plants used in treatment of inflammatory diseases in Ogun State of Nigeria. *European Journal of Scientific Research*, *43*(2), 183–191.

Ogunwande, I. A., Bello, M. O., Olawore, O. N., & Muili, K. A. (n.d.). *Phytochemical and antimicrobial studies on Butyrospermum paradoxum*.

Olanudun, E. A. , Ayoola, M. D. , Famuyiwa, S. O. (2018). the Hyperglycaemia-Lowering Fraction of the Stem Bark of. *Ife Journal of Science Vol. 20, No. 2 (2018) 229*, *20*(2), 229–236.

Panche, A. N., Diwan, A. D., & Chandra, S. R. (2016). Flavonoids: An overview. *Journal of Nutritional Science*, *5*. https://doi.org/10.1017/jns.2016.41

Parekh, J., & Chanda, S. (2006). *In-vitro Antimicrobial Activities of Extracts of Launaea procumbens Roxb . (Labiateae), Vitis vinifera L . (Vitaceae) and Cyperus rotundus L . (Cyperaceae)*. *9*(May).

Patrick, G. (2001). *Instant notes in Medicinal Chemistry* (Original E). BIOS Scientific Publishers Limited.

Petrovska, B. B. (2012). Historical review of medicinal plants' usage. *Pharmacognosy Reviews*, *6*(11), 1–5. https://doi.org/10.4103/0973-7847.95849

Pham, T. D. M., Ziora, Z. M., & Blaskovich, M. A. T. (2019). Quinolone antibiotics. *MedChemComm*, *10*(10), 1719–1739. https://doi.org/10.1039/c9md00120d

Polk, R. E., Fox, C., Mahoney, A., Letcavage, J., & Macdougall, C. (2007). *Measurement of Adult Antibacterial Drug Use in 130 US Hospitals : Comparison of Defined Daily Dose and Days of Therapy*. *23298*. https://doi.org/10.1086/511640

Prescott, J. F. (2013). Classes of Antimicrobial Agents Beta-lactam Antibiotics : Penam Penicillins. *Antimicrobial Therapy in Veterinary Medicine*, 135–153.

Principles of Conventional Soxhlet Use of Conventional Soxhlet. (1997). *1995*.

Ragasa, C. Y., Alimboyoguen, A. B., & Shen, C. C. (2014). Chemical constituents of ficus nota. *Der Pharma Chemica*, *6*(4), 98–101.

Ragasa, C. Y., Ebajo, V. D., De Los Reyes, M. M., Mandia, E. H., Brkljača, R., & Urban, S. (2015). Triterpenes and sterols from Ragasa, C. Y., Ebajo, V. D., De Los Reyes, M. M., Mandia, E. H., Brkljača, R., & Urban, S. (2015). Triterpenes and sterols from Sonneratia alba. International Journal of Current Pharmaceutical Review and Research, 6(6), 256–26. *International Journal of Current Pharmaceutical Review and Research*, *6*(6), 256–261.

Rakotoniaina, E., Donno, D., Randriamampionona, D., Andriamaniraka, H., Soifoini, T., Ramonta, I., Solo, N., Torti, V., Giacoma, C., & Beccaro, G. L. (2020). Insights into an endemic medicinal plant species of Madagascar: The case of famelona (Chrysophyllum boivinianum (Pierre) Baehni). *Acta Horticulturae*, *1267*, 85–90. https://doi.org/10.17660/ActaHortic.2020.1267.14

Ramalivhana, J., Samie, A., Iweriebor, B., Uaboi-Egbenni, P., Idiaghe, J. ., Momba, M. N. B., & Obi, C. (2014). Antibacterial activity of honey and medicinal plant exfile:///C:/Users/hp/Downloads/Documents/document_6.pdftracts against Gram negative microorganisms. *African Journal of Biotechnology*, *13*(4), 616–625. https://doi.org/10.5897/ajb11.892

Rasul, M. G. (2018a). *Conventional Extraction Methods Use in Medicinal Plants , their Advantages and Disadvantages*. *6*, 10–14.

Rasul, M. G. (2018b). *Extraction , Isolation and Characterization of Natural Products from Medicinal Plants*. *6*, 1–6.

Ríos, J. L., & Recio, M. C. (2005). Medicinal plants and antimicrobial activity. *Journal of Ethnopharmacology*, *100*(1–2), 80–84. https://doi.org/10.1016/j.jep.2005.04.025

Rivera, A. M., & Boucher, H. W. (2011). ON THERAPY CURRENT CONCEPTS IN THERAPY AGAINST Current Concepts in Antimicrobial Therapy Against Select. *Mayo Clinic Proceedings*, *86*(12), 1230–1243. https://doi.org/10.4065/mcp.2011.0514

Roberts, J. (2009). *CEPHALOSPORINS*. 7–10.

Roberts, M. C. (2003). Tetracycline therapy: Update. *Clinical Infectious Diseases*, *36*(4), 462–467. https://doi.org/10.1086/367622

Roberts, M. F., Strack, D., & Wink, M. (2018). Biosynthesis of Alkaloids and Betalains. In *Annual Plant Reviews online* (Vol. 40). https://doi.org/10.1002/9781119312994.apr0424

Rossolini, G. M., Arena, F., Pecile, P., & Pollini, S. (2014). Update on the antibiotic resistance crisis. *Current Opinion in Pharmacology*, *18*, 56–60.

Satish, S., Raghavendra, M. P., Mohana, D. C., & Raveesha, K. A. (2008). *Antifungal activity of a known medicinal plant Mimusops elengi L. against grain moulds*.

Sharaibi, O., & Osuntogun, O. (2017). Ethnomedicinal Information and Phytochemical Screening of Medicinal Plants Used in the Treatment of Diarrhea in Lagos State, Nigeria. *European Journal of Medicinal Plants*, *19*(4), 1–7. https://doi.org/10.9734/ejmp/2017/26815

Tacic, A., Nikolic, V., Nikolic, L., & Savic, I. (2017). Antimicrobial sulfonamide drugs. *Advanced Technologies*, *6*(1), 58–71. https://doi.org/10.5937/savteh1701058t

Talebi, A., & Abadi, B. (2019). *World Health Organization Report : Current Crisis of Antibiotic Resistance*. 778–788.

Tiwari, S., & Talreja, S. (2022). Thin Layer Chromatography (TLC) VS. Paper Chromatography: A Review. *Acta Scientific Pharmaceutical Sciences*, *6*(9), 05–09. https://doi.org/10.31080/asps.2022.06.0894

Traore, M. S., Baldé, M. A., Diallo, M. S. T., Baldé, E. S., Diané, S., Camara, A., Diallo, A., Balde, A., Keïta, A., Keita, S. M., Oularé, K., Magassouba, F. B., Diakité, I., Diallo, A., Pieters, L., & Baldé, A. M. (2013). Ethnobotanical survey on medicinal plants used by Guinean traditional healers in the treatment of malaria. *Journal of Ethnopharmacology*, *150*(3), 1145–1153. https://doi.org/10.1016/j.jep.2013.10.048

Vishovan, Y., Ushkalov, V., Kepple, O., & Granate, A. (2020). *ANTIMICROBIAL RESISTANCE AND BIOLOGICAL PROPERTIES OF STAPHYLOCOCCUS SPP . ISOLATED FROM PIGS*. *1*(1). https://doi.org/10.5281/zenodo.3701192

Wansi, J. D., Devkota, K. P., Tshikalange, E., & Kuete, V. (2013). Alkaloids from the Medicinal Plants of Africa. In *Medicinal Plant Research in Africa: Pharmacology and Chemistry*. Elsevier Inc. https://doi.org/10.1016/B978-0-12-405927-6.00014-X

Wood, C. A., Lee, K., Vaisberg, A. J., Kingston, D. G. I., Neto, C. C., & Hammond, G. B. (2001). A bioactive spirolactone iridoid and triterpenoids from Himatanthus sucuuba. *Chemical and Pharmaceutical Bulletin*, *49*(11), 1477–1478. https://doi.org/10.1248/cpb.49.1477

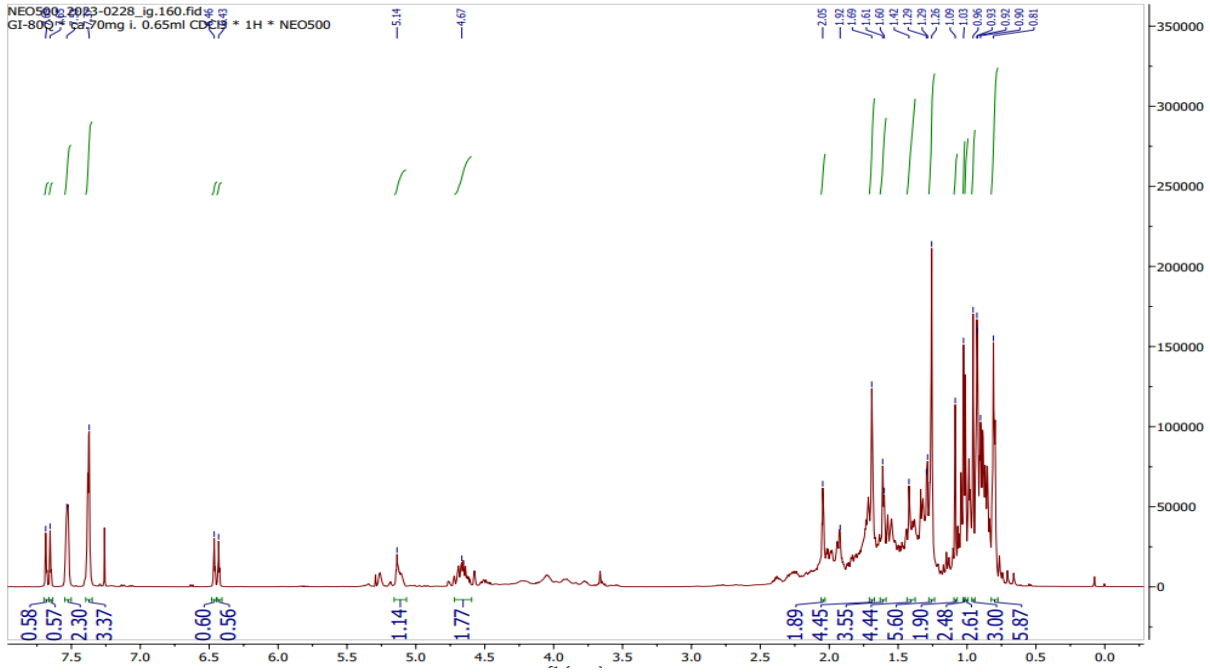
World Health Organization (WHO). (2018). Country cooperation strategy at a glance: Uganda. *Who*, 0–1. https://apps.who.int/iris/bitstream/handle/10665/136975/ccsbrief_uga_en.pdf?sequence=1&isAllowed=y

Xu, C., Wang, B., Pu, Y., Tao, J., & Zhang, T. (2018). Techniques for the analysis of pentacyclic triterpenoids in medicinal plants. *Journal of Separation Science*, *41*(1), 6–19. https://doi.org/10.1002/jssc.201700201

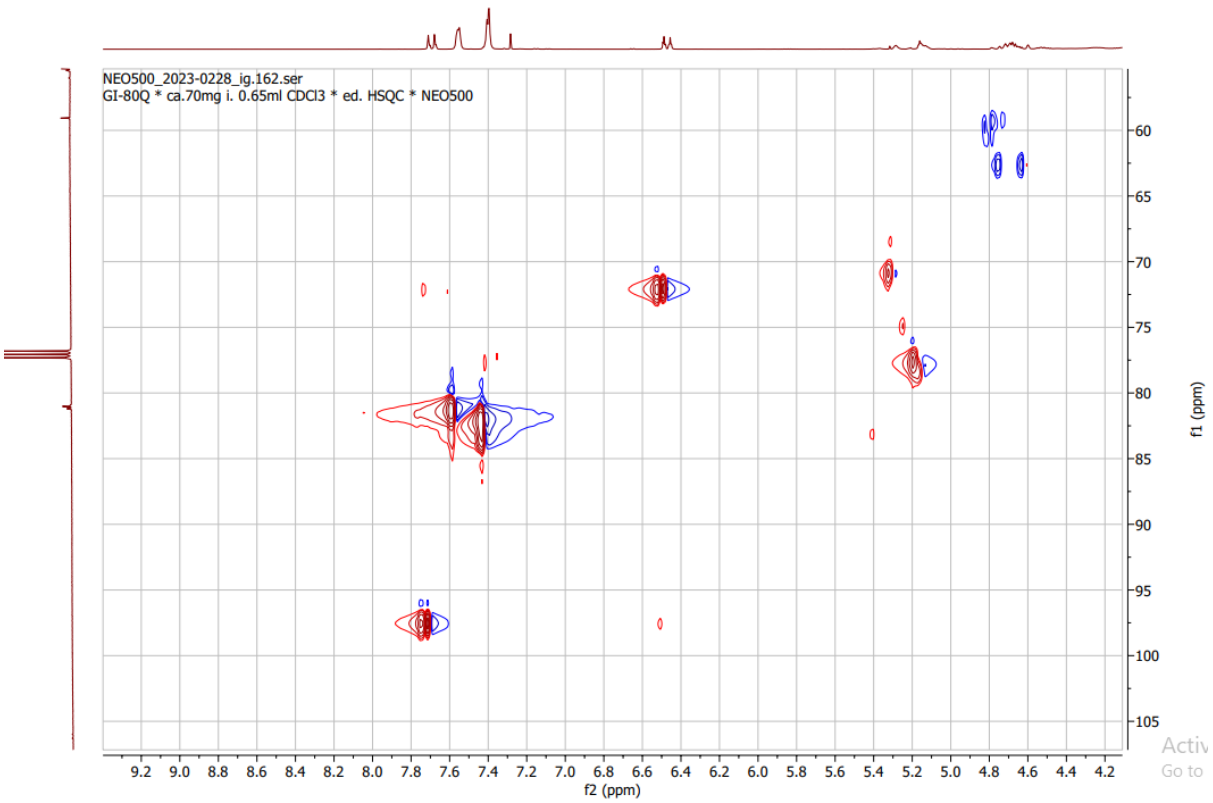
Yao, L. H., Jiang, Y. M., Shi, J., Tom´as, F. A., Tom´as-Barber´an, T., Barber´an, B., Datta, N., Singanusong, R., & Chen, S. S. (2004). Flavonoids in Food and Their Health Benefits. In *Plant Foods for Human Nutrition* (Vol. 59). Springer Science+Business Media, Inc.

APPENDICES

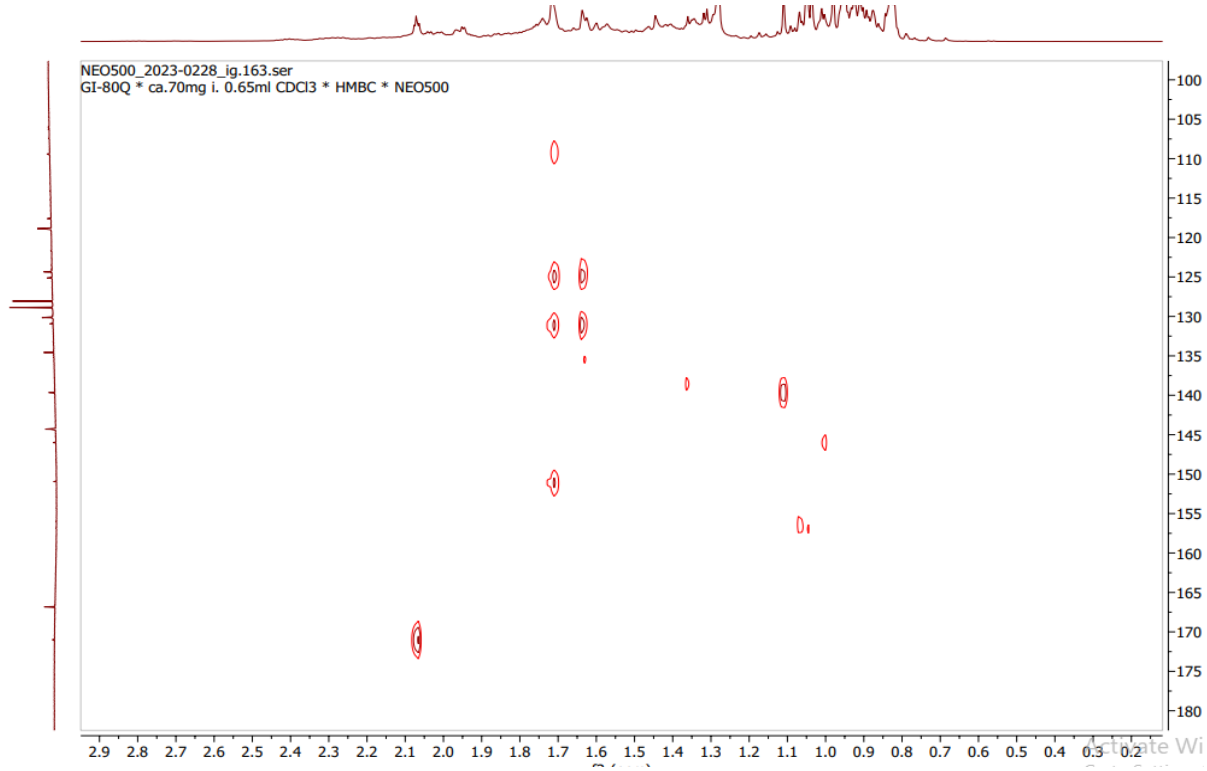
Proton NMR spectrum of compound **48**



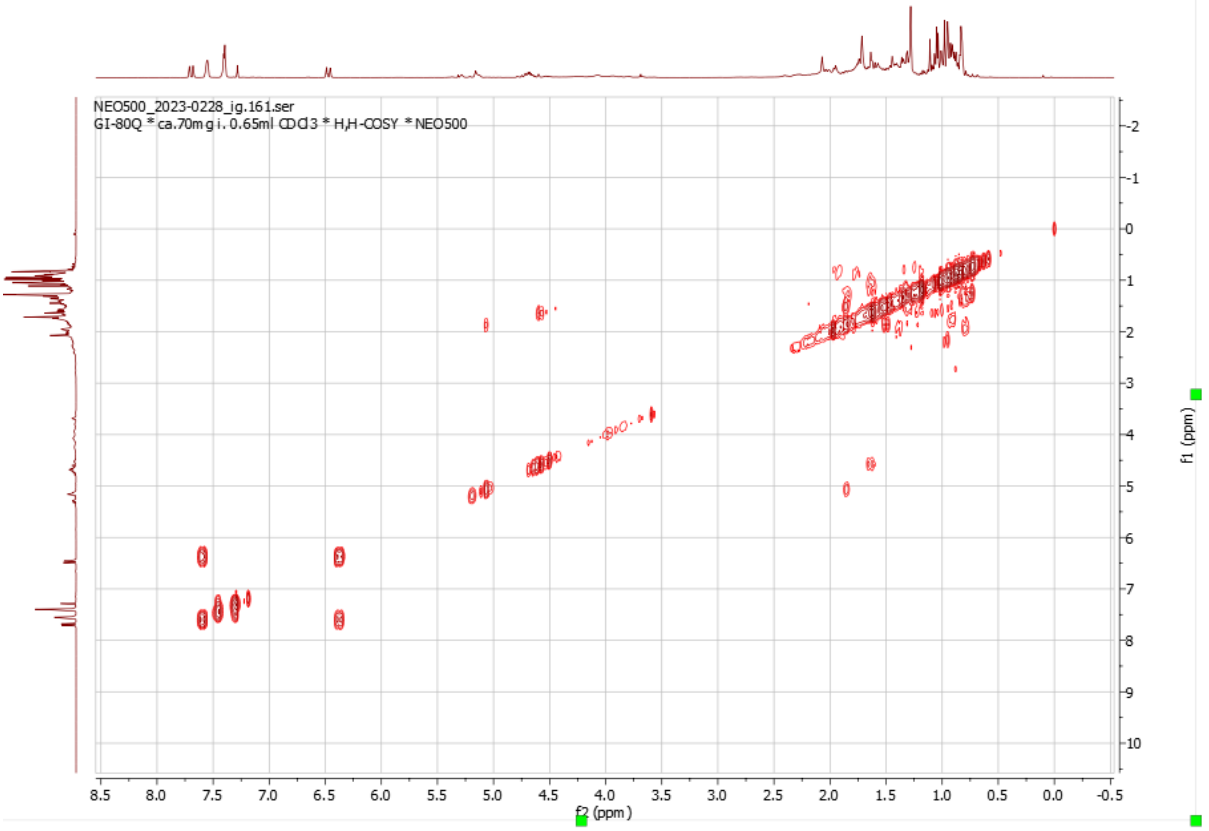
HSQC NMR spectrum of compound **48**



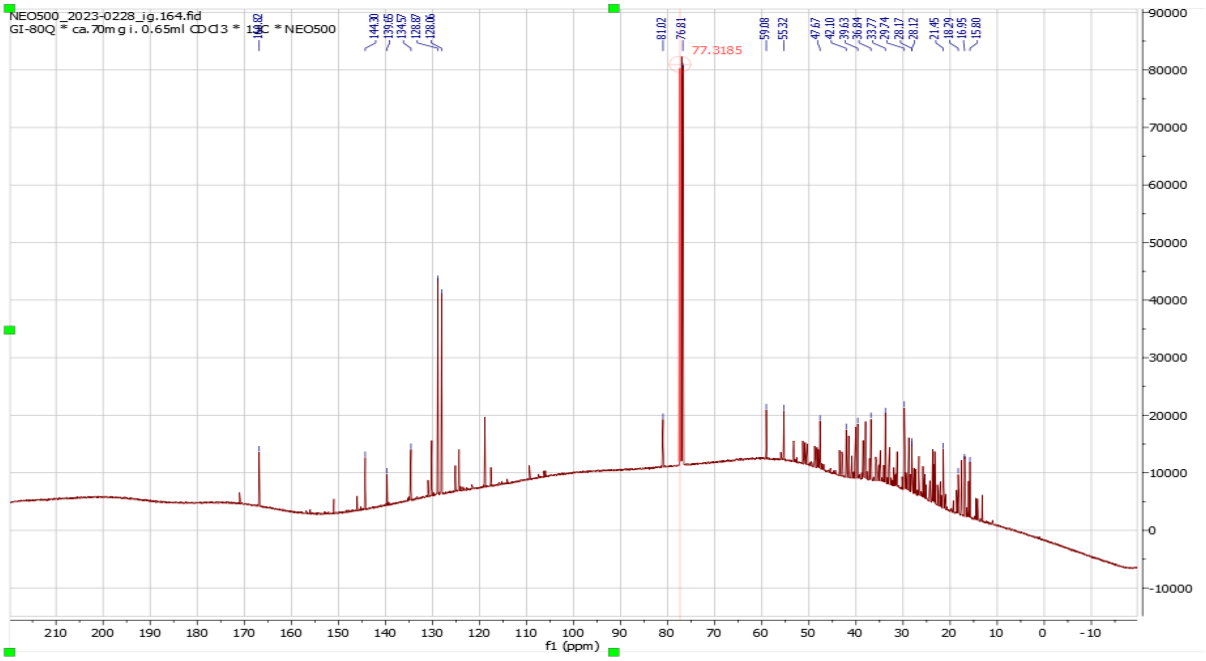
HMBC NMR spectrum of compound **48**



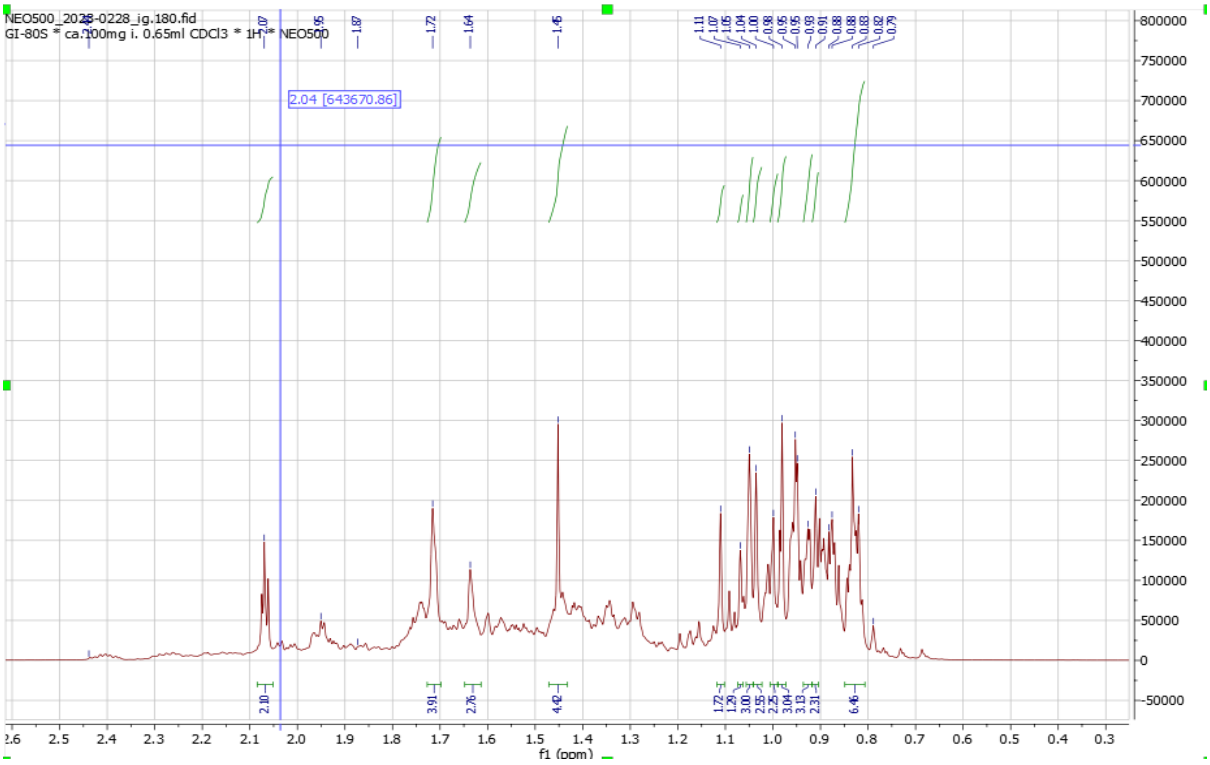
COSY spectrum of compound **48**



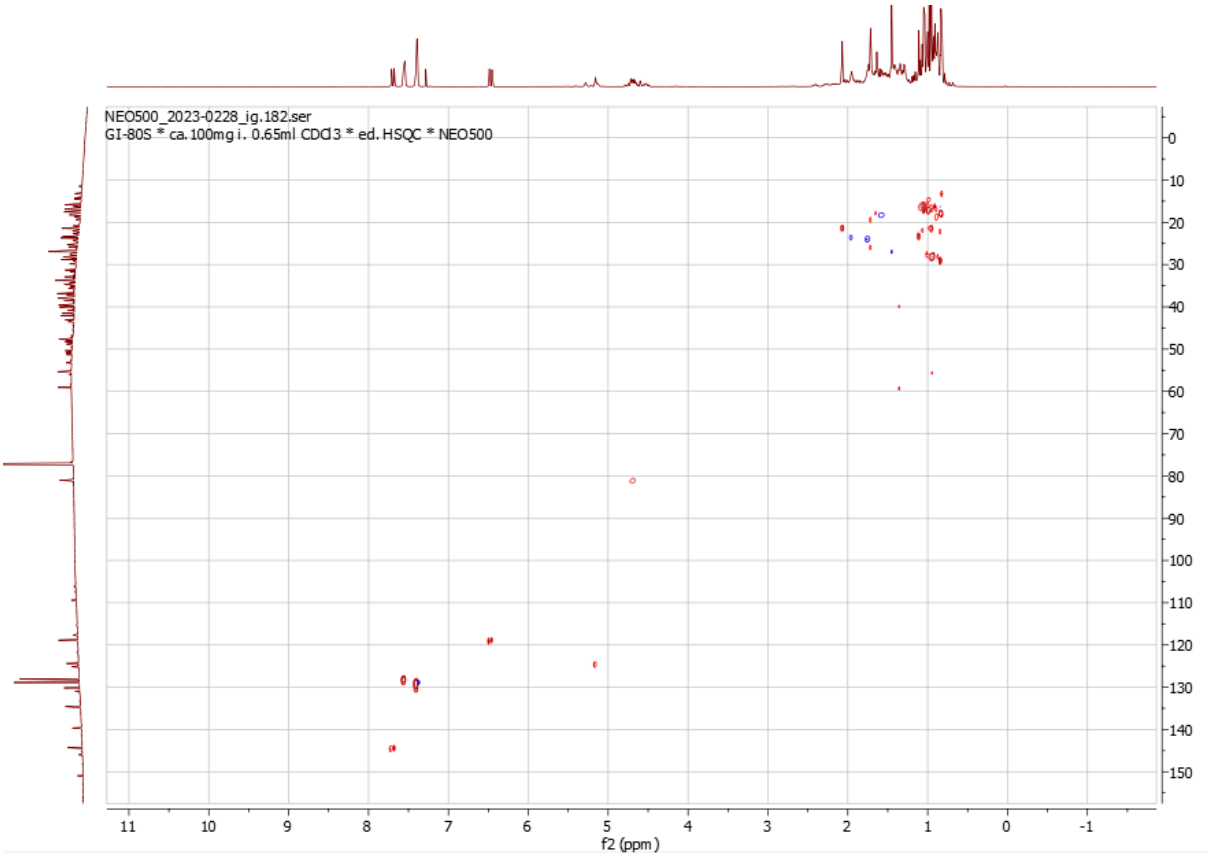
Carbon-13 NMR spectrum of compound **48**



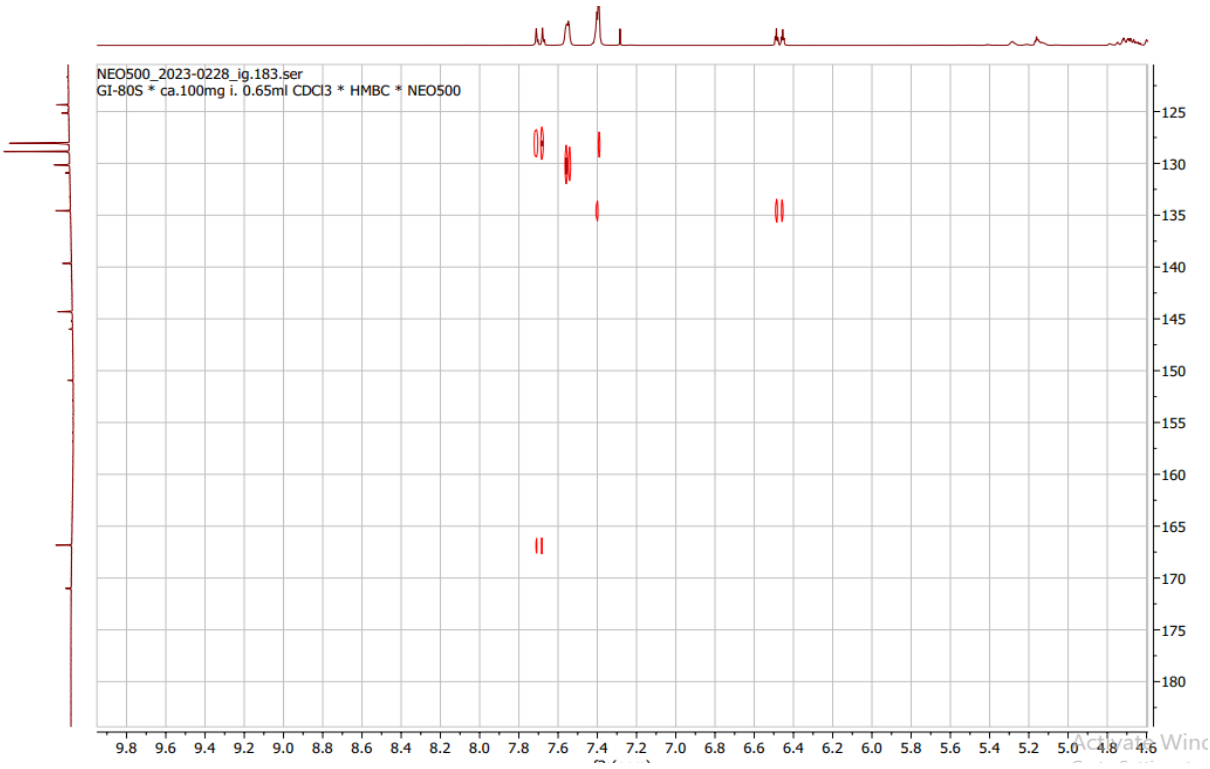
Proton NMR spectrum of compound **49**



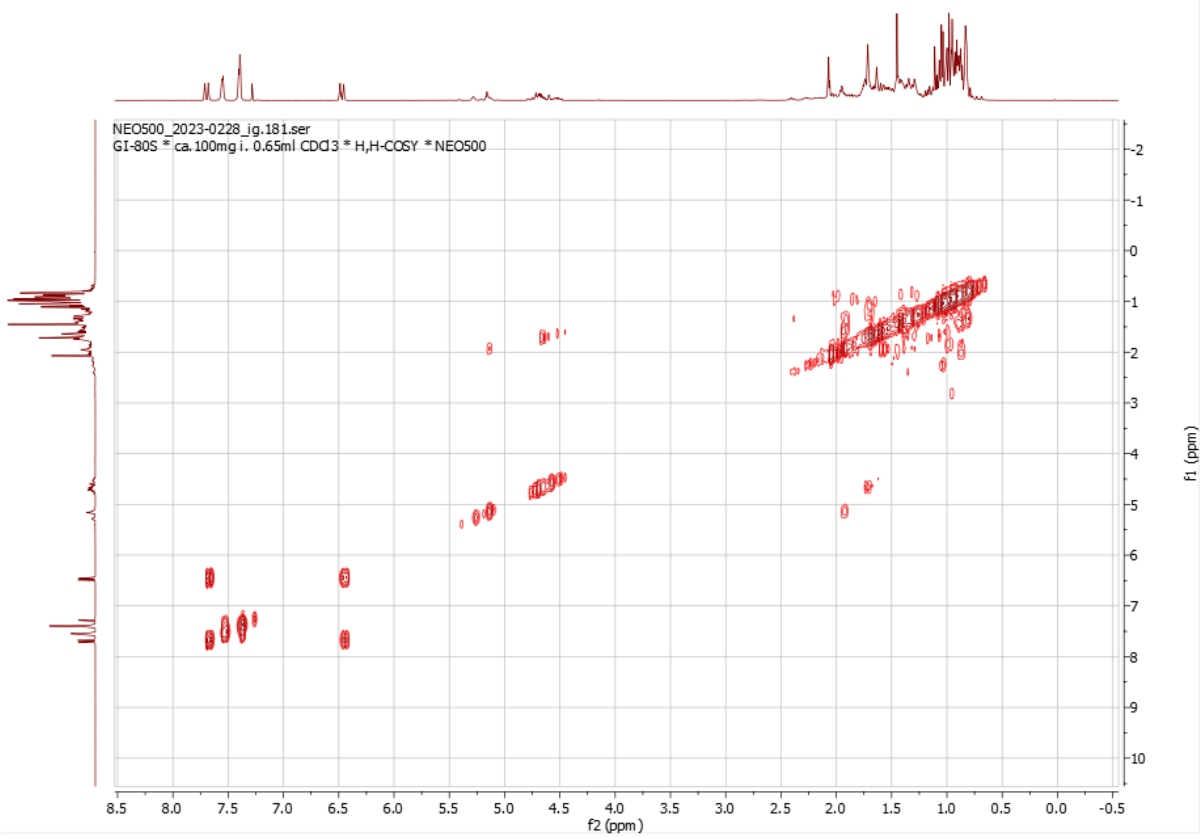
HSQC NMR spectrum of compound **49**



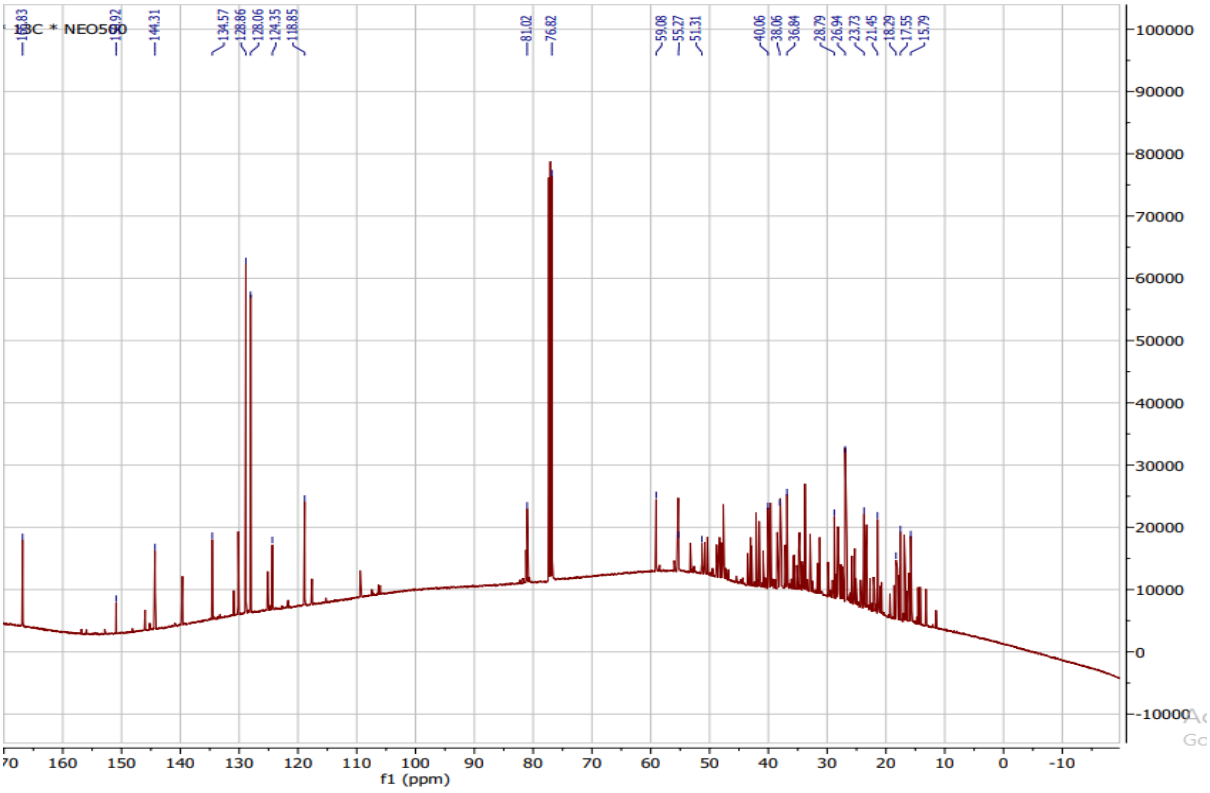
HMBC NMR spectrum of compound **49**



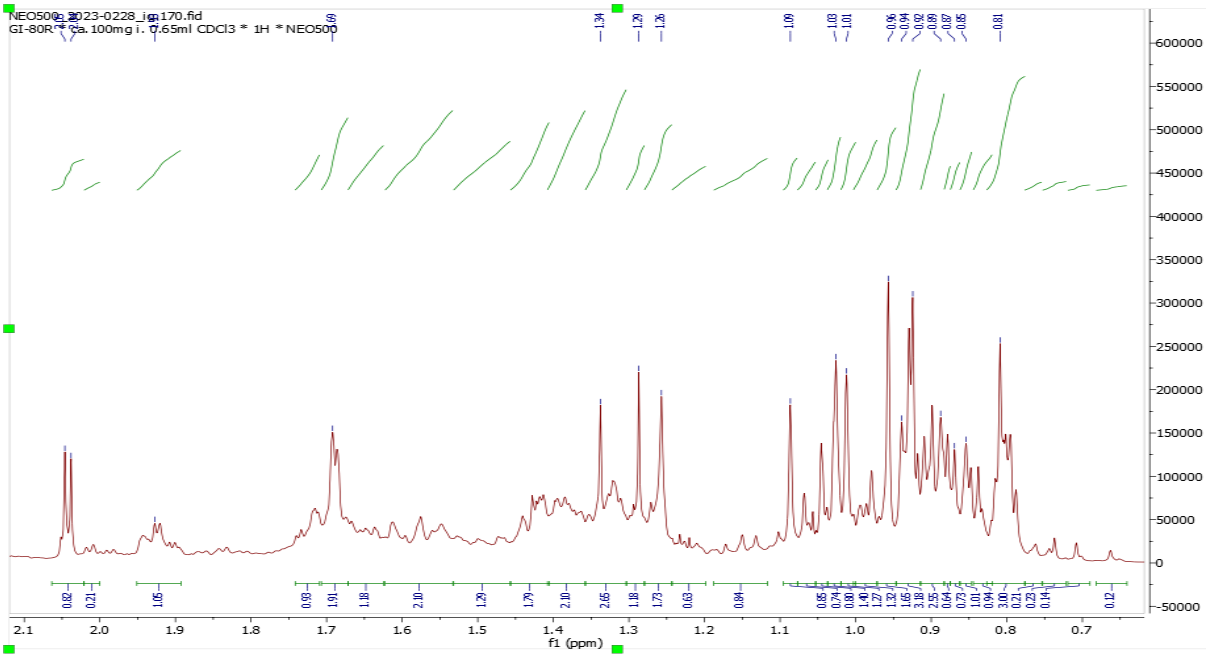
COSY spectrum of compound **49**



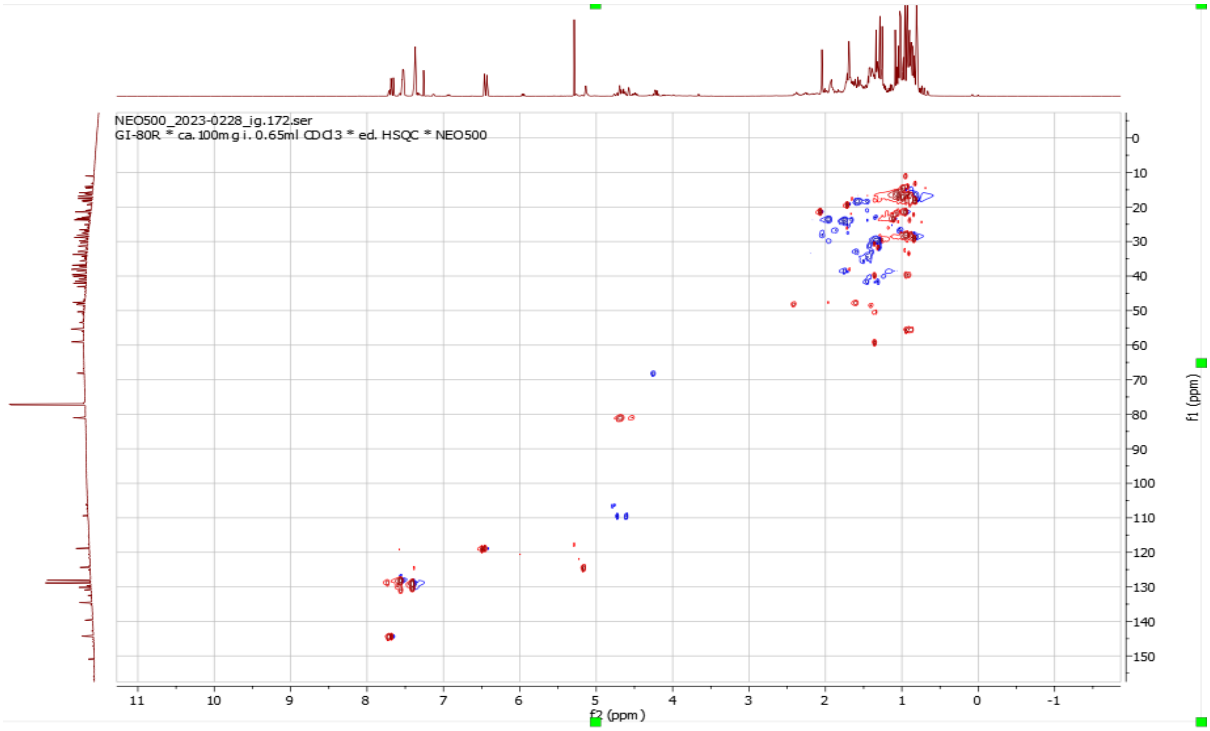
Carbon-13 NMR spectrum of compound **49**



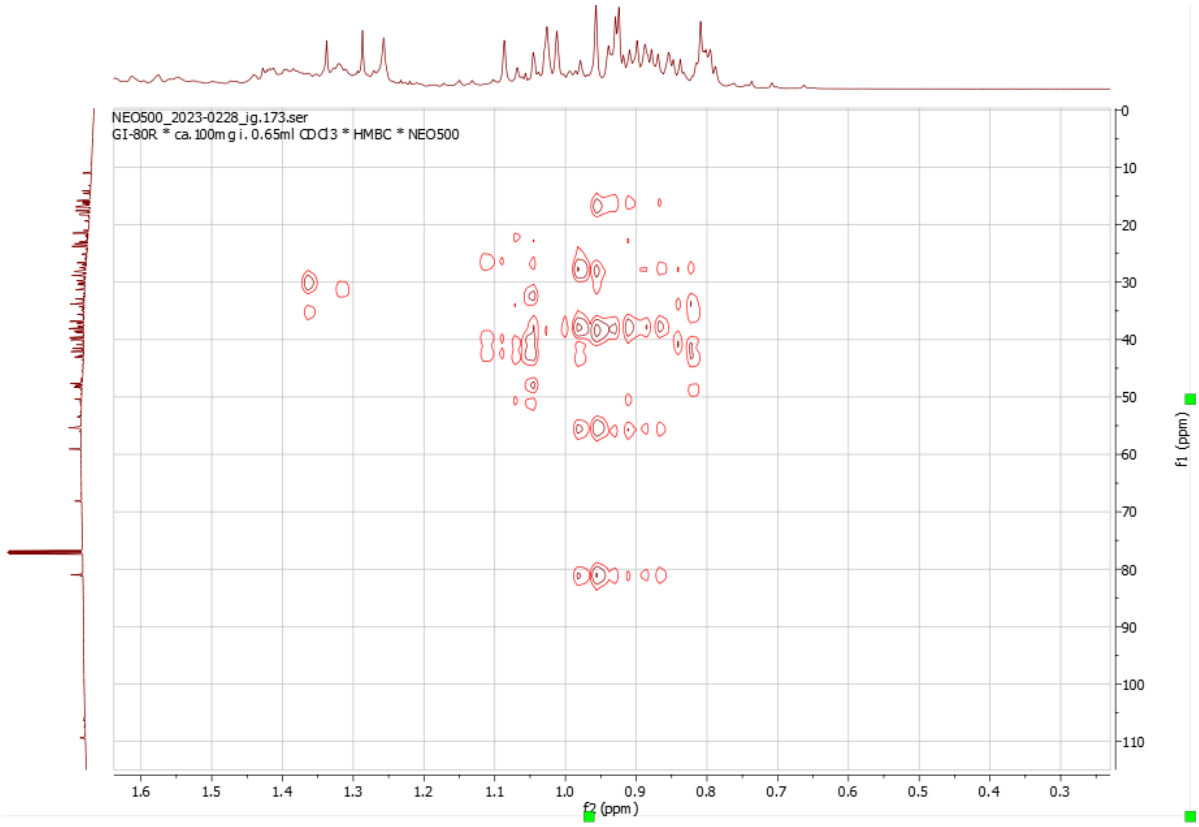
Proton NMR spectrum of compound **50**



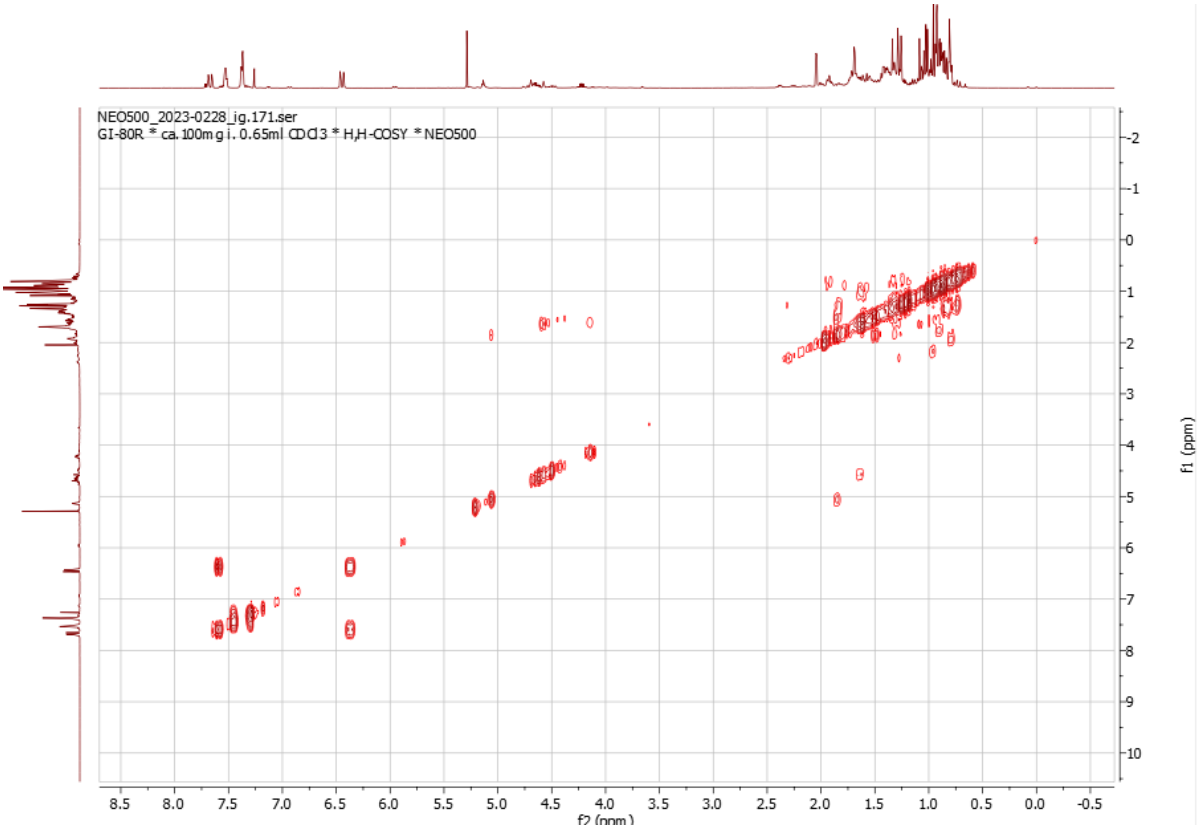
HSQC NMR spectrum of compound **50**



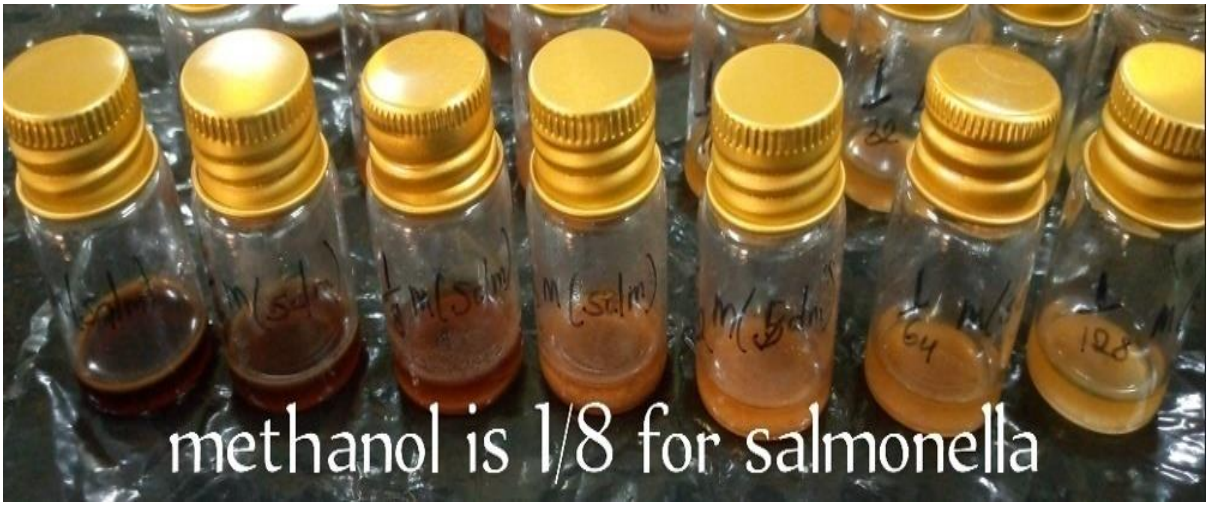
HMBC NMR spectrum of compound **50**



COSY spectrum of compound **50**



MIC of crude methanol extract against *Salmonella typhi*



Diameter of inhibition of DCM and hexane crude extracts on *Pseudomonas aeruginosa*