PHYTOCHEMICAL INVESTIGATION OF *IPOMOEA CAIRICA* FOR ANTIMICROBIAL AGENTS

BY

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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

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DECLARATION

I, Boniface Opio, declare that this dissertation entitled **"Phytochemical investigation of *Ipomoea cairica* for Antimicrobial Agents"** is my own original work, and it hasn't been partially or fully submitted to any other institutions for review, publishing, or the conferral of any degrees. Additionally, in compliance with the regulations, the contributions of others have been appropriately acknowledged and cited in accordance with the requirements of Kyambogo University.

Signature Date

…… ……

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APPROVAL

The Dissertation entitled **"Phytochemical Investigation of *Ipomoea cairica* for antimicrobial agents"** by Mr. Boniface Opio has been written and submitted to the Department of Chemistry, Kyambogo University, with our endorsement as the supervisors.

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DEDICATION

With lots of love, I dedicate this work to my family especially my dear wife Susan Sarah Opio and children for all the support and care rendered to me spiritually, morally and financially that has enabled me to achieve this tedious work. Most importantly for tolerating my absence from home in many occasions.

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"I don't know what tomorrow will bring." (Fernando Pessoa)

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“The day your family stops to be first in your life ... Go back, because you were wrong on the way” (Raul Minh’alma)

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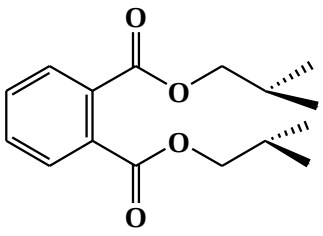
ABSTRACT

The emergence of new infectious diseases and the resurgence of several infections has put the people in Saharan and sub-Saharan Africa to an assiduous risk. This has created the necessity for studies directed towards the development of new alternatives for antimicrobial agents. In this study a portion of the dichloromethane/methanol (1:1, v/v) crude extract from the air dried and pulverized aerial parts of *Ipomoea cairica* was subjected to preliminary phytochemical screening which revealed the presence of alkaloids, sterols, flavonoids, tannins, saponins, terpenoids and phenols. Another portion of the crude extract was subjected to repeated column chromatography over silica gel leading to the identification of two compounds; Diisobutyl phthalate (**53**) and Friedelin **(54)** which were characterized and elucidated using various spectroscopic and spectrometric techniques. The crude extract and the isolated compounds were evaluated for antimicrobial activities against four bacterial strains; *Escherichia coli, Salmonella typhi, Pseudomonas* and *Staphylococcuss aureus,* and three fungal strains; *Aspergillus niger, Penicillum chrysogenum* and *Candida albicans* using well agar diffusion assay and their minimal inhibitory concentration (MIC) values determined using a 2-fold dilution technique. The crude extract exhibited good antimicrobial activities with zones of inhibitions; 20 ± 0.25, 26 ± 0.10, 24 ± 0.12 and 14 ± 0.05 mm for *E. coli, S. typhi, P. aeruginosa* and *S. aureus*, respectively, and the zones of inhibitions for fungal strains were as follows; 16 ± 0.5, 24 ± 0.00 and 20 ± 0.41 mm for *A. niger, P. chrysogenum* and *C. albicans,* respectively. The two isolated pure compounds were only tested against the four strains of bacteria and exhibited relatively weak activities: Compound (**53**); 8.0 ± 0.22, 4.0 ± 0.32, 6.0 ± 0.00 and 6.0 ± 0.55 mm, and Compound (**54**); 8.0 ± 0.05, 5.0 ± 0.50, 8.0 ± 0.12, 10 ± 0.50 mm for *E. coli, S. typhi, P. aeruginosa* and *S. aureus,* respectively. The two isolated pure compounds showed minimum inhibitory concentration (MIC) ranging from 125 to 1000 μg/ml respectively.



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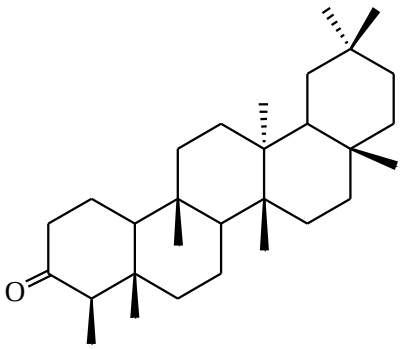

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LIST OF ABBREVIATIONS AND ACRONYMS

BBzP	Butyl Benzyl Phthalate
br s	Broad Singlet
CC	Column Chromatography
DCM	Dichloromethane
dd	Doublet of doublets
ddt	Doublet of doublets of triplets
DIBP	Di-isobutyl phthalte
DMP	Dimethyl Phthalate
DMSO	Dimethyl Sulphoxide
DNA	Deoxyribonucleic Acid
dt	Doublet of triplets
ESI – MS	Electron sprays ionization mass spectrometer
et al	*et alii,* and others
EtOAc	Ethyl Acetate
HCOSY	Homonuclear Correlation Spectroscopy
H-HCOSY	Proton-proton Correlation Spectroscopy
HMBC	Heteronuclear Multiple Bond Correlation
HSQC	Heteronuclear Single Quantum Coherence

Hz	Hertz
I.C	*Ipomoea Cairica*
IC_{50}	50% Inhibition Concentration
J	Coupling constant
m	Multiplet
MBC	Minimum Bactericidal Concentration
MeOH	Methanol
MHz	Mega Hertz
MIC	Minimum Inhibition Concentration
n-hexane	Normal hexane
NMR	Nuclear Magnetic Resonance
NOE	Nuclear Overhauser effect (Enhancement)
q	Quartet
s	Singlet
t	Triplet
TLC	Thin Layer Chromatography
U V	Ultra Violet
W.H.O	World Health Organization
δ	Chemical shift (delta)

CHAPTER ONE: INTRODUCTION

1.1 Background

Despite the significant progress in the various drugs available to treat bacterial and fungal infections, diseases caused by bacteria and fungi remain a major worldwide health concern due the rapid development of resistance to the existing drugs. Microbial diseases rank as number one cause for almost half of the deaths in underdeveloped and tropical countries (Adsul *et al*., 2012). The frequency of life-threatening infections caused by pathogenic microorganisms has increased worldwide and is becoming an important cause of mortality in immune compromised patients even in developed countries (Adsul *et al*., 2012). This is because some microbes have developed resistance to the available antimicrobial drugs (Kumari *et al*., 2016). Changes in the environment have also placed certain human populations in contact with newly identified microbes that are currently causing diseases never seen before. Furthermore, majority of the agents used in the treatment of bacterial and fungal infections are associated with adverse side effects (Elujoba *et al*., 2005). It is therefore imperative to continue searching for compounds that may be safe and with novel modes of actions. In this study, evaluation of the ethnomedicinal and pharmacogical properties of *Ipomoea cairica*, were determined with special emphasis on its antimicrobial activity and chemical analysis.

The medicinal value of this plant lies in bioactive phytochemical constituents that produce definite physiological action on the human body (Akinmoladun *et al*., 2007). Previous work shows that the major bioactive phytochemical

constituents of *I. cairica* are alkaloids, essential oils, flavonoids, tannins, terpenoids, saponins, phenolic compounds (Edeoga *et al*., 2005).

Figure 1: Shows *Ipomoea cairica* (leaves, flowers and stem) plant taken from its natural habitat by Boniface Opio.

The World Health Organization estimated that about three quarters of the population in developing countries relies on plant based medicines as the basic needs for human primary health care (Adsul *et al*., 2012). Antimicrobial agents are abundant in medicinal plants and are substances that are used to either eliminate or inhibit the growth of bacteria (Hassan *et al*., 2015). Microorganisms include bacteria, viruses, protozoans and fungi. Many plants are being used traditionally because of their curative properties and are a source of many potential and powerful drugs. The antibacterial capabilities of hundreds of plant species have been examined, however the bulk of them have not been sufficiently assessed. Therefore, more documentation of this wisdom in either written or other forms is called for (Mandal *et al*., 2015). These natural

compounds form the foundations of modern prescription of drugs as we know today (Lahlou, 2013).

Phytochemicals are natural bioactive compounds found in plants in parts such as; fruits, flowers, leaves, stem barks and roots that work with nutrients and fibers to act as defense system against diseases or to protect against diseases (Hassan *et al*., 2015). These natural plant chemicals provide plants with colour, odour and flavor (Hassan *et al*., 2015). They are produced for defense purposes against infections and predation. Once they are eaten or taken into the body, they can influence the endogenous chemical processes hence, have the potential to: Firstly, stimulate the immune system, secondly, block substances eaten, drunk and breathed from becoming harmful (toxic), thirdly, prevent DNA damage and help with DNA repair, fourthly, reduce the kind of oxidative damage to cells that can spark complications, fifthly, trigger damaged cells to commit suicide before they can reproduce, and lastly help to regulate hormones (Hassan *et al*., 2015)

Ipomoea cairica is a good source of medicine and is used traditionally to treat various bacterial and infections in humans and animals; just like how quinine was first discovered as natural products from the bark cinchona tree, by a Scottish doctor, George Cleghorn to treat malaria. Also, tetracyclines were first discovered as natural products from actinomycetes soil bacteria and reported in the scientific literature in 1948. They were recognized for their interesting broad spectrum antibacterial activity which were observed successfully in the clinical trials (Levy, 2011 & Mikail *et al*., 2022;). There has been continuous evolution of generations of the tetracycline scaffold toward derivatives with improved potency and efficacy against tetracycline-resistant bacteria, with promising

pharmacokinetic and chemical properties (Choudhary, 2022). Therefore, the development of new antibacterial drugs starts by identifying an effective antibacterial plant used by local communities. *Ipomoea cairica,* is used by people in central and eastern Uganda to treat bacterial and fungal infections such as stomachache, cough, scabies, burns, pain relief. It is also used to treat blood disease, sterility in women, urinary tract infection and constipation (Kumari *et al*., 2016). In most cases, the leaves (dry or wet) are crushed and the extract taken as prescribed by the herbalist or an elder while others use the dry leaves as beverages and others put in water use for bathing (Singh *et al*., 2020).

1.2 Statement of the problem

The use of herbal medicines to treat bacterial and fungal infections is getting momentum, yet much of their phytochemical constituents responsible for their therapeutics are not known. Hence, there is need to search for new potential antimicrobial agents from plant origin. This study therefore was directed at investigating, isolating and characterizing the antimicrobial agents from *Ipomoea cairica*.

1.3 Objectives of the study

1.3.1 General objective

The general objective of this study was to isolate and determine the structures of antimicrobial agents from *Ipomoea cairica.*

1.3.2 Specific objectives

The specific objectives of this study were to:

i. Extract and test for the bioactivity of the crude extract.
ii. Isolate the bioactive ingredients.

iii. Carry out antimicrobial assay on the crude and pure isolated compounds.

iv. Elucidate and determine the chemical structures of the compounds isolated.

1.4 Justification of the study

The pharmacological actions of *I. cairica* imply that it contains antimicrobial agents. Some of these compounds may be identified through chromatographic and spectroscopic analyses plus *in-vitro* antimicrobial assays.

1.5 Significance of the study

This study may lead to the discovery of an alternative form of treatment of bacterial and fungal infections. This will be a great move towards reducing the side effects associated with current synthetic antimicrobial treatment methods. The isolated active compounds may be used as templates to synthesize more effective antibacterial drugs with new modes of action which is different from the current antibacterial drugs. Plant products could be included in the primary health care as encouraged by world health organization (Elujoba *et al*., 2005). Furthermore, screening plants for biologically active compounds helps to conserve medicinally useful plant species the population will be sensitized about the importance of the plants.

CHAPTER TWO: LITERATURE REVIEW

2.1 Microbial infections

Microbial infections are ailments or sicknesses caused in animals and humans by the introduction of the following microbes such as bacteria, viruses, fungi and protozoa (Baumgardner, 2012). A person can transmit microbes to someone else through the following ways; First mode of transmission is by sneezing or coughing which can transmit viruses that cause colds or flue or the bacteria that causes tuberculosis, secondly by shaking hands or touching surfaces which are contaminated such as door shutters, computer key boards to mention but a few and lastly, sexual intercourse can transmit microbes such as herpes simplex virus which causes genital herpes, gonorrhea bacteria from one person to another (Chakraborti *et al*., 2019).

2.1.1 Treatment drugs available in the market

Drugs used to treat microbial infections are termed antimicrobials. It includes a group of drugs that are generally classified as; antibiotics, antifungals, antiprotozoals and antivirals like penicillin G, penicillin V, benzathine penicillin, tetracycline, that for centuries have been effective in treating many diseases such as syphilis and other infections caused by Staphylococci and Streptococci bacteria. In developing countries, the World Health Organization estimated that about three quarters of the population relies on plant based medicines as the basic needs for human primary health care (Khatiwora *et al*., 2012). This is because, microbes are developing new properties to resist drug treatment that were once effective at destroying them. Changes in the environment have also placed certain human populations in contact with newly identified microbes that can cause diseases never seen before. Herbal medicine

is the use of herbs for their therapeutic or medicinal values. Herbal plants produce and contain a variety of chemical substances (bioactive ingredients) that act upon the body. Herbalists and community therefore use the leaves, flowers, stem barks, berries, and roots of plants to prevent, relieve, and treat illness of different kinds. Uganda has an immense wealth of rich biodiversity knowledge about ethnomedicine (medicinal plants) which has been accumulated by villagers and tribal people that are unknown to scientists and urban people (Kamatenesi-Mugisha & Oryem-Origa, 2007). People have been using herbs and various plant products for combating diseases from ancient times. Millions of people throughout the world have a greater respect for all things natural as a result of their dissatisfaction with the efficiency and cost of modern medicine. This has led to the usage of plant-based remedies or drugs for the treatment of various ailments. Numerous types of herbs have been well recognized and catalogued by botanists including *I. cairica* (Soewu & Adekanola, 2011).

2.1.2 Limitations of traditional medicine

Although traditional medicine is widely used in management of many di sease ailments, it has some limitations. The main limitations of tradition al remedies are the lack of standardization of raw materials, processing methods and plant products, dosage formulation and the nonexistence of criteria for quality control. The World Health Organization is now acti vely encouraging developing countries to use herbal medicine which hav e been traditionally used for centuries. Global estimates indicate that ov er 75% of world population cannot afford the products of Western phar

maceutical industry and have to rely upon the use of herbal medicines (Kour *et al., 2021).*

The potential of plants as sources for new drugs is still largely unexplo red. Among the estimated 250,000-

500,000 plant species, only a small percentage has been investigated ph ytochemically and the fraction submitted to biological or pharmacologica l screening is even smaller. Although, for example, the National Cancer Institute (NCI) of the United States screened some 35, 000 plant speci es for antitumor activity from 1957 to 1981, these plants will still hav e to be considered as 'uninvestigated' with respect to any other pharma cological activity (Newman & Cragg, 2020). Plants contain hundreds or thousands of metabolites. Thus, any 'phytochemical investigation of a gi ven plant will reveal only a very narrow spectrum of its constituents. T he process that leads from the plant to a pharmacologically active, pure constituent is very long and tedious, and requires a multidisciplinary co llaboration of botanists, pharmacognosists, chemists, Pharmacologists and toxicologists (Ramawat *et al*., 2009). This approach involves the follow ing steps:

The first step is collection, proper botanical identification, authentication and drying of the plant materials. The second step involves preparation of appropriate extracts and preliminary chromatographic analysis by Col umn Chromatography (CC), Thin Layer Chromatography (TLC) and Hig h Pressure Liquid Chromatography (HPLC) where each fraction obtained has to be submitted for bioassay in order to follow the activity (activit y guided fractionation). The third step deals with biological and pharma

cological screening of crude extracts. Fourthly, bio-
assay of pure constituent(s). Most of the bioassays require specialized fa cilities for cell culture and the technical know-
how of a biochemist, biologist or pharmacologist. Finally, structure eluci dation and structure modification of the compounds (Aribi *et al*., 2022; Mroczek *et al*., 2020).

2.2 Botanical information of the plant under study

The *I. cairica* belongs to Convolvulaceae family. It is a climbing herb which is found abundantly in tropical and sub-
tropical regions. It has many common names such as railroad creeper, Cairo morning glory, five fingered-
morning glory, messina creeper, mile-a-
minute vine among others (Sengupta & Dash, 2020). It is a rampant lo ng-
lived (perennial) climber reaching up to 5 m or more in height or creep ing along the ground and fences. It has very distinctive leaves with 5-7 finger-like lobes, large purple, purplish-
pink or whitish tubular flowers (4-6 cm long and 5-
8 cm across) with a darker center. (Srivastava & Shukla, 2015). The *I. cairica* has the following taxonomy as shown in the **Table 2.2.1**.

Table 2.2.1: Taxonomy of *Ipomoea cairica*

Domain	Eukaryota
Kingdom	Plantae
Phylum	Spermatophyta
Sub phylum	Angiospermae
Class	Dicotyledonae

Oder	Solanales
Family	*Convolvulaceae*
Genus	*Ipomoea*
Species	*Ipomoea cairica*

The *Ipomoea* is an ever-

green herbaceous perennial climbing plant, producing slender stem up to five (5) meters long from a tuberous stock. *Ipomoea* is the largest gen us in the flowering plant family Convolvulaceae, with numerous species (Hançerli *et al*., 2018).

The generic name is derived from the Greek words meaning "resemblin g" which refers to their twining habit. The genus *Ipomoea* has over 400 species all over the world from *Ipomoea palma*te forks or *Ipomoea cai rica* L. which grows abundantly in Egypt (Kishore *et al*., 2014).

Below are some of the different species of *ipomoea* other than the *Ipo moea cairica*; *I. batatilla, I. crasscaulis, I. fistulosa, I. albiflora, I. fruti cosa I. gossypioides, I. nicaraguensis, I. transvaalensis and I. texana.*

2.2.1 Plant morphology

Perennial plant with long fusiform tuberous rootstock. Stems are annual, herbaceous, and sub erect or prostrate, up to 1 m long. Leaves are nar rowly deltoid-cordate to broadly cordate-

suborbicular, up to 45 mm long. Corolla is funnel-shaped, 20-

40 mm long, pink to magenta or white with purple center. The most pe culiar aspect is the bright orange fuzzy seeds. The major bioactive const

ituents previously isolated from the genus *Ipomoea* were lipoidal matters and phenolic compounds (John *et al*., 2021).

2.2.2 Habitat

Ipomoea cairica is mainly a weed of waste areas, disturbed sites, rainfo rest margins, open woodlands, bush land, gardens, fences, coastal sand d unes and vegetation growing near waterways (i.e., riparian areas). The g enus *Ipomoea* occurs in the tropics of the world although some species also reaches temperate zones. The species of this genus are mainly distr ibuted throughout the South and Central American countries and Tropica l African territories. It therefore inhabits tropical, subtropical and warmer temperate environments (Kishore *et al*., 2014).

2.2.3 Stems and Leaves

The slender stems are hairless (i.e., glabrous), grow in a twining habit, and sometimes produce roots at the joints (i.e., nodes). The alternately a rranged leaves (3-10 cm long and 3-
10 cm wide) are divided into five or seven narrow lobes, like the finge rs of a hand (i.e., they are palmately lobed). These leaves are hairless (i.e. glabrous) and borne on stalks (i.e., petioles) 2-
6 cm long (Srivastava & Rauniyar, 2020).

2.2.4 Flowers and Fruit

The funnel-shaped (i.e. tubular) flowers are purple to pinkish-
purple (occasionally white) with a darker purple center. They are borne singly or in small clusters on short stalks originating in the leaf forks (i .e. axils). These flowers (4-6 cm long and 5-

8 cm across) have five petals that are fused into a tube (i.e. corolla tub e) and five small sepals (4-
7 mm long). Flowering occurs throughout the year. The fruit capsules a re more or less globular (i.e. sub globose) in shape and turn from gree n to brown in colour as they mature. These capsules (10-
12 mm across) contain four large brown seeds (about 6 mm across) tha t are slightly three-
angled in shape. The seeds have smooth surfaces interspersed with dens e tufts of long silky hairs (Ma *et al*., 2020).

2.2.5 Reproduction and Dispersal

This plant reproduces vegetatively by rooting along its stems and also p roduces seeds. Stem fragments and seeds are often dispersed in dumpe d garden waste and can also be spread by water. It can be planted in gardens, in hedges on walls, mainly for ornamental purpose. Fruiting ho wever is rare and seeds are often not well developed. This explains wh y propagation is mostly by vegetative method (Kishore *et al*., 2014).

2.3 Ethnobotanical uses of *Ipomoea* species

Humans use *Ipomoea* for their contents of medicinal and psychoactive c ompounds mainly the alkaloids to treat various diseases and other disord ers such as gynecological disorders, liver complaints, fever, antioxidant, anti-inflammatory, antimicrobial and anti-
allergic (Londhe *et al*., 2017). It can also be used as food for example, tubers of sweet potatoes and leaves of water spinach are (Mohanraj & Sivasankar, 2014). The various species of *Ipomoea* have wide medical applications. They are used to treat various diseases including; blood dis

ease, sterility in women, urinary tract infection and constipation (Hossan *et al*., 2010; Shah *et al*., 2013). The ethanolic extract of this plant presents an antinociceptive effect i.e., acts as a very strong pain killer (Hossan *et al*., 2010). In Brazilian folk medicine *I. cairica* L. Sweet (Convolvulaceae) is used for the treatment of rheumatism and inflammations (John *et al*., 2021). The aqueous extract from *I. cairica* flower is reported to show anti-

RSV (respiratory syncytial virus) activity *in vitro* (Odimegwu *et al*., 2011). The essential oil of *I. cairica* possesses remarkable larvicidal properties which can induce 100% mortality in the larvae of *Culex tritaeniorhynchus* (100 ppm), *Aedes aegypti* (120 ppm), *Anopheles stephensi* (120 ppm) and *Culex quinquefasciatus* (170 ppm) (Govindarajan *et al*., 2013). Phytochemical composition, antioxidant and other biological activities vary with different environmental conditions and climatic conditions (Bolling *et al*., 2011).

2.3.1 Anti-microbial activity

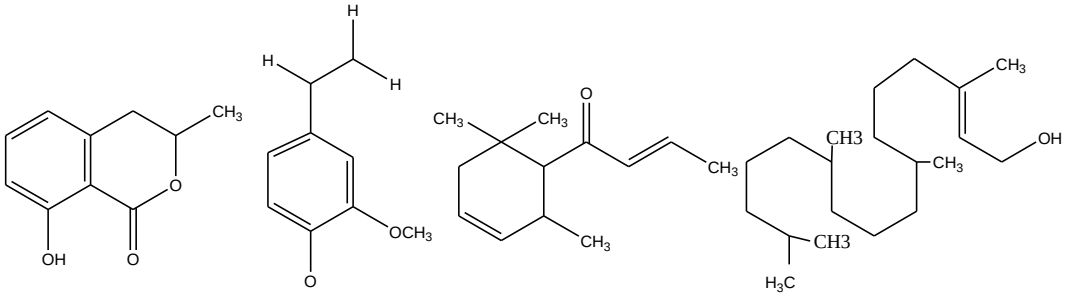
Methanolic extract of leaves of *I. cairica* possesses good anti-

microbial potential presumably because of its phytochemical constituent's correlation with its reductive potentials. The *Ipomoea cairica* leaves are therefore an effective antimicrobial and antioxidant agents that are used as folk medicine to treat and control many diseases; cancer, cardiovascular disorders, diabetes, asthma, and skin infections (Dar *et al*., 2015; Jabborova *et al*., 2019; Shyam Kishore & Upadhyay, 2015).

2.3.2 Anti-inflammatory activity

The aqueous methanol extract of *Ipomoea palmate* proved a remarkable and significant anti-

inflammatory activity. Phytochemical and chromatographic screening of t his bioactive extract by different chromatographic tools (TLC, CC and High Pressure Liquid Chromatography, HPLC) revealed the presence of flavonoids and other compounds (Srivastava & Rauniyar, 2020). The acti ve components were identified as the isoprenoids P-damascenone and E-phytol which showed comparable inhibitory effects to the alkaloid papav erine, a general spasmolytic agent (Pongprayoon *et al*., 1991).



1 **2** **3** **4**

Mellein (**1**), 4-vinylguaiacol (**2**), Pdamascenone (**3**) and E-

phytol (**4**) are used as anti-

inflammatory since they can inhibit the enzymes cyclooxygenase and 5-lipoxygenase which are involved in the synthesis of prostaglandins and l eukotrienes respectively (Paula *et al*., 2003).

2.3.3 Antioxidant activity

The methanol extract of *Ipomoea palmate* (MEIP) flowering tops showe d antioxidant activity by inhibiting DPPH and hydroxyl radical, nitric ox ide and super oxide anion scavenging, hydrogen peroxide scavenging, an d reducing power activities. In addition, the MEIP also contains a notic eable amount of total phenols, which plays a major role in controlling a ntioxidants (Kishore *et al*., 2014).

2.3.4 Mosquitoes Larvicidal activity

The essential oil of *Ipomoea palmata* has remarkable larvicidal propertie s and its use as larvicide against mosquitoes should be explored as this plant grows abundantly in the wild. It is worthwhile to study extensive ly the larvicidal properties of the plants essential oil by isolating and id entifying the active components that cause larval mortality and then use them to assess their potential as an alternative to chemical larvicides (Kishore & Upadhyay, 2015). Plants of the genus *Ipomoea* are widely us ed as medicinal plants for the treatment of various diseases as shown in the **Table 2.3.4**.1

Table 2.3.4.1. Summary of the ethnobotanical uses of the *Ipomoea species*

Species	Plant part	Use	Reference
I. carica	Leaves, Flower, Whole plant	Anti-microbial, anti-inflammatory, anti-allergic, cytostatic and anti-oxidant.	(Singh *et al*., 2020)
I. aquatic	Leaves, Flower, Whole plant	Treatment of diabetes, scorpion venom antidote, ring worm, leprosy, fever, nose bleeding	(Kishore *et al*., 2014)
I. asarifolia	Whole plant	-Itches -antiaging	(Pruenwald, 2006)
I. botata	Whole plant	-Treatment of tumours of mouth and thorat - Eaten raw to treat hypertension, anemia and diabetes.	(Ludvik *et al*., 2004)
I. carnea	Leaves, milky juice. Whole plant	Treat muscle strain. Anti-inflammatory,anti-arthritic, anti-diabetic. Folk medicine on ulcer, fever and rheumatism. Treat immunodeficiency syndrome(AIDS)	(Singh *et al*., 2020).
I. campanulata	Whole plant	Antidote to snake poison	(Singh *et al*., 2003)
I. digitate	Roots	Decoctions against constipation	(Singh *et al*., 2004)
I. indica	Leaves and stems	Used a purgative and treatment of broken bones	(Srivastava & Shukla, 2015)
I. muricata	Whole plant	Treatment of all kinds of skin ailments, wounds, cuts and blisters due burns	(Ysrael, 2003)
I. pes-carpae	Leaves	- useful in fatigue, strain, arthritis,rheumatism, menorrhagia, anti-inflammatory, anti-heamolytic, antispasmodic, anticancer activities and skin diseases. - inhibition of platelet aggregation, diarrhea, vomiting and piles.	(Ko *et al*., 2004)

I. nil		Seeds are useful in anti-inflammatory, carminative, depurative, purgative, vermifuge, inflammations, constipation, dyspepsia bronchitis, fever, skin diseases, scabies and	(Paula *et al*., 2003)
I. purpurea	Whole plant	-stop hemorrhage -treat syphilis	(Camargo, 1998)
I. stoloni fera	Leaves, stems, roots	- treat pain after child birth, stomach problems, inflammations,swelling and wounds	(Paula *et al*., 2003)
I. stan	Leaves, flowers and stems	-Treat epilepticseizures -opthalmic diseases -paralysis	(Pongprayoon *et al*., 1992)
I. muroc oides	Whole plant Leaves and flowers	Burn against mosquitoes in mexico Treatment of inflamation	(Leon *et al*., 2005)
I. pes-tigri dis	Leaves, Roots, Whole plant	- Leaf paste applied twice in a day with coconut oil to cure pimples. - Roots are used as an antidote to snakebite and headache. - Whole plant is used in hemiplegia and used to treat gripe and malarial fever.	(Zhang *et al*., 2014; Clin Res , 2015)
I. quamo clit L.	Whole plant	- Whole plant is applied externally on carbuncles. . Juice of whole plant used along with other ingredients in case of blood dysentery, piles and body weakness.	(Pratyu *et al*., 2011)
I. violac eae	Seeds	- Seeds are used in creation of D-Lysergamide for making psychoactive drugs.	Pan *et al*., 2014
I. ob scur a	Leaves, Seeds	- Leaf juice is administered for Snake bite and dysentery. - Seeds are used as cleaning agent, to improve difficult breathing, relive pain and to improve vision.	(Lovik *et al*., 2004)

I. alba	Leaves, Root bark, Seed, Whole plant	-Root bark is used as a purgative. - Whole herb is used in snakebite. -Leaves used to treat headache.	(Uawangul *et al*,. 2006) (Singh *et al*., 2003)

2.4 Photochemistry of the genus *Ipomoea*

A variety of phytochemicals have been reported from the genus *ipomoea* including alkaloids, terpenoids and phenolic compounds (Pongprayoon *et al*., 1992; Singh *et al*., 2020; Srivastava & Shukla, 2015). These classes of compounds are presented in the sub-sections below.

Table 2.4. 1: Alkaloids of the genus *Ipomoea*

Alkoloids	**Species**	**Activities**	**Reference**
Agroclavine (**5**)	*Ipomoea fistulosa* *Ipomoea muelleri* *Ipomoea violacea*	Antimicrobial Cystotatic	(Srivastava & Shukla, 2015)
Hanoclavine (**6**)	*I. asarifolia* *I.hederacea*	Psychotropic psychotomimetic	(Singh *et al.*, 2020)

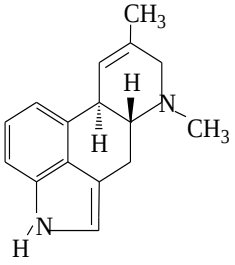
	I.violacea		
Elyamoclavine (**7**)	*Ipomoea muelleri* *Ipomoea violacea* *I.parasitica*	Psychotropic Psychotomimetic	(Mohanraj & Sivasankar, 2014).
Ergine (**8**)	*I. asarifolia* *I. corymbasa* *I. tricolor*	Psychotropic Psychotomimetic	(Pongprayoon *et al*., 1992)
Erginine (**9**)	*I. corymbasa* *I. muelleri* *I. violacea*	Psychotropic Psychotomimetic	(Meira .M 2008)
Ergocrytine (**10**)	*I. tricolor*	Psychotropic psychotomimetic	(Guan *et al*., 2006)

Ergotamine (**11**)			
Ergometrine or ergonovine (**12**)	*I. muelleri* *I. corymbosa* *I. tricolor* *I. violacea*	Psychotropic Psychotomimetic Vasoconstrictor Hemostatic Uterotonic	(Hossan *et al*., 2010; Shah *et al*., 2013)
Ergosinine (**13**)	*I. palmate*	Uterotonic	(Kano *et al*., 2005)
Festuclavine (**14**)	*I. muelleri*	Antimicrobial	(Singh *et al*., 2020)
Lysergol (**15**)	*I. hederacea* *I. muelleri* *I. parasitica* *I. petaloidea* *I. corymbosa*	Psychotropic Psychotomimetic	(Rabah *et al*., 2004).

	I. viola cea		
Penniclavine (**16**)	*I. heder acea* *I. muell eri* *I. cory mbosa* *I. viola cea*	Psychotropic	(Zhao *et al*., 2005)
Ipalbidine (**17**)	*I. alba* *I. muric ata* *I. hard wickki*	Analgesic Antioxidant	(Shaw ***et*** *al*., 2003)
2-*epi*-lentiginosine (**18**)	*I. carne a*	Potent inhibitory activ ity toward rat α-mannosidase	(Ludvik B, *et al*., 2004)
Swainsonine (**19**)	*I. carne a*	Immunomodulatory A ntimetastatic Potent inhibitory activ ity toward rat α-mannosidase	(Kaneshiro, *et al*., 2005).

Calystegine B1 (**20**) Calystegine B2 (**21**) Calystegine C1 (**22**)	*I. aquatic* *I. batatas* *I. hederifolia* *I. eremnobrocha* *I. obscura* *I. pes-caprae* *I. setifera*	Potent inhibitory activity toward ratlysosomal β-glucosidase.	(Hossan *et al*., 2010; Shah *et al*., 2013)
Calystegine B3 (**23**)	*I. aquatic* *I. batatas* *I. carnea* *I. hederifolia*	Moderate inhibitory activity toward rat α- and β-mannosidases	(Pongprayoon *et al*., 1992)

	I. erem nobroch a		



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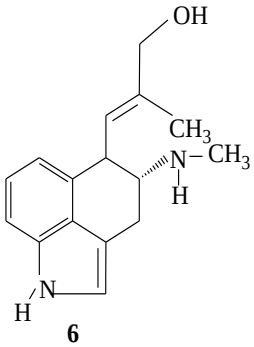

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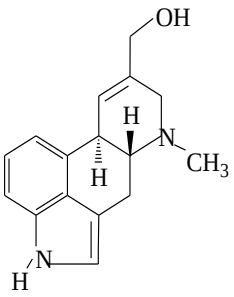

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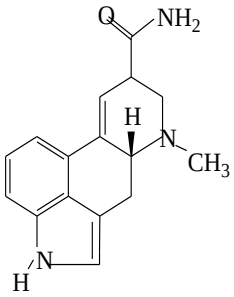

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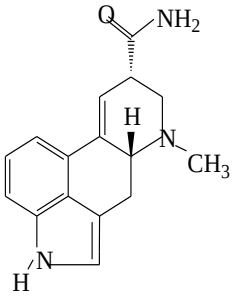

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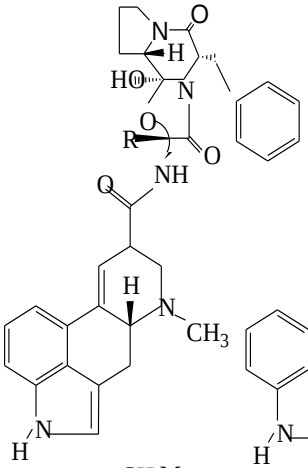

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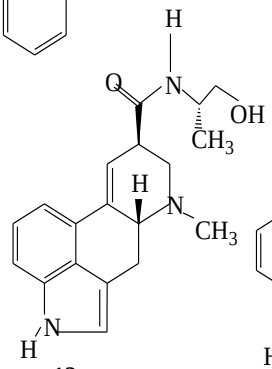
11, R = Me



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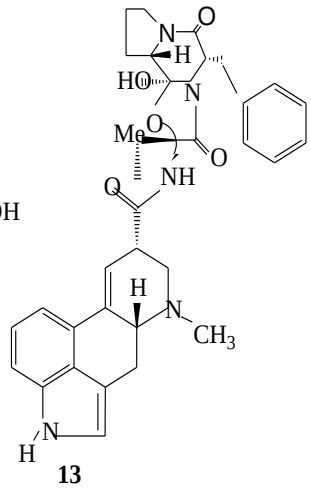

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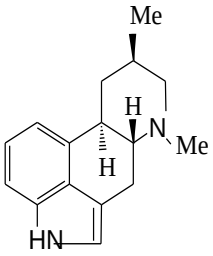

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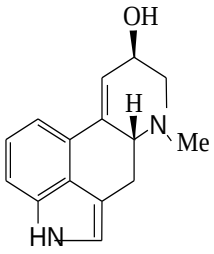

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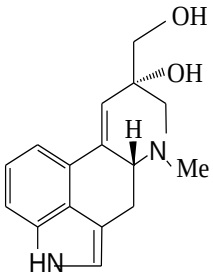

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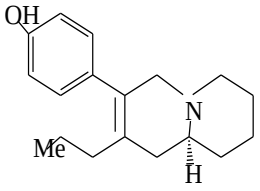

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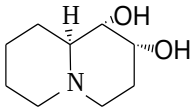

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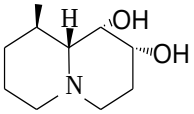

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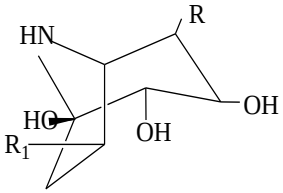

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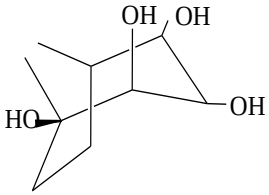
21 R=OH ;R_1= H
22 R =R_1= OH



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2.4.1 Phenolic compounds of the genus Ipomoea

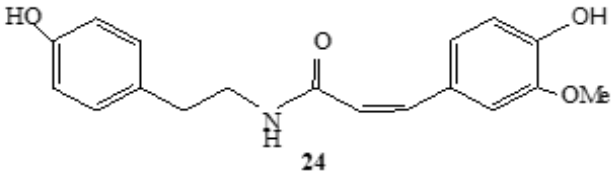
The following Phenolic compounds have been reported from genus *Ipom oea* as shown in the table 2.4.2 below.

Table 2.4. 2: Phenolic compounds of the genus *Ipomoea*

Phenolic compounds	**Species**	**Activities**	**Reference**
N-cis-feruloyl tyramine (**24**) *N-trans*-feruloyl tyramine (**25**)	*I. aquatic*	Inhibition of prostaglandin synthesis	(Singh *et al.*, 2020)
Cafeic acid (**26**)	*I. batatas* *I. muricata*	Antioxidant Antimutagenic	(Islam *et al.*, 2003)
3-*O*-caffeoyl-quinic acid (clorogenic acid) (**27**)	*I. batatas* *I. fistulosa*	Hypoglycemic, antimutagenic antioxidant and inhibition of HIV replication	(Okudaira *et al.*, 2005).
3,5-di-*O*-caffeoyl-quinic acid (**28**) (isoclorogenic acid a)	*I. aquatic* *I. batatas* *I. peas-capra* *I. fistulosa*	Hypoglycemic, antimutagenic antioxidant and inhibition of HIV replication. Antifungal, antispasmodic Collagenase inhibitory	(Meira *et al.*, 2012)
3,4-di-*O*-caffeoyl-quinic acid (**29**)	*I. aquatic* *I. batatas*	Hypoglycemic, antimutagenic antioxidant and inhibition of HIV replication. Collagenase inhibitory	(Choudhary, 2022)

(isoclorogenic acid b)	*I. pes-caprae*		
4,5-di-*O*-caffeoyl-quinic acid (**30**) (isoclorogenic acid c)	*I. aquatic* *I. batatas* *I. pes-caprae* *I. fistulosa*	Hypoglycemic, antimutagenic antioxidant and inhibition of HIV replication. Collagenase inhibitory	(Raj Narayan Roy, 2020)
3,4,5-tri-*O*-caffeoyl-quinic acid (**31**)	*I. batatas*	Hypoglycemic, antimutagenic Antioxidant and inhibition of HIV Replication	(Mahmood *et al*., 1993)
3,5-di-*O*-caffeoyl-4-*O*-coumaroyl-quinic acid (**32**)	*I. pes-caprae*	Collagenase inhibitory	(Zheng & Clifford, 2008).
4,5-di-*O*-caffeoyl-1,3-di-*O*-coumaroylquinic acid (**33**)	*I. pes-caprae*	Inhibition of HIV replication.	(Kusano & Abe, 2000).
4,5-di-*O*-caffeoyl-quinic acid methyl ester (**34**)	*I. pes-caprae*	Collagenase inhibitory	(Mamun *et al*., 2003).

3,4-di-*O*-caffeoyl-quinic acid m ethyl ester (**35**)	*I. pe s-capra e*	Collagenase inhibitory	(Miyazaki *et al.*, 2005).
3,5-di-*O*-caffeoyl-quinic acid m ethyl ester (**36**)	*I. pe s-capra e*	Collagenase inhibitory	(Okudaira *et al.*, 2005)



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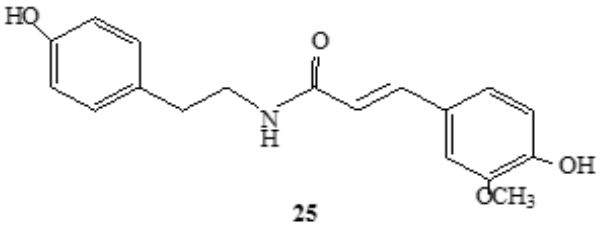

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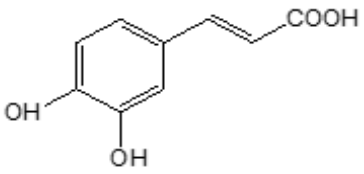

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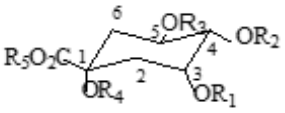

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28 R_1=R_3=Caffeoyl;R_2=R_4=R_5=H
29 R_1=R_2=Caffeoyl;R_3=$R_4$$R_5$=H
30 R_2=R_3=Caffeoyl;R_1=R_4=R_5=H
31 R_1=R_2=R_3=Cffeoyl;R_4=R_5=H
32 R_1=R_3=Caffeoyl;R_2=Coumaroyl;R_4=R_5=H
33 R_1=R_4=coumaroyl;R_2=R_3=caffeoyl;R_5=H
34 R_1=R_4=H;R_2=R_3=caffeoyl;R_5=Me
35 R_1=R_2=caffeoyl;R_3=R_4=H;R_5=Me
36 R_1=R_3=caffeoyl;R_2=R_4=H;R_5=Me

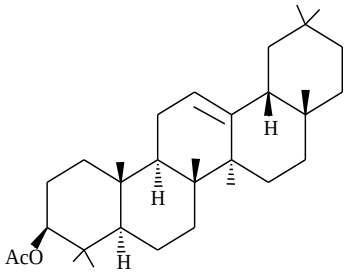
2.4.2 Triterpenes.

A good number of triterpenes have been reported from genus *Ipomoea* as shown in the table 2.4.3 below.

Table 2.4. 3:Triterpenes

Triterpenes	Species	Activities	Reference
β-amirin acetate (**37**)	*I. batatas* *I. pes-capre*	Antinociceptive	(Luo & Kong, 2005)
α-amirin acetate (**38**)	*I. pes-capre*	Antinociceptive	Souza *et al.*, 2005)
Boehmeryl acetate (**39**)	*I. batatas*	Ovopositional stimulant for *Cylas formicarius elegantulus*	(Fatima *et al.*, 2014)
Betulinic acid (**40**) Glochidone (**41**)	*I. pes-capre*	Antinociceptive	(Luo & Kong, 2005)

Friedelin (**42**)	*I. batatas*	Antibacterial against *S. aureus* and antifungal against *P. boydii*	(Meira *et al*., 2012)
Taraxerol (**43**)	*I. digitate*	Acetylcholinesterase inhibitory	(Choudhary, 2022)
Betulin (**44**) **a** and **b**		Antiviral, antiplasmodial, antibacterial as well as antidepressant	(Philpott *et al*., 2009).
Hederagenin (dihydrotriterpene) **(45)**	*I. bolusina*	Antioxidant	(Meira *et al*., 2012)



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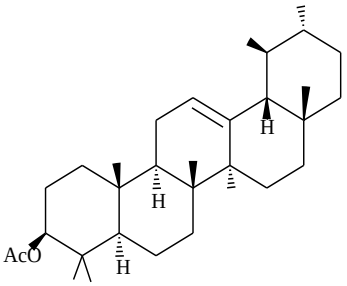

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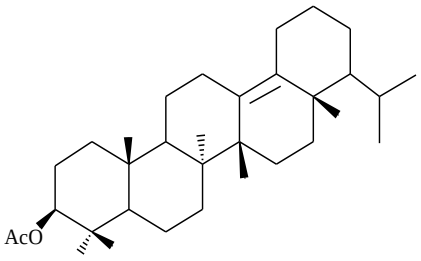

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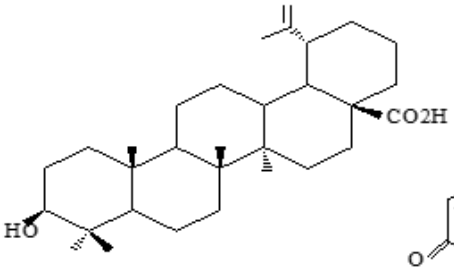

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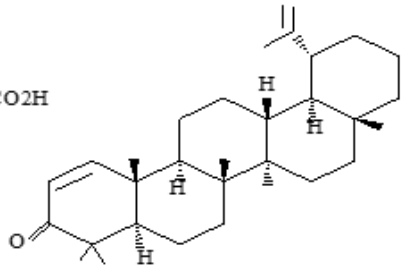

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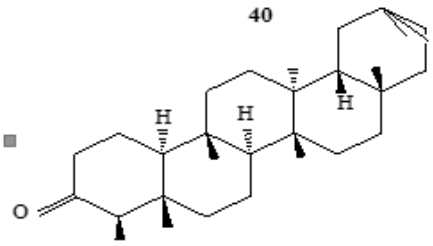

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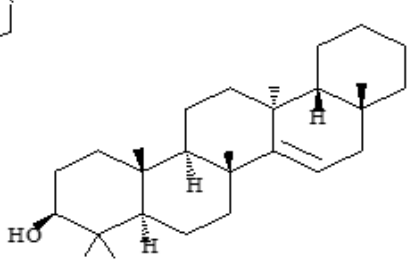

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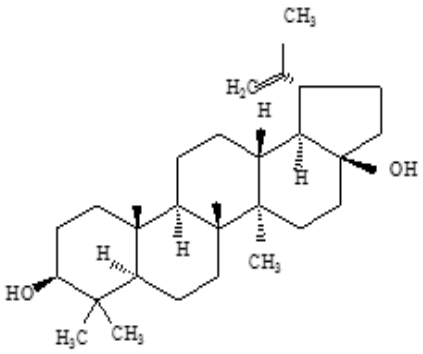

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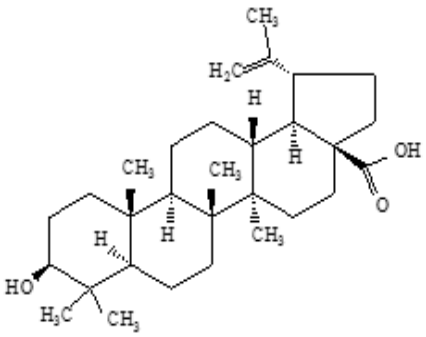

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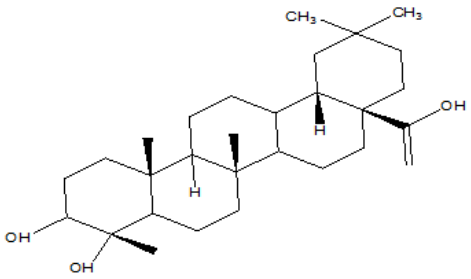

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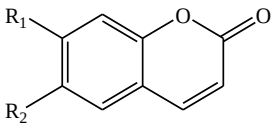
2.4.3 Coumarins isolated from *Ipomoea* species

Coumarins are oxygen containing heterocyclic phytochemicals that occur naturally in several plant species. Some coumarins have been isolated from some species of *ipomoea*. Coumarin (**46**) has been isolated from *I. turpethum* (Meira *et al*., 2012). The compound has cytotoxic, antibacterial (Ojala *et al*., 2000), as well as antifungal properties (Souza *et al*., 2011; & Souza *et al*., 2005). Coumarin (**47**), scopoletin has been isolated from a number of species of *Ipomoea* including *I. batatas, I. cairica, I. digitata, I. stans* and *I. turpethum* (Meira *et al*., 2012). The Coumarin exhibits a number of properties that include inhibition of prostate cancer proliferation, acetyl cholinesterase inhibition, antioxidant, anticoagulant and anti-

HIV activities (Meira *et al*., 2012). Esculetin (**48**) has been isolated rom *I. batatas* (Meira *et al*., 2012). The compound has antioxidant, anticoagulant as well as anti-

HIV properties (Meira *et al*., 2012). Umbelliferone (**49**) isolated from *I. batatas, I. cairica* and *I. digitata* is known for its anticoagulant as well as anti-HIV properties (Meira *et al*., 2012).



46 $R_1 = R_2 = H$
47 $R_1 = OH; R_2 = OMe$
48 $R_1 = R_2 = OH$
49 $R_1 = OH; R_2 = H$

2.4.4. The phthalates isolated from the genus *Ipomoea*

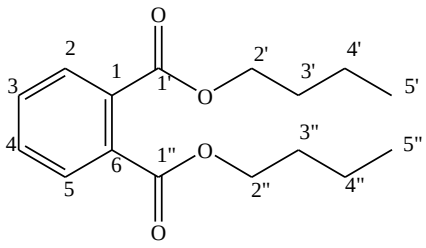
Diesters of 1, 2-benzenedicarboxylic acid (phthalic acid), commonly known as phthalates, are a group of man-made chemicals with a wide spectrum of industrial applications. High molecular weight phthalates (for example, di(2-ethylhexyl) phthalate [DEHP], di-isononyl phthalate [DiNP], di-n-octyl phthalate [DnOP]), are primarily used as plasticizers in the manufacture of flexible vinyl which, in turn, is used in consumer products, flooring and wall coverings, food contact applications, and medical devices.

Manufacturers use low molecular weight phthalates (for example, diethyl phthalate [DEP] and dibutyl phthalate [DBP]) in personal-care products (for example, perfumes, lotions, cosmetics), as solvents and plasticizers for cellulose acetate, and in making lacquers, varnishes, and coatings, including those used to provide timed releases in some pharmaceuticals (Fatima *et al.*, 2014; Raj Narayan Roy, 2020).

Table 2.4. 4:Phthalates

Phthalates	Species	Activity	Reference

Dibutyl Phthalate (**50)**	*I. carne a* stem	Antibacterial, ink and ton er products	(Islam *et al*., 2002)
Butyl benzyl pht halate (BBzP) (**51)**	*I. barta ta*	Adhesive and sealants, Paints and coatings.	(Raj Narayan Roy, 2020)
Dimthyl phthalat e (DMP) (**52)**	*I. carne a*	Safety glasses, laquers,sol and rocket propellants	(Fatima *et al*., 2014)



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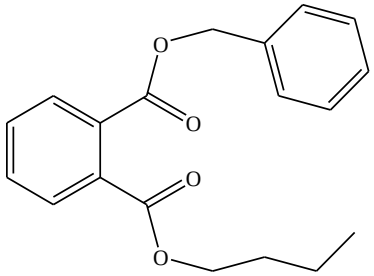

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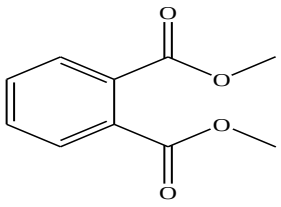

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2.5 Analytical techniques utilized in natural product research are r eviewed.

The choice of appropriate methodologies is fundamental to both qualitati ve and quantitative research of plant-

derived bioactive chemicals. This section discusses a few of the method ologies that are frequently employed in studies on natural products.

2.5.1 Extraction

Any study using medicinal plants must begin with extraction, which has a big impact on the study's results. In some cases, "sample preparation techniques" are used to describe extraction methods. It is true that the development of current chromatographic and spectrometric techniques has made bioactive compound analysis simpler than ever, but the success s till depends on the extraction processes, input variables, and the actual plant parts employed. The matrix characteristics of the plant part, the so lvents employed, temperature, and extraction time are the most frequent variables impacting extraction procedures. Therefore, if the extraction pro cess is carried out properly, it is possible to conduct additional separatio n, identification, and characterization of bioactive chemicals. Several trad itional extraction procedures can be used to separate bioactive chemicals from plant sources. The majority of these approaches rely on the abilit y of the various solvents to remove material while also applying heat a nd/or mixing. Traditional methods that are frequently employed include soxhlet extraction, maceration, hydro distillation, and cold percolation to produce a crude extract that is then concentrated using a rotary evapor ator under low pressure (Azmir *et al.*, 2013).

2.5.2 Column Chromatography

In column chromatography, there are two phases; the stationary phase (a solid adsorbent) is placed in a vertical glass column and the mobile ph ase (a liquid) is added to the top and flows down through the column (

by either gravity or external pressure). Column Chromatography is generally used as a purification technique to isolate desired compounds from a mixture (Kenkel, 2002). The crude extract to be purified by column chromatography is applied at the top of the column. The liquid solvent (the eluent) is passed through the column by gravity or by the application of air pressure. Equilibrium is established between the solute adsorbed on the adsorbent and the eluting solvent flowing down through the column. Because the different components in the mixture have different interactions with the stationary and mobile phases, they will be carried along with the mobile phase to varying degrees and a separation will be achieved. The individual components, or elutants, are collected as the solvent drips from the bottom of the column (Harvey, 2007). Silica gel (SiO_2) and alumina (Al_2O_3) are the two adsorbents commonly used for column chromatography. These adsorbents are sold in different mesh sizes, indicated by a number on the bottle label. The polarity of the solvent which is passed through the column affects the relative rates at which compounds move through the column (Harvey, 2008). Polar solvents can compete more effectively with the polar molecules of a mixture for the polar sites on the adsorbent surface and will also solvate the polar constituents better. As a result, a highly polar solvent will move even highly polar molecules rapidly through the column. If a solvent is too polar, movement becomes too rapid, and little or no separation of the components of a mixture will be achieved. If a solvent is not polar enough, no compounds will elute from the column. Proper choice of an eluting solvent is therefore paramount for the successful application of column c

hromatography as a separation technique. Often a series of increasingly polar solvent systems are used to elute a column. A non-polar or less polar solvent example hexane is first used to elute the less-polar compounds. Once the less-polar compounds are out of the column, a more-polar solvent (ethyl acetate) is added to the column to elute the more-polar compounds (Kenkel, 2002).

2.5.3 Thin layer Chromatography

Thin layer chromatography (TLC) is often used to analyze the fractions obtained from column chromatography to establish whether the fraction collected contains more than one component and if fractions can be combined without affecting their purities (Kenkel, 2002). The separation by TLC basically depends on the relative affinity of compounds towards stationary and mobile phases. The compound which is under the influence of mobile phase (driven by capillary action) travels over the surface of the stationary phase. During this movement, the compound with a higher affinity to the stationary phase travels slowly while that with a lower affinity to the stationary phase travels faster. Thus separation of components in the mixture is thus achieved. Once separation is done, the individual components are then seen (visualized) as spots on the plate after staining with iodine vapour under UV light (Harvey, 2007).

2.5.4 Nuclear magnetic resonance (NMR) spectroscopy

Nuclear magnetic resonance spectroscopy depends on the absorption of energy when the nucleus of an atom is excited from its lowest energy spin state to the next higher one. Many elements are difficult to study b

y NMR, and some cannot be studied at all. However, the two elements that are the most common in organic molecules (carbon and hydrogen) have isotopes (^{1}H and ^{13}C) capable of giving NMR spectra that are ric h in structural information. A proton nuclear magnetic resonance (^{1}H N MR) spectrum tells us about the environments of the various hydrogen atoms in a molecule while a carbon-

13 nuclear magnetic resonance (^{13}C NMR) spectrum does the same for t he carbon atoms (Carey, 2000). Therefore, to determine a substance's m olecular structure, both ^{1}H and ^{13}C NMR are used. It is often used in c onjunction with other spectrometric techniques such as FTIR and Mass Spectrometry for better results. The main differences between ^{1}H & ^{13}C NMR include: The operating frequency (the gyromagnetic ratio) for ^{13}C is about one-

fourth that of proton, so the resonance frequency of ^{13}C is about one-fourth. Thus, a spectrometer requiring a 300 MHz transmitter for ^{1}H wil l require a frequency of 75.6 MHz for ^{13}C resonance (300/75.6 = ca 4). The scale for chemical shift, δ, in ^{13}C NMR is from 0 to around 240 ppm whereas the ranges for δ is from 0 to ca. 15 ppm in ^{1}H NMR s pectra. In the C-

C Coupling only 1% of the carbon atoms are magnetic, so there is onl y a small probability that an observed ^{13}C nucleus is adjacent to anothe r ^{13}C nucleus. Therefore, carbon-

carbon splitting/coupling is ignored in ^{13}C NMR. In the ^{13}C NMR the s pectrometers are less sensitive compared to when carrying out ^{1}H NMR experiment. The peak areas/Integrations or areas under the ^{13}C NMR p

eaks are not proportional to the number of carbon atoms giving rise to the peaks. Usually methyl carbons (CH_3) and methylene carbons (CH_2) t end to give strongest absorptions (due to NOE effect), and quaternary c arbons tend to give weak signals (due to absence of NOE effect) and t herefore, ^{13}C NMR spectral peaks are not integrated.

The HMBC locates setterlite peaks (long range protons) i.e., protons wh ich are 2-

3 bonds away. It gives connectivity information by showing the correlati ons between protons and carbons that are separated by multiple bonds. The HSQC on the other hand determines proton-

carbon single bond correlations. The protons lie along the x-

axis and the carbon are along the y-axis. The H-

H COSY gives the correlation between protons which are coupled to ea ch other in the ^1H NMR spectrum. It is 2D spectrum that shows scalar coupling between vicinal H-

atoms and can be used to determine the signals arising from the neighb ouring protons.

CHAPTER THREE: MATERIALS AND METHODS

3.1 General

The solvents (n-Hexane and Ethyl acetate) used in the extraction and chromatographic separations were double glass distilled, methanol, dichloromethane and acetone were Analytical grade reagents. The proton and carbon spectra were obtained on a Bruker Avance 500 MHz spectrometer using Chloroform ($CDCl_3$) signals as the reference. The spectra were processed using standard soft wear, MestReNova version 10. Structural elucidation and NMR assignments were based on two-dimensional H-H COSY, HSQC and HMBC. Analytical TLC was done on silica gel 60 (F_{254} Merck) pre-coated Aluminium plates. The visualization of the spots on the TLC was carried out using the UV light (254 or 366 nm), iodine vapour and/or Draggendroff's reagent sprayed to view unclear or faint spots clearly.

3.2 Collection of Plant materials

The plant material (aerial parts) was collected from Kyambogo University ward, Nakawa division, Kampala district – Central Uganda in April 2019 and dried under shade for one month. . The plant materials were thoroughly cleaned under water as described by Ogwal-Okeng *et al.*(2003) and Lamorde *et al.* (2010).The sample specimen was taken to Makerere University for proper identification and authentication in the Herbarium. The identification was done by Dr. Paul Ssegawa o

f the department of Botany, Makerere University where a voucher speci men Number (IG005) was deposited.

3.3 Extraction

Extraction was done in the laboratory, at the Department of Chemistry, Kyambogo University. The air-

dried material (aerial parts), were crushed into powdered form by using a blender and 700 grams were obtained. The material's powdered form was kept in an airtight glass as advised by Sofowora, (1993). Grounded sample (620 g) of the plant was soaked in a mixture of dichlorometha ne (DCM) and Methanol (MeOH) in the ratio of 1:1. The initial extract ion required 1.3 litres of each solvent, and it was carried out after a 24 -

hour soaking period. The crude extract was filtered twice using a filter funnel packed with cotton wool as recommended by Yenesew *et al*., (2 012). Second and third extractions were done to ensure exhaustive remo val of the bioactive compounds present as described by Azmir *et al*., (2 013). Concentration of the crude extract was carried out under low pres sure using a rotary evaporator and yielded the crude (52.6082 g) (Appe ndix 1).

3.4 Isolation

The crude extract was subjected to fractionation for isolation using; Col umn Chromatography, (CC), thin layer chromatography, (TLC) and Ultra violet (UV) lamp to view the TLC plates. About 200 g of silica gel (70-

230 mesh ASTM) was packed in a column, and 35 g of the crude extr

act was adsorbed in 35 g of silica gel eluted with hexane with increasi ng polarity of ethyl acetate. The column was run with different solvent gradients of hexane and ethyl acetate mixture starting with 100 % hexa ne to 100 % Ethyl acetate. Thin layer chromatography was conducted o n each fraction and those with similar spot-

movement; retention factor (RF) were combined. Purification and washin g of the fractions obtained was conducted using methanol, and dichloro methane solvent was used for dissolving the samples, small columns we re also packed to further isolate the fractions with multiple spots as gui ded by TLC results (Minor *et al*., 2014). Repeated fractionation resulted in to 52 fractions based on their TLC profile and a total of 12 compo unds were isolated after purification by washing using methanol and use of columns of very small diameters (packing of small columns).

3.5 Qualitative Phytochemical test

Qualitative determinations of the phytochemical constituents was carried out on the methanol extracts of *I. cairica* as described by Prashant *et a l.*, (2011). The different extracts of the aerial parts of *I. cairica* were te sted for various components by their specific tests such as; Mayer's test, Dragendroff's test, for alkaloids; Ferric chloride test, Lead acetate test for phenolic compounds, Acetic anhydride and concentrated sulphuric a cid test for terpenoids; Benedict's test, Fehling’s solution test for redu cing sugars and Ninhydrine test for amino acids.

3.5.1 Test for Phenolic Compounds

a) The extract (50 mg) was dissolved in distilled water (5 mL). A few drops of neutral ferric chloride solution (5%) was added. A dark green colour indicated the presence of phenolic compounds (Banu & Cathrine, 2015).

b) Lead Acetate Test: The crude extract was dissolved in distilled w ater and lead acetate solution (3 mL,10%) was added. A bulky white precipitate indicated the presence of phenolic compounds (Ramos & Bandiola, 2017).

3.5.2 Test for flavonoids (Alkaline reagent test)

An aqueous solution of the extract was treated with ammonium hydrox ide solution (10%). Yellow fluorescence was seen as a sign of flavonoi ds. (Saeed *et al*., 2012).

3.5.3 Test for Alkaloids

The crude extract was dissolved in dilute hydrochloric acid and filtered. The filtrate was further tested with following reagents for the presence of alkaloids.

a) Dragendroff's test

The filtrate was treated with potassium bismuth iodide solution followed by heating. The development of an orange-
red precipitate served as evidence that alkaloids were present (Jasim *et al.*, 2015).

b) Mayer's test

The extract was treated with potassium mercuric iodide solution. Formati on of a whitish yellow or cream coloured precipitate indicated the prese nce of alkaloids (Culvenor & Fitzgerald, 1963; Vats *et al.*, 2011).

3.5.4 Test for quinones

The extract (0.5 g) was mixed with toluene (10 mL) before filtering. T he filtrate was then mixed with ammonia solution (5 mL,10%). After sh aking the mixture, pink, crimson, or violet colorations appeared, indicati ng the presence of quinones (McIntosh, 1976).

3.5.5 Test for Saponins

The solid extract (1.0 g) was boiled with distil led water (5 mL), filtere d. To the filtrate, distilled water (3 mL) was added and shaken vigorou sly for about 5 minutes. Frothing which persists on warming showed th e presence of saponins (Kareru *et al*., 2008).

3.5.6 Test for terpenoids

A little of the crude extract was dissolved in ethanol. To it, acetic anhy dride (1ml) was added followed by the addition of concentrated sulphuri c acid (conc H_2SO_4). A change in colour from pink to violet was an in dication of the presence of terpenoids (Sofowora, 1993).

3.5.7 Test for Amino acids

Ninhydrin Test was used to carry out this test where extract solution was treated with Ninhydrin (Tri-ketohydrindene hy-

drate) at the pH range of 4 -

8. Development of purple colour indicated the positive response for am ino acids (Friedman, 2004).

3.5.8 Test for reducing sugars

a) Fehling's test for free reducing sugar:

Extract (0.5 g) was dissolved in distilled water and filtered. The filtrate was heated with equal volumes of Fehling's solution A and B (5 mL).

Formation of a red precipitate of cuprous oxide was an indication of the presence of reducing sugars (Wadood *et al.*, 2013).

b) Benedict's Test:

To the extract solution (5 mL) of Benedict's solution (5 mL) was added in a test tube and boiled for few minutes. Development of brick red precipitate confirmed the presence of reducing sugars (Faizy *et al.*, 2021).

3.6 Antimicrobial Activity Tests

Using the agar well diffusion method, the antibacterial activity of crude extract and isolated compounds was evaluated against a few selected species of gram positive and gram negative bacteria and fungi.

3.6.1 Anti- bacterial Activity Test

The antibacterial assay of the crude extract was carried in the Biology laboratory, department of Biology, Kyambogo University. The bacterial cultures used in the study were obtained from the Department of Biology and Natural Sciences, Kyambogo University. The antibacterial activity was determined after incubation by measuring the inhibition zone on the selected common human pathogenic bacterial strains. The antibacterial assay involves growing of the organisms (culturing), preparation of the e

xtract to be used and carrying out the test on the selected micro-organisms (bacterial stains)

Culturing, the stock organisms were cultured in broth bacteriological w ater. Sub-culturing was done in Nutrient Agar by streak plate technique and incub ated at 37 ^{0}C for 24 hours.

Preparation of the extract, the crude extract (1 g) was weighed and dis solved in Dimethyl sulphoxide (DMSO) (2 mL).

Test method, Agar well Diffusion method was used. Muller Hinton Aga r (MHA) (15 mLs) was dispensed on a clean petri-dish and left to cool before the test organisms were inoculated. Four w ells of about 5 mm were made using sterile cork borer. Approximately 1 mL of each; crude extract and the pure isolated sample was dispense d in wells, tetracycline and DMSO as positive and negative control for antimicrobial activity respectively. After 24 hours of incubation at 37 ^{0}C , the sensitivity was determined by measuring the zones of inhibition. (Khatiwora *et al*., 2012).

3.6.2 Antifungal activity of leaves and flower (aerial parts) extracts

In this study, the antifungal activity was studied against the microorgani sm viz. *Aspergillus niger, Penicillum chrysogenum* and *Candida albicans* . The cultures were obtained from the standard cultures maintained in th e Microbiology Department of Biology, Kyambogo University. These cul tures were maintained on Sabouraud Dextrose Agar (SDA) at first being incubated at 25 ^{0}C for about 72-

96 hours and then stored at 4 ^{0}C as stock cultures for further antifungal activity. Fresh cultures were obtained by transferring a loop full of cul tures into sabouraud dextrose broth and then incubated at 25 ^{0}C for 72 hrs.

To test antifungal activity, the well diffusion method was used. Here c ulture media was prepared in sabouraud dextrose agar (SDA) and incuba ted for a period is 72 hours at 25 ^{0}C. The rest of the method is the sa me as that of antibacterial activity. The concentration used for antifunga l activity was 200 mg/ml (Agarry & Osho, 2005).

3.6.3 Minimum inhibitory concentration (MIC)

The minimum inhibitory concentration is a test aimed at determining lo west concentration of the drug compound that will inhibit the growth an d activity of the microorganisms. This was carried out within a 24 hr, period. The concentration for MIC was done by serial diluting each isol ated compounds using a 2-

fold method; 1/2, 1/4, 1/8, 1//16, 1/32, 1/ 64, 1/128 and 1/256 by foll owing a procedure previously described by Rabe *et al*. (2002), with so me modifications. An inoculum of 100µl (0.5 McFarland standard) of o vernight microbial cultures of each type bacteria; *E. coli, S. typhi, S. au reus,* and *P. aeruginosa* were added in each of the vails. Triplicate of e ach vails was made and the procedure repeated for each of the test org anisms. The value of each dilution in $\mu g/$

ml was obtained, a volume of each was taken and made up to 20 mL with a corresponding volume of nutrient agar, which together was poure d aseptically into Petri dish and allowed to set after swirling. The agar

plates were then taken to the oven for dryness at 60 ℃ for 20 minutes. Then the microorganisms present in the broth media were then sub cultured to reduce their viable count to about 1 in 10^6. To each vail was then added 10 μl of each organism. Then the vails were incubated for 24 hours. The MIC was taken based on the inhibition of organisms added (Rabe *et al*., 2002).

3.6 Structure Determination

The respective chemical structures of the isolated compounds was determined by a combination of spectroscopic & spectrometric techniques; Mass spectrometer (MS), Infra-red (IR), Ultraviolet Visible (UV Vis) and Nuclear magnetic resonance (NMR)) (Anderson *et al.*, 2004; Boughendjioua & Boughendjioua, 2017; Minor *et al.*, 2014). The proton and carbon spectra were obtained on a Bruker Avance 500 MHz spectrometer using a residual solvent signal of Chloroform ($CDCl_3$) as the reference. The spectra were processed using standard soft wear, MestReNova. Structural elucidation and NMR assignments were based on two-dimensional H-H COSY, HSQC and HMBC.

CHAPTER FOUR: RESULTS AND DISCUSSION

4.0 Introduction

The pulverized aerial parts (620 g) of *Ipomoea cairica* yielded sizeable amount of the crude extract (52.6082 g). Therefore, the Percentage yield of the extract = 52.6082/620 x 100% = 8.4852 % per the first three e

xtractions. The extract was analyzed by TLC which showed the presenc e of several compounds as judged from the many spots on the TLC pla te visualized under UV light (254 and 366 nm).

4.1 Results of Qualitative Phytochemical Test

The **table 4.1** shows the results of the phytochemical tests of the vario us bioactive compounds of *Ipomoea cairica* methanol/DCM extract.

Table 4.1. Phytochemical Tests of the Bioactive Compounds.

Plant Constituents	Test/Reagents	Present / Absent
Alkaloids	Dragendorff's	++
	Mayer's	+
Sterols	Sulphuric acid	+
Flavanoids	NaOH Solution	+
	H_2SO_4	+
Reducing Sugars	Fehling solution	-
	Benedicts Solution	-
Tannins	Dichromate	+
	Lead Acetate	+
	$FeCl_3$	+
Saponins	Distilled water	+
Terpenoids	Acetic acid + H_2SO_4	+
Phenols	FCl_3	+
	Lead Acetate	+
Amino Acids	Ninhydrin	-

The phytochemical compounds observed in the extracts of the plants are known to play important roles in bioactivity of medicinal plants and these secondary metabolites exert antimicrobial activity through different mechanisms. The medicinal values of medicinal plants lie in these phytochemical compounds, and as such, produce definite physiological actions on the human body. Tannins, which are part of the phytochemical constituents, have been found to form irreversible complexes with proline rich protein resulting in the inhibition of cell protein synthesis. Parekh and Chanda (2007) reported that tannins are known to react with proteins to provide typical tanning effect which is important for the treatment of inflamed or ulcerated tissues. Herbs that have tannins as their main components are astringent in nature and are used for treating intestinal disorders such as diarrhea and dysentery. Hence supportive of the use of *I. cairica* as herbal medicine (Choudhary, 2022) . Li and Wang (2003) reviewed the biological activities of tannins and observed that tannins have anticancer activity and can be used in cancer prevention, thus suggesting that *I. cairica* has potential as a source of important bio-active molecules for the treatment and prevention of cancer. Another secondary metabolite compound observed were alkaloids which are one of the largest groups of phytochemicals in *I. cairica* with amazing effects on humans and this has led to the development of powerful antinociceptive agents. One of the most common biological properties of alkaloids is their toxicity against cells of foreign organisms. These activities have been widely studied for their potential use in the elimination and reduction of human cancer cell lines. It is documented that the presence of Sap

onins can control human cardiovascular disease and reduce cholesterol, a lso tannins may provide protection against microbiological degradation of dietary proteins in the semen (Just *et al*.,1998) revealed the inhibitory effect of saponins on inflamed cells. Saponin was found to be present i n MeOH/DCM extract of the aerial parts of *I. cairica* and has supporte d the usefulness of this plant in managing inflammation (Hammuel *et al* ., 2011; Ralte, 2014). Steroidal compounds present in the extracts are of importance and interest due to their relationship with various anabolic hormones including sex hormones (Quinlan *et al*., 2000), worked on ster oidal extracts from some medicinal plants which exhibited antibacterial a ctivities on some bacterial isolates (Neumann *et al*., 2004), also confirm ed the antiviral property of steroids. Flavonoids, another constituent of *I. cairica* leaves and flower extracts exhibited a wide range of biological activities like antimicrobial, anti-inflammatory, anti-angionic, analgesic, antiallergic, cytostatic and antioxidant properties. One of the ability of flavonoids is their ability to scavenge for hydroxyl ra dicals, and superoxide anion radicals and thus health promoting in actio n.

4.2 Characterization of the compounds isolated from *Ipomoea cairic a*

The crude extract (35 g) was fractionated by column chromatography ov er silica gel using increasing amounts of EtOAc in *n*-hexane as the solvent, resulting into isolation of two compounds after re peated column chromatography. Diisobutyl phthalate (27.9 mg), which w as obtained as a yellow amorphous solid from the fraction eluted with

EtOAc (2%) in *n*-

hexane. From the combined fractions eluted with EtOAc (1%) in *n*-hexane yielded 58.4 mg of Friedelin (58.4 g) which precipitated as a white amorphous solid.

4.2.1 Diisobutyl phthalate (**53**)

Compound **53** was isolated as a yellow amorphous solid from the fraction eluted with EtOAc (2%) in *n*-

hexane and it was active on TLC visualized under UV light (254 nm). The ESI-

MS analysis showed an $[M+H]^+$ peak at *m/z* 279 corresponding to the molecular formula $C_{16}H_{22}O_4$.

The ^1H NMR analysis in 500 MHZ spectrometer with $CDCl_3$ as the solvent system (Appendix 7) gave a multiplet at δ_H 7.56 which exhibited a correlation, in the ^1H-

^1H COSY, with a pair of protons resonating at δ_H 7.73 (2H, m, H-2/5) typical of an AA′XX′ spin system, thus, suggestive of a symmetrically di ortho-

substituted aromatic ring. Furthermore, the presence of a phthalate moiety was deduced from the ^{13}C NMR spectrum (Table 4.2.1) which gave a carbonyl peak at δ_C 167.7 showing cross peaks, in the HMBC spectrum, with protons at δ^H 7.73 (H-2/5) and 4.23 (2H, m, H-3/3′). In addition, the ESI-

MS spectrum gave fragmentation peaks at *m/z* 43, 57, 69, 71, and (167) which could be attributed to ions such as $CH_3CH^+CH_3$ (43), $CH_3CH_2CH_2CH_2^+$, $CH_3CH_2CH{=}CHCH2^+$, $CH_3CH_2CH_2CO^+$ (71) and C_6H_4COOHC

$(OH)_2$ (167), respectively. The fact that the base peak was at *m/z* 43 is suggestive of the presence of an isobutyl group responsible for a stable secondary carbocation ($CH_3CH^+CH_3$).

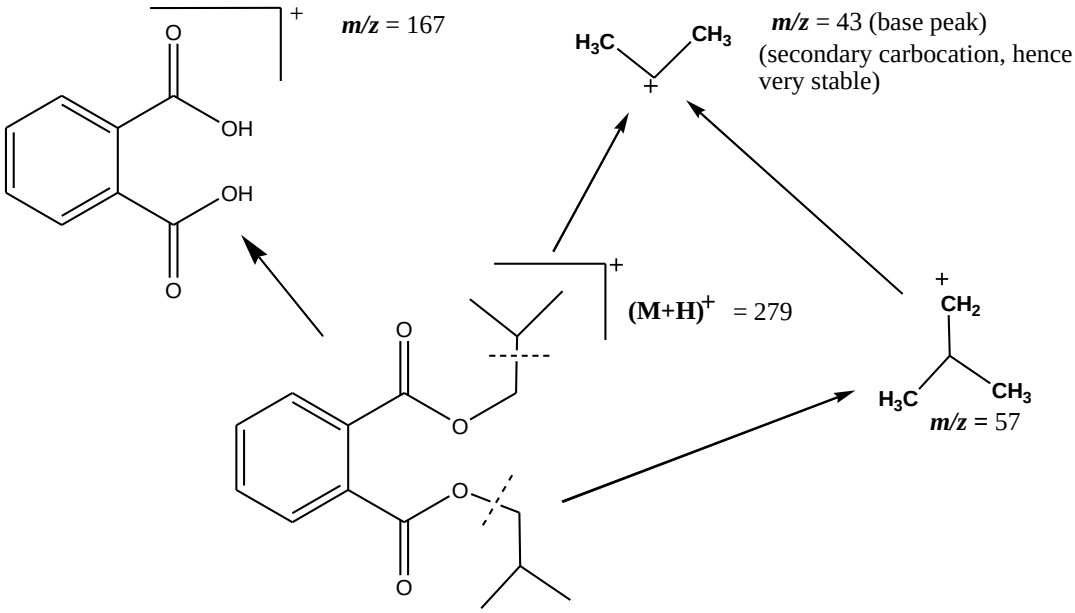


Figure 2:ESI-

MS Fragmentation pattern of compound 53 (Appendix 7 page 87).

The signals at 4.26 ppm (in the ^1H NMR spectrum) due to oxymethele ne protons showed an interaction, in ^1H-

^1H COSY, with a methine proton at 1.28 ppm further supported the pre sence of an isobutyl group. The splitting in H NMR from the CH_2O ca n be attributed to an ABX system in which AB are the two protons on CH_2O (H-2′/H-

2″) and X is the proton in the neighbouring CH group (H-3′/H-

3″). Each proton in an ABX system couples with the other two protons due to a locking brought about by the steric hindrance from the two i sobutyl arms.

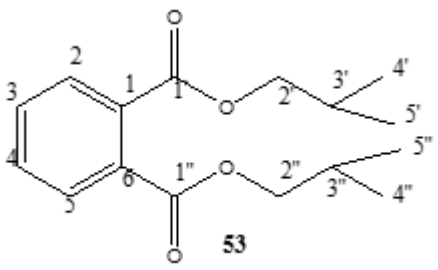
The connectivity in the molecule was fully determined using 2D NMR (H-H COSY, HSQC and HMBC) techniques (see **Table 4.2.1**).

Table 4.2. 1: ^{13}CNMR, ^{1}HNMR, H-H COSY, and HMBC spectral data for compound 53 ($CDCl_3$) (500 MHz); δ in ppm

C No	δ^{13}C a (ppm)	δ^{13}C b	δ^{1}H (ppm	Mult. (*J* in Hz)	^{1}H-^{1}H COSY	HMBC
1'/1''	167.9	167.7	-	-	-	2',4
1/6	132.6	132.4	-	-	-	3
3/5	131.0	131.6	7.56 (2H)	(m)	4	
2/4	129.0	128.8	7.73 (2H)	(m)	3	
2'/2''	68.3	65.5	4.23 (2H)	(m)	3'	
3'/3''	32.1	30.6	1.28 (H)	(m)	4',2'	
4'/4''	23.9	19.18	1.45 (3H)	(m)	4',5'	2'
5'/5'	11.1	13.7	0.93(3H)	(m)	4'	3' 4'
,						

^{13}C a: Diisobutyl data from literature (Ruikar *et al*., 2011)

δ^{13}C b: data from the isolated compound.



Using the above NMR data and literature (Ruikar *et al*., 2011), compou nd **53** was identified as Diisobutyl phthalate. It has previously been isol ated from some plants (Egorov *et al.*, 1981; Ruikar *et al.*, 2011; Shobi & Viswanathan, 2018), some bacteria (Dahari *et al*., 2016; Roy *et al*., 2006), some fungi and marine algae (Adsul *et al.*, 2012). Diisobutyl pht halate was found to be biologically active against bacteria (Khatiwora *et al*., 2012).

4.2.2 Friedelin (**54**)

From the combined fractions eluted with EtOAc (1%) in *n*-hexane yielded compound **54** (58.4 mg) obtained as a white amorphous solid [active on TLC, visualized under UV light (254 nm]. The NMR spectral features (Table 4.2.2) shows that the compound is highly saturat ed and by comparison with data published in literature, the compound was identified as Friedelin (Akihisa *et al.*, 1992; Gaysinski *et al*., 2015; Habib *et al*., 2020; Mann *et al*., 2011. The ^{13}C NMR spectrum display ed a peak at δ_C 213.4 (C-1) assignable to a carbonyl carbon, which showed a correlation, in the HMBC, with a set of protons, at δ_H 2.27 (1H, m, H-3) and δ_H 2.38 (1H, m, H-7). Furthermore, carbon signals at δ_C 59.6 (C-2), δ 58.3 (C-3), δC 53.3 (C-4) and δ_C 42.9 (C-5) were attributed to the presence of methine (CH) carbons having corre lations, in the HSQC, with protons, at (δ_H 1.53, 2.27, 1.40, and1.53, res pectively. Carbons at δ_C 42.2 (C-6), δ_C 39.8 (C-9), δ_C 38.4 (C-11), δ_C 37.4 (C-12), δ_C 30.1 (C-22) and δ_C 28.1 (C-

23) assigned to quaternary carbons, carbons at δ_C 41.6 (C-7), δ_C 41.4 (C-8), δ_C 39.4 (C-10), δ_C 36.1 (C-13), δ_C 35.7 (C-14), δ_C 35.4 (C-15), δ_C 32.1 (C-17), δ_C 32.0 (C-18), δ_C 30.5 (C-21), δ_C 22.4 (C-24) and δ_C 18.3 (C-27) assigned to CH_2 carbons. Eight methyl groups could be identified f rom the ^{1}H NMR spectrum; [δ_H 1.00 (3H, H-25), δ_H 1.18 (3H, H-19), δ_H 0.99 (3H, H-16), δ_H 1.50 (3H, H-20), δ_H 1.05 (3H, H-26), δ_H 0.72 (3H, H-28), δ_H 0.91 (3H, H-29) and δ_H 0.88 (3H, H-30)] assigned to the following carbons; δ_C 20.4 (C-25), δ_C 31.9 (C-19), δ_C 35.1 (C-16), δ_C 30.6 (C-20), δ_C 18.8 (C-26), δ_C 18.1 (C-28), δ_C 14.8 (C-29) and δ_C 6.9 (C-30) respectively.

The ^{1}H NMR displayed the following peaks; multiplets at δ_H 1.53 (3H, H-2, H-5, H^a-10), δ_H 2.27 (1H, H-3), δ_H 1.40 (1H, H-4), δ_H 2.38 (1H, H^a-7), δ_H 2.31(1H, H^b-7), δ_H 1.28(3H, H^a-8, H^a-14, H^a-17), δ_H 1.76 (1H, H^b-8), δ_H 0.93 (1H, H^b-10), δ_H 1.38 (1H, H^a-13), δ_H 1.57 (1H, H^b-13), δ_H 1.46 (1H, H^b-14), δ_H 1.39 (1H, H^a-15), δ_H 1.20 (1H, H^b-15), δ_H 1.43 (1H, H^b-17), δ_H 1.31 (1H, H^a-18), δ_H 1.50 (1H, H^b-18,), δ_H 1.38 (2H, H-21), δ_H 1.95 (1H, H^a-24), δ_H 1.68 (1H, H^b-24), δ_H 1.46 (1H, H^a-27) and δ_H 1.36 (1H, H^b-27), and a doublet at δ_H 0.88 (3H, *J* = 6.8 Hz, H-30 as shown in the **table 4.2.2** below.

Table 4.2. 2:13CNMR, 1HNMR, H-H COSY and HMBC spectral data for compound 54, ($CDCl_3$) (500 MHz); δ in ppm

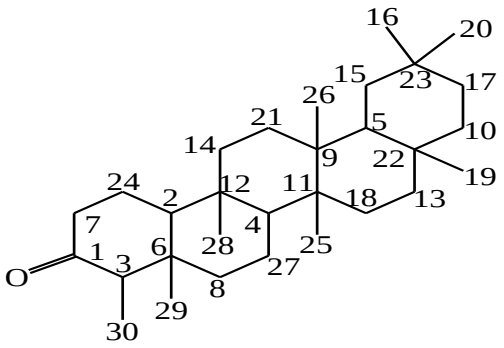
Peak	δ ^{13}CNMR*	δ^{13}CNMR#	δ ^{1}HNMR[ppm] (J [Hz])	H-H COSY	HMBC
1	213.4	213.2			7,3
2	59.6	59.5	1.53 (1H, m)	24	24,7,3,8,4
3	58.3	58.2	2.27 (1H, m)	30	7,8,29
4	53.3	53.1	1.40 (1H, m)	27	25,8,14,2,28
5	42.9	42.8	1.53 (1H, m)	15	26,19,10,13
6	42.2	42.1			24,2,3,30,29,27
7	41.6	41.5	2.38 (1H, m)	24	2,3
			2.31 (1H, m)		
8	41.4	41.3	1.28 (1H, m)	27	4,29
			1.76 (1H, m)		
9	39.8	39.7			21,26,5,25,4
10	39.4	39.2	1.53 (1H, m)	17	19,5
			0.93 (1H, m)		
11	38.4	38.3			25,18,13,4,26
12	37.4	37.4			2,14,28,4,21
13	36.1	36.0	1.38 (1H, m)	18	19,10,5
			1.57 (1H, m)		
14	35.7	35.6	1.28 (1H, m)	21	28,4,2
			1.46 (1H, m)		
15	35.4	35.3	1.39 (1H, m)	5	16,20,17
			1.20 (1H, m)		
16	35.1	35.0	0.99 (s)		15,17,20
17	32.2	32.8	1.28 (1H, m)	10	20,16,15
			1.43 (1H, m)		
18	32.0	32.4	1.31 (1H, m)	13	4,25
19	31.9	32.1	1.18 (s)		5,10,13
20	30.6	31.8	1.50 (s)		16,17,15
21	30.5	30.5	1.38 (2H, m)	14	26,5
22	30.1	30.0			19,17,18,13,10,5
23	28.1	28.2			20,16,15,17,5,10

24	22.4	22.3	1.95 , m)	(1H	7,2	7
			1.68 H, m)	(1		
25	20.4	20.2	1.00	(s)		4,18
26	18.8	18.6	1.05	(s)		21,5
27	18.3	18.2	1.46 m)	(1H,	8,4	4
			1.36 m)	(1H,		
28	18.1	17.9	0.72	(s)		14,4,24,2
29	14.8	14.6	0.91	(s)		2,8,3
30	6.9	7.0	0.88 .8)	(d, 6	3	3

a and **b** represent H bonded to same carbon in a specified position.

*→ data from the isolated compound,

→Friedelin data from literature (Meira *et al.*, 2012)



54

Using the NMR data from the spectra above and literature (Akihisa *et al.*, 1992; Gaysinski *et al.*, 2015; Habib *et al.*, 2020; Mann *et al.*, 2011), the compound was identified to be friedelin (**54**). Friedelin (**54**) was f irst isolated from *I. batatas* and is known to exhibit antibacterial activit y against *Staphylococcus aureus* and it is also antifungal against *Psedall escheria boydii* (Meira *et al.*, 2012). According to literature, Friedelin i solated from *Azima tetracantha lam* was found to have anti-

diahoreal effect (Kumar *et al.*, 2020; Tabassum *et al.*, 2019) when used on wistar rats. It also anti-diabetic effects (Mohsina *et al.*, 2018; Sunil *et al.*, 2021) and anti-bacterial effects (Ogunnusi *et al.*, 2010).

4.3 Results of the antimicrobial test of the crude extract.

The **Table 4.3.1** shows the results of the bioassay of the methanol /DCM crude extract of *I. cairica* on four different strains of bacteria. The crude extract (1 mL) was dispensed in wells for antimicrobial activity, followed by a positive control using tetracycline and a negative control using DMSO. The average diameter of zones of inhibition were measured in millimeter (mm).

Table 4.3. 1: Antibacterial activity of the crude extract on selected strains of bacteria.

Test organisms	Diameter of zone of inhibition (mm)		
	Crude extract	Positive control (Tetracycline)	Negative control (DMSO)
S. typhi	20 ± 0.25	30 ± 0.10	0.00
E. Coli	26 ± 0.10	45 ± 0.32	0.00
P. aeruginosa	24 ± 0.12	35 ± 0.45	0.00
S. aureus	14 ± 0.05	40 ± 0.00	0.00

All the bacterial strains were found to be susceptible to the methanol/DCM extract as the zone of inhibition diameters (20 mm, 26 mm, 23 mm 13 mm for *S. typhi*, *E.coli* , *P.aeruginosa* and *S. aureus* respecti

vely,(**Table 4.2.1**) were within the range for standard antibiotics such as ampicillin (inhibition diameter 16-
22 mm), doxycycline (inhibition diameter 18-
24 mm) and tetracycline (inhibition diameter 18-
45 mm), as reported by the Clinical and Laboratory Standards Institute (Wayne, 2010).

4.3.1 Antifungal activity of the crude extract

The table below shows the results of the bioassay of the methanol/DCM crude extract of *I. cairica* on three different strains of fungi (**Table 4. 3.2**). The extract (1 ml) of concentration 200 $\mu g/ml$ was dispensed in wells for antifungal activity, followed by a positive control using ketoconazole and a negative control using DMSO. The average diameter of zones of inhibitions were measured in millimeter (mm).

Table 4.3. 2: Antifungal activity of the crude extract

Fungal strains	Diameter of zone of inhibition (mm)		
	DCM:MeOH extract	Ketoconazole/positive control	DMSO Negative control
A.nigar	16 ± 0.50	21 ± 0.10	0.00
C. albicans	24 ± 0.00	18 ± 0.00	0.00
P. chrysogenum	20 ± 0.41	28 ± 0.50	0.00

The extract showed a strong UV absorption (298-
380 nm), a typical of the occurrence of aromatic compounds. The results are in agreement with Convolvulaceae chemistry that the metabolites a

re well known for their biological and pharmacological potentials. This i s due to the presence of ; alkaloids, terpenoids, flavonoids, saponins, ta nnins and other bioactive molecules.

The antibacterial studies of the methanol/DCM extract of the aerial parts of *1.Cairaca* showed inhibition zone against bacterial strains *E. coli* (2 6 mm), *S. typhi* (20 mm), *P. aeruginosa* (24 mm) and *S. aureus* (13 m m) in comparison with the standard drug tetracycline with inhibition zon e against *E. coli* (30 mm), *S. typhi* (45 mm), *P. aeruginosa* (35 mm) a nd *S aureus* (40 mm) as given in **table 4.3.1**.

The antifungal results of the crude extract of the aerial parts of I.Cairac a revealed the inhibition zone of three different strains of fungus, i.e., *A . nigar* (16 mm), *C. albicans* (22 mm) and *P. chrysogenum* (20 mm) in comparison with the standard drug, ketoconazole which showed the inh ibition zone against; *A. nigar* (21 mm), *C. albicans* (18 mm) and *P. ch rysogenum* (28 mm) as given in **table 4.3.2**. The bioassay of the crude extract of *I.Cairaca* provides evidence of the occurrence of the anti-pathogenic natural products against both bacteria and fungi.

Table 4.4.1: Antibacterial activity of compound 53 against selected strains of bacteria.

Bacterial str ains	Diameter of zone of inhibition (mm)		
	Compound 53	Positive control (chloramph enicol)	Negative control (D MSO)
E. coli	8.0 ± 0.22	25 ± 0.23	0.00
P. aeruginos a	4.0 ± 0.32	34 ± 0.65	0.00
S. aureus	6.0 ± 0.00	35 ± 0.18	0.00
S. typhi	6.0 ± 0.55	36 ± 0.35	0.00

Table 4.4.2. Antibacterial activity of compound 54 against selected strains of bacteria.

Bacterial strains	Diameter of zone of inhibition (mm)		
	Compound 54	Positive control (chloramphenicol)	Negative control (DMSO)
E. coli	8.0 ± 0.05	38± 0.00	0.00
P. aeruginosa	5.0 ± 0.50	14 ± 0.25	0.00
S. aureus	8.0 ± 0.12	30 ± 0.04	0.00
S. typhi	10 ± 0.50	32 ± 0.85	0.00

Antibacterial activity of the isolated compounds (1000 $\mu g/ml$) each in dimethyl sulphoxide were tested against the four different strains of bacteria. The diameters of zone of inhibition showed that the organisms were susceptible to the isolated compounds while both results indicated that *P. aeruginosa* was resistant to the compounds since it gave the lowest zones of inhibition (5.0 mm) as compared to the other strains. All the average zones of inhibitions formed by the isolated compounds were significantly different to those formed by chloramphenicol, the standard antibiotic used as a positive control. Dimethyl sulphoxide (DMSO) was used as a negative control (**Appendix 2**).

4.4 Minimum inhibitory concentration (MIC)

In the case where there was no bacterial growth and also not greater than the minimum inhibitory concentration was taken as the minimum bacterial concentration.

Table 4.5.1: Minimum Inhibitory Concentration (MIC) of isolated compound 53

Concentration ($\mu g/ml$)	500.00	250.00	125.00	62.50	31.25	15.63	7.82	3.91
E. coli	-	-	-	+	+	+	+	+
P. aeruginosa	-	-	+	+	+	+	+	+
S. typhi	-	-	+	+	+	+	+	+
S. aureus	-	-	-	+	+	+	+	+

Where: The minus sign (-

) showed no growth of organisms occurred (colourless).

The positive sign (+) indicated growth occurred (turbid ity)

The pure isolated compound 53 showed a minimum inhibitory concentra tion (MIC) of 125 $\mu g/ml$ on *E. coli,* 250 $\mu g/$

ml on *P. aeruginosa,* 250 $\mu g/ml$ on *S. typhi and* 125 $\mu g/$

ml on *S. aureus*. This showed that compound 49 was more effective ag ainst *E. coli* and *S. aureus* as compared to *P. aeruginosa* and *S. typhi* (**Appendix 4**).

Table 4.5.2: Minimum Inhibitory Concentration (MIC) of isolated c ompound 54

Concentratio n ($\mu g/ml$)	500.0 0	250.0 0	125.0 0	62.5 0	31.2 5	15.6 3	7.8 2	3.9 1
E. coli	-	-	-	+	+	+	+	+
P. aeruginos a	-	-	+	+	+	+	+	+
S. typhi	-	-	+	+	+	+	+	+
S. aureus	-	-	+	+	+	+	+	+

Where: The minus sign (-) showed no growth of organisms occurred.

The positive sign (+) indicated growth occurred.

The results indicated that, the *E. coli* with MIC value of 125 $\mu g/ml$ is more susceptible to the compound **54** as compared to *P. aerugino sa, S. typhi* and *S. aureus* with each having MIC value of 250 $\mu g/ml$ (**Appendix 5**)

4.5 Physical and Spectroscopic Properties of Compounds Isolated/Discussed

4.5.1 Di-isobutyl phthalate (**53**)

Yellow amorphous powder, visible under UV$\lambda\ max$ 254 nm, ^{1}H NMR and ^{13}C NMR (**Table 4.2.1**), ESI-MS at *m/z* 279 $[M+H]^{+}$ (29), 262 (35), 183 (20), 167 (54), 71 (25), 69 (30), 57 (70),and 43 (100) as the base peak. Using the above data and literature (Ruikar *et al*., 2011), the compound was identified as Diisobutyl phthalate with a general molecular formula $C_{16}H_{22}O_{4}$. The NMR spectra for compound **53** are shown in **appendix 7**.

4.5.2 Friedelin (**54**)

Isolated as an amorphous white solid, UV$\lambda\ max$ 254 nm, ^{1}H NMR and ^{13}C NMR (Table 4.2.2). Upon comparing the data with what is reported in literatures [Akihisa *et al* (1992), and Habib *et al* (2020)], the compound was identified as Friedelin with *m/z* 426 with a general formula $C_{30}H_{50}O$ (**Appendix 8**).

CHAPTER FIVE: CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusion

Two pure compounds were isolated and characterized from the aerial parts of *I. cairica* namely; Diisobutyl phthalate (**53**) and Friedelin (**54**). The crude extract from the aerial parts of *I. cairica* showed good antimicrobial activities against all the selected microbial strains for both bacteria and fungi. The isolated compounds showed less antimicrobial activity as compared to the crude extract, which means that the isolated compounds would give better results when subjected synergistically (Egorov *et al*., 1981).

5.2 Recommendations

1. The aerials parts of *I. cairica* should be investigated further using the different modern separation techniques such as MPLC or HPLC to exhaustively isolate most of phytoconstituents.
2. These isolated compounds should then be evaluated for their antimicrobial potential against some other pathogenic strains which are purposively relevant in the clinical investigations.
3. Structural modification of the two isolated compounds; Diisobutyl phthalate (**53**) and Friedelin (**54**) leading to analogues, significantly with improved antimicrobial activities should be considered.
4. More safety/cytotoxicity studies should be carried out about extracts and the isolated compounds.

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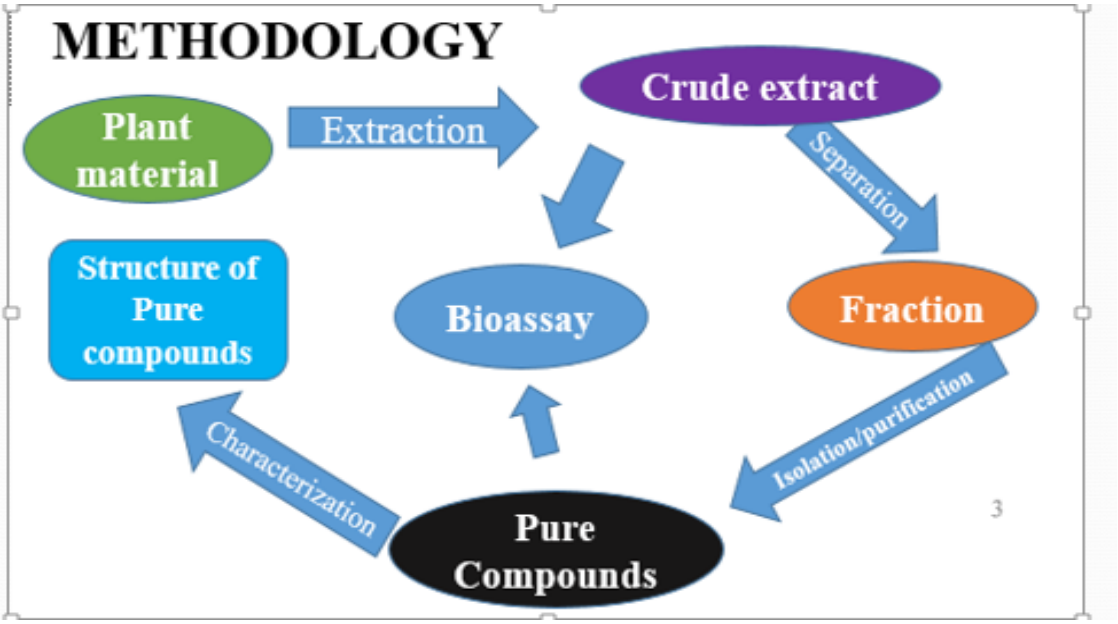
APPENDICES

APPENDIX 1: LABORATORY PROCESSES



Addition of adsorbed crude extract in to the column

Running of column using an increasing solvent gradient

Compounds isolated from *Ipomoea cairica* extract

Ipomoea cairica identified at the herbarium T.L.C in progres

s

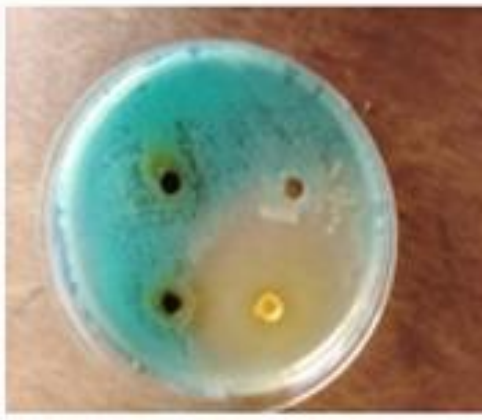
Concentration of the fractions collected using a rotary evaporator under reduced pressure.

APPENDIX 2: RESULTS OF BIOACTIVITY OF THE METHANO L/DCM CRUDE EXTRACT OF *IPOMOEA CAIRICA*

Bacterial strains used

Escherichia coli

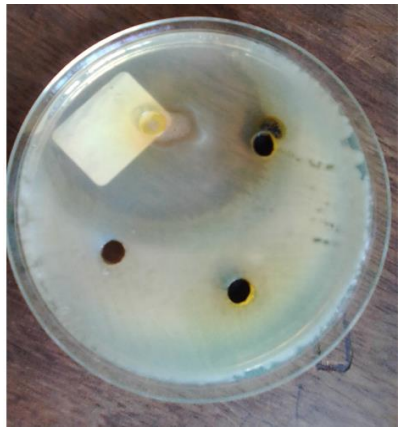
Pseudomonas aeruginosa



Salmonella typhi

Staphylococcus-aureus

APPENDIX 3: RESULTS OF BIOACTIVITY OF THE ISOLATED COMPOUNDS 53 AND 54



Staphylococcus aureus

Pseudomonas aeruginosa

Staphylococcus aureus

Pseudomona aeruginosa

APPENDIX 4: RESULTS OF THE MINIMUM INHIBITORY CON CENTRATION (MIC) OF COMPOUND (53) ON SELECTED BAC TERIA

MIC for Escherichia coli

MIC for Pseudomonas aeruginosa

MIC for Salmonella typhi

MIC for Staphylococcus aureus

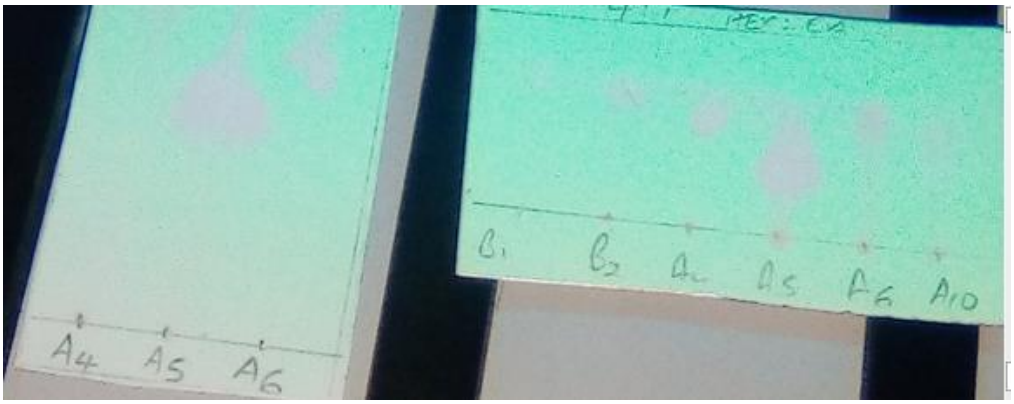
APPENDIX 5: RESULTS OF THE MINIMUM INHIBITORY CON CENTRATION (MIC) OF COMPOUND (54) ON SELECTED BAC TERIA.

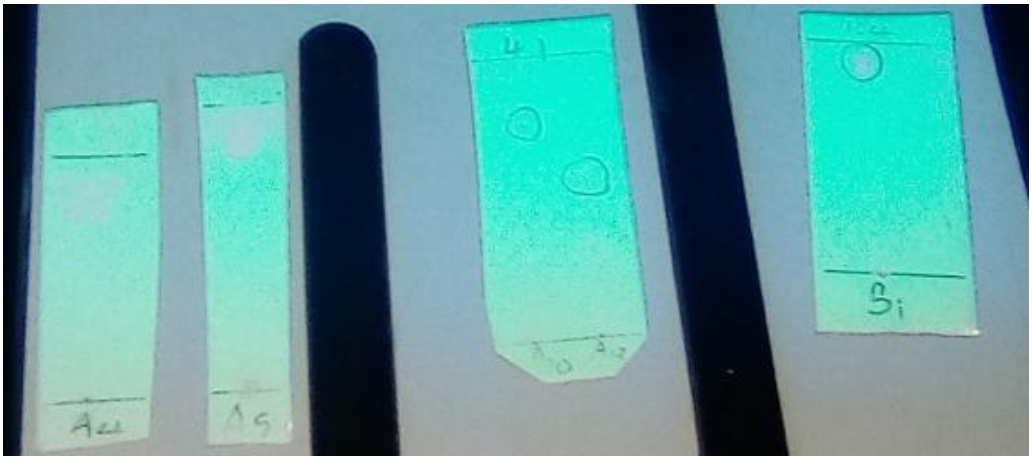
Escherichia coli
Pseudomonas aeruginosa

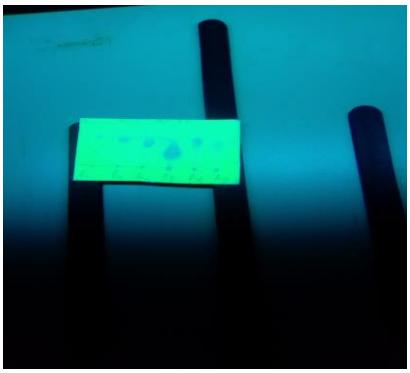
Staphylococcus aureus *Sa*

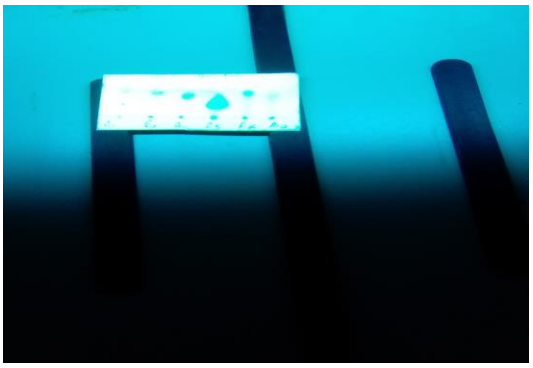
lmonella typhi

APPENDIX 6: TLC RESULTS FOR THE ISOLATED COMPOUNDS



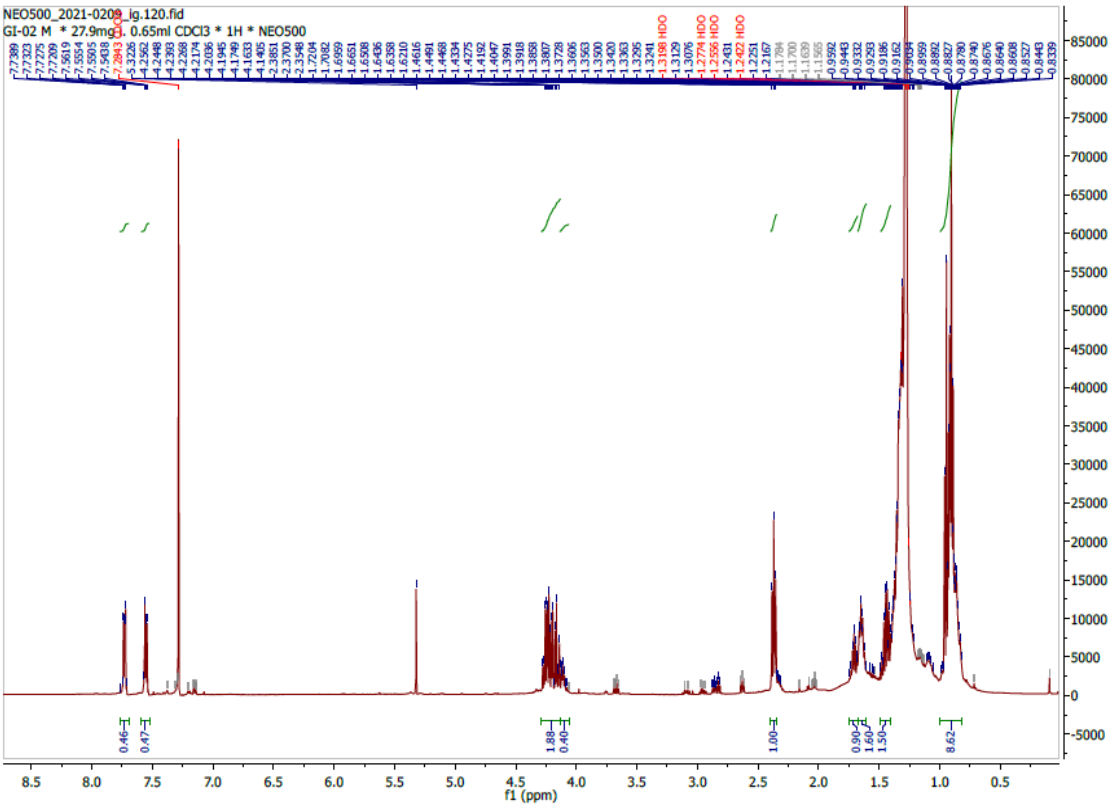


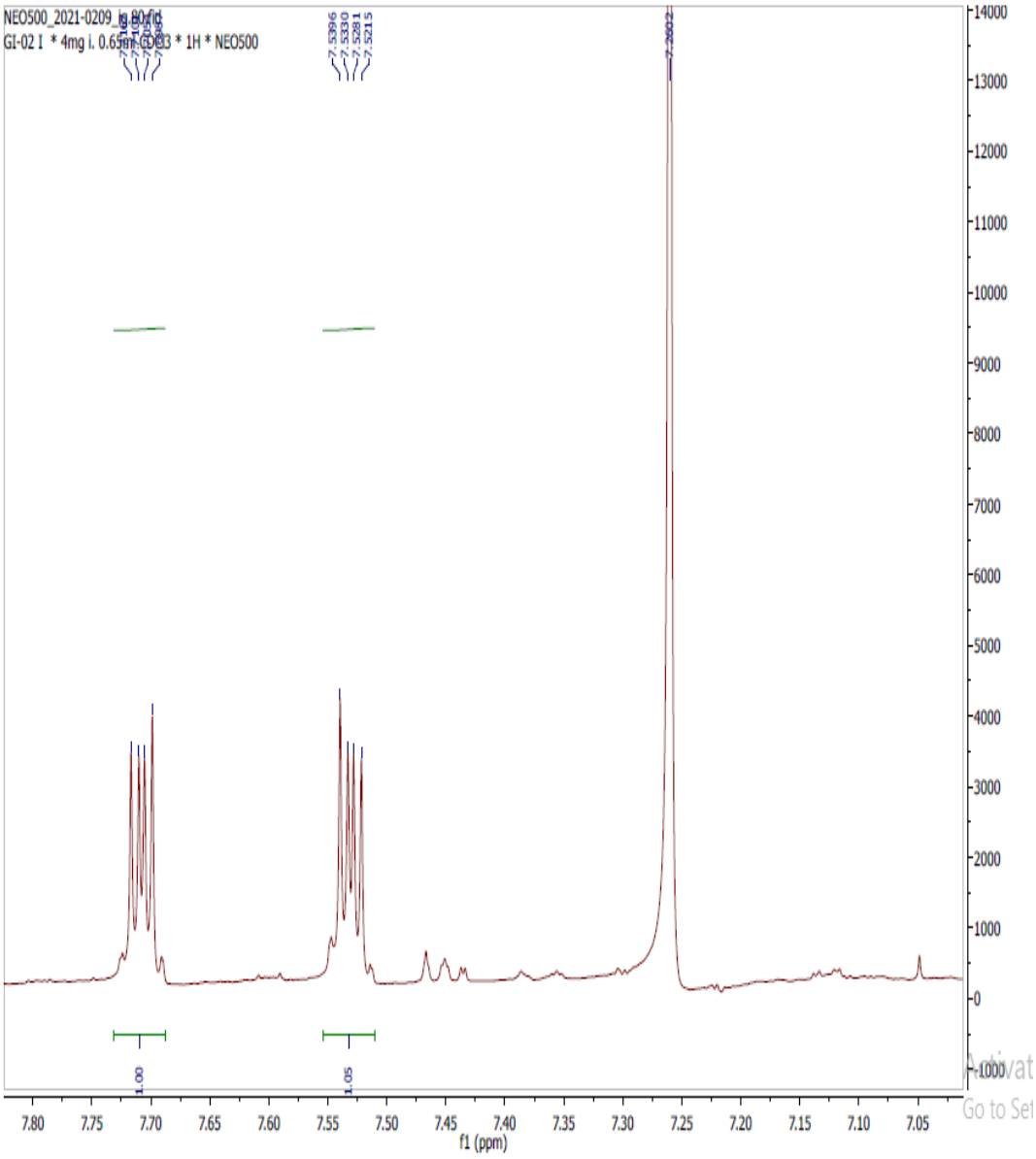


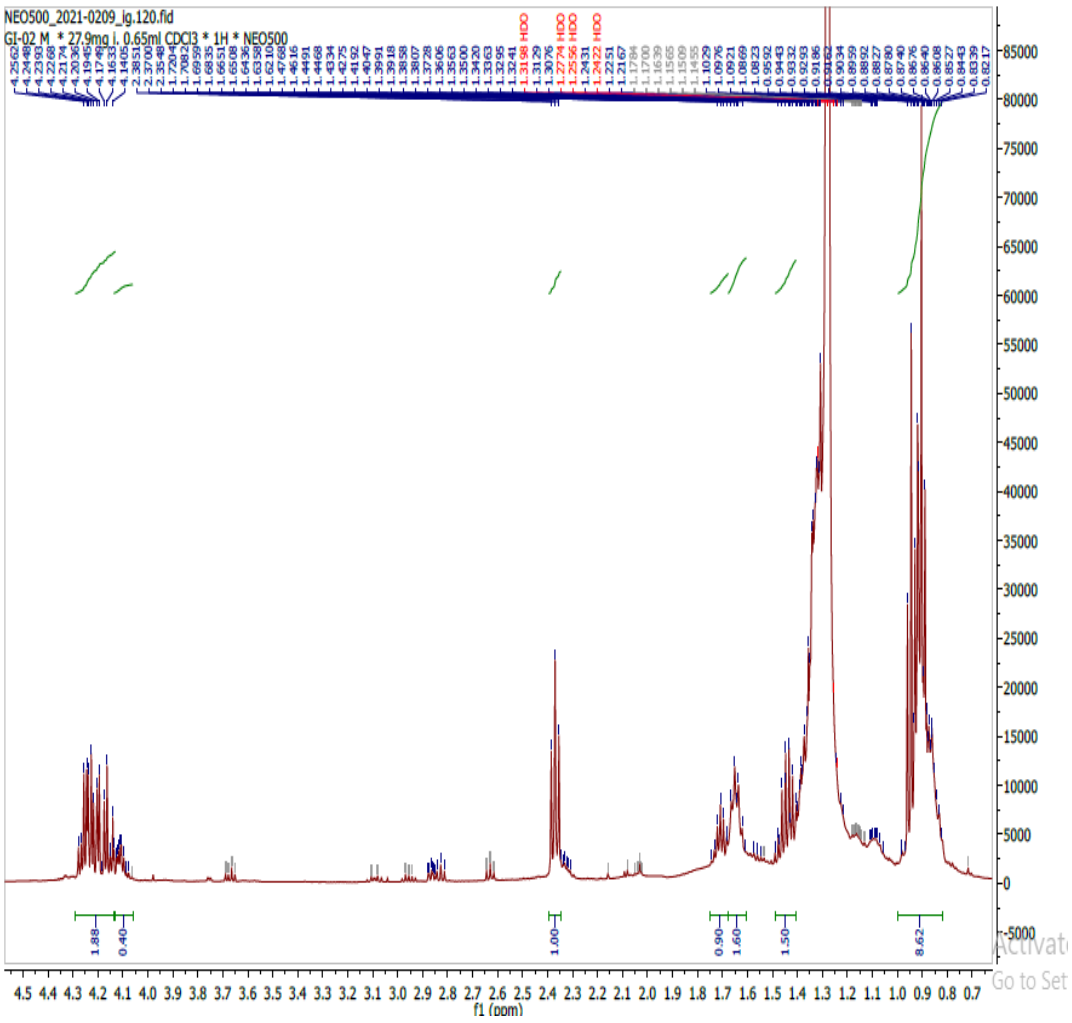


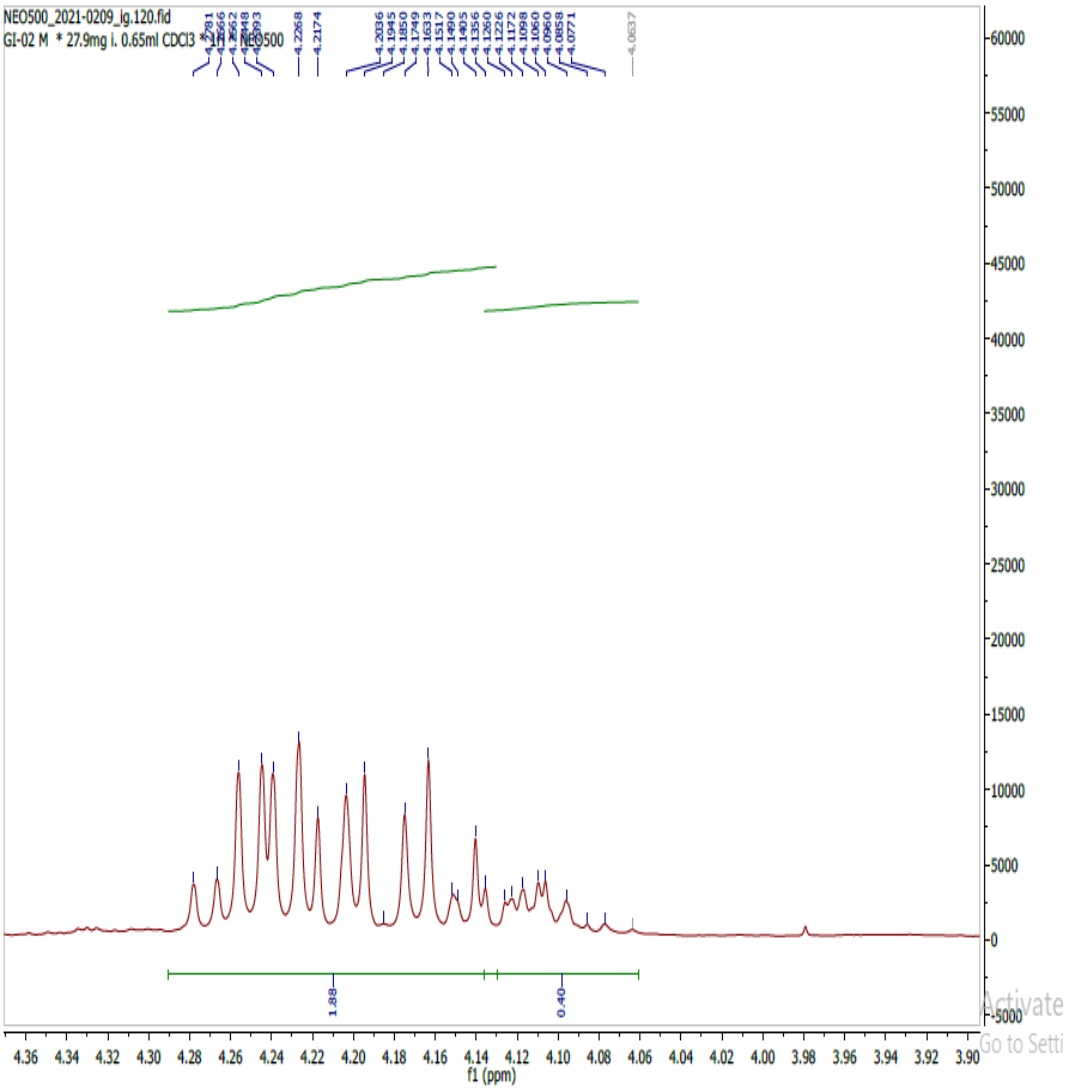
APPENDIX 7: SPECTRA FOR COMPOUND 53

^{1}H NMR FOR COMPOUND **53** ($CDCl_3$, 500Hz)

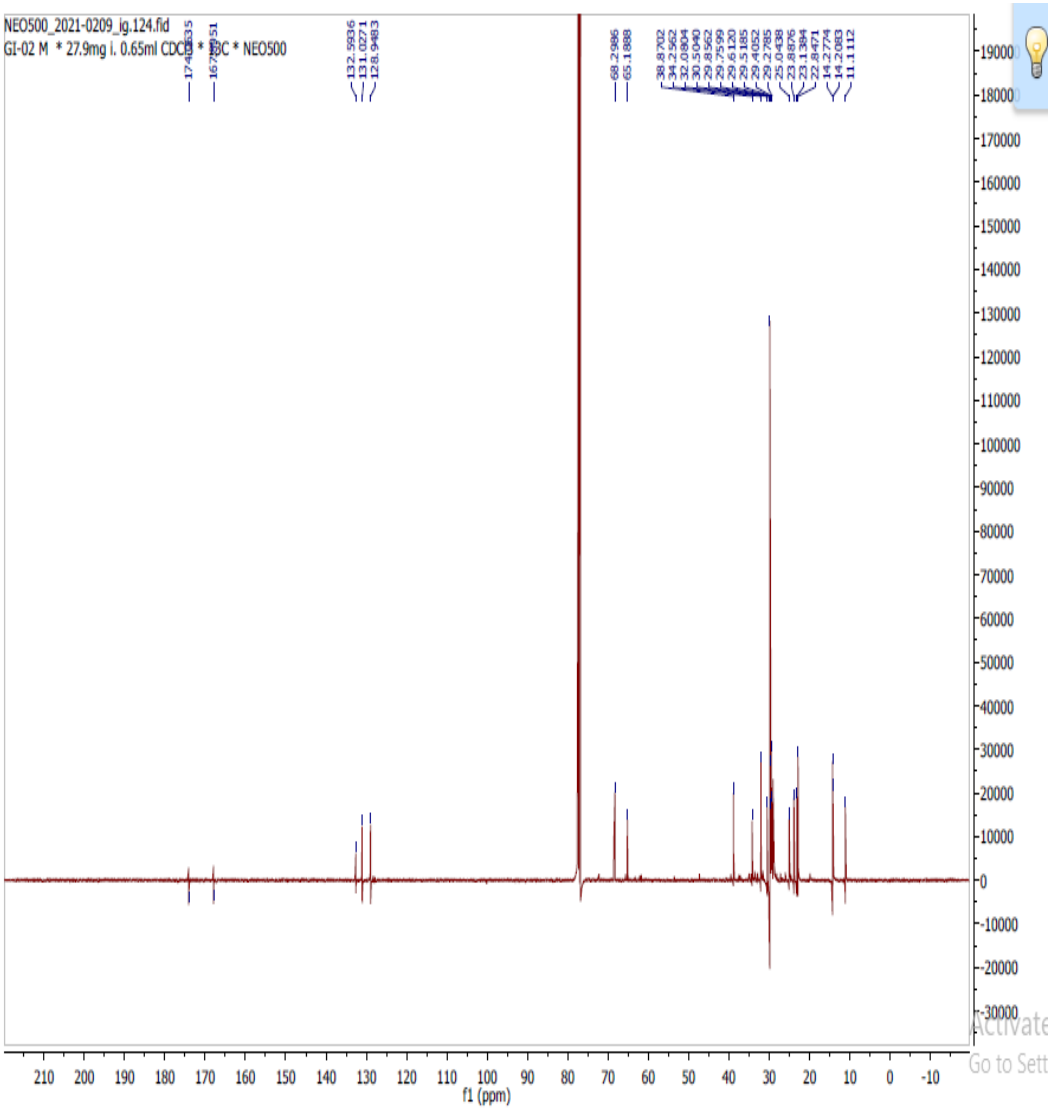



NEO500_2021-0209_ig_80.fid
GI-02 I * 4mg i. 0.65ml CDCl3 * 1H * NEO500
7.5396
7.5330
7.5281
7.5215
7.2602
1.00
1.05
f1 (ppm)

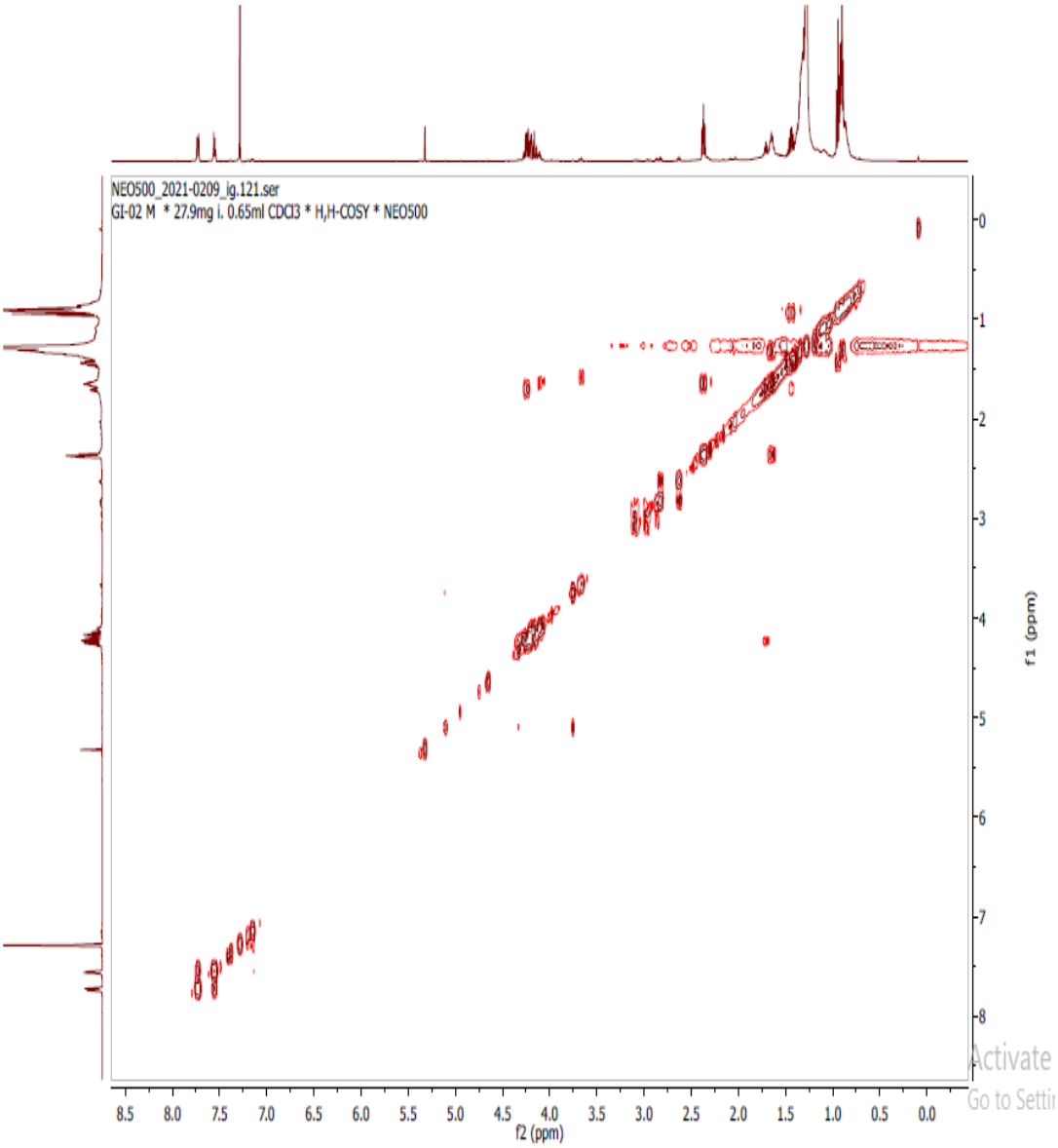

NEO500_2021-0209_ig.120.fid
GI-02 M * 27.9mg i. 0.65ml CDCl3 * 1H * NEO500
1.88
0.40
1.00
0.90
1.60
1.50
8.62
f1 (ppm)


NEO500_2021-0209_lg.120.fid
GI-02 M * 27.9mg i. 0.65ml CDCl3
NEO500
4.2268
4.2174
4.0637
1.88
0.40
f1 (ppm)

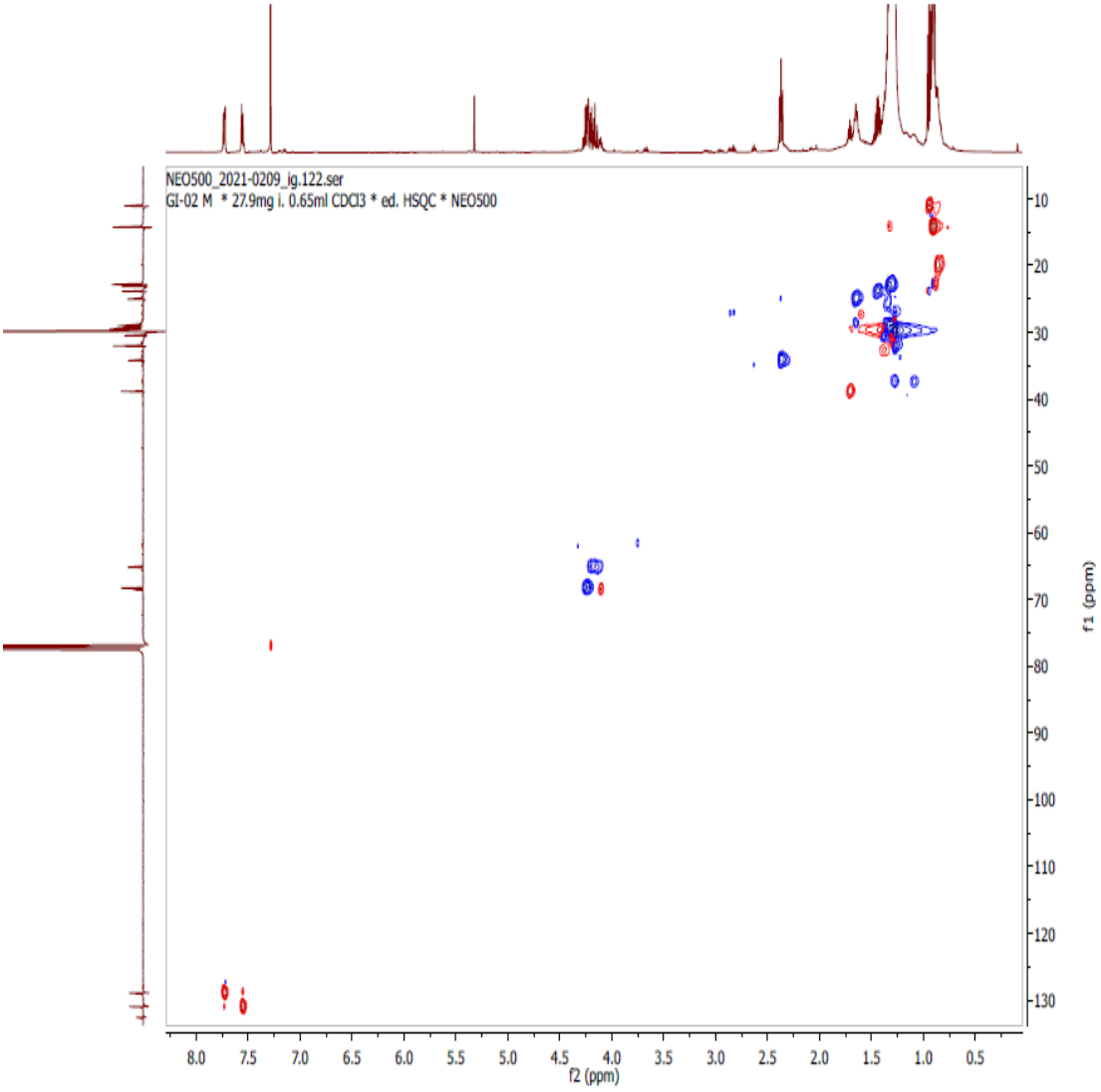
^{13}C NMR SPECTRUM FOR COMPOUND **53** ($CDCl_3$, 500 Hz)

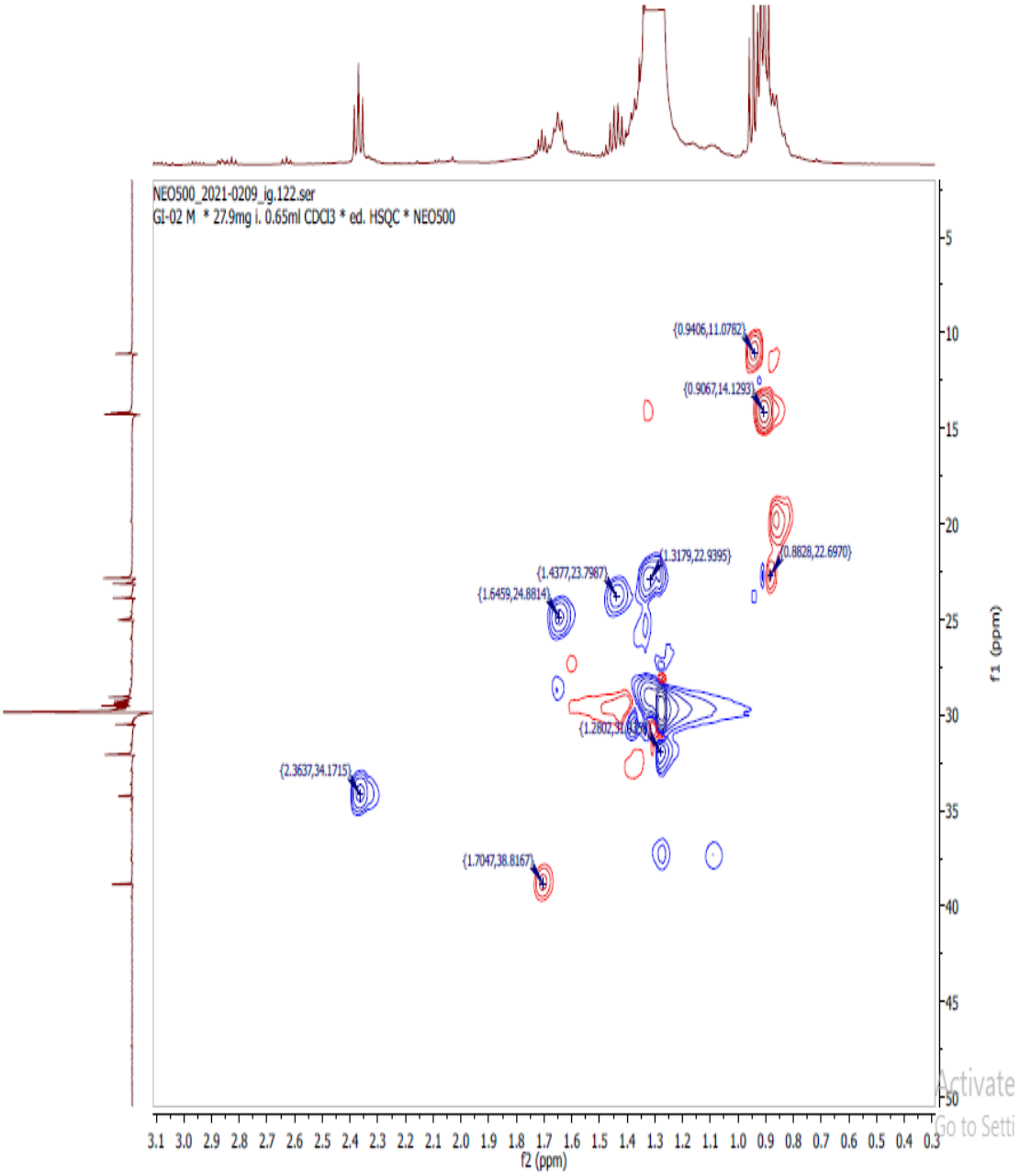


H-H COSY NMR SPECTRUM FOR COMPOUND **53** ($CDCl_3$, 500 Hz)

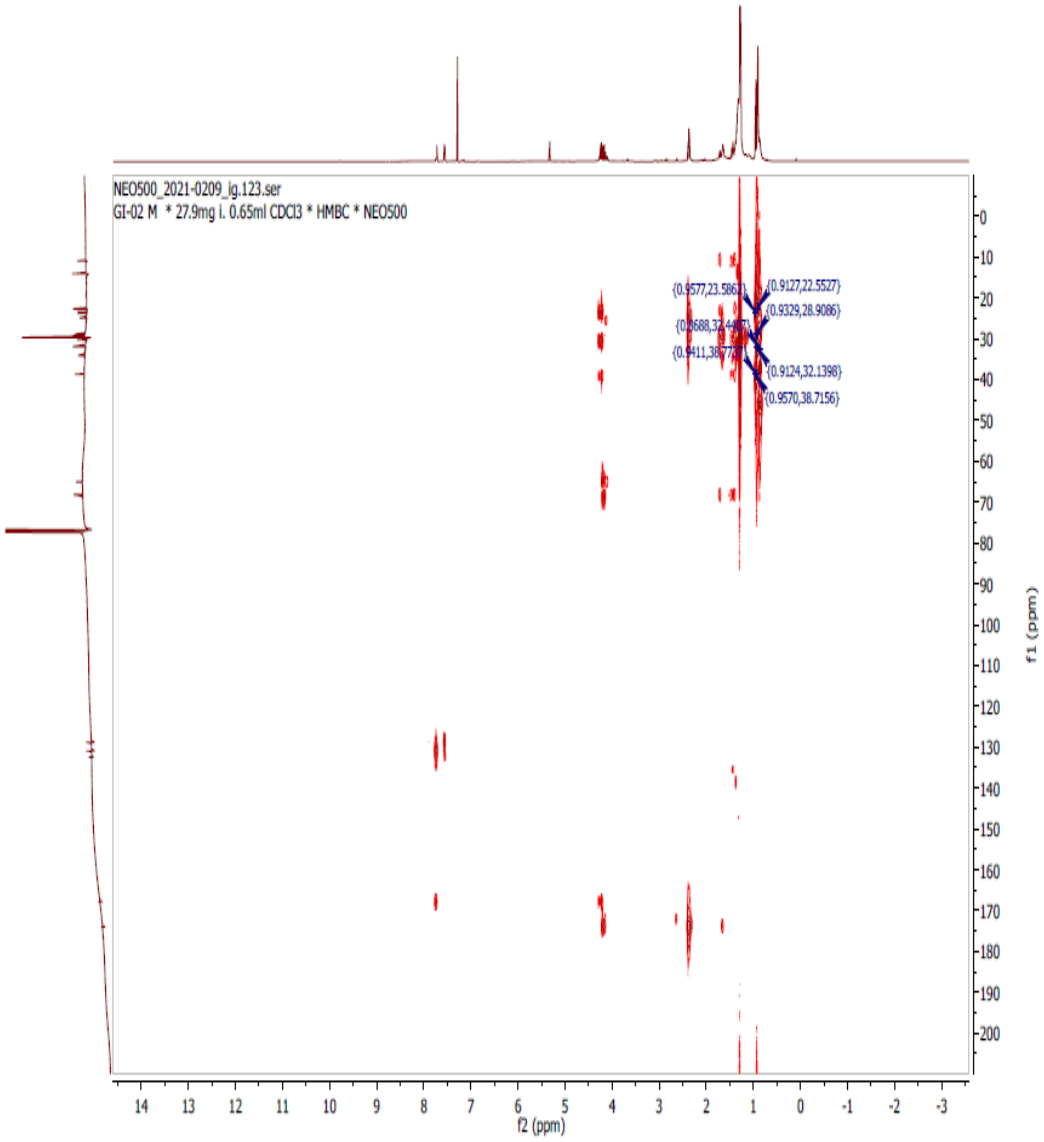


HSQC SPECTRUM FOR COMPOUND **53** ($CDCl_3$, 500 Hz)

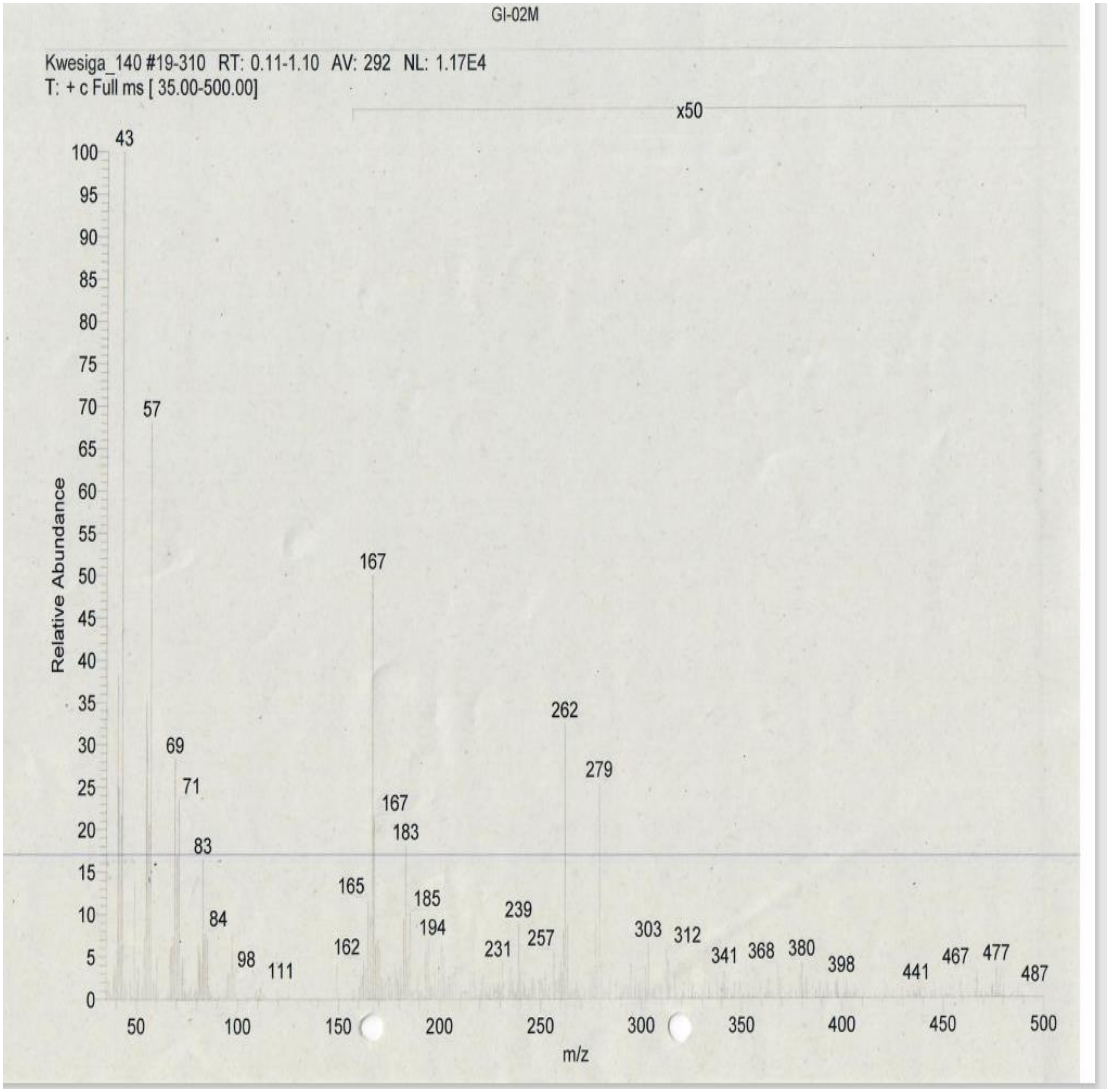



NEO500_2021-0209_ig.122.ser
GI-02 M * 27.9mg i. 0.65ml CDCl3 * ed. HSQC * NEO500
{0.9406,11.0782}
{0.9067,14.1293}
{1.3179,22.9395}
{0.8828,22.6970}
{1.4377,23.7987}
{1.6459,24.8814}
{2.3637,34.1715}
{1.7047,38.8167}
f1 (ppm)
f2 (ppm)

HMBC SPECTRUM FOR COMPOUND **53** ($CDCl_3$, 500 Hz)

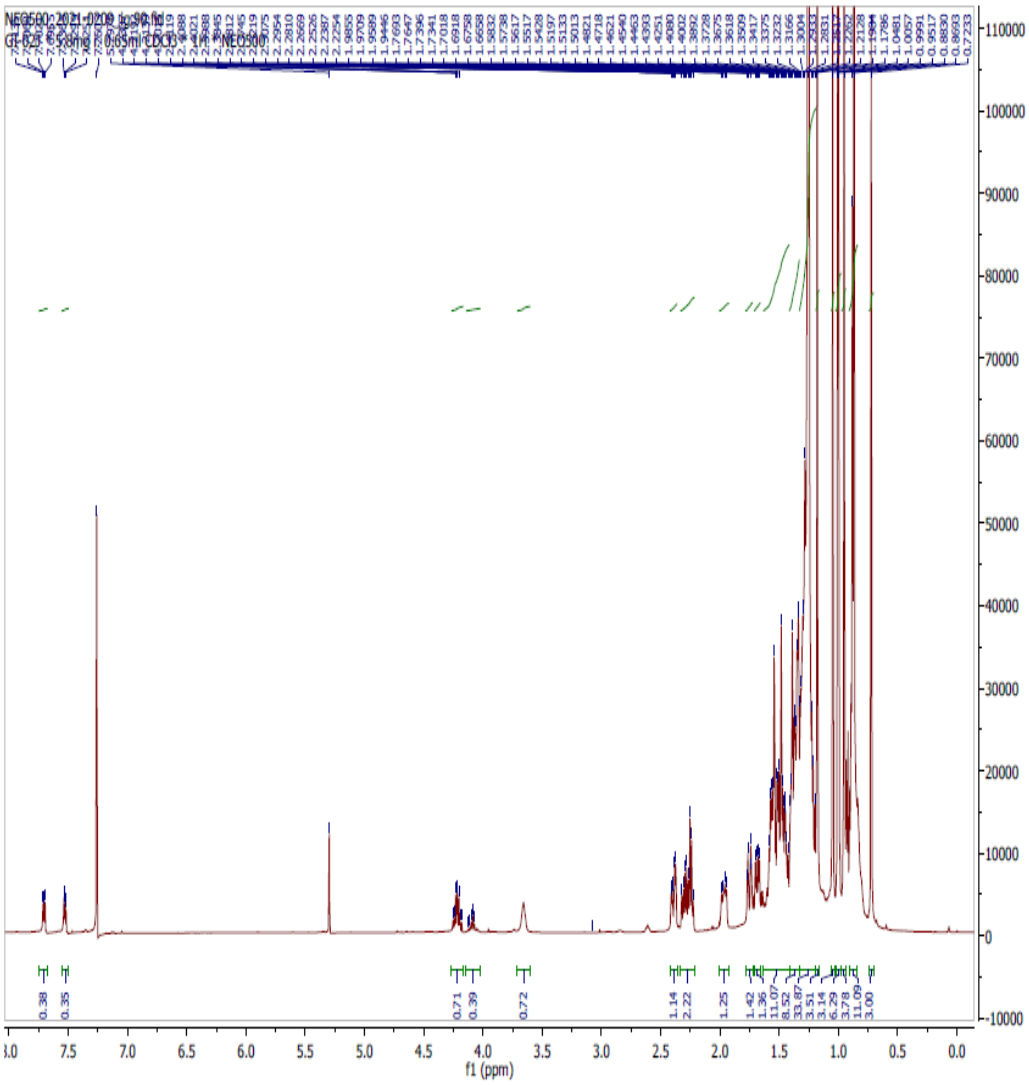


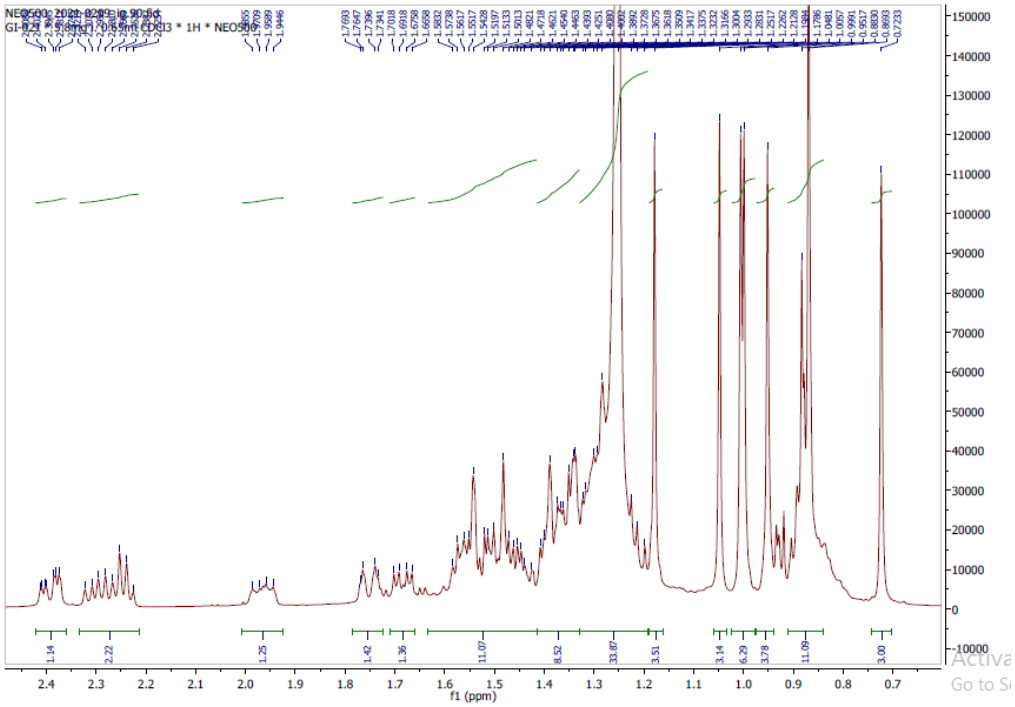
THE **ESI- MS** SPECTRUM OF COMPOUND **53**

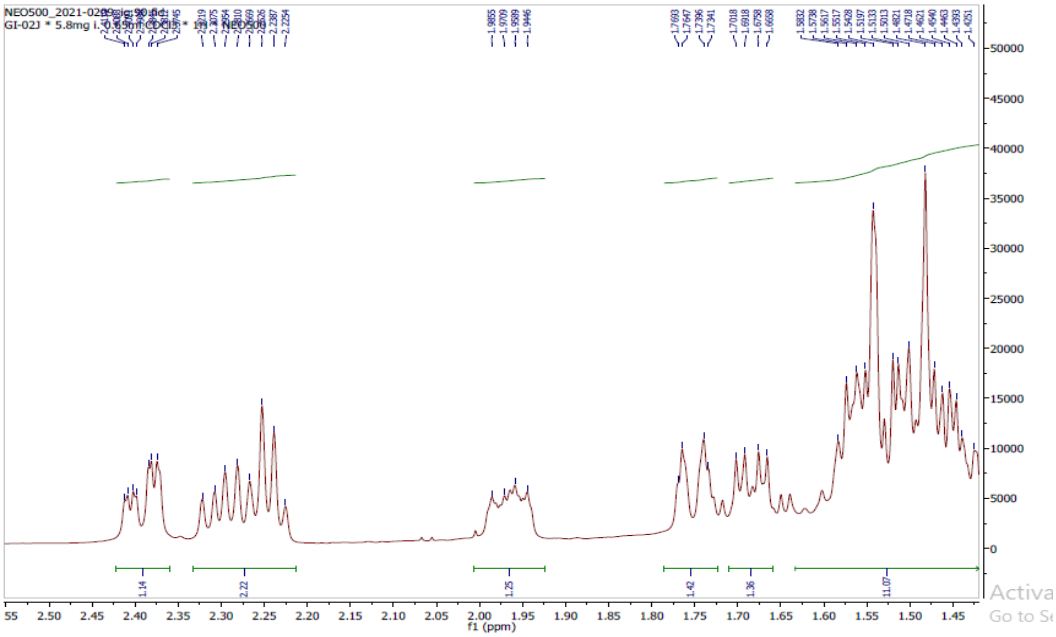


APPENDIX 8: SPECTRA FOR COMPOUND 54

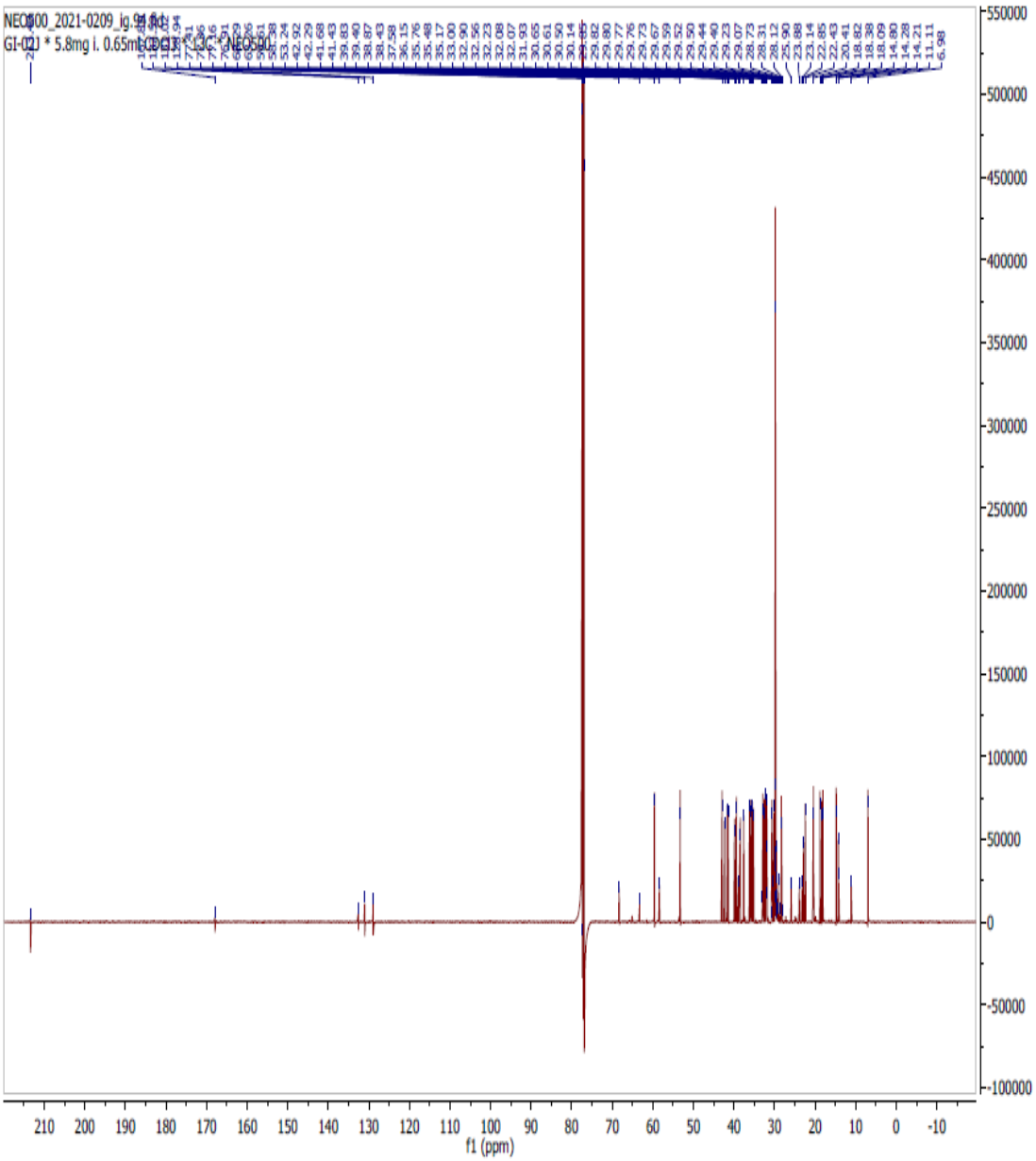
^{1}H NMR FOR COMPOUND **54** ($CDCl_3$, 500Hz)

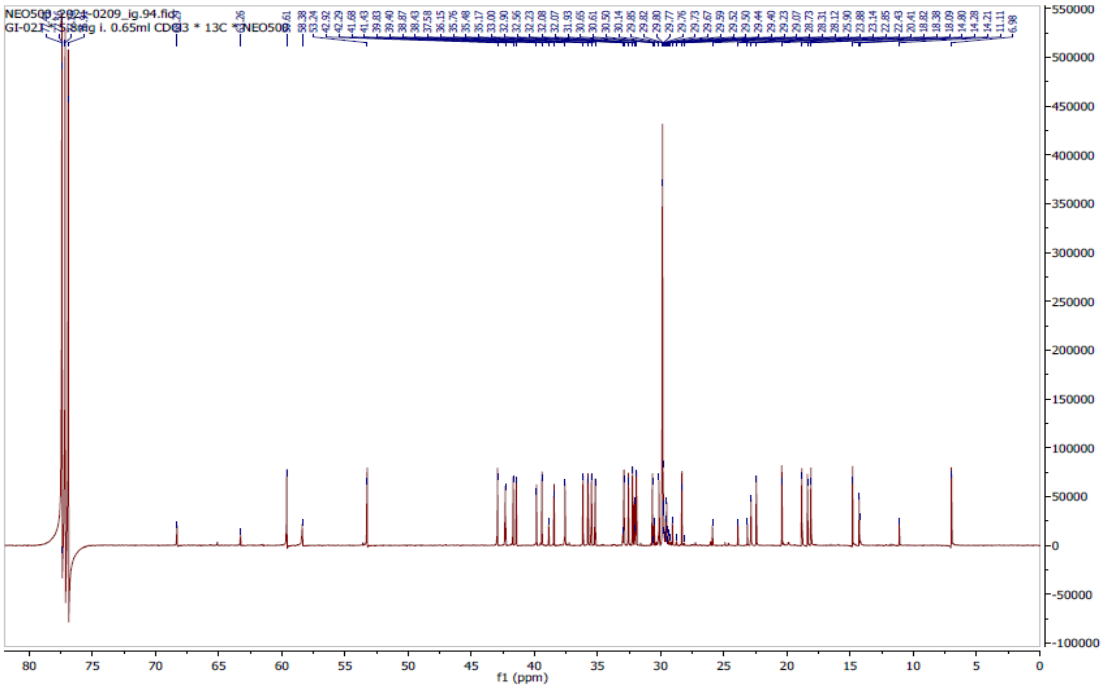


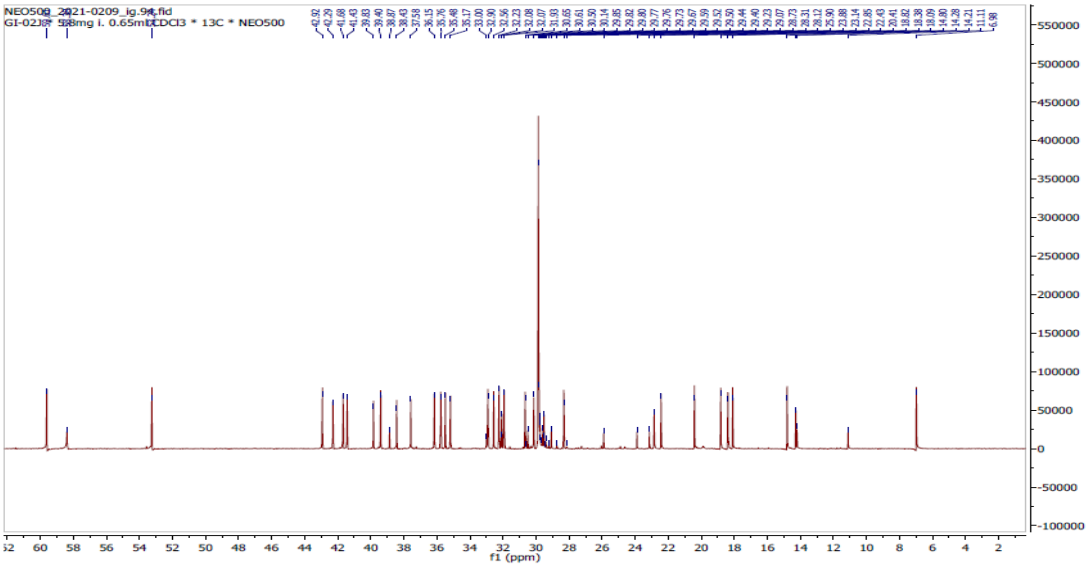

f1 (ppm)


f1 (ppm)

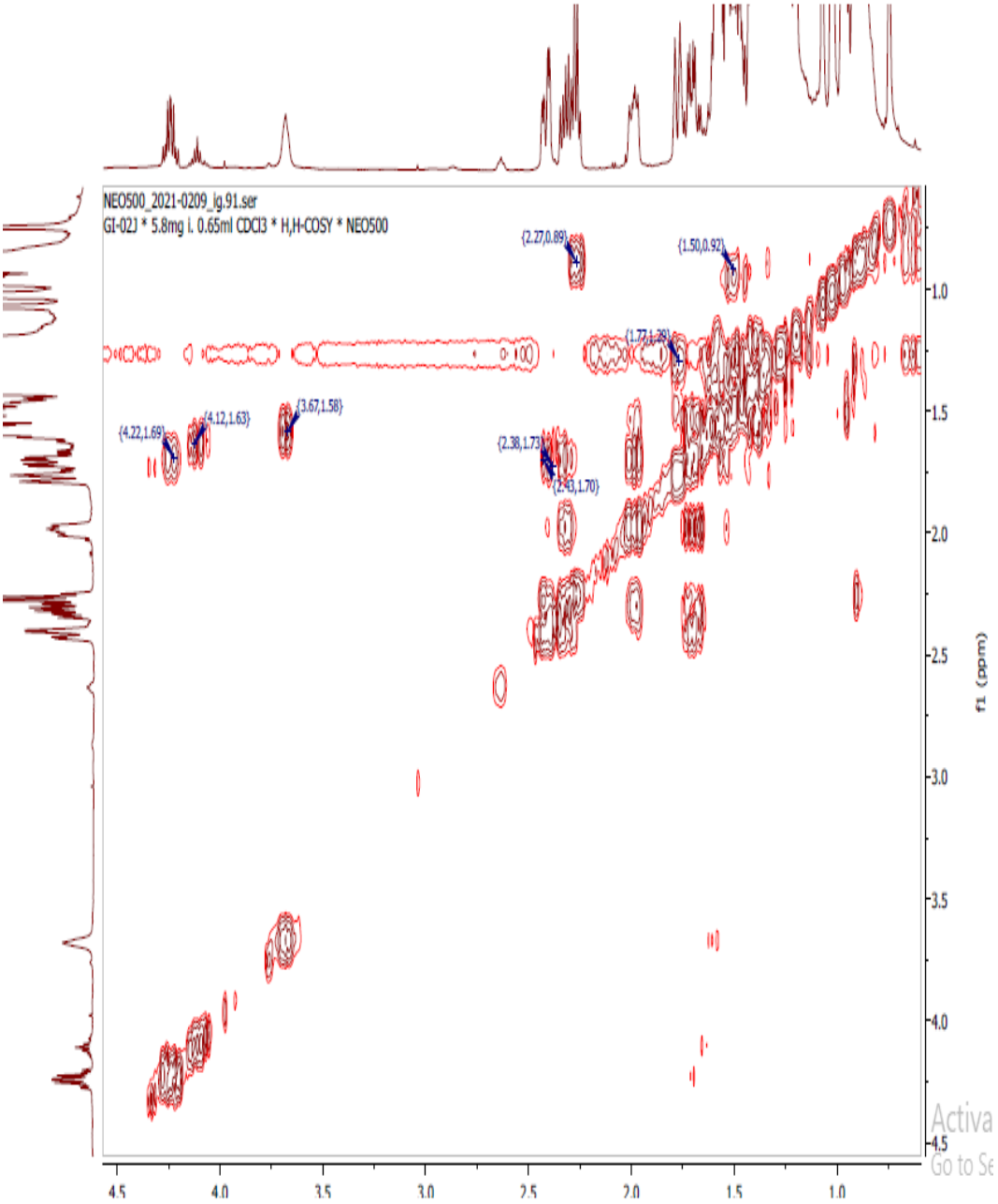
^{13}C NMR SPECTRUM FOR COMPOUND **54** ($CDCl_3$, 500 Hz)



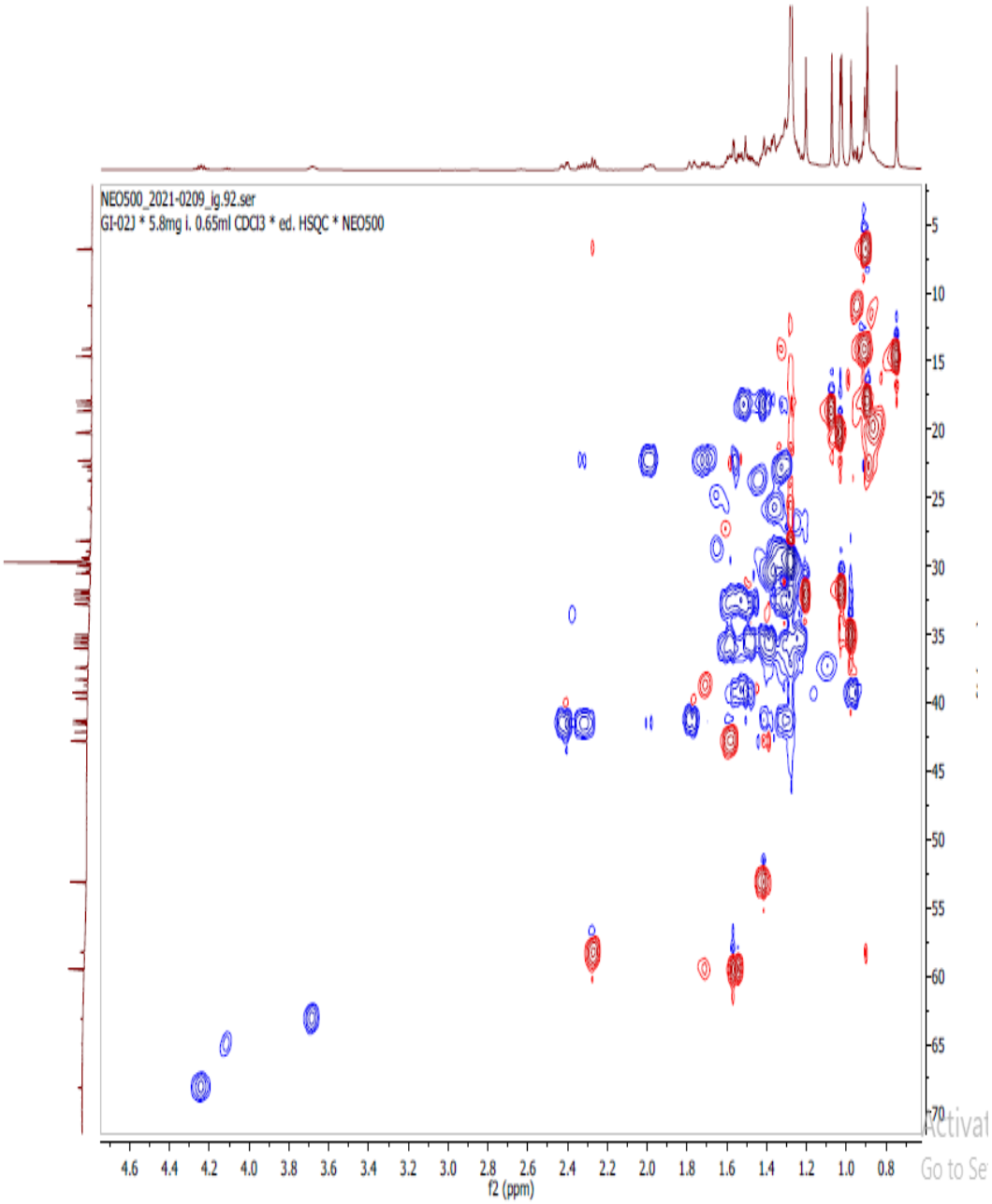

f1 (ppm)

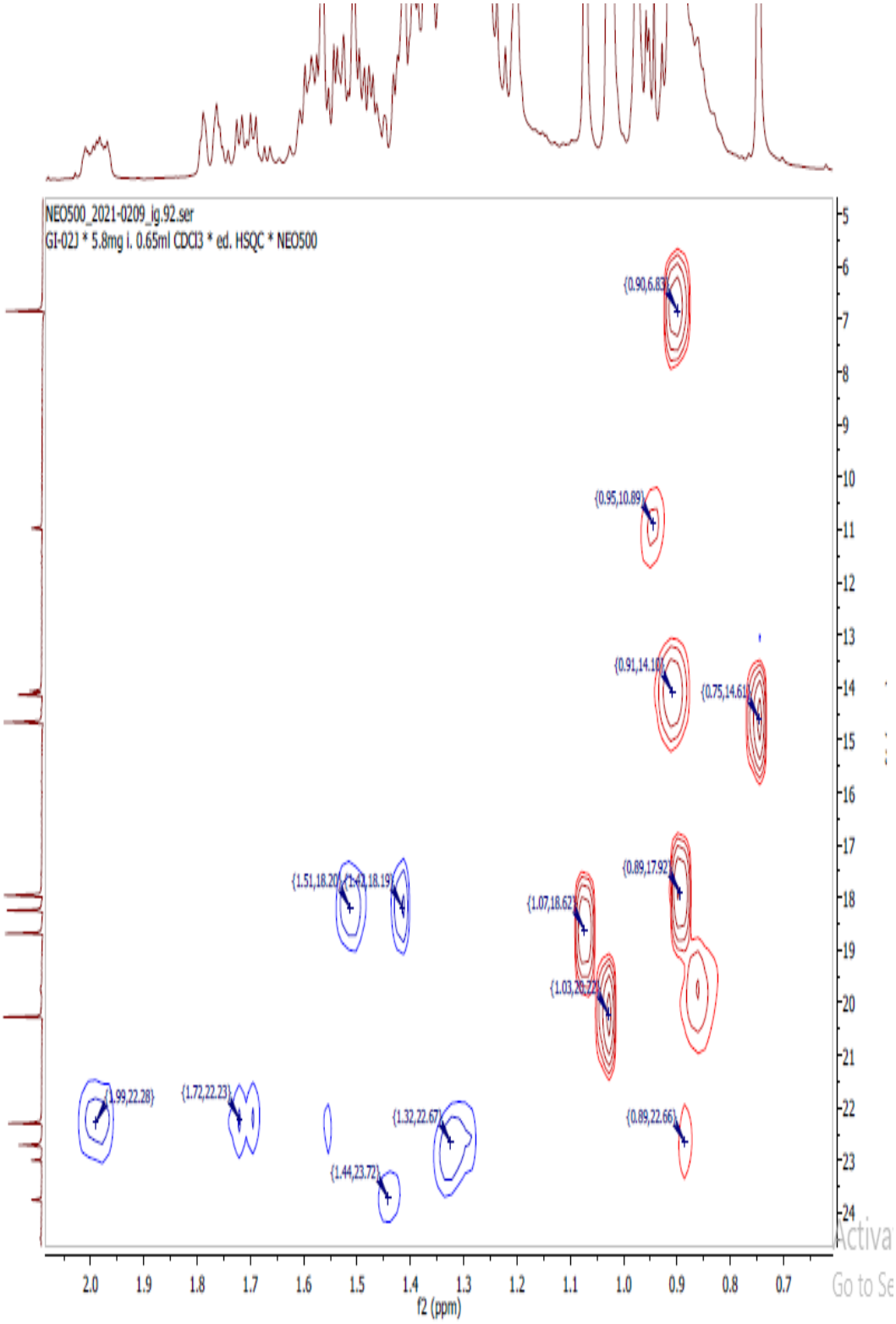

f1 (ppm)

H-H COSY NMR SPECTRUM FOR COMPOUND **54** ($CDCl_3$, 500 Hz)

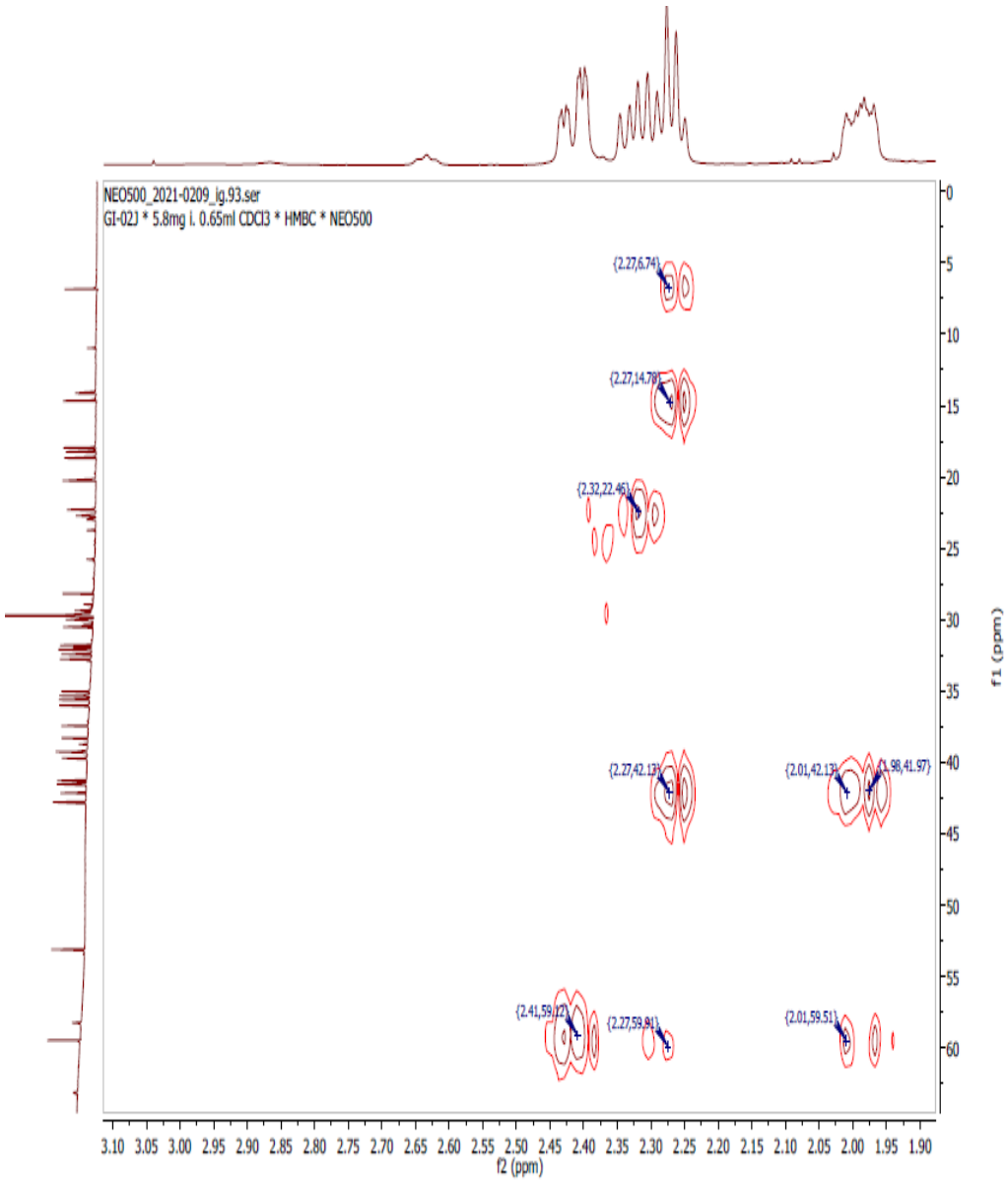


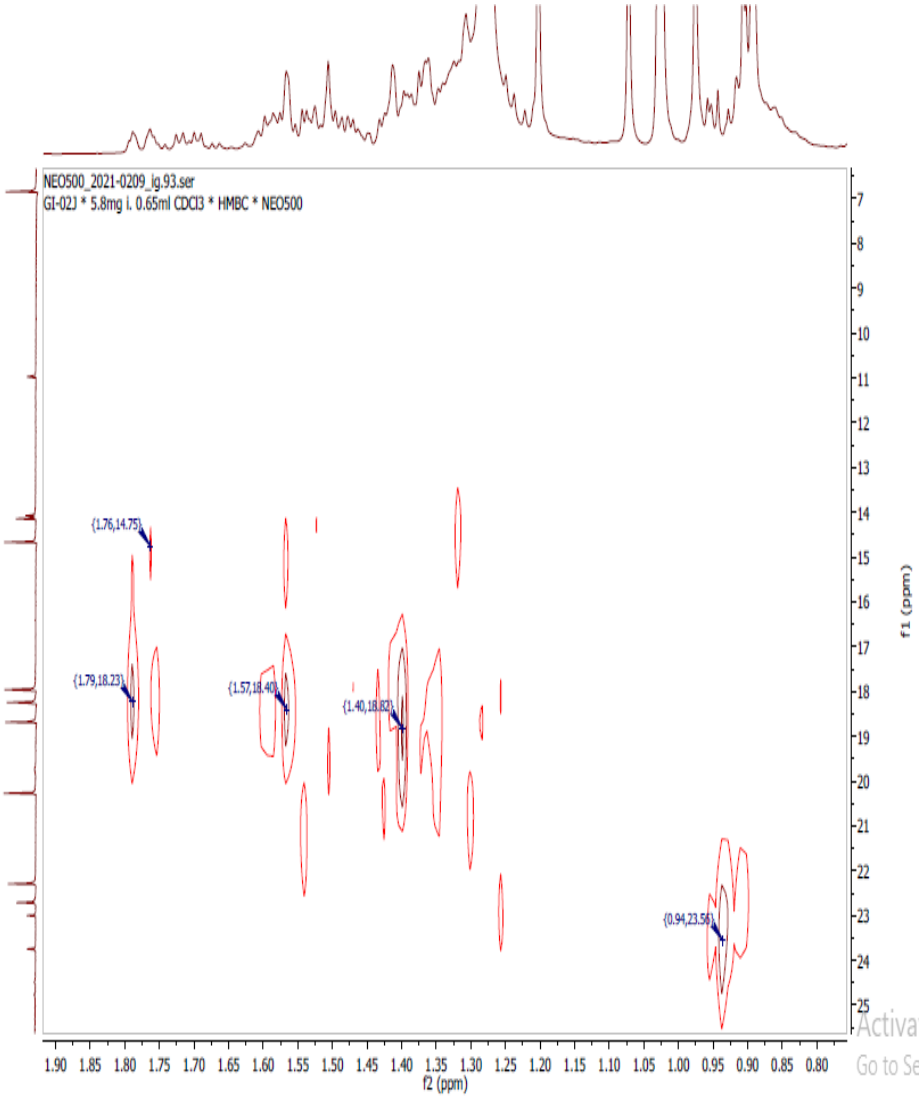
HSQC SPECTRUM FOR COMPOUND **54** ($CDCl_3$, 500 Hz)




NEO500_2021-0209_jg.92.ser
GI-021 * 5.8mg i. 0.65ml CDCl3 * ed. HSQC * NEO500
{0.90,6.83}
{0.95,10.89}
{0.91,14.10}
{0.75,14.61}
{1.51,18.20}
{1.42,18.19}
{1.07,18.62}
{1.03,20.72}
{0.89,17.92}
{1.99,22.28}
{1.72,22.23}
{1.44,23.72}
{1.32,22.67}
{0.89,22.66}
f2 (ppm)

HMBC SPECTRUM FOR COMPOUND **54** ($CDCl_3$, 500 Hz)




NEO500_2021-0209_ig.93.ser
GI-02J * 5.8mg i. 0.65ml CDCl3 * HMBC * NEO500
{1.76,14.75}
{1.79,18.23}
{1.57,18.40}
{1.40,18.82}
{0.94,23.56}
f1 (ppm)
f2 (ppm)