PHYTOCHEMICAL INVESTIGATION OF *HARRISONIA ABYSSINICA* AND *THESPESIA GARCKEANA* FOR ANTIPLASMODIAL AND ANTIMICROBIAL COMPOUNDS.

BY

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THIS THESIS IS SUBMITTED IN PARTIAL FULFILMENT FOR THE MASTER OF SCIENCE IN CHEMISTRY IN THE DEPARTMENT OF CHEMISTRY, UNIVERSITY OF NAIROBI.

2014.

DECLARATION

This thesis is my original work and has not been presented for a degree in any other University.

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DEDICATION

This thesis is dedicated to my dear friend and sister Everlyne Mueni Nthale.

ACKNOWLEDGEMENT

ABSTRACT

Harrisonia abyssinica (Simaroubacea) and *Thespesia garckeana* (Malvaceae) have been used in folk medicine but there is no scientific data on them. This study therefore sought to investigate the phytochemical and microbial principles in these plants so as to improve the knowledge base and hopefully produce lead compounds. The stem bark of *H. abyssinica,* the roots and stems of *T. garckeana* were subjected to solvent extraction by cold percolation. The crude extracts underwent chromatographic separation and a total of seven compounds were isolated. The seven compounds were characterized using spectroscopic techniques and identified as obacunone (**1**), from *H. abyssinica* stem barks, gossypol (**2**), 6,6´-dimethoxygossypol (**3**), 6-methoxygossypol (**4**), stigmasterol (**5**) from *T. garckeana* roots, *E*-docosyl 3-(3,4-dihydroxyphenyl) acrylate (**6**) and betulinic acid (**7**) from *T. garckeana* stems. The crude extracts and all the seven compounds were tested for antiplasmodial and antimicrobial activity. The crude extract of *H. abyssinica* exhibited antiplasmodial activities with IC_{50} values of 5.6 and 4.4 µg/ml against the (D6) and (W2) strains of *Plasmodium falciparum* respectively. The crude extract from the roots of *T. garckeana* showed no antiplasmodial activity at concentrations less than 50 µg/ml, but exhibited 100 % inhibition against *Candida glabrata* at concentrations of 50 µg/ml. Obacunone (**1**) was found to have a moderate activity against D6 and W2 strains of *P. falciparum* with IC_{50} values > 4.76 µg/ml. Some compounds including gossypol (**2**) showed strong activity of IC_{50} value of 0.89 µg/ml against vancomycin resistant Enterococcus (VRE) ATCC 700221 and antimicrobial activities against *Candida glabrata*, *Staphylococcus aureus* and methicillin resistant *Staphylococcus* (MRS) with IC_{50} values of 3.21, 6.98 and 4.19 µg/ml respectively. However, its monomethoxylated derivative, 6-methoxygossypol (**4**) showed interesting activities against *C. glabrata*, MRS and vancomycin resistant *Enterococcus* (VRE) ATCC 51299 and ATCC 700221 strains with IC_{50} values of 0.8, 9.37, 2.31 and 1.45 µg/ml respectively
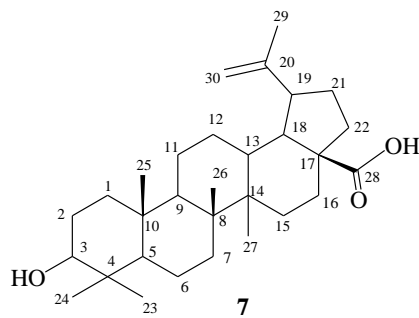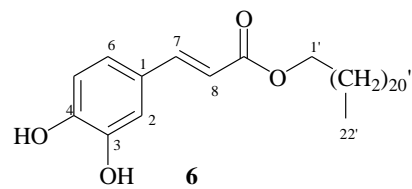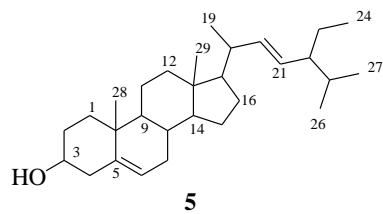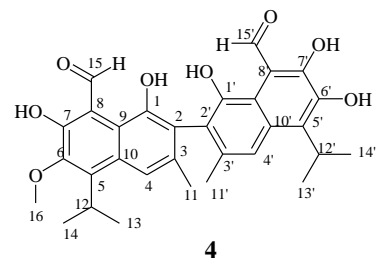
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LIST OF ABBREVIATIONS

ACT   Artemisinin combination therapy

ATCC   American type culture collection

CC   Column chromatography

CH_2Cl_2 Dichloromethane

CLSI   Clinical and laboratory standards institute

COSY   Correlation spectroscopy

D6   Chloroquine sensitive *Plasmodium falciparum*

DMSO   Dimethyl sulfoxide

EIMS   Electron impact mass spectrometry

EtOAc   Ethyl acetate

HMBC   Heteronuclear multiple bond correlation spectroscopy

HMQC   Heteronuclear multiple quantum correlation spectroscopy

IC_{50} Inhibition concentration, concentration of substance that produce 50% inhibition
of certain process

MBC   Minimum bacterial concentration

MFC   Minimum fungicidal concentration

NMR   Nuclear magnetic resonance

RPMI   Roswell park memorial institute

TLC   Thin layer chromatography

UV   Ultra violet

W2   Chloroquine resistant *plasmodium falciparum*

LIST OF APPENDICES

1.1   General Introduction

Plants have been an integral part of life in many indigenous communities including Africa [Sidigia *et al.,* 1995]. Most of Africa's biodiversity plays major roles in the cultural evolution of the human societies habiting the continent [Mugabe and Clark, 1998]. Apart from other ethno botanical uses, plants are especially important for their ethno-medicinal applications and among the many diseases traditionally treated with medicinal plants, malaria ranks as the single most important condition treated with herbal remedies. About 80 % of the rural population in Africa depends on traditional herbal remedies [WHO, 2002 and Zirihi *et al.,* 2005]. There is widespread use of traditional herbal remedies in the management of malaria [Gessler *et al.,* 1995] and microbial infections [Ahmed *et al*., 2014], however, scientific analysis of the plants is largely unexplored [WHO, 2002]. There is an urgent need to study the phytochemistry of anti-malarial and anti-microbial plants in the quest of drug discovery for these disease problems which are largely tropical in nature.

Historically, majority of anti-malarial drugs have been derived from medicinal plants or from structures modeled on plant compounds [Klayman, 1985]. Research on medicinal plant extracts used in folk medicine represents a suitable approach for the development of new drugs [Calixito, 1996]. The World Health Organization (WHO) recognizes that the centuries-old use of certain plants as therapeutic resources should be taken into account in developing them for use [Gilbert *et al.,* 1997]. Thus, it considers phytotherapy in its health programs and suggests basic procedures for the validation of drugs from plant origin in developing countries [Vulto and Smet, 1998].

The ongoing malaria control problems have been aggravated by inadequate health structures, poor socio-economic conditions and with the increase in resistance to antimalarial drugs [Muthaura *et al.,* 2007a]. Some of the antimalarial drugs of plant origin include quinine (**8**) from *Cinchona succiriba* and artemisinin (**9**) from Chinese herb *Artemisia annua* which has been used for malaria therapy in China for over 1000 years. The artemisinin modelled compounds,

artesunate-mefloquine, artemether-lumefantrine, artesunate-amodiaquine and artesunate-sulfadoxine/pyrimethamine are currently used for the treatment of uncomplicated form of malaria and work by reducing the parasites load rapidly [White, 2008, Olliarop and Wells, 2009, Craft, 2008 and Sinclair *et al*., 2009]. Other antimalarial natural products include: sergeolide (**10**) a quassinoid isolated from *Picrolemma pseudocoffea*, glaucarubin (**11**), chaparrin (**12**), brusatol (**13**) isolated from *Brucea javanica*, and tehranolide (**14**) isolated from *Artemisia diffusa*.
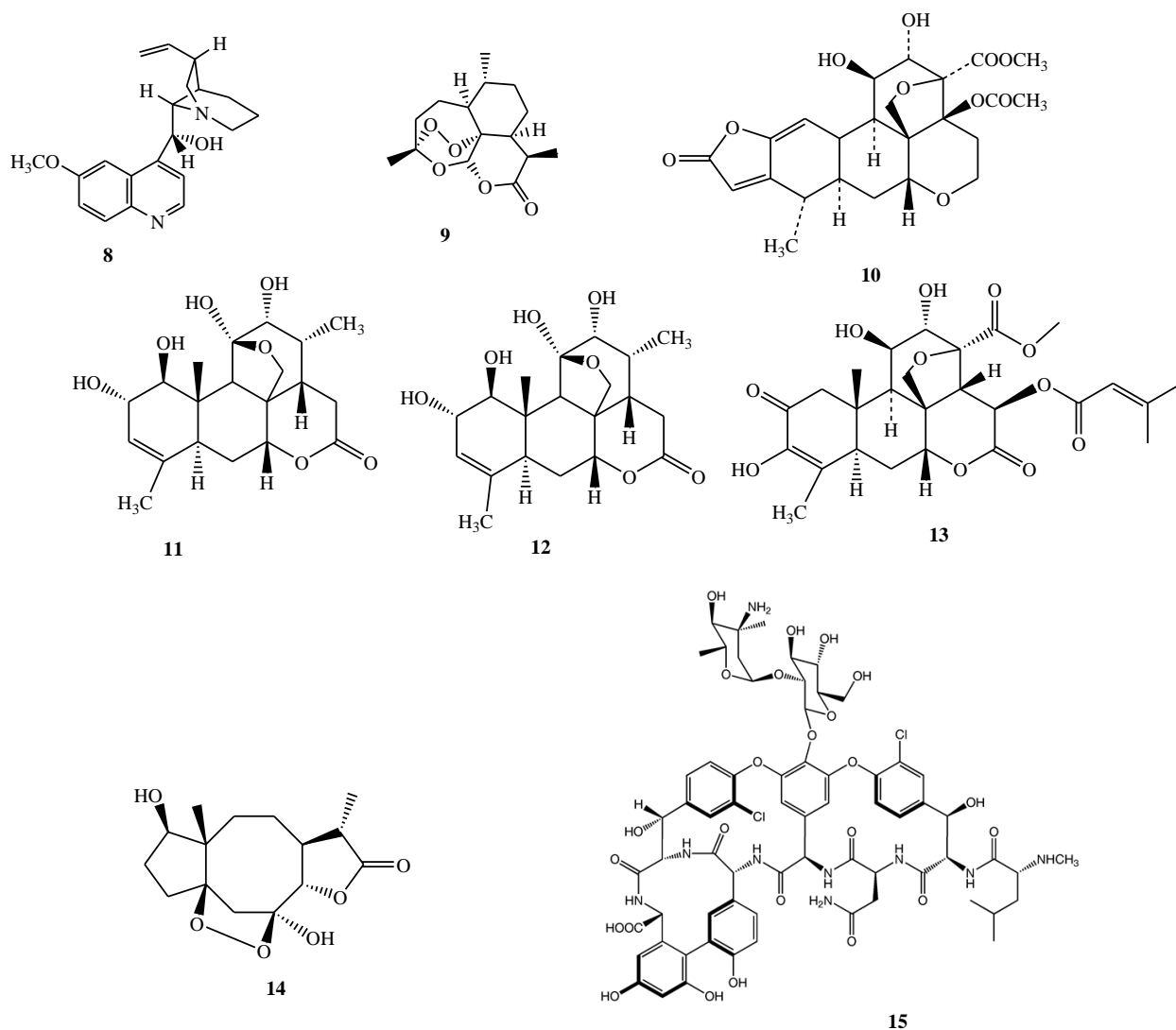
Figure 1: Some antimalarial and antimicrobial natural products.

The World Health Organization has advocated traditional medicine as safe remedies for ailments of both microbial and non microbial origins [WHO, 1978]. Several herbs are known to possess medicinal values including antimicrobial properties [Mahesh and Satish, 2008] with most plants producing polyphenols which exhibit biological activities such as antibacterial, antiviral, antiallergic, anti-inflammatory, anticancer and immunostimulant [Scalbert *et al*., 2005]. Antimicrobial drug resistance, compromising the treatment of bacteria, viral, fungal and parasitic infections has become a grave global health issue [Dancer, 2001 and Berger-Bachi, 2002]. The inevitable use of antibiotics guarantees the development of resistance in microbes hence need for new antimicrobial agents to treat resistant drug infections .This can only be achieved by exploring non-antibiotic compounds from the plants which can act as drug leads.

Data from the National Nosocomial Infection Surveillance has identified enterococcus as the second most frequently encountered nosocomial pathogen causing 12 % of all hospital acquired infections [Udo *et al*., 2003]. The enterococcus is a bacterium found in the digestive and genital tracts, blood stream and wounds with tolerance to a wide range of environments and persistent on hospital surfaces for a long period of time [Kramer *et al*., 2006]. The enterococcus is known to exhibit intrinsic resistance to known antibiotics such as vancomycin (**15**) [Moellering, 1992, Murray, 1990]. Rapid spread of vancomycin resistant *Enterococcus* is attributed to the bacteria's ability to colonise the gastrointestinal tract and the skin and persist in the human host [Austin *et al*., 1999]. Mortality rates attributed to VRE infections are estimated to be 70 % of the worlds' population [Edmond *et al*., 1996, Erlandson *et al*., 2008 and Oliveir and Bettcher, 2010].

In the search for new anti-malarial and anti-microbial principles from Kenyan flora, the crude extracts and some compounds from *H. abyssinica* and *T. garckeana* were obtained and investigated for their *in vitro* antiplasmodial activities against D6 and W2 strains of *P. falciparum* and for their anti-microbial potencies against *Candida glabrata*, *Staphylococcus aureus*, methicillin resistant *Staphylococcus* (MRS) and vancomycin resistant *Enterococcus* (VRE) based on the traditional uses of these plants.

1.2   Statement of the Problem

In Kenya, a large population in the rural set up uses medicinal plants to treat many diseases including malaria and microbial infections which are of national concern [Muthaura *et al.,* 2007]. In view of development of resistant strains of microbes such as *Enterococci* species to the vancomycin (**15**) drugs and *Plasmodium falciparum* to the current drugs such as artemisinin (**9**) along Cambodia borders, there is need for alternative, new and affordable therapies. The extracts of the two plants in the study had shown good antiplasmodial and anti-microbial activities in preliminary studies, hence the motivation to investigate the phytochemistry and activities of the constituent compounds with respect to malaria and microbial infections.

1.3   General Objective

To prepare organic solvent extracts of *Harrisonia abyssinica* and *Thespesia garckeana*, isolate compounds in them and investigate them for anti-plasmodial and selected anti-microbial effects.

1.4   Specific Objectives

i)   To extract the stem of *H. abyssinica*, roots and stems of *T. garckeana* using 1:1 MeOH in CH_2Cl_2.

ii) To isolate compounds from the extracts in (i) above.

iii) To characterize compounds from the extracts in (i) above.

iv) To test the extracts for *in vitro* anti-plasmodial and anti-microbial activities.

v)   To test the isolated pure compounds for *in vitro* anti-plasmodial and anti-microbial activities.

CHAPTER TWO

LITERATURE REVIEW

2.1   Malaria

Parasitic infections including malaria and microbial diseases are major health problems in Africa today [Microbial Infection and immune defence, 2000]. Malaria is caused by the lethal causative parasitic species the *Plasmodium falciparum* and is transmitted by *Anopheles gambiae* [Khaemba *et al.,* 1994] which is the most efficient vector. The World Health Organization describes malaria as a dangerous infection affecting 300-500 million people with up to 1 million deaths yearly, majority being children under the age of 5 years and pregnant women. According to World Health Organization, ninety percent (90 %) of the world's malaria infections and deaths occur in Africa.

In Kenya, malaria is the leading cause of morbidity and mortality, accounting for 30-50 % of the outpatient attendance and 20 % of all admissions to health facilities [Ministry of Public Health Services, 2005-2010]. The high infectious rate would be attributed to inadequate medical care; failure to use insecticide treated nets and increased resistance of the parasite to the drugs. Artemisinin combination therapy (ACT) is currently used as the first line drugs for treatment of malaria. There are some recent concerns that the efficacy of ACT therapies have declined on the Thai- Cambodian border due to occurrence of resistant strains of the parasite [Dondorp *et al* ., 2009]. However, there are many compounds in the literature that have *in vivo* and *in vitro* activities towards the malarial parasites that can be used as drug leads such as sergeolide (**10**) glaucarubin (**11**), chaparrin (**12**), brusatol (**13**) and tehranolide (**14**). It is in the view of this reported sporadic resistance that it is pertinent that the quest for identification of new antiplasmodials which can be developed to new antimalarials be persued.

2.2   Microbial Infections

Most microbial infections are caused by viruses, bacteria, fungi and protozoa [Ahmed *et al*., 2014]. The microbial pathogens infect animals and plants causing enormous economic losses. The infections caused by these organisms are classified into three major groups: acute infections which are severe and last for a short period, chronic infections which develop from acute infections and last for days to months to a life time while the latent infections are hidden or silent and may not cause symptoms again after the first acute episode. Most of the microbes have developed drug resistance and survive in the hospital environment due to either intrinsic resistance to the commonly used drugs, by mutation or through receipt of foreign genetic material as a result of the transfer of plasmids and trasposons. The main known resistant microbes are the methicillin resistant *Staphylococcus aureus* (MRSA) and vancomycin resistant *Enterococci* (VRE) and these possess potential threats to human health [Ahmed *et al*., 2014].

The most known resistant microbe in the world is the VRE. The *Enterococci* species were traditionally regarded as low grade pathogens but recently they have emerged as an increasingly important cause of nosocomial infections with only two species being responsible for majority of human infections *E. faecalis* and *E. faecium.* Infections caused by these pathogens include urinary tract infections, bacteraemia, endocarditis, neonatal sepsis and menegitis [Seema *et al*., 2008]. The VRE infections are difficult to control and resistant to drugs such as naturally occurring drug, vancomycin (**15**), ampicillin, gentamicin and streptomycin [Edwards, 2000], therefore this research will attempt to find potential anti-VRE drug, and other anti-microbial leads from natural sources.

2.3   Botanical Information on Genus *Harrissonia*

The genus *Harrisonia* belongs to the family Simaroubacea which has around 30 genera and 150 species of trees and shrubs widely distributed in the tropics and sub tropics [Dutra *et al*., 1992]. The stem, root bark and leaves in this family are often bitter to taste due to triterpenoid lactone compounds called quassinoids. The plants in the family Simaroubacea are used in traditional

medicine as anti-helmintic, anti-viral, anti-leukemic, anti-feedant, anti- tuberculosis, anti-malarial agents and for treatment of cancer [Joshi *et al*., 2003]. Some of the well known genera include *Ailanthus*, *Amaroria*, *Harrisonia*, *Kirkia, Simarouba*, *Picrolemma* and *Alvaradoa.*

Plants in the genus *Harrisonia* are shrubs or small trees. The genus comprises of three species namely; *H. abyssinica, H. perforata* and *H. brownii. H. abyssinica* is found in tropical Africa including East Africa and grows widely along the coastal region of East Africa and in Western Kenya [Rajab *et al.,* 1999]. *H. brownii* is found in the Southern and South Eastern Asia, mountains of North and middle Vietnam [White, 1973 and Ridle, 1976]. *H. perforata* is found in North Australia and China [Chen *et al*., 1997].

Harrisonia abyssinica is found in tropical Africa and some of the local names include: pedo in Luo, mkidunya in Luhya, ekalale in Turkana and msamburi in Swahili. It is an evergreen shrub or tree which grows up to 6 m tall. The branches have straight or curved spines which are usually in pairs. Flowers are cream or yellow and fruits are red when ripe. It grows mainly in riverine, dry bush land, wooded grassland and on coastal forest margins. The species is ever threatened due to over exploitation for medicinal purposes [Balde *et al.,* 1995].

2.3.2   Ethnomedicinal Information on the Genus *Harrisonia*

Harrisonia species have worldwide importance in traditional natural medicine and some of these are shown in Table 2.1

Table 2. 1: Ethno-Medicinal Information on the Genus *Harrisonia*.

Harrisonia abyssinica			
Country	**Plant part**	**Uses**	**Reference**
Kenya	Root	Root decoction drunk as remedy for dizziness Insomnia, nausea, vomiting, bubonic plague, swollen testicles, induce abortion and tuberculosis.	Schmelzer *et al*., 2010
	Root bark	Decoction used for fever and venereal diseases.	Kokwaro, 1993 Beentje, 1994
	Stem bark	Treatment of skin diseases, gonorrhea and tuberculosis.	Balde *et al*., 2001
Kenya and Tanzania	Root	Root decoction drunk to manage cancer.	Schmelzer *et al*., 2010
	Leaves and twigs	Decoction of leaves and twigs is drunk to treat haemorrhoids.	
Tanzania	Leaves	Hot leaf decoction is used as inhalation to treat head ache.	
	Root bark	Nyamwezi people swallow the smoke from burning root bark to treat hookworm infection. Crushed and soaked roots and bark in water is drunk to purify and strengthen the body. Hot water extract of fresh and dried root bark is used to treat skin diseases.	
	Leaves	Nyamwezi people apply the pulped leaves to snake bites.	
Uganda	Roots	Root decoction is used to treat livestock against east coast fever and lumpy skin disease. Root infusion used to treat snake bites, used as nose drops to treat insanity. Root powder applied to incisions to treat migraine.	Schmelzer *et al*., 2010 Balde *et al*., 1995
Togo	Leaves	Leaf decoction used to treat diabetes.	
Ghana	Root	Pounded and boiled root is taken to treat cough and whooping cough.	
Ethiopia	Root	The Shinasha (Boro) people use decoction of roots treat to dysmenorrhea.	
	Leaves	Leaf decoction or infusion used as a wash to disinfect wounds and abscesses.	
Kenya	Root	Root powder, decoction or infusion used to treat venereal diseases, fever and malaria, diarrhoea, urinary problems and intestinal worms.	

Harrisonia perforata			
Country	**Part**	**Uses**	**Reference**
Indonesia	Young shoots	Remedy against diarrhoea.	Kiew, 2001
Philippines	Root bark	Remedy against diarrhoea, dysentery and cholera.	
Indo- China	Leaves	Ashes of roasted leaves mixed with oil are applied to relieve itch.	
Thailand	Root	Dried root is considered antipyretic and anti-inflammatory, wound healing and treatment of diarrhea.	
South China	Root	Used for prevention and treatment of malaria and boils.	White, 1973

Harrisonia brownii			
Country	**Part**	**Uses**	**Reference**
Papua New Guinea	Leaves	Bitter decoction of leaves is used in the treatment of diarrhea, malaria, coughs and asthma.	Kiew, 2001

2.3.3 Biological Activities of the Genus *Harrisonia*

The crude extract of the root bark of *H. abyssinica* showed antifeedant, cytotoxicity and plant growth inhibitory activities [Kubo *et al*., 1976 and Liu *et al*., 1982]. This was attributed to the presence of four tetranortriterpenes; harrrisonin (**21**) and obacunone (**22**) both of which shows antifeedant activity against the larvae of East African monophagous crop pest *Spodoptera exempta* [Kubo *et al*., 1976 and Hassanali *et al.,* 1986], acetoxyharrisonin (**23**) which is active against the southern armyworm, *Spodoptera eridania* [Liu *et al*., 1982] and pedonin (**26**) which has potent antifeeding activity against the African crop pests *Eldana saccharina* and M*aruca tastulalis* [Hassanali *et al*., 1987].

Preliminary studies have shown that the dichloromethane extract of the stem bark of *H. abyssinica* exhibited anti-plasmodial activities with IC_{50} value of 4.4 µg/ml and 5.6 µg/ml against chloroquine resistant and chloroquine sensitive strains respectively [Irungu *et al*., 2007]. The activity was attributed to the presence of quassinoids such as perforaquassin C (**16**),

perforaquassin A (**17**), perforaquassin B (**18**) and perforaquassin (**19**) [El Tahir *et al.,* 1999], limonoid harrisonin (**21**) [Kubo *et al*., 1976] and chromones such as peucenin 7-methyl ether (**30**), *O*-methylalloptaeroxylin (**28**) and perforatic acid (**29**).

The ether extract of the root bark of *H. abyssinica* exhibited antimicrobial activity against *Trichophyton mentagrophytes* and *Neisseria gonorrhoea* [Sofowora, 1982]. The chloroform extract of the stem bark of the same plant was found to have antifungal activity against *Aspergillus niger, Microsporum canis, Trichophyton mentagrophytes* and *Aspergillus fumigatus* [Balde *et al.,* 1995].

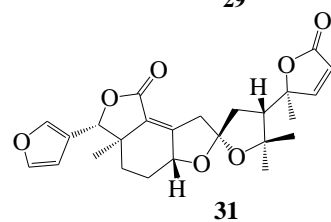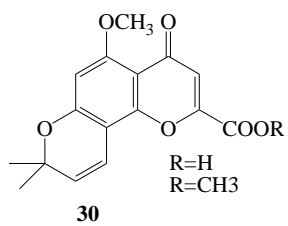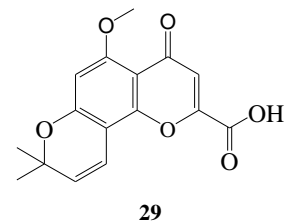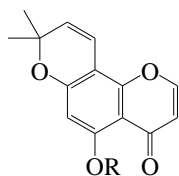### 2.3.4   Compounds Reported from Genus *Harrisonia*

The genus *Harrisonia* is known to produce limonoids, terpenoids and chromones. Table 2.2 shows different classes of compounds isolated from this genus in previous studies.

Table 2. 2: Compounds isolated from genus *Harrisonia*.

Compound	Biological Source and Use	Reference
Perforaquassin C (**16**)	*H. perforata*	Tuntiwachwuttikul *et al.*, 2006
Perforaquassin A (**17**)		
Perforaquassin B (**18**)	*H. abyssinica*	Balde *et al.,* 2001
Perforaquassin (**19**)		
Atalantolide (**20**)		
Harrisonin (**21**)		Hassanali *et al.,* 1987
Obacunone (**22**)		Kubo *et al.,* 1976
11β, 12β diacetoxyharrisonin (**23**)		Liu *et al.,* 1982
		Epe and Mondon, 1979
		Kamuichi *et al*., 1996
2-methylalloptaeroxylin (**24**)		Rugutt *et al.,* 2001
2-hydroxymethyl alloptaeroxylin (**25**)		Balde *et al.,* 1987
Pedonin (**26**)	Roots of *H. abyssinica.* Shows antifeeding activity against the *Maruca tastulalis.*	Ahmed *et al*., 1987
Alloptaeroxylin (**27**),	Roots of *H. abyssinica*	Okorie, 1982
O-methyl alloptaeroxylin(**28**)		Okorie*.,* 1982
Perforatic acid (**29**),		
Peucenin-7-methyl ether (**30**)	Fruits of *H. perforata*	Kamuichi *et al.,* 1996
Harrpernoid C (**31**)		Rajab *et al.*, 1999
Performone C (**32**)	*H. perforata*	Tuntiwachwuttikul *et al*., 2006
Harperforin C2 (**33**)		Khuong-Huu *et al*., 2000
Brownin A (**34**)	*H. brownii*	Koike *et al*., 1993
5- hydroxyl- 7 methoxy- 2 methyl -8- prenyl chromene (**35**)	*H. perforata*	Tanaka *et al*., 1995
Umatin (**36**)		Dean and Robinson, 1971
Grieveichromenol (**37**)		Tuntiwachwuttikul *et al*., 2006
Pachymic acid (**38**)		Zhou *et al.,* 2008
Pinoresinol (**39**)		Ouyang *et al.,* 2007
Gallic acid (**40**)		
Methyl gallate (**41**).		
Happernoid B (**42**)		Okorie, 1982
Rutaevin (**43**)		Sugimoto, *et al* 1988
Peucenin (**44**)		Thadaniti *et al.,* 1994

Compound		Reference
5,7 dimethoxy, 2-methyl, 8-prenyl chromone (**45**)	*H. perforata*	Tanaka *et al* .,1995
Aissatone (**46**)	Stem bark of *H. abyssinica*	Balde *et al*., 1999
Bissaone(**47**)		
Foritin (**48**)	*H. perforata*	Thu *et al*., 2006
Harperforin B$_1$ (**49**)		Chiaroni *et al*., 2000
Harperforin B$_3$ (**50**)		Khuong-Huu *et al*., 2001
Harperforin C$_2$ (**51**)		
Harperforin D (**52**)	*H. perforata*	Chiaroni *et al*., 2000
Harperforin E (**53**)		Khuong-Huu *et al*., 2000
Harperforin F (**54**)		
Harperforin G (**55**)		Khuong-Huu *et al*., 2001
Harrisonol A (**56**)		Yin *et al*., 2009
Harrisotone A (**57**)		
Harrisotone E (**58**)		
Perforatin D (**59**		Tanaka *et al*., 1995
Perforatin C (**60**)		Tuntiwachwuttikul *et al*., 2006
Perforatin B (**61**)		
Perforamone A(**62**)		Kamiuchi *et al*., 1996
Perforamone B (**63**)		Rajab *et al*., 1999
Cycloabyssinone (**64**)		
Perforin A (**65**)		Kamiuchi *et al*., 1996
Pterochromenol/perforamone D (**66**)		Tuntiwachwuttikul *et al*., 2006
Perforatin G (**67**)		
Greveichromenol (**68**)		Tanaka *et al*., 1995

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24 R=Methyl
25 R=CH₂OH

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27 R = H
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R=H
R=CH3

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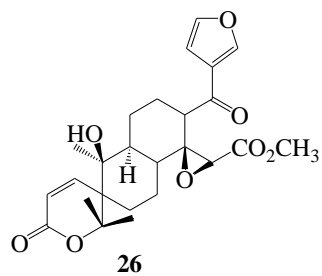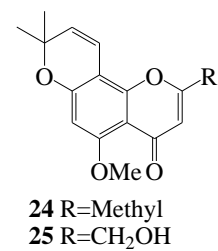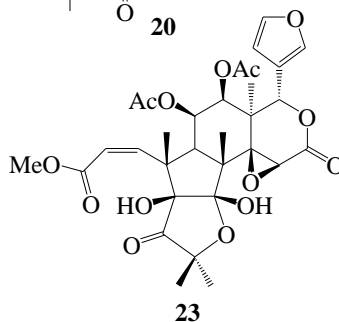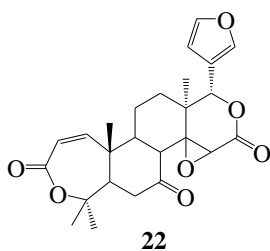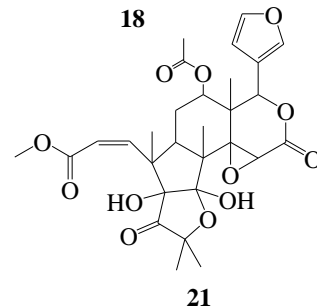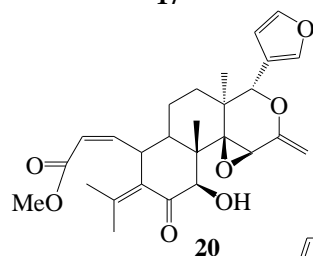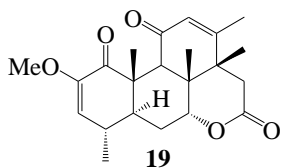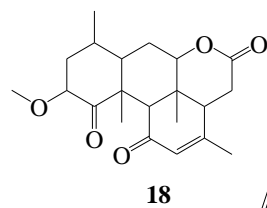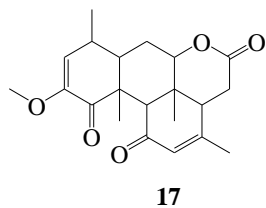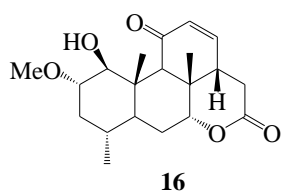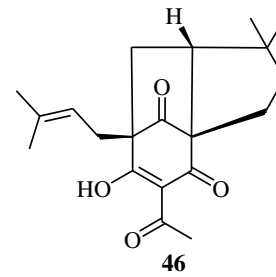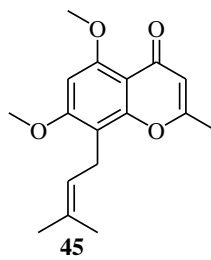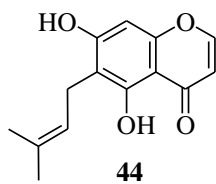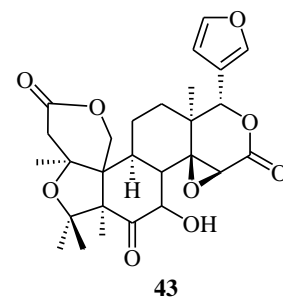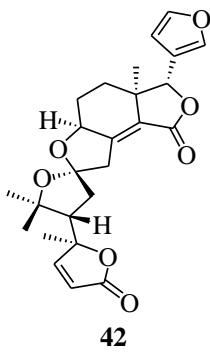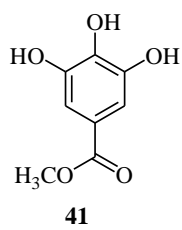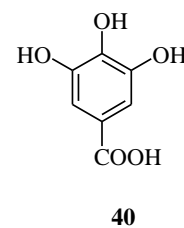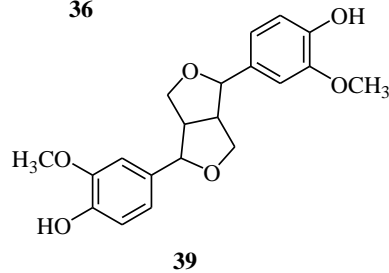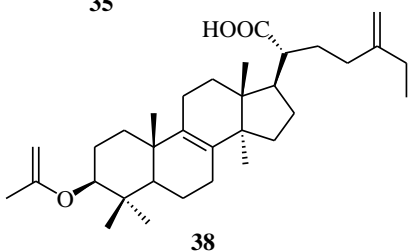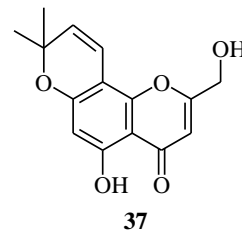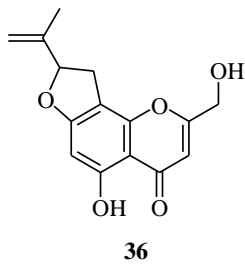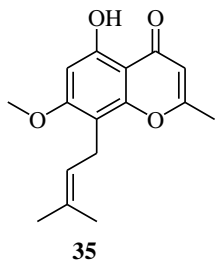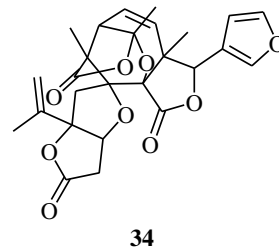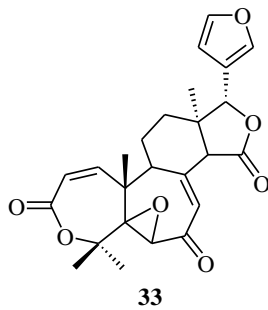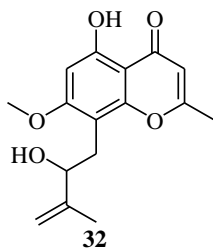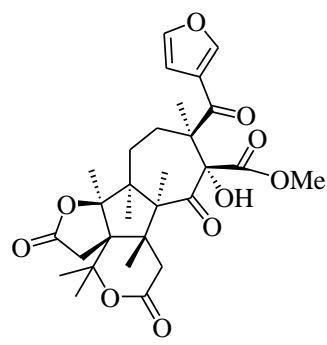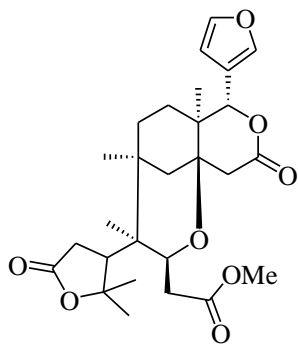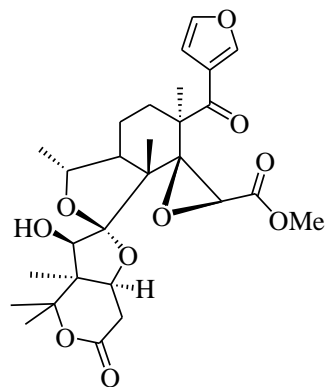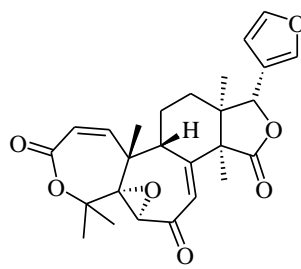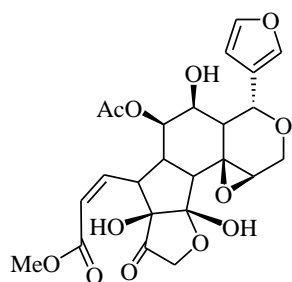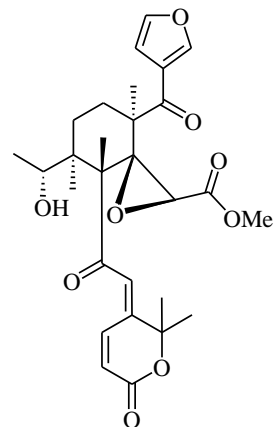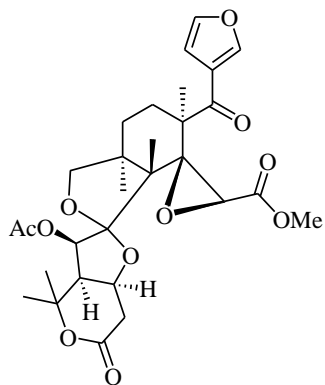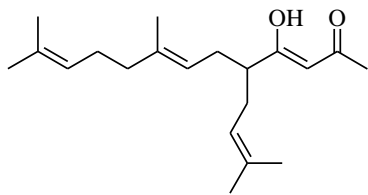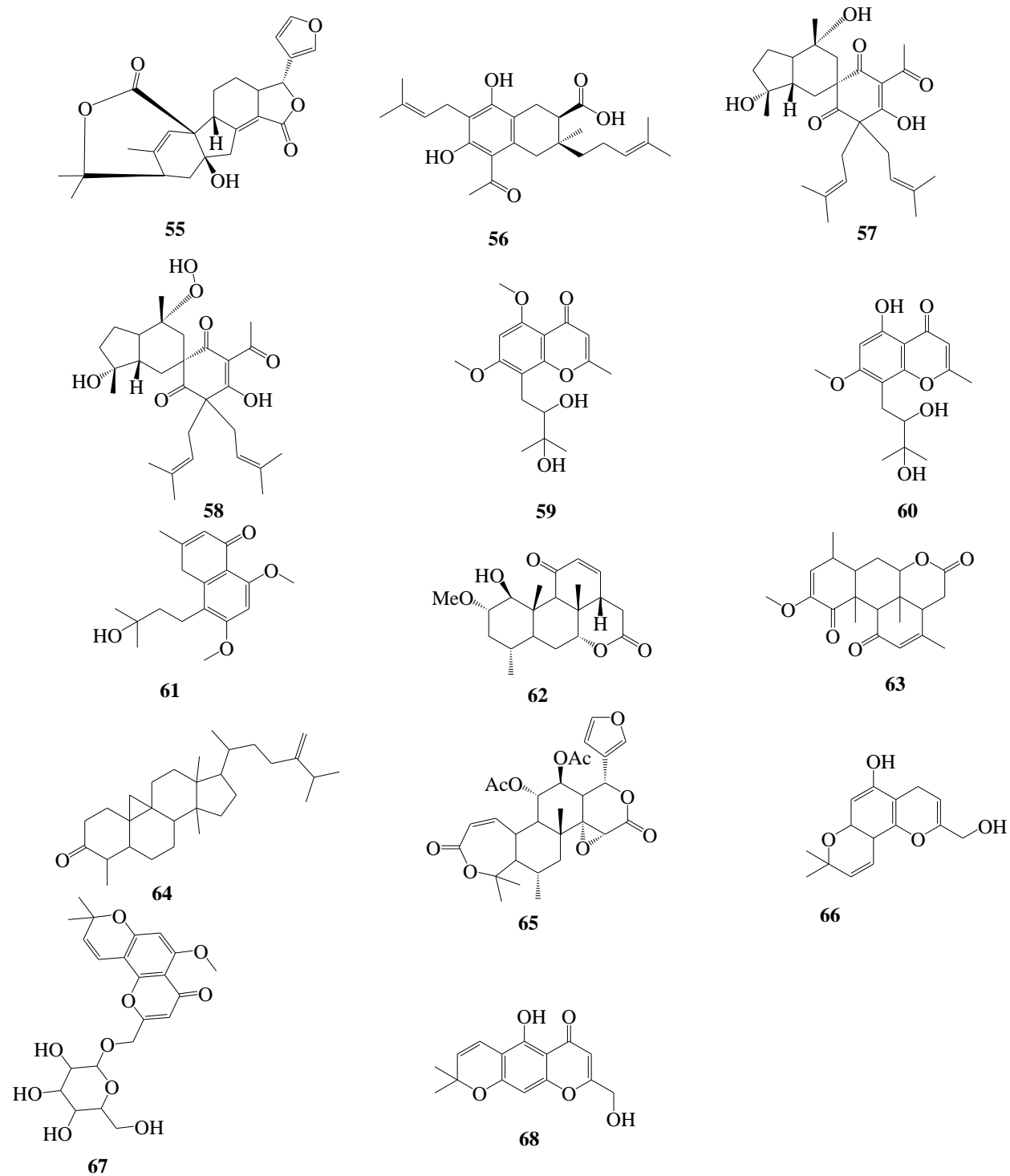
Figure 2: Compounds Isolated from Genus *Harrisonia*

2.4   Botanical Information on Genus *Thespesia*

2.4 1   The Family Malvaceae

It is a family of flowering plants consisting of approximately 119 genera with close to 4200 species, mainly consisting of herbs, shrubs and trees [Watson and Dallwitz, 1992]. Plants in this family are useful in provision of natural fibres, food, beverage, timber, traditional medicine and in horticulture. The most common genus is *Gossypium* that is used in provision of natural fibres [Brubaker *et al*., 1999b]. The other well known genera include *Thespesia* (*Azanza*)*, Theobroma* (source of chocolate), *Hibiscus, Palva, Malva, Lavatera,* and *Malope*.

2.4.1.1   The Genus *Thespesia*

The genus *Thespesia* belonging to the family Malvaceae is distributed worldwide in the tropical and subtropical regions. It is closely related to cotton (genus *Gossypium*) with which it shares, among other characteristics, the presence of gossypol glands in many plant parts [http://rubens.anu.edu.au/htdocs/surveys/charlotte/byartist/display00422.html], with the main compound gossypol (**81**) produced to protect the plant against predators and if taken in large quantities is toxic to all mammals. Gossypol leads to production of immature spermatozoa, detached sperm heads and decrease in the number and mobility of the spermatozoa [Ruttle, 1989]. In Kenya this genus is represented by *T. garckeana* [ICRAF, 1992] and the other members worldwide: ***Thespesia garckeana*** also known as *Azanza garckeana,* which is an evergreen medium sized woodland tree normally 3-10 m in height, distributed in South Eastern tropical Africa is found in wooded grasslands, open woodland and thickets [Beentje, 1994]. The plant is found mainly in South Africa, Mozambique, Botswana, Ghana, Zimbabwe, Malawi, Kenya, Namimbia, Tanzania, Zambia, Burundi and Democratic Republic of the Congo [ICRAF, 1992 and Palmer and pitman, 1972].

The fruit is encased in a reddish, hard, roundish capsule and has very high energy content 8.1 kJ/g and when ripe it is chewed like gum, producing a sweet glutinous thick gloop [Palmer and Pitman, 1972]. The jelly, syrup and relish from the fruits are added into indigenous soups or made into porridge and occasionally dried to be reconstituted later [Storrs, 1979].

The wood is used in building of house frames, poles and oxen or donkey yokes. It is also used in making smaller items such as spoons, carvings, combs and tool handles, used as fire wood and charcoal, the inner bark is used to produce good quality rope fibre [Storrs, 1979 and Palgrave, 1988, ICRAF, 1992 and Mulofwa *et al*., 1994]. The leaves are used as fodder for cattle though their digestibility is too low due to the secondary metabolites produced. The leaves are harvested and turned to green manure [Excell and Wild, 1961; Krog, 2003 and Palgrave, 2000]. The common names of *T. garckeana* include: snotappel (Afrikaans); chinga, mukole (Bemba); African chewing gum, azanza, quarters, snot apple, tree hibiscus, wild hibiscus (English); muneko (Lozi); mukole (Lunda); uxhakuxhaku (Ndebele); mkole (Nyanja); mutohwe (Shona); mtobo (Swahili); muneko (Tongan); morajwa (Tswana); kitoo (Kamba) [Anonymous].

Thespesia populnea: is widely distributed in Hawaii, California, Florida, Africa, the Caribbean islands, and Asia [Milbrodt *et al*., 1997]. Various parts of this plant are found to possess useful medicinal properties, such as anti-fertility, anti-bacterial, anti-inflammatory, anti-oxidant, purgative, and hepatoprotective activities [Vasudevan *et al.*, 2007]. The species produces a valuable wood, somewhat similar in appearance and working quality to old growth mahogany [Little and Wadsworth 1964]. The wood is durable and highly resistant to dry-wood termites [Little and Wadsworth, 1964], is used for furniture, crafts, and musical instruments.

Thespesia lampas is also known as *Hibiscus lampas:* It is a medicinal plant found throughout India, in Eastern Tropical Africa and Australia [Nadkarni, 2007]. It is an annual shrub with reddish brownish coloured bark. It is found in humid as well as seasonally dry regions with average annual rainfall of 1500-1700 mm [Nadkarni, 2007].

Thespesia grandiflora: It is endemic to Puerto Rico and is planted as an ornamental in Florida, Hawaii, Honduras, and on several of the Caribbean Islands [Francis, 1989b, Little and Wadsworth, 1964]. It is an attractive, small to medium sized tree with dark green foliage and large, dark pink or red flowers. The wood is highly durable and resistant to dry wood termites and is used for furniture, crafts and musical instruments [Little and Wadsworth, 1964].

2.4.2   Ethnomedicinal Information on the Genus *Thespesia*

Members of *Thespesia* have been used for medicinal purposes by indigenous people in several parts of the world. Table 2.3 gives a summary of different *Thespesia* species and their traditional uses by different communities.

Table 2. 3: Ethnomedicinal Uses of Genus *Thespesia*

Thespesia populnea			
Country	Part	Use	Reference
India	Ground up bark	Treatment of cutaneous infections such as scabies, ring worm, guinea worm and eczema.	Chopra *et al.,* 1956
Mauritius	Ground up bark	Treatment of dysentery and haemorrhoids.	Nagappa and Cheriyan, 2001
South India	Leaves	Applied to inflamed and swollen joints.	Vasudevan and Parle, 2006
South India	Young fruit	Secretes a yellow sticky sap used to treat worm and other skin diseases.	
India	Various parts	Found to possess useful medicinal properties, such as anti-fertility, anti-microbial, anti-inflammatory, anti-oxidant, and purgative and hepatoprotective activity.	Shirwaikarkumar *et al.*, 1995
Fiji	Leaves	A decoction of the leaves is used in treating coughs and headaches.	Sheela and Kannan, 2003
Samoa	Stem bark	An infusion of the stem bark is used to treat intestinal diseases (Tonga).	
Tonga	Leaves	A drink made from the leaves and bark is used to treat fever in teething children.	
Thespesia (Hibiscus) lampas			
Nepal	Roots	Root paste used to cure jaundice in Korku tribe of Amravati District of Maharashtra.	Jagtap *et al.*, 2006, Tamang., 2003
	Roots	Used as anti- diabetic, anti-helmintic and antioxidant.	Sangameswaran., *et al* 2008 Kumaraswamy and Satish, 2008 Sangameswaran *et al.,* 2009 Kosalge and Fursule, 2009
Nepal	Stem	Used in treatment of inflammation, acidity, bleeding nose, bronchitis, cough, dysentery, fever, sun stroke, urinary complaints, anti-helmintic and carbuncle.	Adhikari *et al*., 2007
India	Roots and fruits	Used for treatment of gonorrrhoea, jaundice, and syphilis. Shows anti-microbial and hepatoprotective activity.	Vasaraj *et al*., 1997 Sangameswaran *et al*., 2008
South India	Seeds	Crushed and made into a paste and taken orally along with jiggery thrice for two days to get relief from whooping cough.	Jeyaprakash *et al*., 2011

2.4.3   Biological Activities Reported for the Genus *Thespesia*

The sesquiterpenoid quinones; mansonone E (**69**) and F (**70**), thespesone (**89**) and thespone (**90**), isolated from *T. populnea* have been found to induce contact dermatitis in man, to possess tumor formation capacity and to have antifungal properties. They also affect lipid peroxidation and cytochrome P450 activity [Thomson, 1971, Sandermann and Dietrichs, 1959, Tanaka *et al.,* 1966 and Chen *et al.*, 1990]. Mansonone E (**69**), mansonone D (**78**) and populene D (**84**) showed IC_{50} values of 0.05, 1.85 and 0.08 µg/ml respectively, against breast cancer cell line [Boonsri *et al.*, 2008].

Gossypol (**81**) isolated from flowers of *T. populnea* was found to exhibit male-fertility [Matlin *et al*., 1985], anti-tumor [Joseph *et al*., 1986, Band *et al*., 1989 and Benz *et al*., 1990], anti-amoebic [Gonzalez-Garza *et al*., 1989] and anti-HIV [Lin *et al*., 1989] activities. This compound also showed potent cytotoxicity activity against cervical and oral cavity cancer cell lines with IC_{50} values of 0.08 and 0.04 µg/ ml respectively [Boonsri *et al*., 2008].

The ethanolic and aqueous extracts of *T. populnea* aerial parts showed antibacterial and antifungal activities [Shastry *et al.,* 2005] which could be attributed to gossypetin (**99**) from the flowers of *T. populnea*.

2.4.4   Compounds Reported from Genus *Thespesia*

The genus *Thespesia* is represented in Africa by two species *T. garckeana* and *T. populnea.* From previous studies *O*-naphthoquinones have been isolated from these two species. *T. populnea* has been of great interest to scientists due to production of milo (beverage) hence its phytochemistry is well known but little phytochemistry on *T. garckeana* has been reported. This information is given in Table 2.4.

Table 2. 4: Compounds Isolated from Genus *Thespesia*.

Compound	Biological Source and Use	Reference
Mansonone E (**69**)	*T. garckeana*	Boonsri *et al*., 2008
Mansonone F (**70**)		
Mansonone G(**71**)		
Mansonone H(**72**)	*T. garckeana*/ *T. populnea*	
Azanzone B (**73**)		
Azanzone A (**74**)	*T. garckeana*	
Dehydrooxoperezinone (**75**)	*T. populnea*	Puckhaber and Stipanovic, 2004
Populneol (**76**)	*T. populnea* used as hepatic oxidase inhibitor	Shivastva *et al*., 1963
Populene A(**77**)	*T. populnea*	Boonsri *et al*., 2008
Mansonone D(**78**)	T. *populnea* used for cytotoxicity and super oxide anion generation properties	Puckhaber and Stipanovic, 2004
Thespesenone (**79**)	*T. populnea*	
5,12-epoxy-2-hydroxyl-1,4,6-cadinatriene-3,8-dione	*T. populnea*	Milbrodt *et al.,* 1997
Gossypol (**81**)	*T. populnea*	Bhakuni *et al*., 1968
7-hydroxyl isoflavone (**82**)		
Populene C(**83**)	*T. populnea*	Boonsri *et al*., 2008
Populene D (**84**)		
Populene F (**85**)		
Populene E (**86**)		
Populene G (**87**)		
Kaempferol 5- glucoside (**88**),	*T. populnea*	Datta *et al* .,1973
Thespesone (**89**)	*T. populnea*	Neelakantan *et al*., 1983
Thespone (**90**)		
Nonacosane (**91**)	*T. populnea*	Seshadri and Sharma, 1975
Myricyl alcohol (**92**)		
Lupenone (**93**)		
Lupeol (**94**)		
β- sitosterol (**95**)		
β- sitosterol β- D- Glucoside(**96**)		
Rutin (**97**)	*T. populnea*	Datta *et al* .,1973
Quercetin (**98**)	*T. populnea*	Kasim *et al.,* 1975
Gossypetin (**99**)	*T. populnea*	Kasim *et al.,* 1975
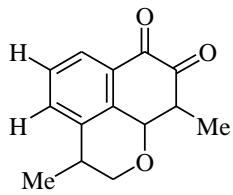
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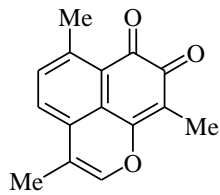
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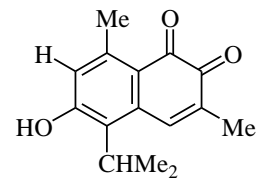
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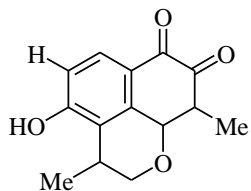
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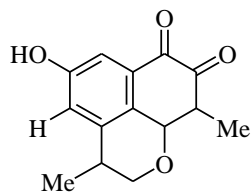
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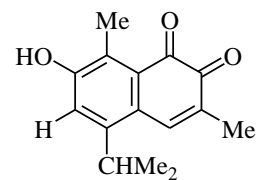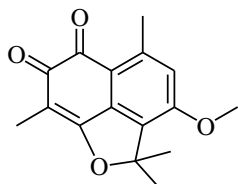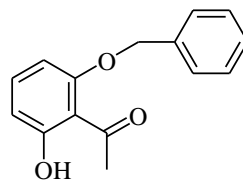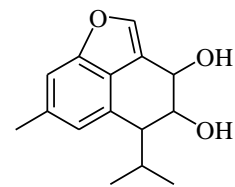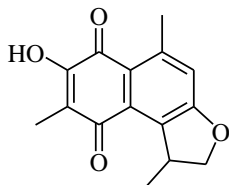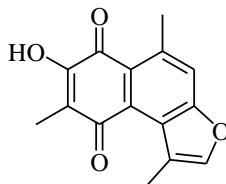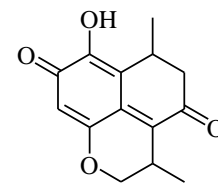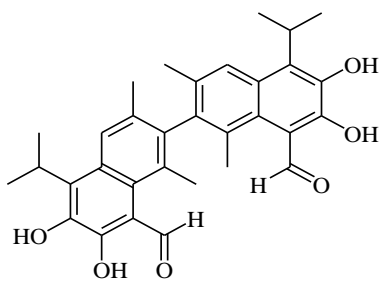
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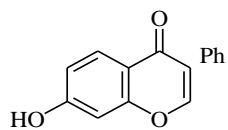
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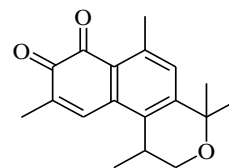
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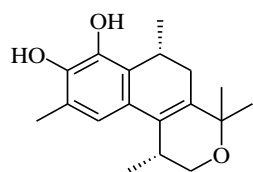
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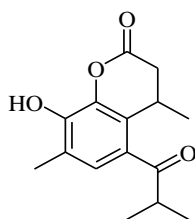
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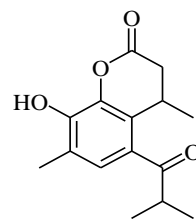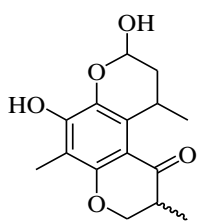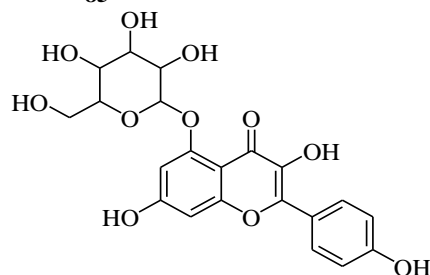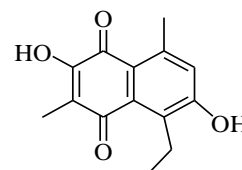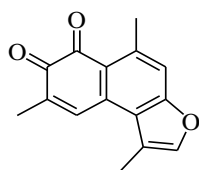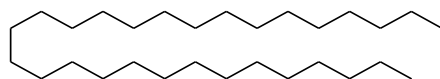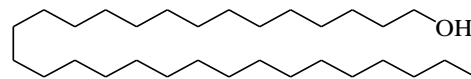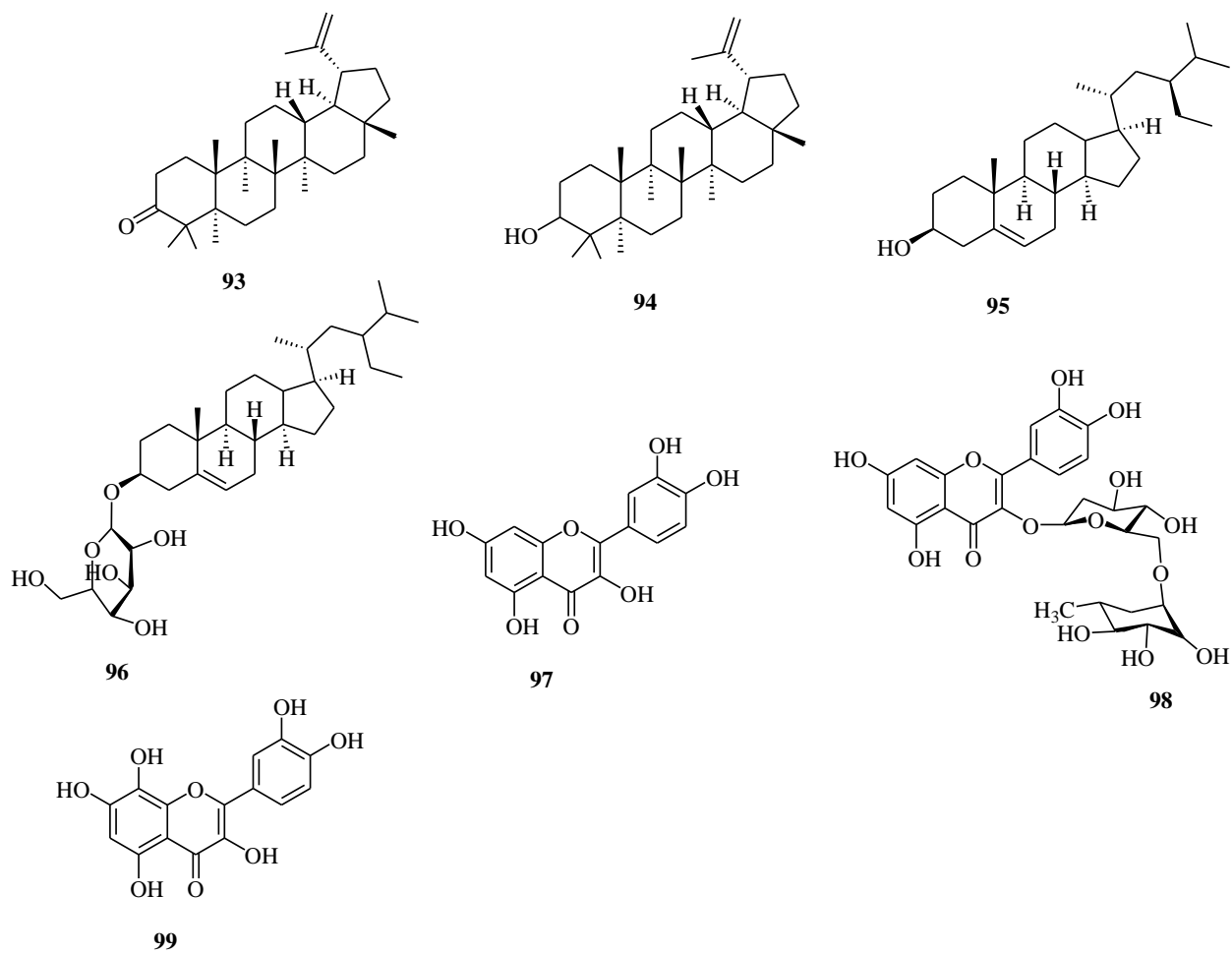
Figure 3: Compounds Isolated from Genus *Thespesia*.

CHAPTER THREE

EXPERIMENTAL

3.1   General

The ^{1}H NMR and ^{13}C NMR spectra were recorded on a Varian 400 MHz spectrometer and the chemical shifts values were determined in ppm (δ) with TMS as the internal standard. Both the positive and negative mode of the EIMS was used. The compounds were visualized under UV light at 254 and 366 nm and exposure to iodine. Silica gel 60-120 mesh with gradient elution was used to carry out column chromatography while Kieselgel 60 H was used to make the preparative thin layer chromatography (TLC) plates. The solvents used for extraction and column chromatography were purified by fractional distillation process. Preparative TLC plates were prepared on glass plates (20 cm by 20 cm) by making a suspension with 80 g of preparative silica gel in 200 ml of water for 6 plates. The resultant slurry (40 ml) was poured onto each plate and spread uniformly using a flat spatula and left overnight to dry resulting to plates of 2 mm thickness. The plates were then activated in an oven set at 110 °C for 30 minutes and allowed to cool before use.

3.2   Plant Material

The stem bark of *H. abyssinica* was collected from Ngogoni forest in Kwale County in June 2012 while the roots and stems of *T. garckeana* were collected from Muthetheni, Machakos County in March 2013. The two plant materials were identified by Mr. Patrick Mutiso from the University of Nairobi herbarium, School of Biological Sciences where voucher specimens of the two plants MVM 2012/UoN 01 and MVM 2013/UoN 02 are deposited.

3.3   Extraction of the Stem Bark of *Harrisonia abyssinica*

The stem bark of *H. abyssinica* was cut into small pieces and air dried under shade for one week. The air dried and ground stem bark (2.6 kg) was extracted exhaustively with 1:1 MeOH in CH_2Cl_2 by cold percolation at room temperature for 48 hours. The resultant extracts were filtered and concentrated using rotary evaporator under reduced pressure to give the crude extracts of 50 g.

3.3.1   Isolation of Compounds from the Stem of *H. abyssinica*

The crude extract of the stems of *H. abyssinica* obtained using 1:1 MeOH/CH_2Cl_2 (45 g) was adsorbed onto 45 g of silica gel, dried *in vacuo*, ground into fine powder and loaded onto a column packed with 450 g of silica gel under *n*-hexane. This was subjected to column chromatography by gradient elution initially with *n*-hexane with increasing amounts of CH_2Cl_2 up to 100 % followed by 2 % MeOH in CH_2Cl_2 up to 8 % MeOH. This yielded 80 fractions of 450 ml each which were combined based on the similarities of their TLC profiles (CH_2Cl_2, 5 % MeOH in CH_2Cl_2), dried and weighed resulting to only six fractions (17A-17E).

The fraction of the major column 17C and 17D (2 g) eluted with 100 % CH_2Cl_2 were dried *in vacuo*, dissolved in CH_2Cl_2 and adsorbed on 2.0 g of silica gel. The adsorbed and ground material was then loaded onto a column of 60 g of silica gel packed under 3 % EtOAc in *n*-hexane. Initial elution was achieved using the same solvent system (3 % EtOAc in *n*-hexane) and subsequently increasing the amounts of EtOAc up to 40 % resulting into 20 fractions of 60 ml each. The fractions which revealed similar TLC profiles were combined to give only seven fractions labeled as 21A-21G. Fraction 21E, F and G (1.013g) were further combined. Chromatographic separation of the fraction of the minor column eluted with 20 % and 40 % EtOAc in *n*- hexane (21E-21G, 1.013 G) on silica gel (10 g) under 5 % EtOAc in *n*-hexane, followed by gradient elution up to 60 % EtOAc in *n*- hexane was achieved. This resulted to thirty fractions of 50 ml each. The fractions were combined based on similarities of their TLC profiles into seven fractions (22A-22G). Fraction 22 F was concentrated *in vacuo* and recrystallized from

1 % MeOH in CH_2Cl_2 to yield white amorphous powder compound (**1**) (10 mg, R_f 0.40 (4 % MeOH in CH_2Cl_2).

3.4   Extraction of the Roots of *Thespesia Garckeana*

The ground plant material (2.6 kg) of *T. garckeana* was exhaustively extracted by cold percolation with 50 % MeOH in CH_2Cl_2 for 72 hours at room temperature. The solvent was filtered to remove debris and concentrated *in vacuo* using rotary evaporator to yield 70 g of extract.

3.4.1   Isolation of Compounds from the Roots of *T. Garckeana*

The root extract (65 g) of *T. garckeana* was dissolved in 100 ml of CH_2Cl_2 and adsorbed onto equal quantity (65 g) of silica gel. The adsorbed material was loaded onto a column packed with 650 g of silica gel under 30 % CH_2Cl_2 in *n*-hexane. Gradient elution using increasing amounts of CH_2Cl_2 up to 100 % followed by 1 % MeOH in CH_2Cl_2 up to 3 % MeOH in CH_2Cl_2 yielded 80 fractions of 650 ml each. The fractions that revealed similar TLC profiles were combined, resulting to only twelve fractions (29A-29L).

Purification of the fraction eluted with 50 % CH_2Cl_2 in *n*- hexane and 100 % CH_2Cl_2 (29E and 29 F, 3 g) by column chromatography using silica gel (30 g) under *n*-hexane and subsequently increasing solvent polarities with increasing amounts of CH_2Cl_2 led to 50 fractions of 20 ml each which were combined in to five fractions 30A-30E. Recrystallization of the fraction of the minor column eluted with 80 % CH_2Cl_2 in *n*-hexane (30B) using 5 % CH_2Cl_2 in *n*-hexane resulted to colorless needle like crystals of compound (**5**) (10 mg, R_f 0.35 (15 % EtOAc in *n*-hexane). Recrystallization of fraction 29 I from the main column eluted with 1 % MeOH in CH_2Cl_2 under 50% CH_2Cl_2 in *n*-hexane formed yellow, UV active crystals, compound (**2**) (35 mg, R_f 0.35 (15 % EtOAc in *n*-hexane).

The fraction 29J (4 g) from the main column eluted with 1 % MeOH in CH_2Cl_2 was purified further by column chromatography using silica gel 40 g eluting initially with 5 % EtOAc in *n*-hexane and subsequently with increasing amounts of EtOAc up to 40 % EtOAc in *n*-hexane yielding a total of 58 fractions 100 ml each. These fractions were combined depending on the similarities of their TLC profiles (33A-33F). Fraction 33A from the minor column eluted with 10 % EtOAc in *n*-hexane was dried and recrystallized under 50 % CH_2Cl_2 in *n*-hexane to form light yellow crystals of compound (**3**) (35 mg, R_f 0.4 (15% EtOAc/*n*-hexane). Fraction 33B from the small column eluted with 10 % EtOAc in *n*-hexane recrystallized out of this solvent system to form yellow crystals of compound (**4**) (20 mg, R_f value of 0.32 (10 % EtOAc/*n*-hexane).

3.5 Extraction of the Stem of *Thespesia Garckeana*

The air dried and ground stem of *T. garckeana* (2.8 kg) was exhaustively extracted with 50 % MeOH in CH_2Cl_2 for 48 hours at room temperature. The resultant crude extract was filtered to remove debris and concentrated using a rotary evaporator under reduced pressure yielding 80 g of crude extract.

3.5.1 Isolation of Compounds from the Stems of *T. Garckeana*

Isolation of constituents of the crude extract of *T. garckeana* was achieved by column chromatography using silica gel matrix. Briefly, 75 g of the crude extract was adsorbed onto 75 g of silica gel and loaded on a column of 750 g of silica gel packed under 2.5 % EtOAc in *n*-hexane. Gradient elution was achieved with increasing amounts of EtOAc in *n*-hexane from 2.5 % to 40 % EtOAc collecting 500 ml of the eluant. A total of 70 fractions were collected and combined based on similarities of their TLC profiles to four fractions (36A- 36D). The fraction of the major column eluted with 20 % EtOAc in *n*-hexane (36 B) was recrystallized from 50 % CH_2Cl_2 in *n*-hexane to form amorphous solids of compound (**6**) (20 mg, R_f 0.37 (20 % EtOAc in *n*-hexane). Fraction eluted from the main column with 15 % EtOAc in *n*-hexane (36 A) recrystallized under 50 % CH_2Cl_2 in *n*-hexane to form white needle like crystals, which on spotting showed similar TLC profile to compound (**5**) and therefore were combined. The mother

liquor of fraction 36 A was dried and recrystallized under 50 % CH_2Cl_2 in *n*-hexane to form an amorphous powder of compound (**7**) (10 mg, R_f value of 0.30 (15 % EtOAc in *n*-hexane).

3.6   Biological Activities

3.6.1   Antiplasmodial Test

The *in vitro* anti-plasmodial tests were done at the National Centre for Natural Products Research, Research Institute of Pharmaceutical Sciences, University of Mississippi, using the modified version of the CLSI methods [Samoylenko *et al*., 2009]. Briefly, the anti-plasmodial activities of the pure compounds were carried out on two strains of *Plasmodium falciparum*; Indochina W2 (chloroquine resistant) and Sierra Leone D6 (chloroquine sensitive). Pure compounds, 2 mg/ml were dissolved in DMSO and serially diluted to make 4.76, 1.59 and 0.53 µg/ml and subsequently transferred into 96-well microplates. Two strains *P. falciparum*; Sierra Leone D6 (chloroquine sensitive) and Indochina W2 (chloroquine resistant) were added onto the 96 wells containing the test samples.

A suspension of red blood cells infected with *P. falciparum* (D6 or W2) strains, (200 µl, with 2 % parasitemia and 2 % hematocrit in RPMI-1640 medium supplemented with 10 % human serum and 60 µg/ml amikacin) was added to the wells containing 10 µl of test samples at various concentrations. The plate was flushed with a gas mixture of 90 % nitrogen, 5 % oxygen and 5 % carbon IV oxide in a modular incubation chamber and incubated at 37 °C for 72 hours. The IC_{50} values were computed from the dose response curves by plotting percent growth against concentrations. The control drugs used were artemisinin and chloroquine (98 % purity assessed by HPLC).

3.6.2   Antimicrobial Test

The *in vitro* anti microbial test was performed using a modified version of the CLSI methods [Samoylenko *et al*., 2009], at the National Centre for Natural Products Research, University of Mississippi. All the organisms were obtained from the American Type Culture Collection and

include the fungi *Candida glabrata* (ATCC 90030)*, Candida albicans* (ATCC 90028)*, Candida krusei* (ATCC6258)*, Aspergillus fumigatus* (ATCC 90906) and the bacteria *Staphylococcus aureus,* methicillin-resistant *Staphylococcus aureus* (ATCC 33591)*, Eschericia coli* (ATCC 35218) *Enterococcus faecium* ATCC 700221, *Enterococcus faecalis* ATCC 51299 and *Enterococcus faecalis* ATCC 29212.

Pure compounds (2 mg) were dissolved in DMSO and serially diluted in 20 % DMSO/saline to make 20, 10, 5, 2.5, 1.25, 0.675, upto 0.02 µg/ml and transferred in duplicate into 96-well flat bottom microplates. Microbial innocula were prepared in assay medium to afford targetful CFU/ml after addition to the samples. Growth, solvent and media controls were included in each test plate. Assay plates were read at 630 nm before and after incubation using Biotek Power Wave XS plate reader or the Polarstar Galaxy Plate Reader respectively. Percent growth was plotted versus test concentration to afford the IC_{50} values or concentration that affords 50 % growth relative to controls. The minimum fungicidal or bacterial concentrations (MFC/MBCs) were determined by removing 5µl from each clear well, transferring to agar and incubating until growth is seen. Drug controls used were ciprofloxacin [ICN Biomedicals, Ohio] for bacteria and amphotericin B [ICN Biomedicals, Ohio] for fungi.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Compounds Isolated from *Harrisonia abyssinica*

From this species, only one compound (obacunone **1**) was isolated.

4.1.1 Obacunone (1)

Compound (**1**) was isolated as white amorphous solid with melting point of 230-231 °C and an R_f value of 0.40 (4 % MeOH in CH_2Cl_2). The ^1H NMR spectrum (Table 4.1) exhibited two downfield shifted aromatic protons due to the presence of the ether bond in the furan ring at δ 7.51 (br, 1H) and 8.03 (*br*, 1H) assigned to H-22 and 23, respectively. Furthermore, two olefinic protons that were coupling with each other, appeared at δ 6.69 (*d*, *J*=12 Hz, 1H) and 5.82 (*d*, *J*=12 Hz, 1H) attributed to H-1 and 2. Methyl protons were observed at δ 1.06 (H-18), δ 1.16 (H-30), δ 1.32 (H-28), 1.35 (H-19), δ 1.40 (H-29). Presence of these methyl protons was confirmed by the HMQC spectra where the protons were attached to the following carbons respectively; δ 19.9, δ 16.7, δ 32.0, δ 16.7, and δ 26.8. The ^{13}C NMR (Table 4.1) showed characteristic peaks for a limonoid skeleton from the presence of one deshielded carbonyl carbon at δ 208.7 (C-7); two shielded carbonyls at δ 167.3 (C-16) and 167.0 (C-3) and oxygenated carbons of an epoxide group at δ 65.7 (C-14) and 53.2 (C-15).

Based on the above spectroscopic evidence (1D and 2D NMR) and comparison with literature [Okorie *et al*., 1982] compound (**1**) was identified as obacunone previously isolated from the roots of *H. abyssinica.*
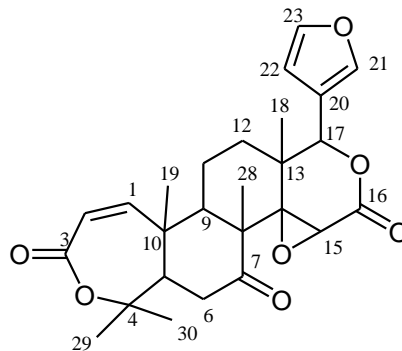
1

Table 4. 1: ^{1}H (CDCl$_3$ and DMSO at 400 MHz) and ^{13}C (CDCl$_3$, DMSO, 400 MHz) NMR chemical shift data, together with HMBC correlations for compound (1).

C, Position	^{1}H NMR δ, ppm	^{13}C NMR δ, ppm	HMQC δ, ppm	HMBC ^{2}J	HMBC ^{3}J
1	6.69, *d*, 12Hz	158.3	158.3	C-10	C-3, C-5, C-9, C-19
2	5.82, *d*, 12Hz	121.9	121.9		C-3, C-10
3		167.0			
4		84.4			
5	2.70, *m*	55.9	55.9	C-4, C-6, C-10	C-1, C-19
6	α-2.22 β-3.04	40.1	40.1	α: C-5, C-7 β: C-5, C-7	α: C-8, C-10 β: C-4, C-10
7		208.7			
8		52.5			
9	2.11	48.3	48.3	C-8, C-10, C-11	C-19, C-30
10		43.2			
11	1.35	16.9	16.9		C-9, C-10, C-13
12		29.9	29.9	C-13	C-14, C-18
13		37.8			
14		65.7			
15	3.79, *s*	53.2	53.2	C-14, C-16	C-8
16		167.3			
17	5.20, *s*	75.5	75.5		C-12, C-14, C-16, C-18, C-22
18	1.06, *s*	19.9	19.9	C-13	C-14, C-17
19	1.35, *s*	16.7	16.7	C-10	C-5, C-9
20		131.1			
21	6.20, *s*	121.6			
22	7.51, *s*	157.9	157.9	C-20	C-23, C-17
23	8.03, *br s*	158.0	158.0		
28	1.32, *s*	32.0	32.0	C-4	C-5, C-29
29	1.40,*s*	26.8	26.8	C-4	C-5, C-28
30	1.16, *s*	16.7	16.7	C-8	C-7, C-9, C-14

4.2 Compounds Isolated from *Thespesia garckeana*

4.2.1 Gossypol (2)

Compound (**2**) was isolated as a yellow amorphous solid with melting point of 177-182 °C and R_f value of 0.35 (15 % EtOAc in *n*-hexane).

The ^{1}H NMR spectrum (Table 4.2) exhibited characteristic peak for aldehydic proton at δ 11.03 (*s*) assigned to H-15 and 15´, downfield shifted chelated hydroxyl proton at δ 14.26 (*s*) assigned to H-7 and 7´ and aromatic proton δ 7.71 (*s*) assigned to H-4 and 4´. Furthermore, the ^{1}H NMR spectrum showed proton resonance at δ 3.82 due to methine protons flagged by two methyl groups assigned to H-12 and 12´, δ 1.49 (*m*) assigned to a set of isopropyl methyl protons at 13, 14, 13´ and 14´ positions. There was a well resolved peak at δ 2.16 integrating for only three protons assigned to methyl protons at H-11 and 11´. The presence of the isopropyl unit in the molecule was confirmed from 2D, ^{1}H NMR and COSY spectrum which exhibited correlation between H-12/12´ with H-13/13´ and H-14/14´.

The ^{13}C NMR spectrum (Table 4.2) corroborated the presence of the downfield shifted carbonyl and aromatic carbon from the carbon resonances appearing at δ 199.1 and δ 117.2 assigned to carbon 15, 15´ and 4, 4´ positions respectively. Furthermore, the ^{13}C NMR spectrum showed peaks for three oxygenated carbons δ 151.1, 142.6, 155.4 assigned to the aromatic carbons at 1, 1´6, 6´, 7 and 7´ positions respectively. The other peaks appeared at δ 27.8 for a set of methyne carbons at positions 12 and 12´, δ 20.4 for methyl carbons at position 11/11´ and δ 20.2 for a set of methyl carbons 13/14, 13´ and 14´.

The mass spectrum [EIMS] of compound (**2**) showed the molecular ion peak of $C_{30}H_{30}O_8$ at $[M]^+$, *m/z* 518 and other major peaks at *m/z* 500 (loss of H_2O), 482 (loss of $2H_2O$), 467 (loss of -CH_3) and 439 (loss of -$CH(CH_3)_2$).

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Table 4. 2: ^{1}H (CDCl$_3$ at 400 MHz) and ^{13}C (CDCl$_3$ at 400 MHz) NMR chemical shift data, together with HMBC correlations for compound (2).

C, Position	^{1}H NMR δ, ppm	^{13}C NMR δ, ppm	HMQC δ, ppm	HMBC ^2J	HMBC ^3J
1/1´	6.05 (*s*)	151.1			
2/2´		117.5			
3/3´		133.9			
4/4´	7.71 (*s*)	117.2	117.2	C-3, C-10	C-2, C-11
5/5´		134.3			
6/6´		142.6			
7/7´	14.26 (*s*)	155.4			
8/8´		111.6			
9/9´		114.8			
10/10´		129.3			
11/11´	2.16 (*s*)	20.2	20.2	C-3	C-4, C-2
12/12´	3.82 (*s*)	27.8	27.8	C-5	C-6
13/13´	1.49	20.4	20.4		C-5, C-12
14/14´	1.49	20.4	20.4		
15/15´	11.03 (*s*)	199.1		C-8	C-7
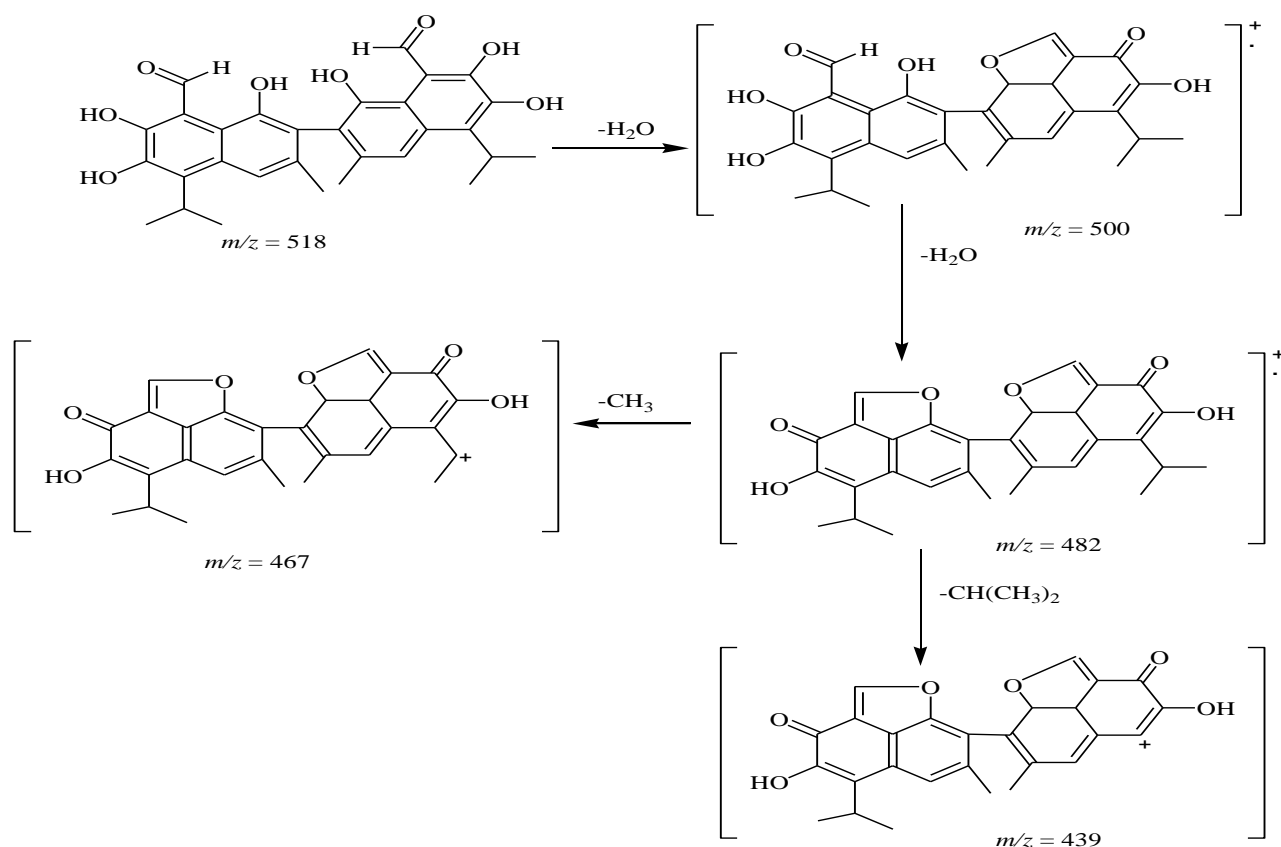
Scheme 1: Mass Fragmentation Pattern for Gossypol (2)

Thus based on the above spectroscopic data and correlation with literature [Datta *et al*., 1972] this compound was a symmetrical binaphtyl dialdehyde disesquiterpene named as 1,1´,6,6´,7,7´-Hexahydroxy-3,3´-dimethyl-5,5´-*bis*(1-methylethyl)-[2,2´-binaphthalene]-8,8´-dicarboxaldehyde trivial name gossypol (**2**). This compound has previously been reported as a natural product [Bhakuni *et al*., 1968] and it is a symmetrical binaphthyl dialdehyde disesquiterpene, $C_{30}H_{30}O_8$.

4.2.2   6,6´-dimethoxygossypol (3)

Compound (**3**) was isolated as luminous yellow crystals with melting point of 164-167 °C and R_f value of 0.4 (15 % EtOAc in *n*-hexane). The ^{1}H and ^{13}C NMR spectra (Table 4.3) of this compound were similar to that of compound (**2**) except for the presence of two methoxy groups in place of two hydroxyl groups at carbon 6 and 6´ positions. The ^{1}H NMR exhibited characteristic peaks attributed to an aldehydic protons, and aromatic protons at δ 11.17 (*s*) and δ 7.88 (*s*) assigned to H-15, 15´and H-4, H-4´ respectively. The hydroxyl group at carbon 6 and

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6´were methoxylated leading to the appearance of two identical methoxy groups at δ 3.99 assigned to CH₃ protons at 16 and 16´ in compound (**3**).

The ^{13}C NMR spectrum (Table 4.3) confirmed the presence of a set of the following carbons; carbonyl groups, two methoxys; hydroxylated aromatic carbons, methynes, methyls attached to aromatic rings and two chemically equivalent methyls of an isoprene system at δ199.1 (C-15, 15´); 60.7 (C-16 and 16´); 150.1 (C-1, 1´), 144.6 (C-6, 6´), 160.9 (C-7, 7´); 27.8 (C-12, 12´); 19.9 (C-11, 11´); 21.4 (C-13, 13´ and C-14, 14´) respectively.

The mass spectrum [EIMS] of compound **3** showed the molecular ion peak of $C_{32}H_{34}O_8$ at *m/z* 569 [M+ Na]$^{+}$.
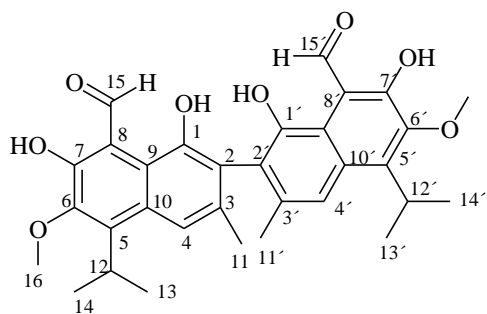
Based on the above spectroscopic data and comparison with literature to the related compound (**2**) the compound was therefore identified as 6,6´-dimethoxygossypol (**3**) previously isolated from one week old roots of *Gossypium hirsutum* and *Gossypium barbadense* [Stipanovic *et al.,* 1975]. This is the first report of this compound from this genus.
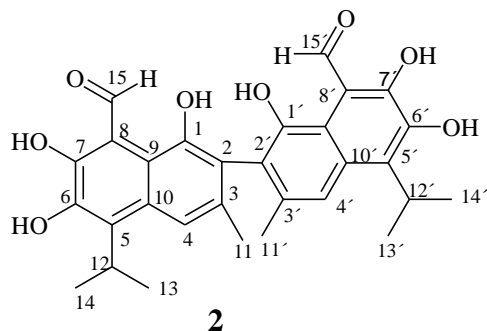
Table 4. 3: ^{1}H (CDCl$_3$ at 400 MHz) and ^{13}C (CDCl$_3$ at 400 MHz) NMR chemical shift data, together with HMBC correlations for compound (3).

C, Position	Stipanovic *et al.,* 1975	^{1}H NMR δ, ppm	^{13}C NMR δ, ppm	HMQC δ, ppm	HMBC ^2J	HMBC ^3J
1/1´	5.78	5.99 (*s*)	150.1		C-1	C-2
2/2´			117.3			
3/3´			133.3			
4/4´	7.75	7.88 (*s*)	119.1	117.3	C-1, C-10	C-2, C-11
5/5´			147.5			
6/6´			144.6			
7/7´	14.51	14.55/14.17	160.9			
8/8´			105.0			
9/9´			113.2			
10/10´			129.3			
11/11´	2.12	2.17 (*s*)	19.9	19.9		C-2, C-5
12/12´	3.90	3.99	27.8	27.8		C-5
13/13´	1.53	1.57 (*m*)	21.4		C-12	C-5
14/14´	1.53	1.57 (*m*)	21.4		C-12	C-5
15/15´	11.10	11.17 (*s*)	199.1		C-9	C-7
OMe - 16,16´	3.94	3.99 (*s*)	60.7	60.7		

4.2.3   6-methoxygossypol (4)

Compound (**4**) was similarly isolated as yellow crystals with melting point of 146-149 ^{o}C and R$_f$ value of 0.32 (10 % EtOAc in *n*-hexane).

The chemical shift values of this compound in the ^{1}H NMR and ^{13}C NMR spectra (Table 4.4) were similar to those of compounds (**2**) and (**3**) except for the presence of only one methoxy group. This was evidenced from the ^{1}H NMR which showed a sharp singlet at δ 3.87 and the corresponding ^{13}C NMR signal at δ 60.7 an indication of a di-*ortho* substituted methoxy group, which was assigned to H-16. The placement of the methoxy group at C-6 was confirmed by the presence of chelated hydroxyl protons with the carbonyl group at C-15, consistent with a free hydroxyl group at the neighbouring C-7 carbon. The ^{13}C NMR spectrum showed a set of peaks for each carbon indicating that compound (**4**) was not a dimer as was the case with compounds (**2**) and (**3**). This compound would have resulted from methoxylation of the hydroxyl group at C-6 of gossypol or oxidative coupling of two different monomers which differed from gossypol monomer only at the C-6 position which has a methoxy group in place of a hydroxyl group.

Based on spectroscopic data coupled with literature values compound (**4**) was named 6-methoxygossypol. This compound was previously isolated from one week old roots of *Gossypium hirsutum* and *Gossypium barbadense* [Stipanovic *et al.,* 1975]. However, this is the first report of this compound from *T. garckeana*.

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Table 4. 4: ^{1}H (CDCl$_3$ at 400 MHz) and ^{13}C (CDCl$_3$ at 400 MHz) NMR chemical shift data, together with HMBC correlations for compound (4).

C, Position	Stipanovic *et al.,* 1975	^{1}H NMR δ, ppm	^{13}C NMR δ, ppm	HMQC δ, ppm	HMBC ^2J	HMBC ^3J
1/1´	5.68/5.78(*s*)	6.53	150.9/ 150.6			C-2, C-2´
2/2´			116.6/ 116.7			
3/3´			136.3			
4/4´	7.75(*s*)	7.75/7.77 (*s*)	117.7/ 117.4	117.7/ 117.4		C-2, C-2´, C-5, C-5´, C-11, C-11´
5/5´			134.3			
6/6´	6.37	6.20	144.6/ 142.6		C-6´	C-5´, C-7´
7/7´		14.55/14.17 (*s*)	160.8/ 155.6			
8/8´			113.1/ 111.6			
9/9´			114.7/ 114.7			
10/10´			129.5			
11/11´	2.12(*s*)	2.15/2.14 (*s*)	21.7	21.7		C-2, C-2´, C-4, C-4´
12/12´	3.90	3.85	27.8/ 27.7	27.7/ 27.8	C-13/C-14	C-6
13/13´	1.53(*s*)	1.51 (*s*)	20.4/20.3	20.4/20.3/20.2	C-12, C-12´	
14/14´	1.53(*s*)	1.51 (*s*)	20.2			
15/15´	11.17(*s*)	11.0 (*s*) 11.11 (*s*)	209.2/ 199.2		C-8, C-8´,	C-7 , C-7´
16 O-Me	3.95(*s*)	3.87 (*s*)	60.7	60.7		

4.2.4 Stigmasterol (5)

This compound was isolated as white needle like crystals with melting point of 160-164 $^{\circ}$C and R_f of 0.35 (15 % EtOAc in *n*-hexane). Compound **5** was not sensitive to UV_{254} light and therefore was visualized using iodine vapour. The ^{1}H NMR (Table 4.5) exhibited two olefinic protons, a hydroxymethine proton and a vinylic proton at δ 5.05 (*m*), 5.18 (*m*, 1H), 3.53 and 5.35 (*t*) assigned to protons at H-20, 21, 3 and 6 positions respectively. The presence of these groups was confirmed by the ^{13}C NMR spectrum which showed peaks corresponding to δ 129.2 and 138.3 for olefinic carbons, 71.8 for hydroxymethine carbon and 121.9 for the vinyl carbon assigned to carbons at C-20, 21, 3 and 6 positions respectively. Methyl protons were observed at δ 1.01 (*s*) H-29, δ 0.93 (*m*) H-19, δ 0.85 (*m*) H-24, δ 0.82 (*m*) H-26 and δ 0.80 (*m*) H-27.

The ^{13}C NMR (Table 4.5) indicated presence of a quaternary carbon δ140.9 (C-5) and six methyls; δ 12.0 (C-29), δ 12.2 (C-24), δ 19.0 (C-28), δ 19.6 (C-27), δ 20.1 (C-26). Based on 1D and 2D spectra as well as correlation with literature [Prakash and Prakash, 2012] compound (**5**) was identified as (3*S*,8*S*,9*S*,10*R*,13*R*,14*S*,17*R*)-17-[(*E*,2*R*,5*S*)-5-ethyl-6-methylhept-3-en-2-yl]-10,13-dimethyl-2,3,4,7,8,9,11,12,14,15,16,17-dodecahydro-1H-cyclopenta[α]phenanthren-3-ol trivial name stigmasterol with a molecular formular $C_{29}H_{48}O$. This compound has previously been isolated from many plants including *Rubus suavissimus* [Prakash and Prakash, 2012], *Phaseolus vulgaris* [Ott and Ball, 1944] and *Spillanthes acmella* [Isah *et al*., 2012].
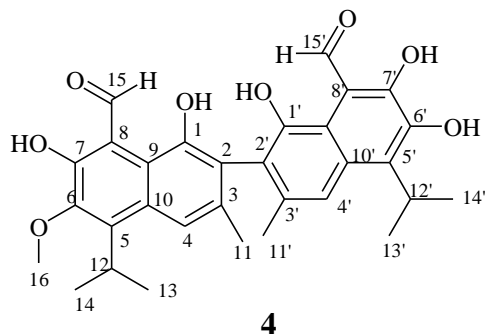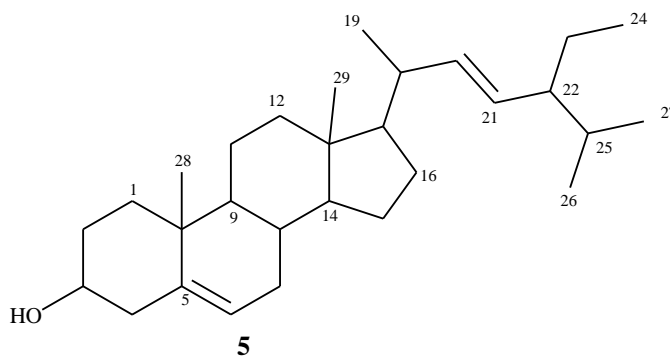

5

Table 4. 5: ^{1}H (CDCl$_3$ at 400 MHz) and ^{13}C (CDCl$_3$ at 400 MHz) NMR chemical shift data, together with HMBC correlations for compound (5).

C, Position	^{1}H NMR δ, ppm	^{13}C NMR δ, ppm	HMQC δ, ppm	HMBC ^2J	HMBC ^3J
1	1.85 (*m*)	37.5	37.5	C-2, C-10	C-3, C-5
2		31.9			
3	3.53 (*m*)	71.8	71.8		
4	2.29	42.4	42.5	C-3, C-5	C-6
5		140.9			
6	5.35 (*t*)	121.9	121.7		C-4, C-8, C-10
7	1.99 (*m*)	32.1	32.1	C-6	C-5, C-9
8	1.54	32.1	32.1	C-7, C-9	
9		50.3			
10		36.7			
11		21.3			
12		39.9			
13		42.6			
14		56.9			
15		26.3			
16		28.5			
17		56.3			
18		36.3			
19	0.93 (*d*)	19.2	19.2	C-18	C-17, C-20
20	5.05 (*m*)	129.2	138.3		
21	5.18 (*m*)	138.3	129.2		
22		46.1			
23		23.3			
24	0.85 (*dd*)	12.2	12.2		
25		29.4			
26	0.82 (*d*)	20.1	20.1		
27	0.80 (*d*)	19.6	19.6		
28	0.67(*s*)	19.0			
29	1.01 (*s*)	12.0	12.0	C-13	C-17

4.2.5 *E*-docosyl 3-(3,4-dihydroxyphenyl) acrylate (6)

This compound was isolated as white amorphous powder with R$_f$ value of 0.37 (20 % EtOAc in *n*-hexane).

The ^{1}H NMR spectrum (Table 4.6) showed the presence of two *trans* oriented olefinic protons at δ 7.55 (*d*, *J*=16 Hz) and 6.25 (*d*, *J*=16 Hz) assigned to H-7 and H-8 respectively. Furthermore, there were peaks attributed to aromatic protons; in *ortho* orientation at δ 6.76 (*d*, *J*=8Hz) and 6.85 (*d*, *J*=8 Hz) assigned to H-5 and 6 respectively, in *meta* orientation to one of the aromatic protons at δ 6.99 (*s*) assigned to H-2. The other peaks at δ 0.86 (*t,* 3H, 12Hz) were attributed to

the terminal methyl protons at the end of the aliphatic chain assigned to H-22′ and 1.61(m, 2H) assigned to H-2′.

The ^{13}C NMR spectrum (Table 4.6) showed presence of two oxygenated carbons; δ 148.9 (C-3), δ 151.5 (C-4), a carbonyl peak at δ 172.2 (C-9), a methyl δ 17.8 (C-22′), and a carbon attached to an ester bond δ 68.6 (C-1′).

The mass spectrum, negative EIMS of compound (**6**) showed the molecular ion peak of $C_{31}H_{52}O_4$ at $[M]^+$ m/z 488. The other major peak appeared at m/z 459 (loss of a -CH_2CH_3 group). The spectra data were similar to that of C_{22} n-alkyl docosyl derivative isolated from *Halocnemum strobilaceum* hence the compound deduced to be *E*-docosyl 3-(3,4-dihydroxyphenyl) acrylate. This is the first report of this compound from genus *Thespesia*.
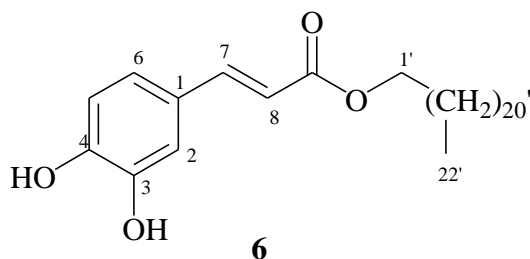

6

Table 4. 6: ^{1}H (CDCl$_3$ and MeOD at 400 MHz) and ^{13}C (CDCl$_3$ and MeOD at 400 MHz) NMR chemical shift data, together with HMBC correlations for compound (6).

C, Position	^{1}H NMR δ, ppm, m	^{13}C NMR δ, ppm	HMQC δ, ppm	HMBC ^2J	HMBC ^3J
1		125.8			
2	6.99 (*s*)	117.9		C-3	C-4, C-6
3		148.9			
4		151.5			
5	6.76 (*d*, 8 Hz)	118.5		C-4, C-6	
6	6.85 (*d*, 8Hz)	130.6		C-4	C-2
7	7.55 (*d*, 16Hz)	136.4		C-1, C-8	C=O
8	6.25 (*d*, 16Hz)	119.1		C=O	
9, C=O		172.2			
1′	4.18 (*t*, 4Hz)	68.6	68.6	C=O, C-2′	C-3′
2′	1.61 (*m*, 8 Hz, 8 Hz)	32.6			
3′		29.8			
20′		26.5			
21′		32.7			
22′	0.86 (*t*, 12 Hz)	17.8	17.8	C-21′	C-20′

4.2.6 Betulinic acid (7)

Compound (**7**) was isolated as white/cream amorphous powder (10 mg) with an R_f value of 0.3 (20%EtOAc in *n*- hexane). It was soluble in MeOH and UV in active. ^{1}H NMR (Table 4.7) showed presence of a hydroxymethine proton δ 3.09, *dd* 4 Hz, 4Hz (H-3), olefinic proton δ 4.64/4.61, *d* (H-29), methyl protons δ 1.62, *s* (H-30), δ 0.89, *s* (H-26), δ 0.78, *s* (H-24), δ 0.67, *s* (H-23), methyne protons δ 1.55, *s* (H-18) and δ 2.95, *m* (H-19).

The ^{13}C NMR spectra data (Table 4.7), carbonyl peak was observed at δ 182.8 (C-28), sp^3 hybridized oxygenated carbon δ 82.3 (C-3), two olefinic carbons δ 154.4 (C-20) and δ 112.97 (C-29). Methyl carbon peaks were also evident δ 29.4 (C-27/C-25), δ 22.1 (C-30), δ 18.9 (C-24) and δ 18.1 (C-23/26). The negative electrospray ionization mode showed a deprotonated ion [M-H] $^-$ at 455 with a strong intensity. Since the carboxyl group is a strong proton donor, the deprotonated ion *m/z: 455 [M]* $^-$ can be generated more easily than protonated species [Shin *et al*., 1999].

The compound was therefore identified as betulinic acid from the above data and comparison with authentic sample.
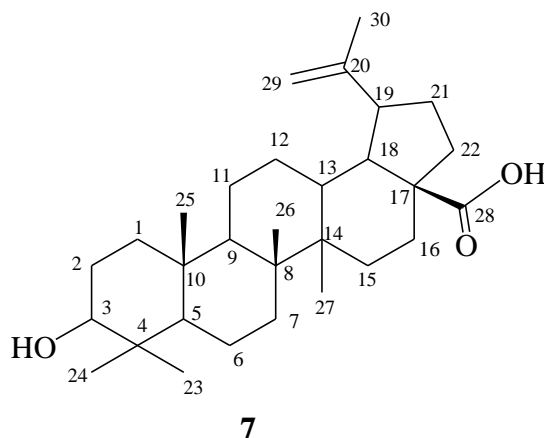

7

Table 4. 7: ^1H (CDCl$_3$ and MeOD at 400 MHz) and ^{13}C (CDCl$_3$ and MeOD at 400 MHz) NMR chemical shift data, together with HMBC correlations for compound (7).

C, Position	^1H NMR δ, ppm	^{13}C NMR δ, ppm	HMQC δ, ppm	HMBC ^2J	HMBC ^3J
1	0.89 (*m*)	33.5	33.5		C-3, C-5, C-25
2	1.55 (*m*)	31.4	31.4		
3	3.09 (*dd*, 4 Hz, 4Hz)	82.3	82.3	C-4	C-23, C-2
4		42.6			
5	1.23 (*m*)	54.5	54.5	C-4, C-6	C-7
6		19.6			
7		24.7			
8		42.7			
9		59.4			
10		44.5			
11		22.2			
12		19.4			
13	1.36 (*m*)	38.2	38.2		C-17, C-19, C-28
14		46.2			
15	1.12 (*m*)	34.3	34.3	C-16	C-8, C-14, C-17
16	2.22 (*m*)	36.0	36.0		C-14, C-18
17		60.1			
18	1.55 (*m*)	53.0	53.0	C-17	C-14, C-19, C-20, C-28
19	2.95 (*m*)	50.9	50.9	C-20, C-21	C-29, C-30
20		154.4			
21		30.6			
22	1.84 (*dd*)	40.9	40.9	C-17, C-21	C-18, C-19, C-28
23	0.67 (*s*)	18.1	18.1	C-4	C-5, C-3
24	0.78 (*s*)	18.9	18.9	C-4,	C-5, C-23
25	0.83 (*s*)	29.4			
26	0.89 (*s*)	18.1	18.1	C-8	C-7, C-9, C-14
27		29.4			
28		182.8			
29	4.64/4.61 (*d*)	112.9			C-19, C-30
30	1.62 (*s*)	22.1	22.1	C-20	C-19, C-29

4.3   Biological Activities

4.3.1   Antiplasmodial Activities

Obacunone (**1**) had a moderate activity with an IC$_{50}$ value greater than 4.76 µg/ml against the *P. falciparum* D6 and W2 strain.

4.3.2 Antimicrobial Activities

Table 4. 8: Anti-VRE activities (IC$_{50}$/MIC/MBC) in µg/ml of isolated compounds; gossypol (2), 6,6´-dimethoxygossypol (3) and 6-methoxygossypol (4).

	Enterococcus faecium ATCC 700221 IC$_{50}$/MIC/MBC	*Enterococcus faecalis* ATCC 29212 IC$_{50}$/MIC/MBC	*Enterococcus faecalis* ATCC 51299 IC$_{50}$/MIC/MBC
Vancomycin		1.428/2.5/-	2.45/10.0/-
Gossypol (2)	0.89/2.50/10.00	2.45/5.00/10.00	2.71/10.00/10.00
Compound (3)	3.35/10.00/10.00	10.78/20.00/20.00	10.81/20.00/20.00
Compound (4)	1.45/2.50/5.00	5.50/10.0/10.0	2.31/5.00/5.00

Enterococcus faecium ATCC 700221- resistant strain, *Enterococcus faecalis* ATCC 51299-resistant strain and *Enterococcus faecalis* ATCC 29212- sensitive strain

Compound (2) had a strong activity with IC$_{50}$ values of 0.89 and 2.45 µg/ml against ATCC 700221 and ATCC 29212 respectively, compound (4) displayed a strong activity against VRE ATCC 700221 and 51299 strains with IC$_{50}$ values of 1.45 and 2.31 µg/ml while compound (3) exhibited an IC$_{50}$ value of 3.35 µg/ml against ATCC 700221. The strong anti-VRE activity of these compounds rationalizes its use for the treatment of VRE infections.

Compound (4) showed a very strong activity against *Candida glabrata* with an IC$_{50}$ values of <0.8 µg/ml, with gossypol (2) showing activity against *C. glabrata* and methicillin resistant staphylococcus aureus. All the compounds were inactive towards *Candida albicans*, *Eschericia coli, Aspergillus fumigatus, Pseudomonas aeruginosa* and *Mycobacterium intracellulare* with IC$_{50}$ values > 20 µg/ml.

Table 4. 9: Anti-microbial activities (IC$_{50}$ in µg/ml) of isolated compounds; gossypol (2), 6,6´-dimethoxygossypol (3), 6-methoxygossypol (4), stigmasterol (5) and betulinic acid (7).

	C. glabrata IC$_{50}$ µg/ml	*S. aureus* IC$_{50}$ µg/ml	MRS IC$_{50}$ µg/ml
Gossypol (2)	3.21	6.98	4.19
6,6´-dimethoxygossypol (3)	>20	>20	>20
6-methoxygossypol (4)	<0.8	>20	9.37
Stigmasterol (5)	>20	>20	>20
Betulinic acid (7)	>20	>20	>20

CHAPTER FIVE

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

The limonoid obacunone (**2**) was isolated from *Harrisonia abyssinica.* The crude extract of 1:1 MeOH/CH$_2$Cl$_2$ stem bark exhibited antimalarial activity of IC$_{50}$ value of 5.6 µg/ ml against the sensitive *Plasmodium falciparum* D6 strain and 4.4 µg/ ml against resistant strain W2.
Thespesia garckeana crude root extract showed strong antimicrobial activity of 100 % inhibition against *Candida glabrata* at a concentration of 50 µg/ ml. From this plant, anti-fertility phenolic compound disesquiterpene aldehyde gossypol (**2**), 6,6´-dimethoxygossypol (**3**) and 6-methoxygossypol (**4**), stigmasterol (**5**), compound (**6**) and betulinic acid (**7**) were isolated.

The conclunsions drawn from this study are summarized below:

1. The crude extract of *Harrisonia abyssinica* exhibited strong antimalarial activity which may be attributed to presence of limonoids known to have high activity, combined with the synergistic effect of obacunone (**1**) which were not isolated due to their ability to decompose at room temperature.

2. Gossypol (**2**), a symmetrical dimer with two naphthyl units has previously been reported in *Thespesia populnea* and the genus *Gossypium*. However, this is the first report of the occurrence of Gossypol in *Thespesia garckeana* and appears to be the major compound in the roots.

3. This is the first report of the occurrence of 6,6´-dimethoxygossypol (**3**) and 6-methoxygossypol (**4**) in genus *Thespesia* and may have been formed through methoxylation of hydroxyl groups at positions 6 and 6´of gossypol.

4. *E*-docosyl 3-(3,4-dihydroxyphenyl) acrylate (**6**) and betulinic acid (**7**) are reported here for the first time in genus *Thespesia.*

5.2 Recommendations

1. Compounds isolated from *Harrisonia abyssinica* were very unstable may be due to the presence of the oxirane ring in the limonoids which is highly unstable. Since the plant produced a very interesting compound, it is recommended that further phytochemical studies on this species be pursued that would lead to isolation and characterization of more compounds with high bioactivities.

2. The roots of *Thespesia garkeana* have potential use as antimicrobial agent in communities of rural Africa. Therefore the crude extracts can be formulated for anti-microbial applications as in soaps and other disinfectants.

3. Thorough phytochemical investigations on the stem and the leaves of *Thespesia garckeana* that could result in the isolation and characterization of other compounds with interesting bioactivities should be done.

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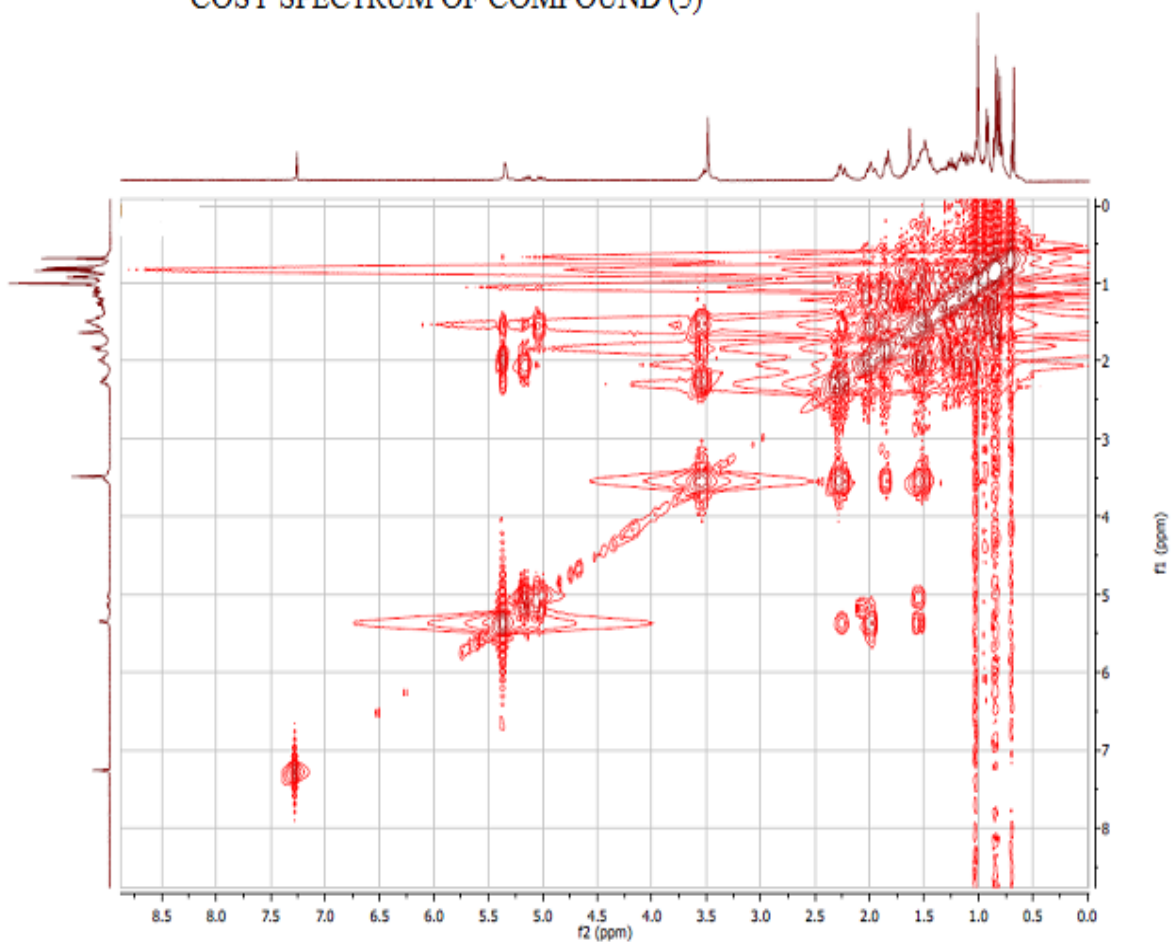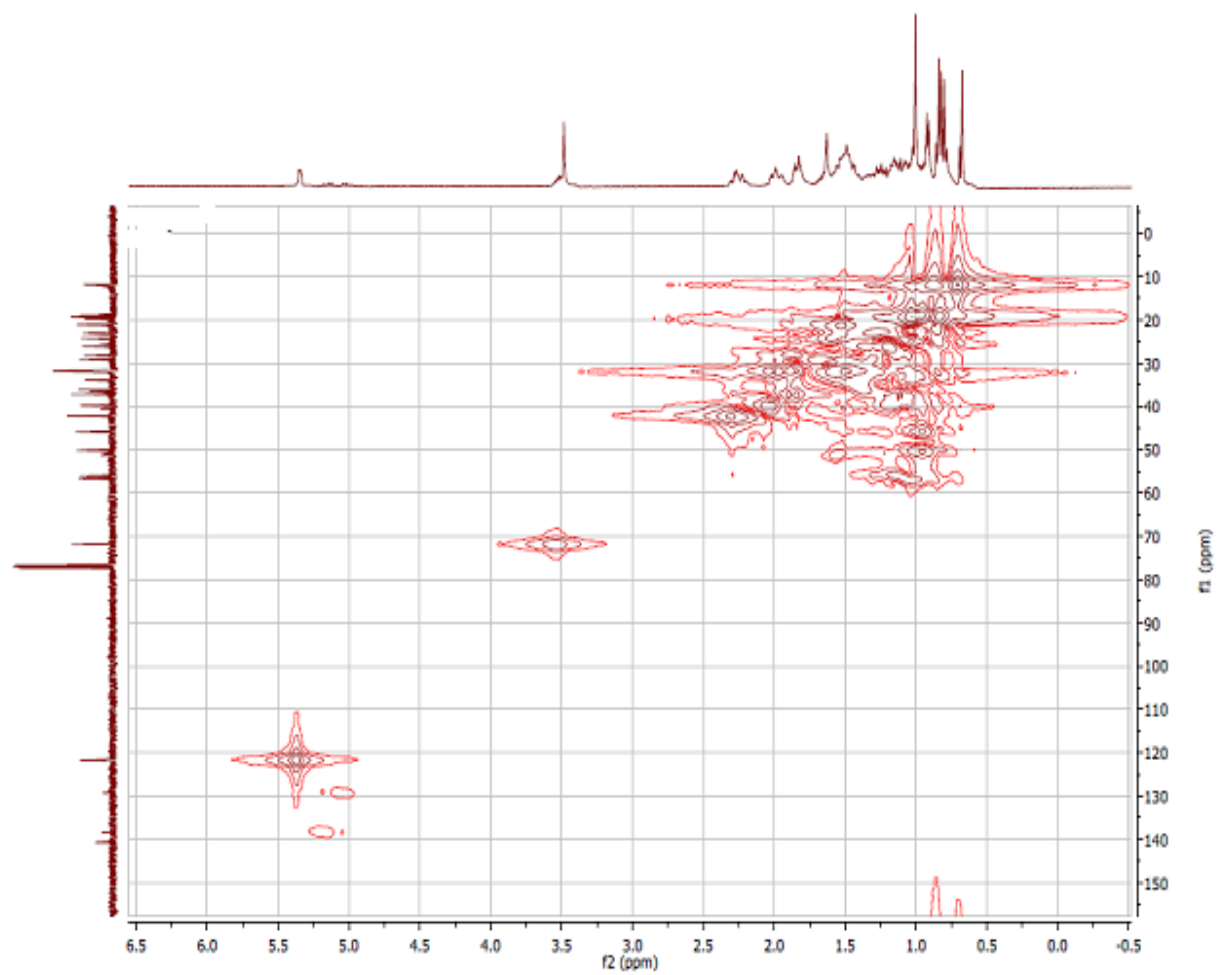
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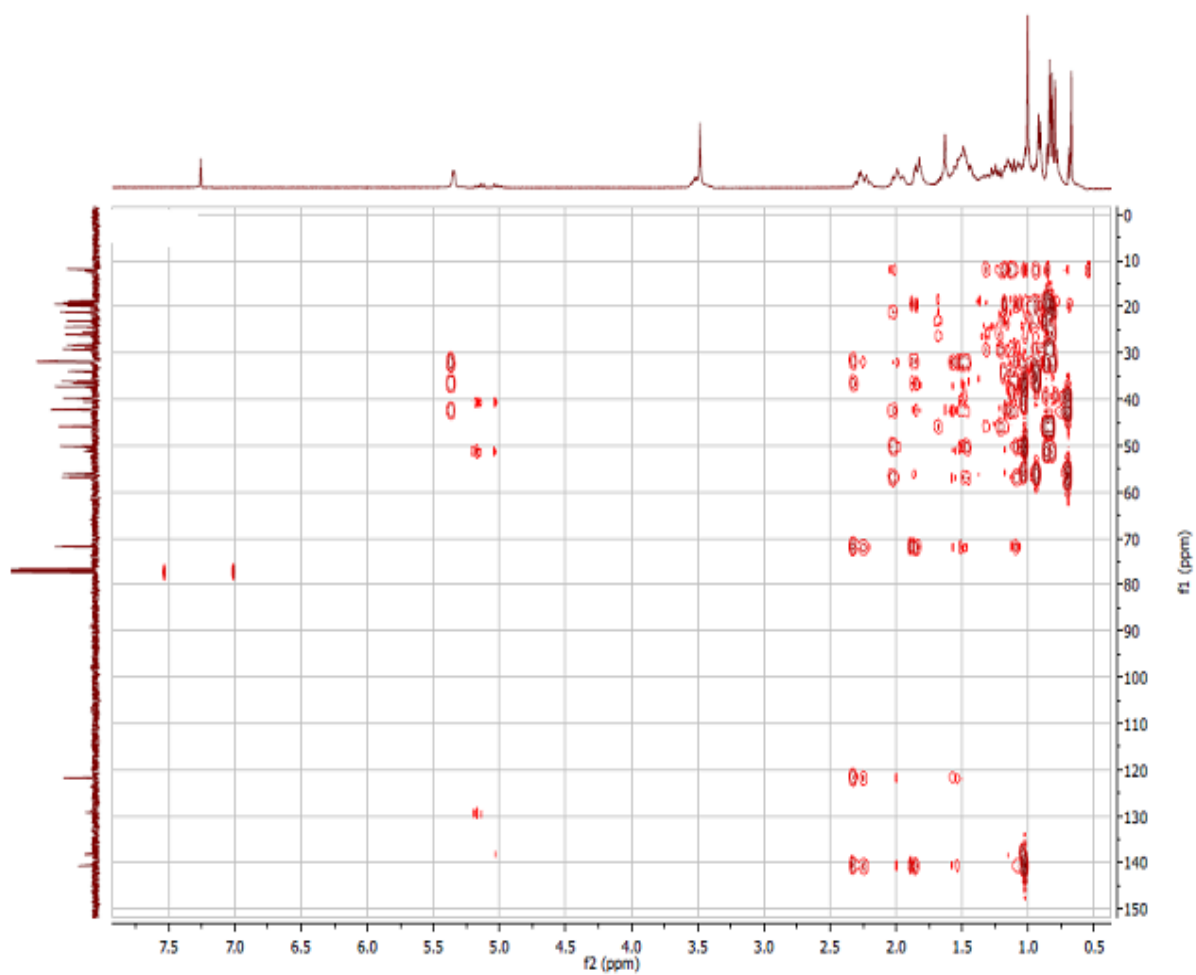
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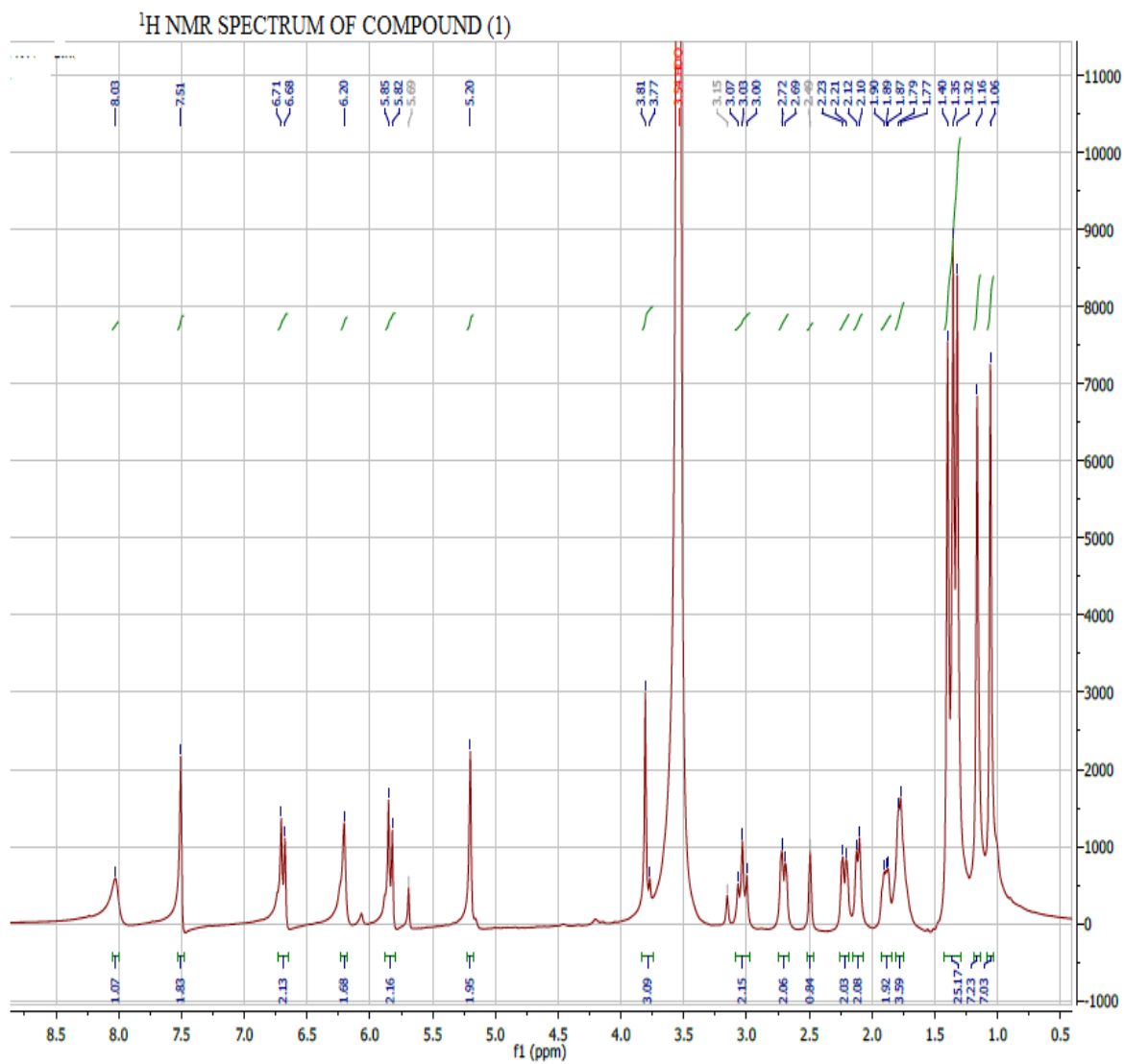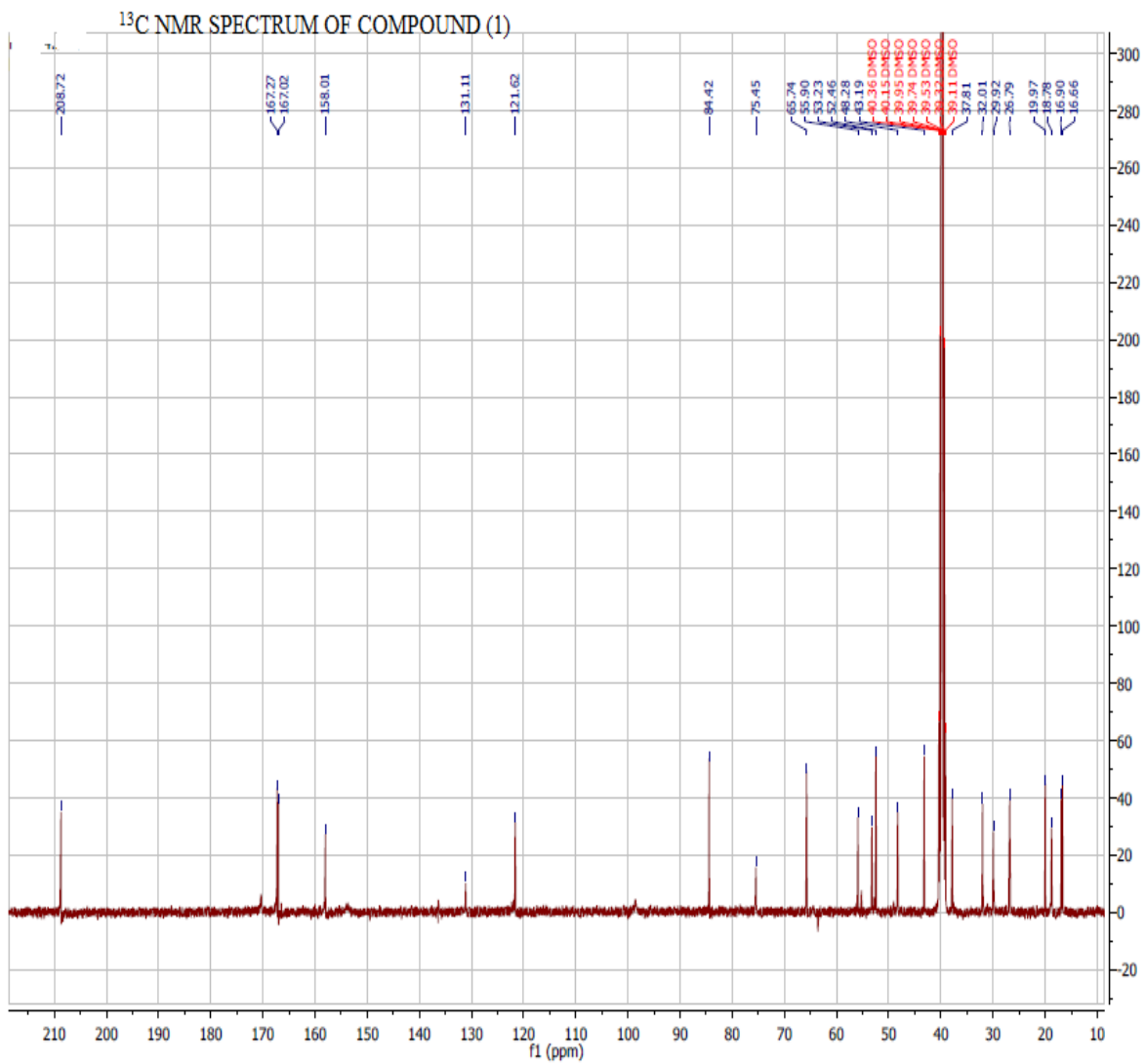
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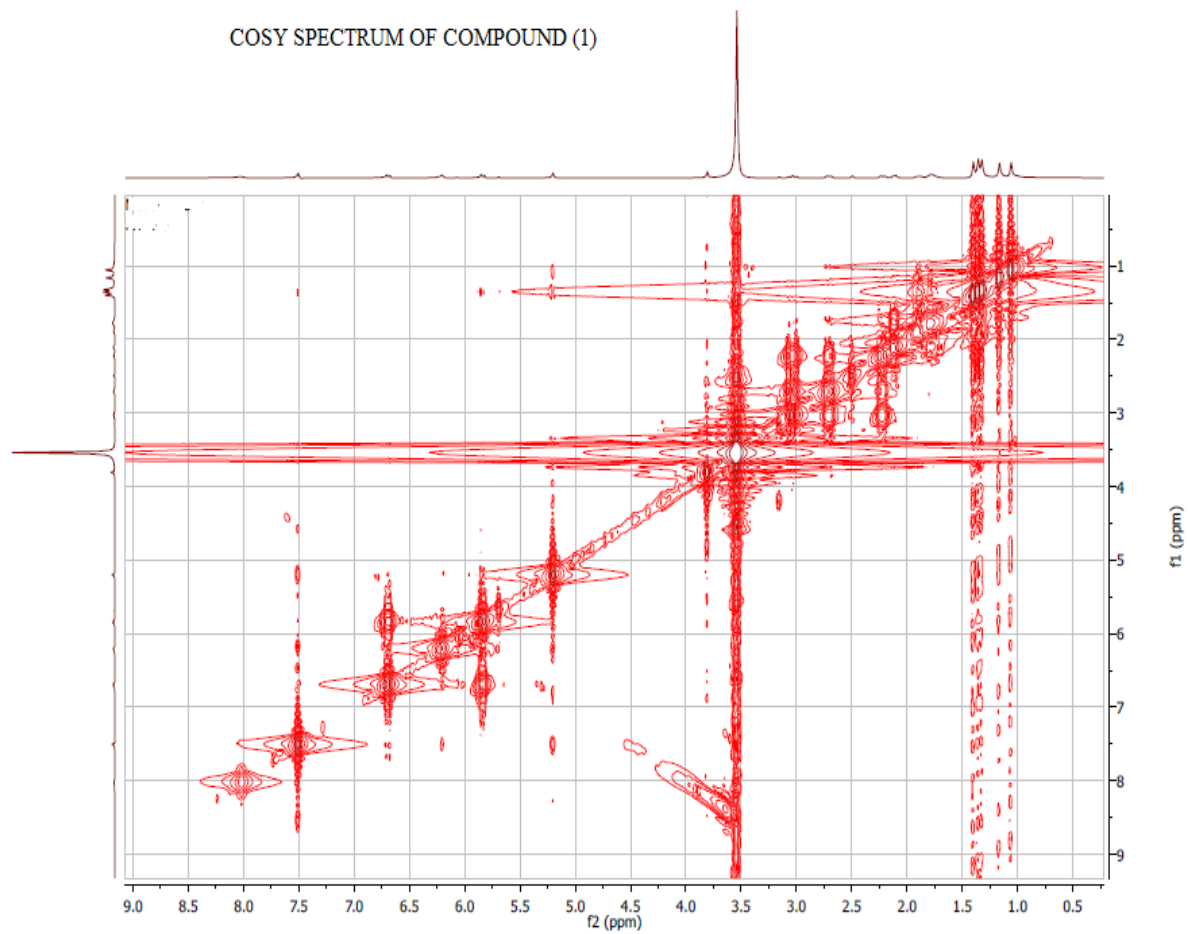
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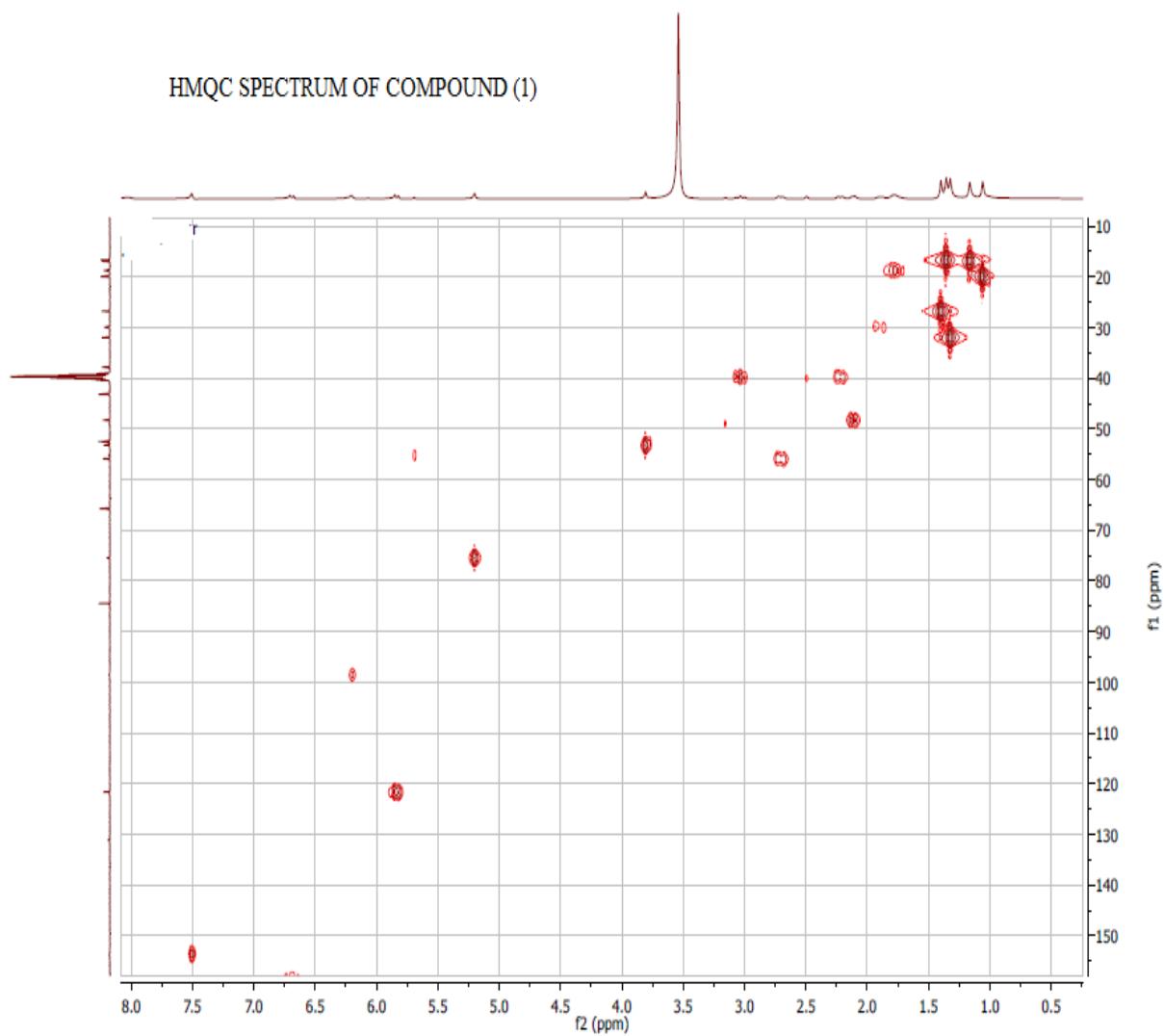
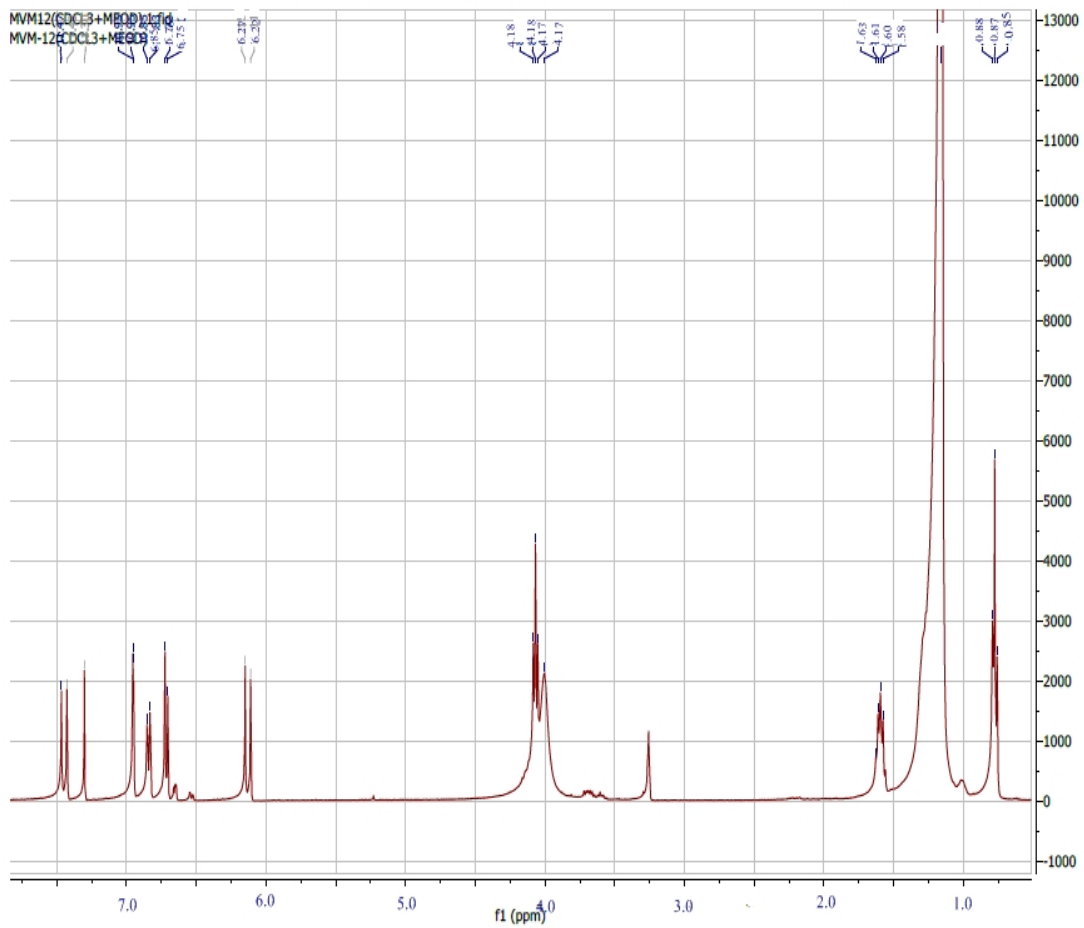HMBC SPECTRA OF COMPOUND (1)
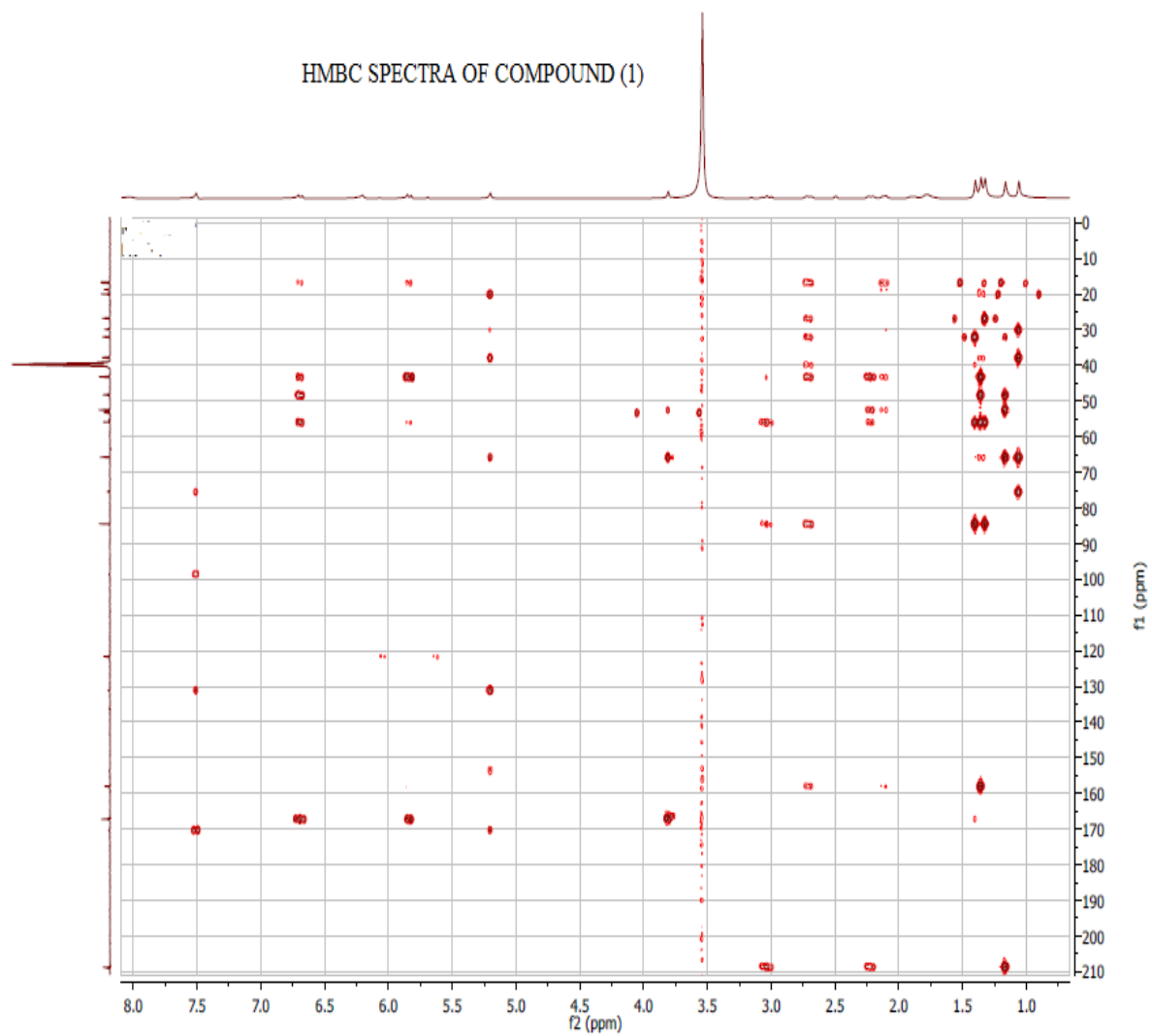
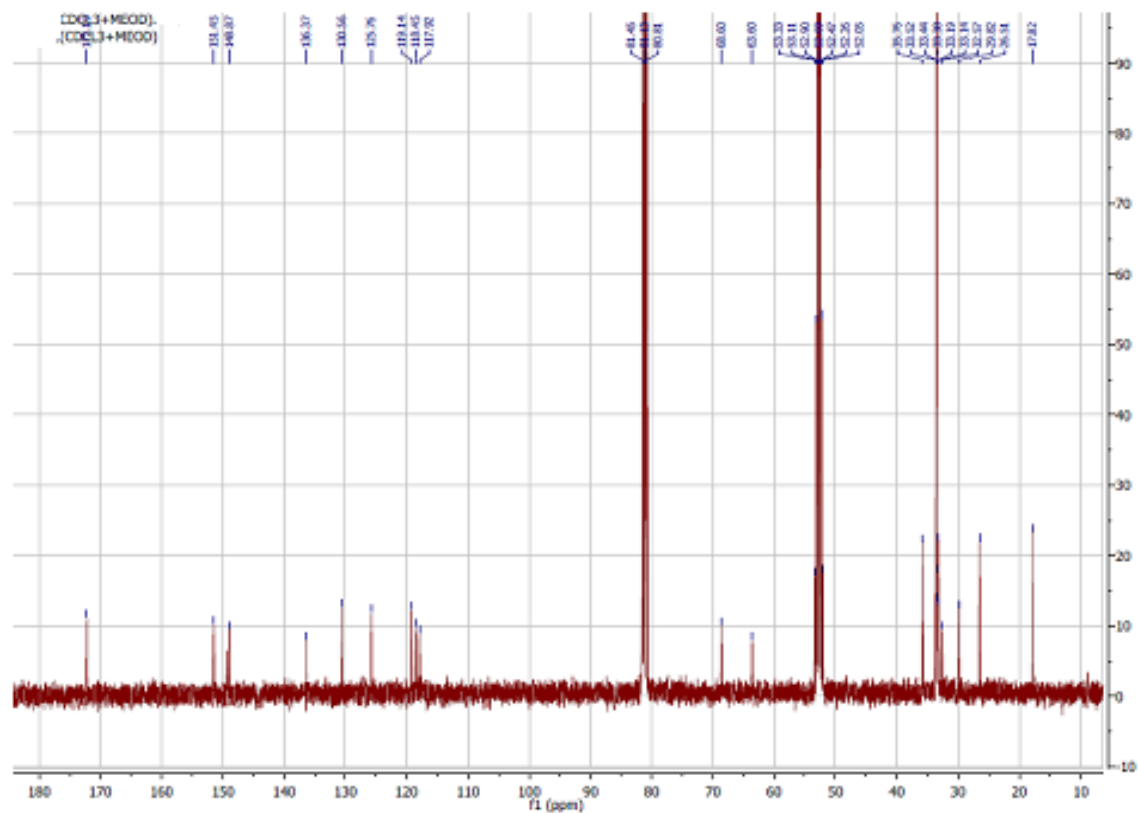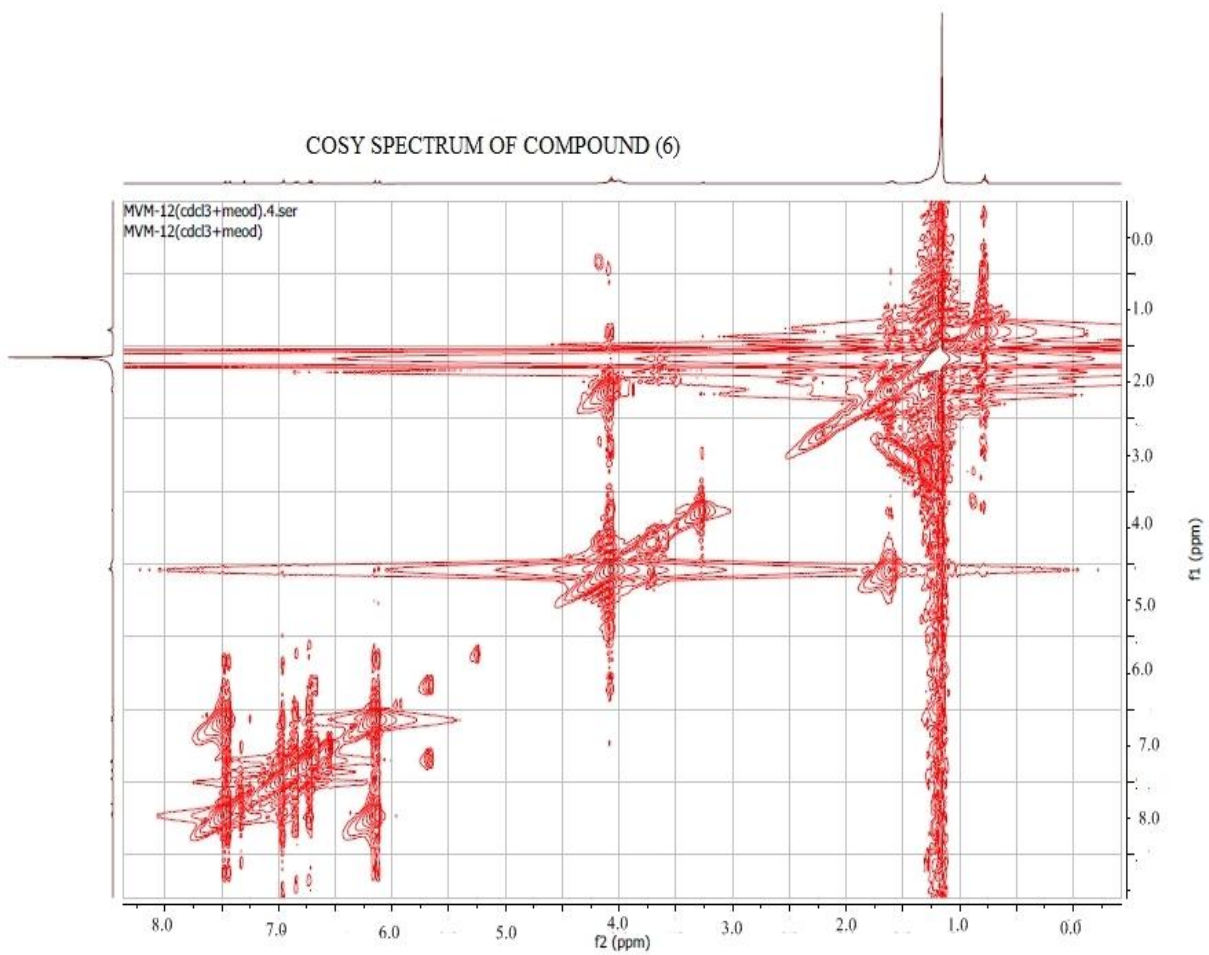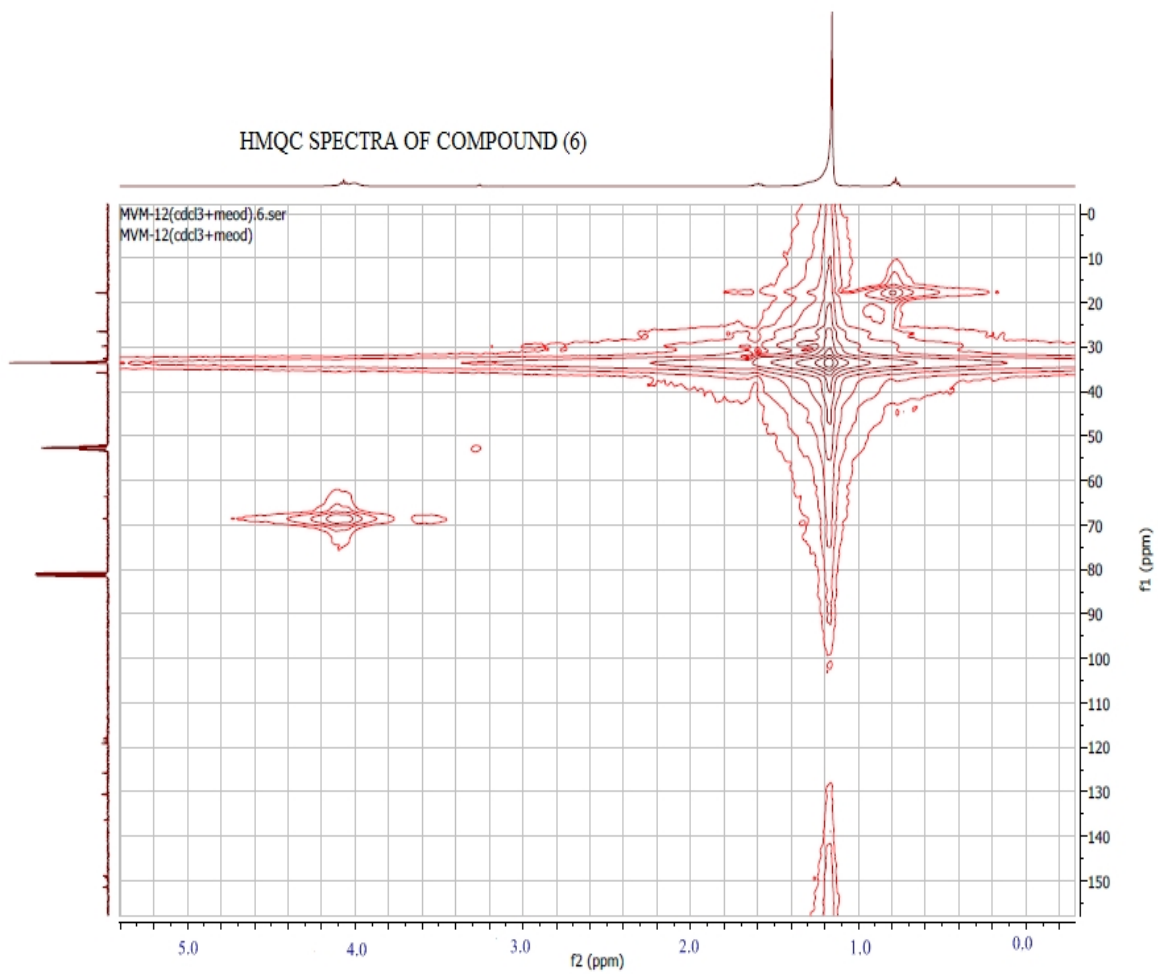HMBC SPECTRUM OF COMPOUND (1)
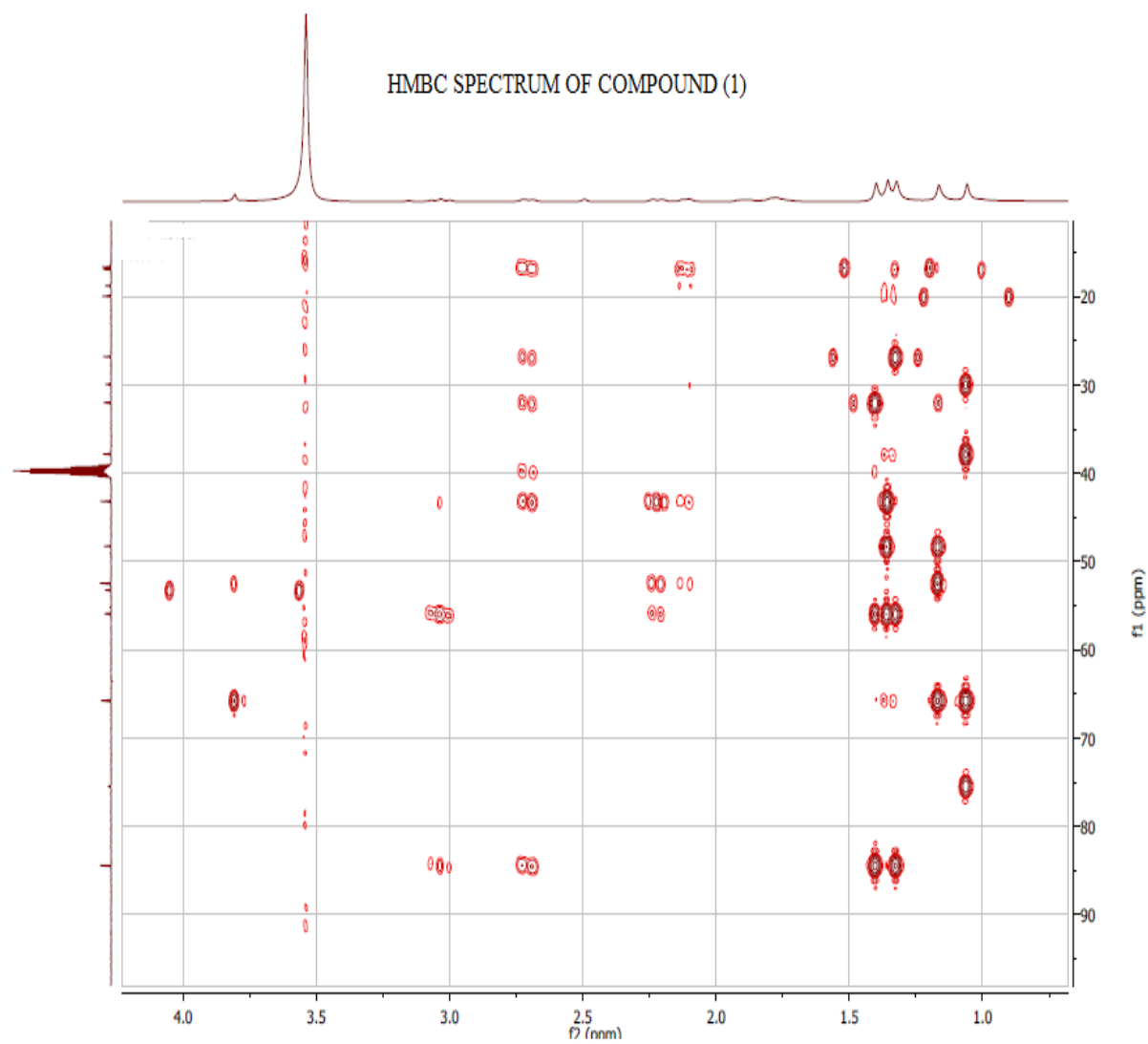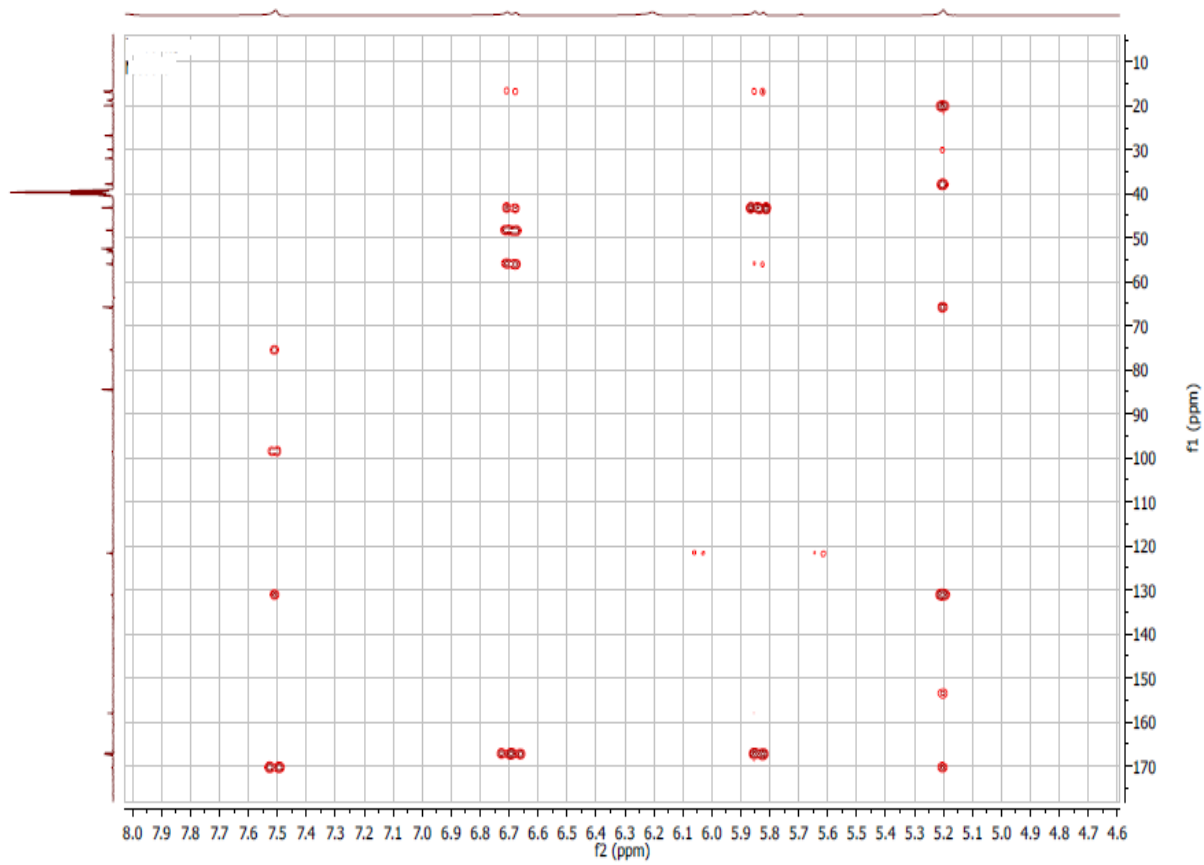
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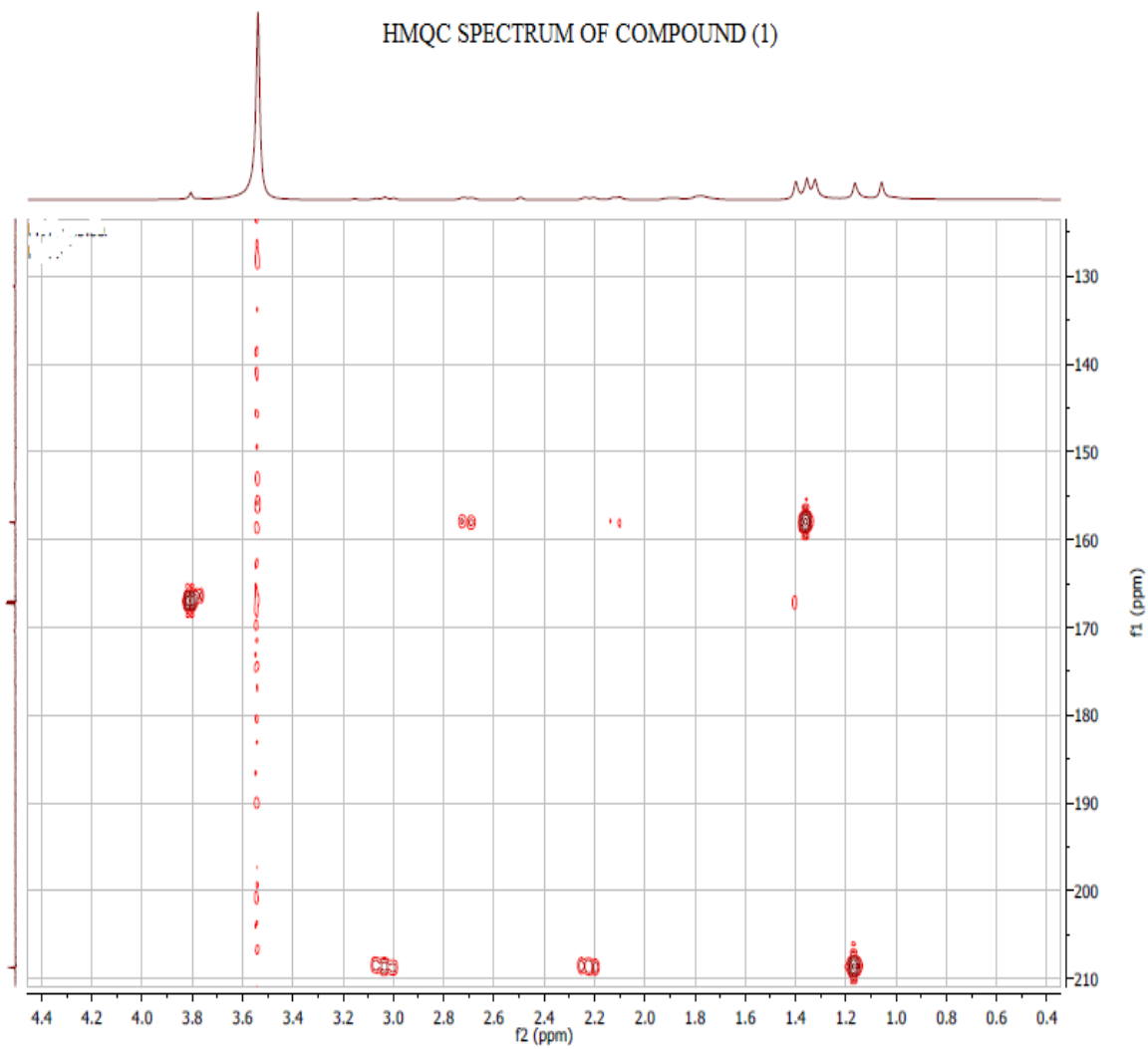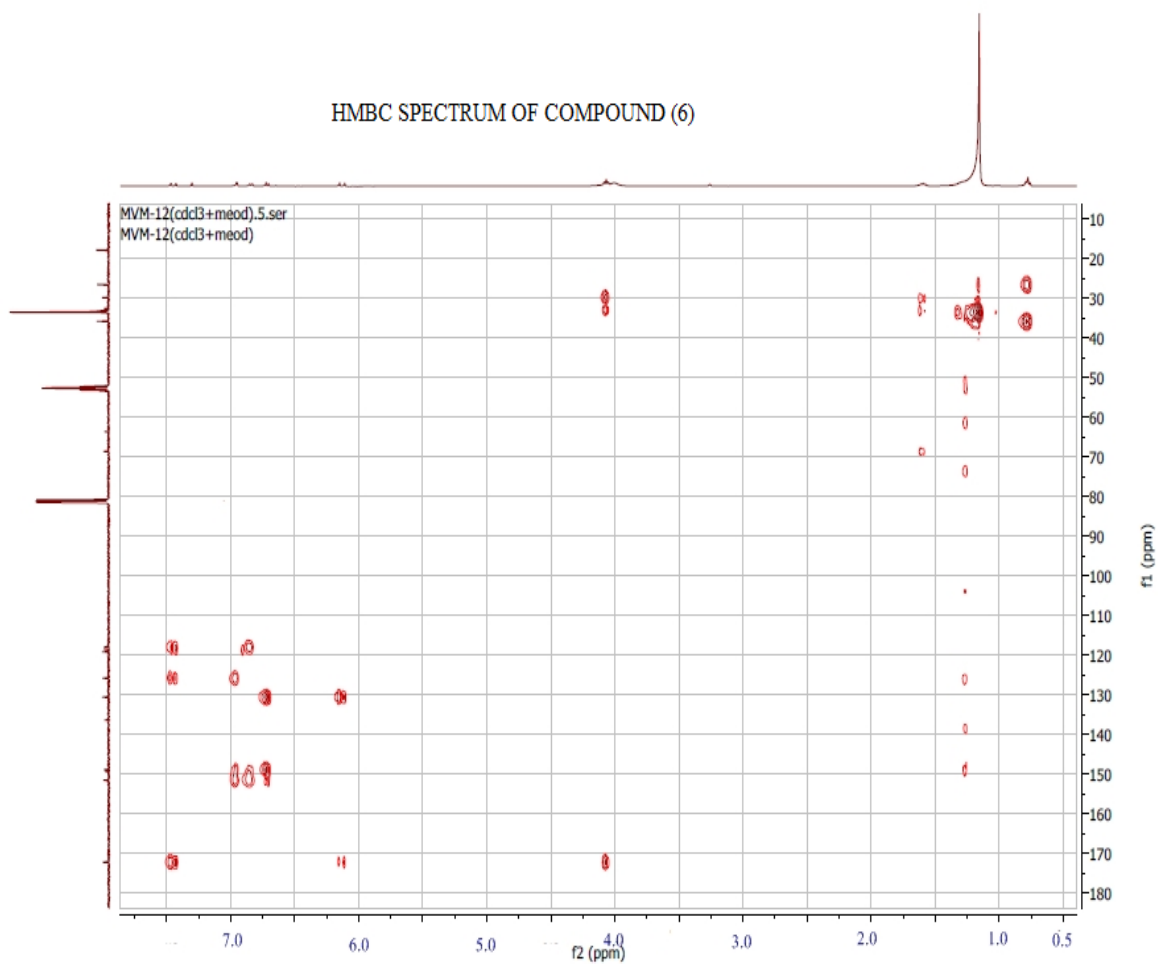
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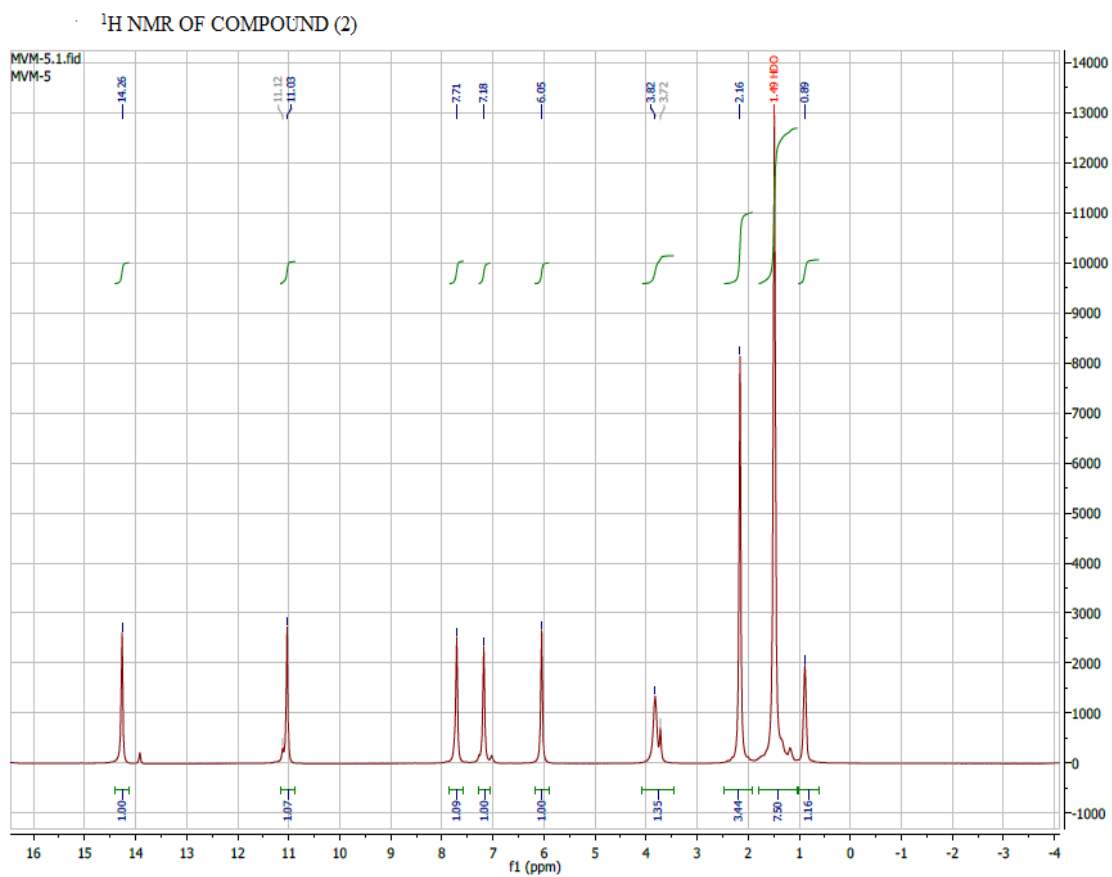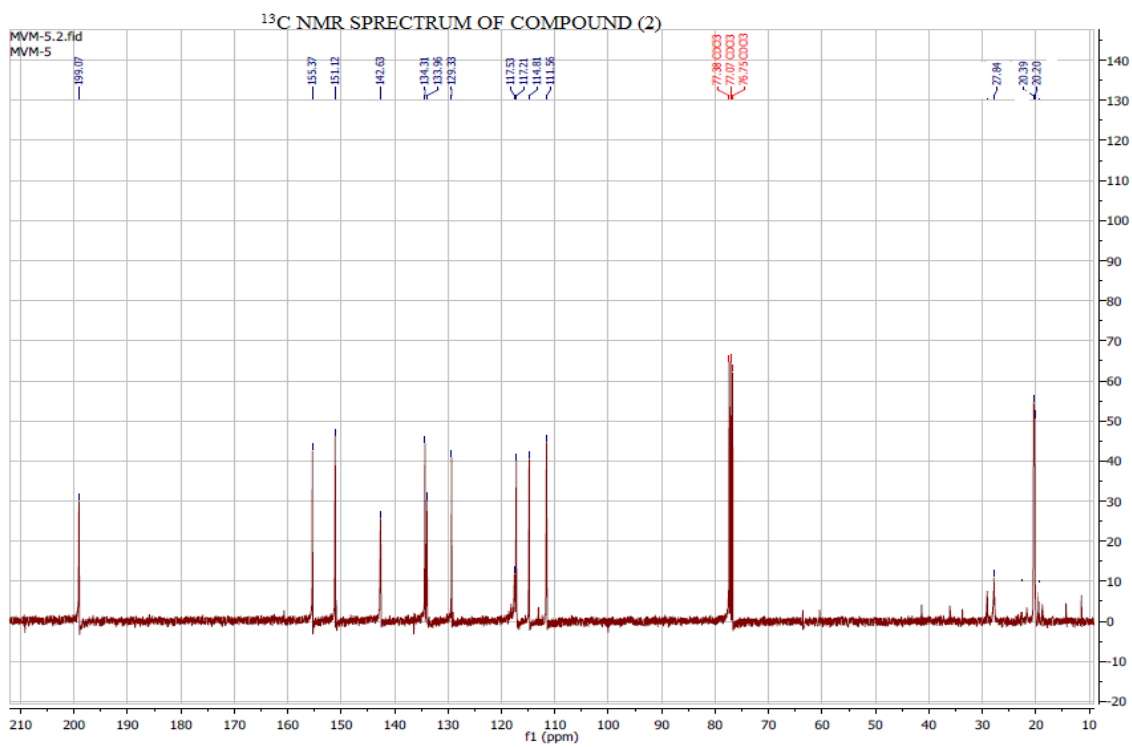
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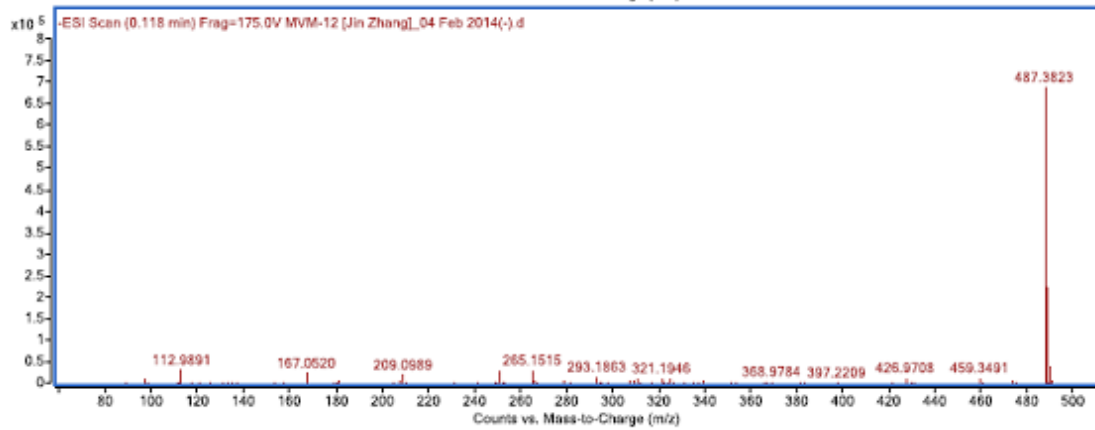
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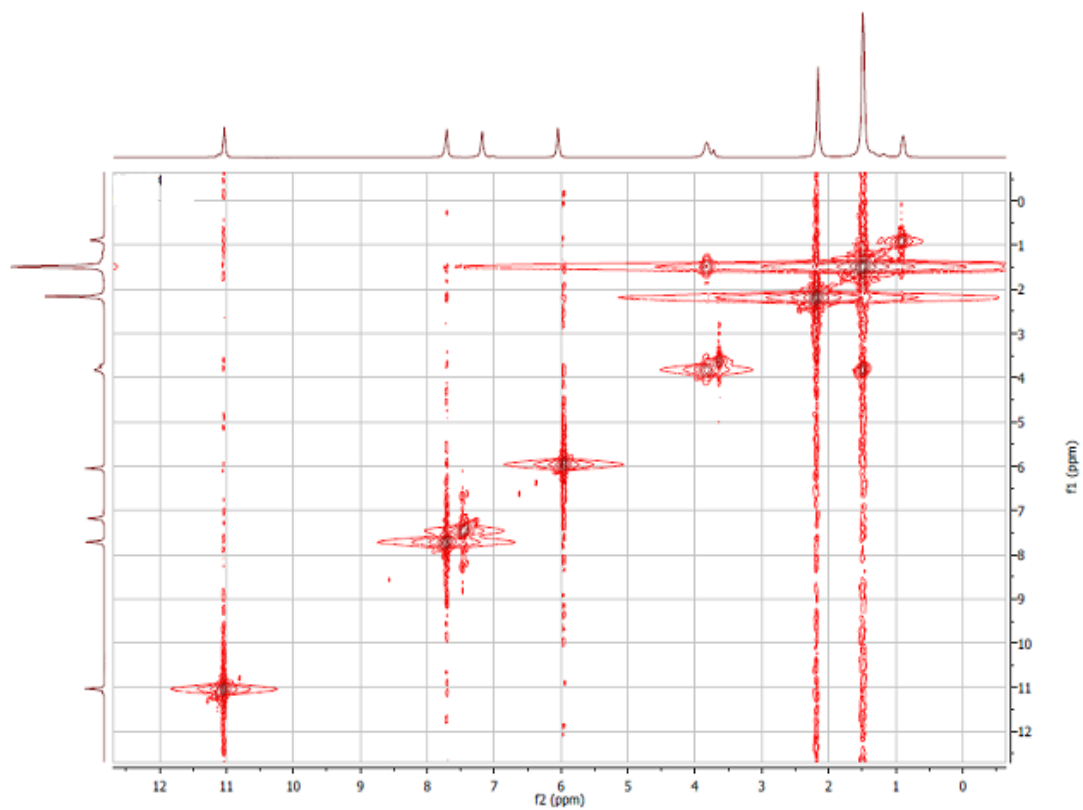
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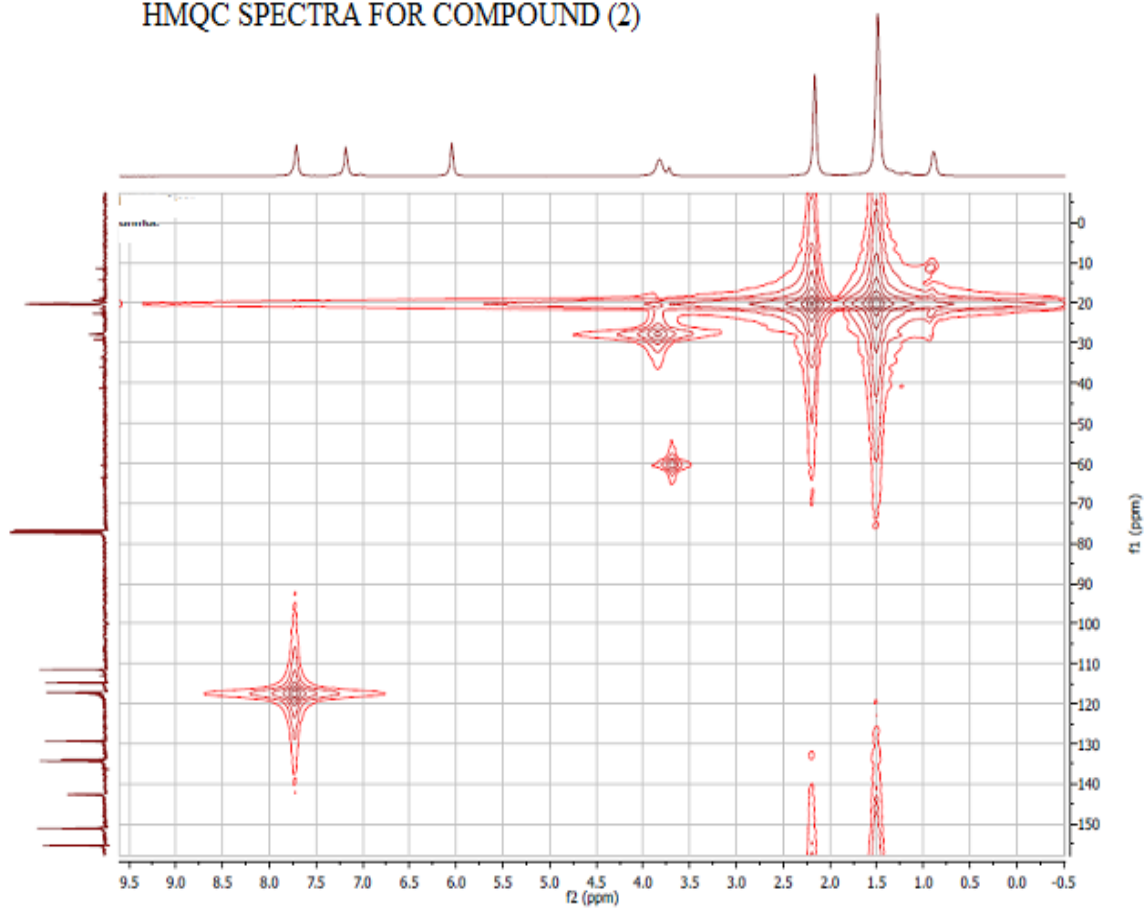
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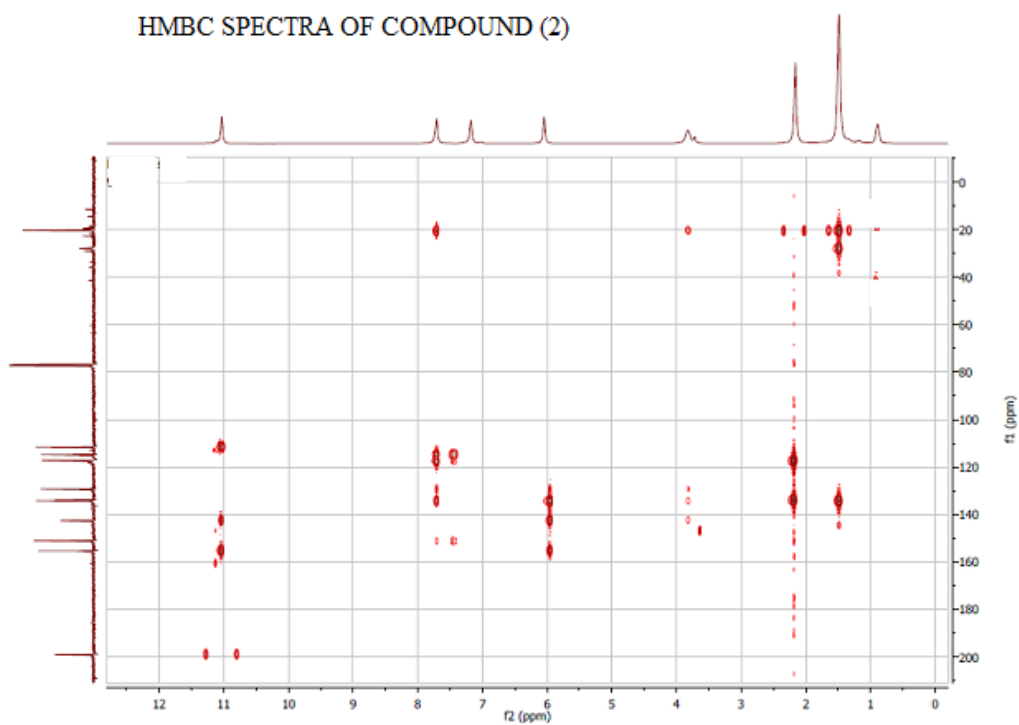
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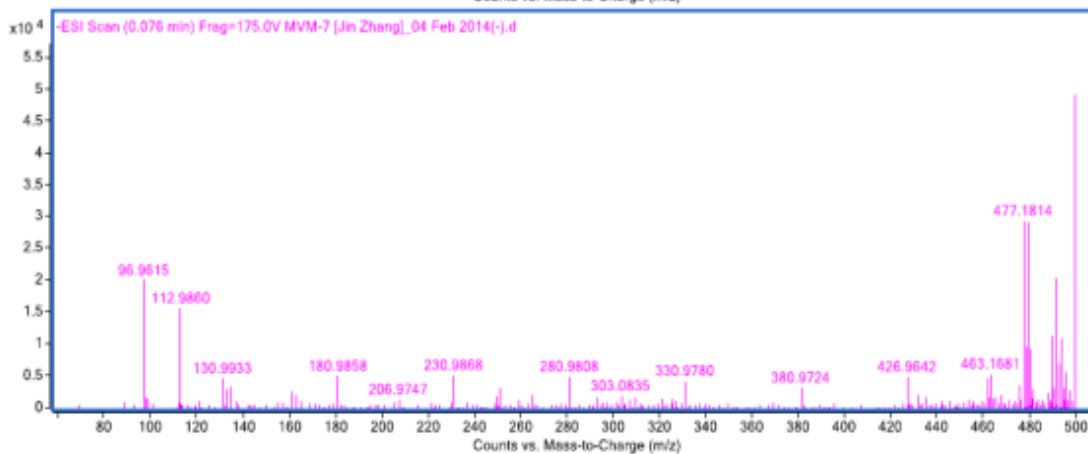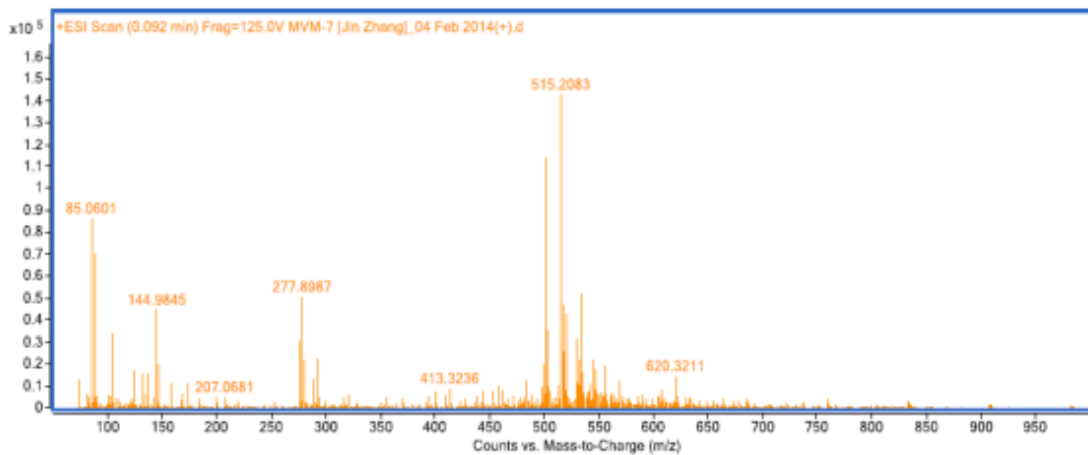
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x10 5

85.0601
144.9845
277.8987
207.0681
413.3236
515.2083
620.3211

Counts vs. Mass-to-Charge (m/z)

-ESI Scan (0.076 min) Frag=175.0V MVM-7 [Jin Zhang]_04 Feb 2014(-).d

x10 4

96.9615
112.9860
130.9933
180.9858
206.9747
230.9868
280.9808
303.0835
330.9780
380.9724
426.9642
463.1681
477.1814

Counts vs. Mass-to-Charge (m/z)

75

COSY SPECTRUM OF COMPOUND (3)

MVM-9.4.ser
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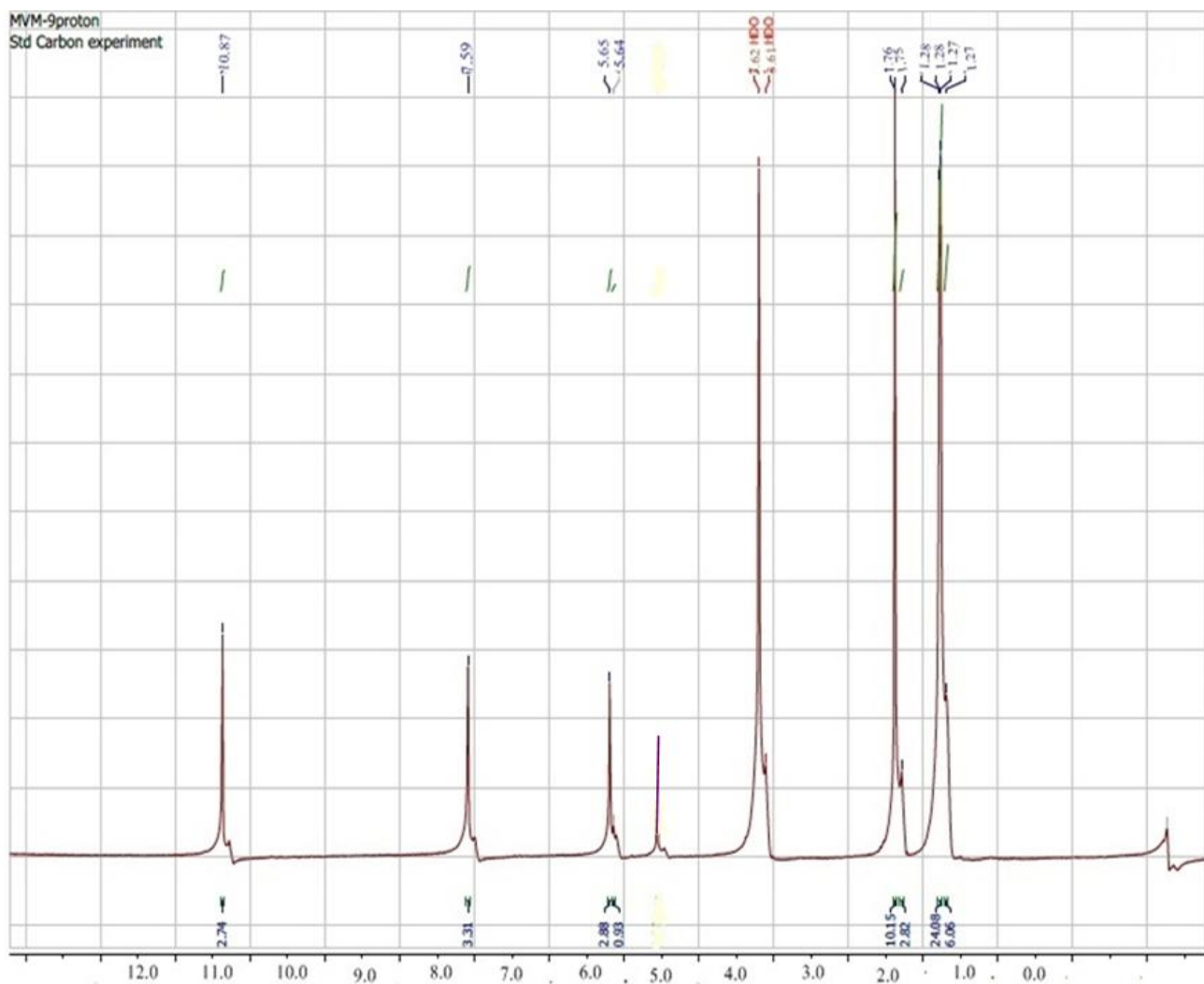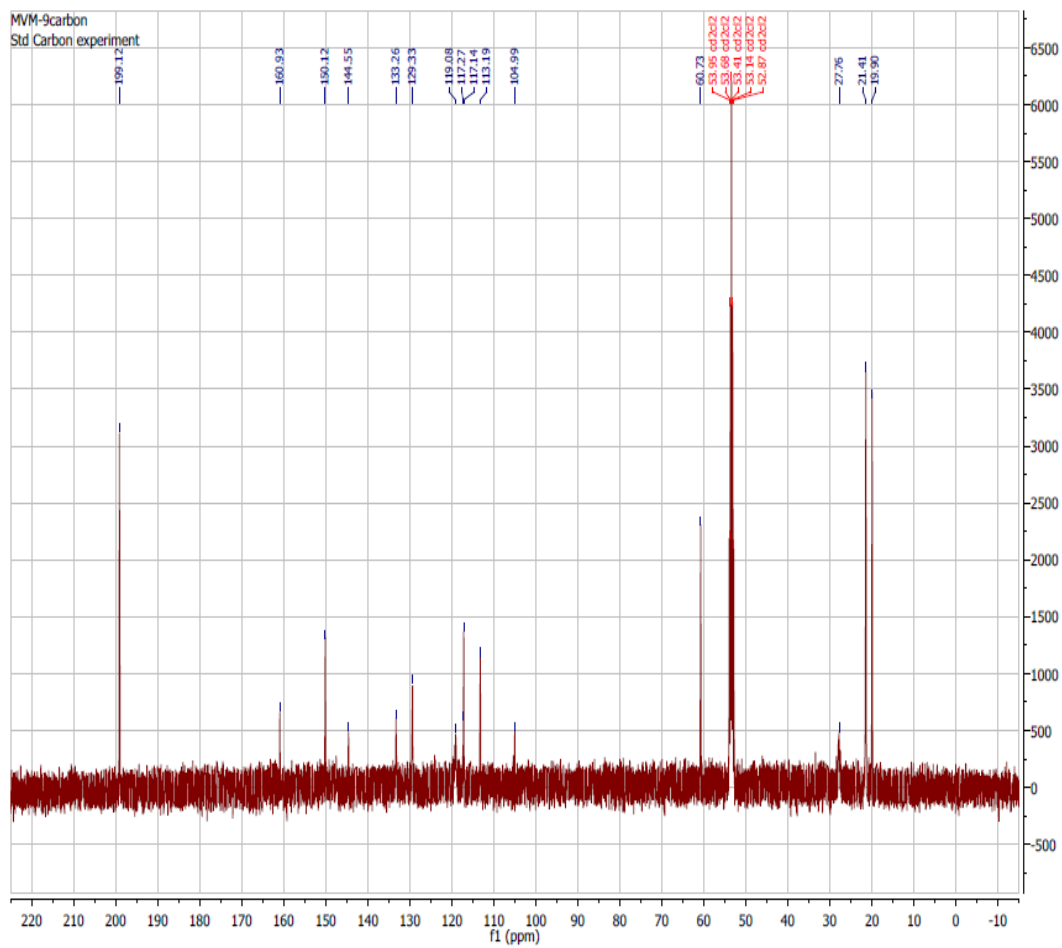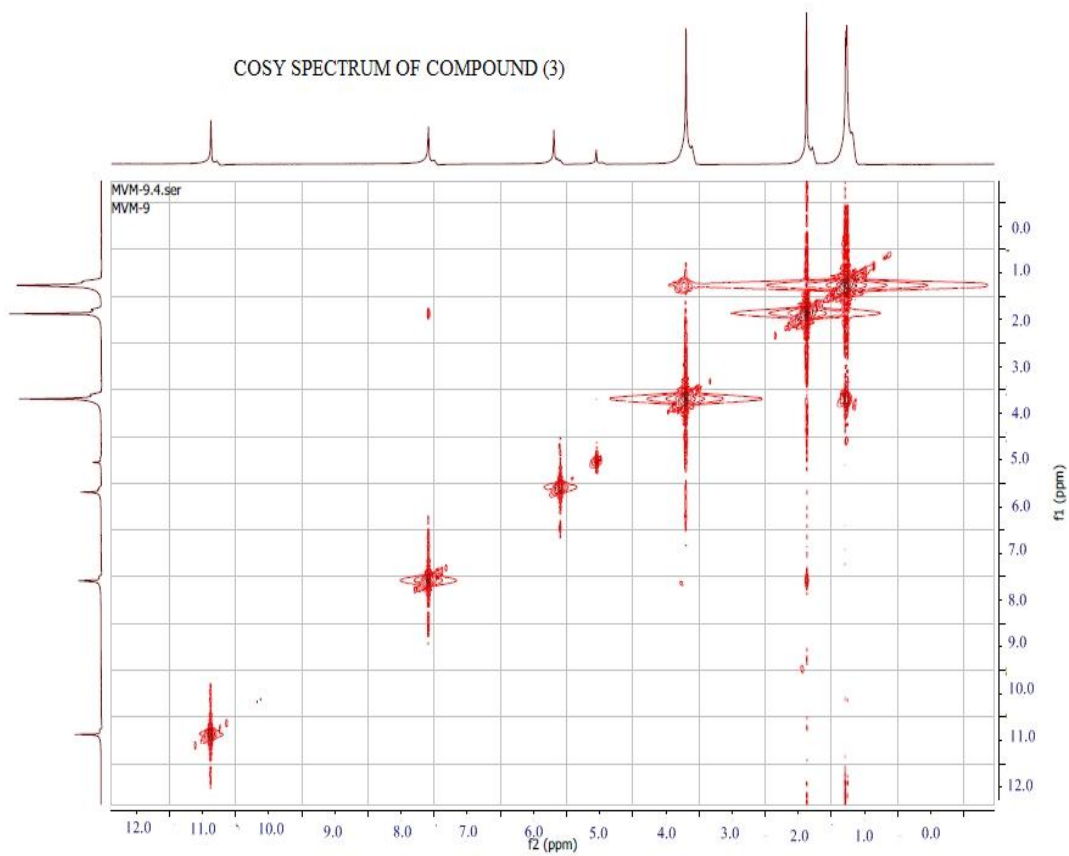
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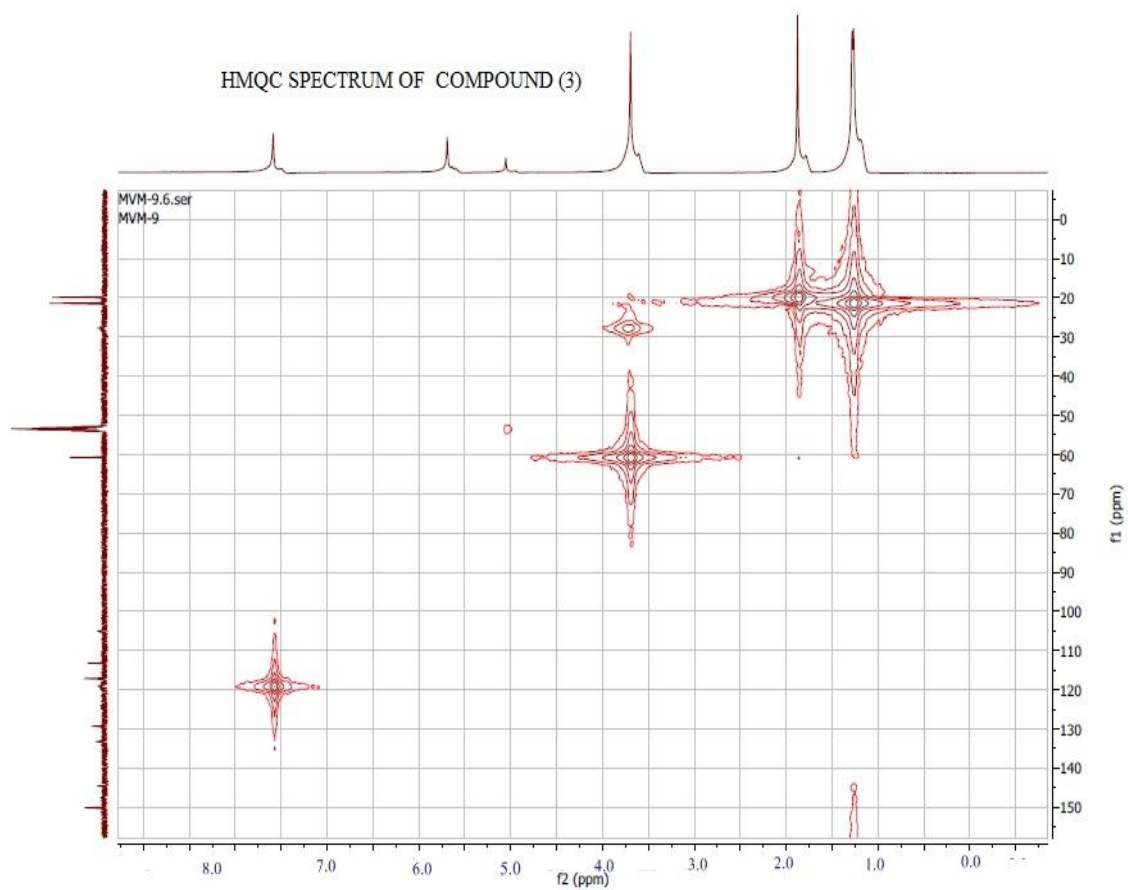
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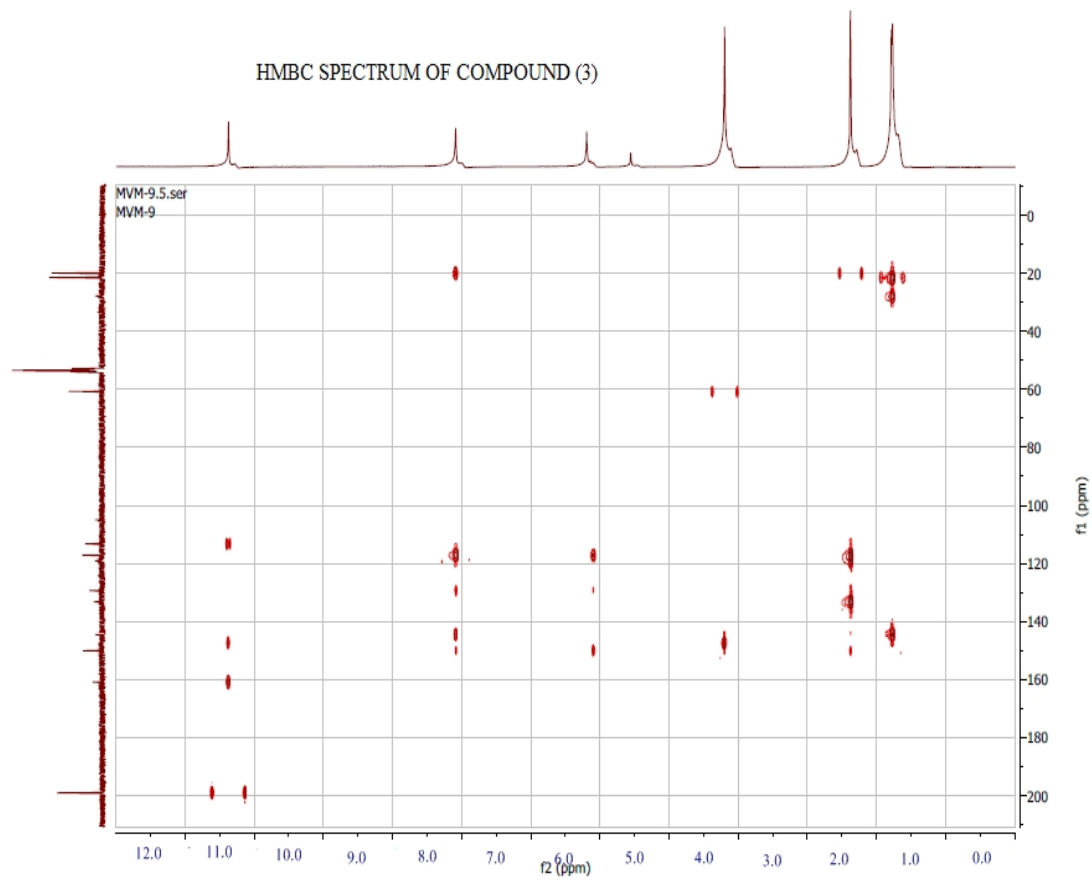
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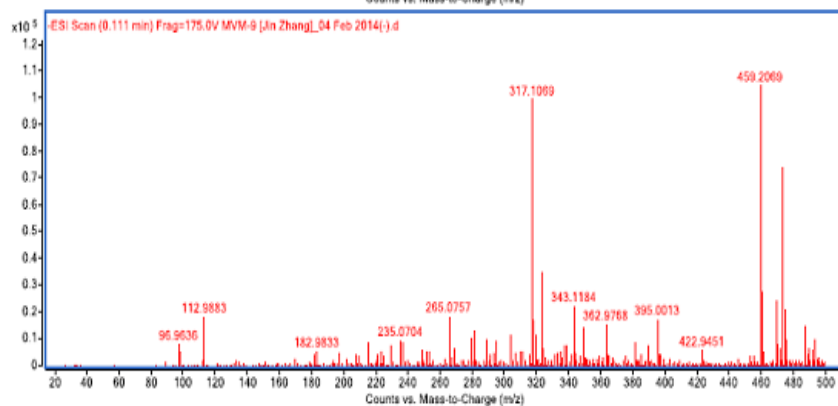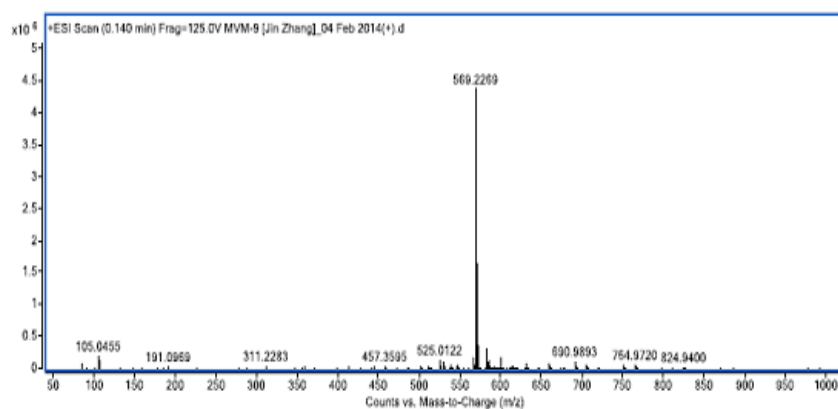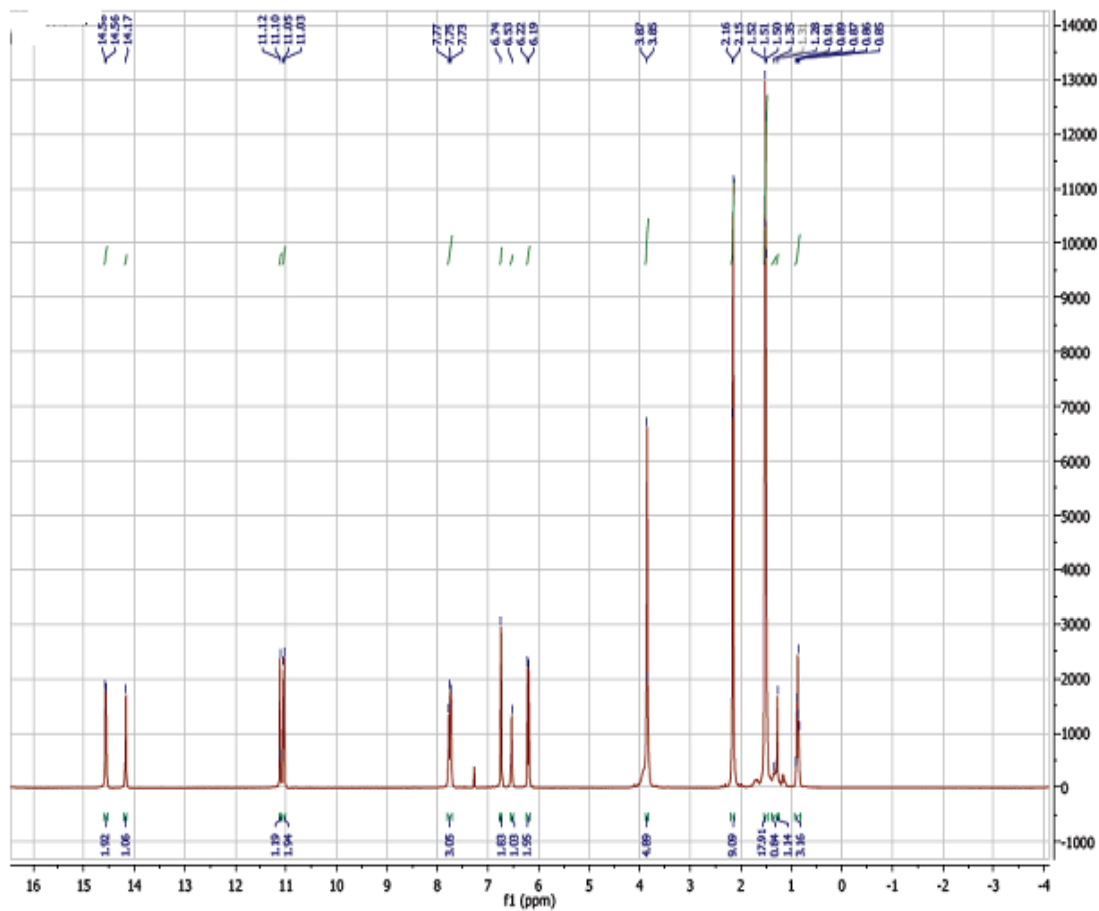
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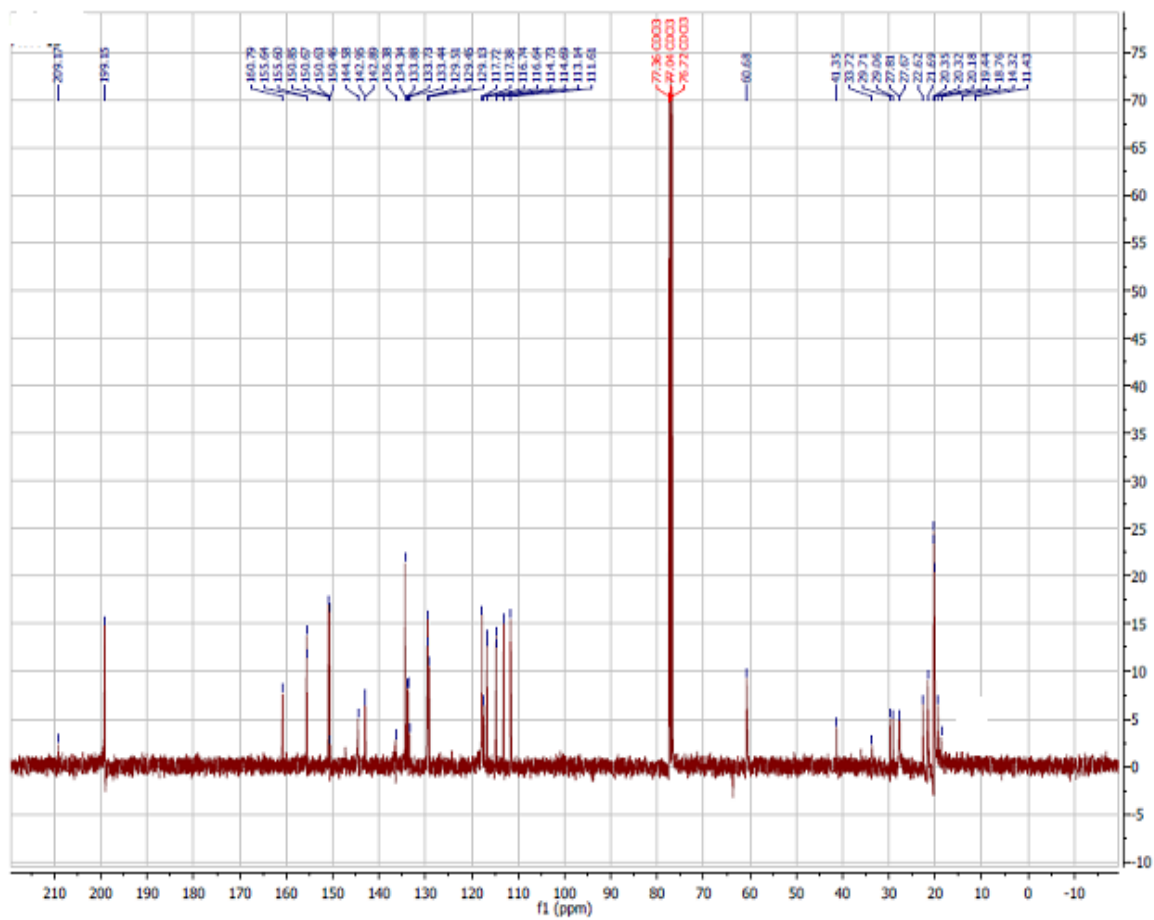
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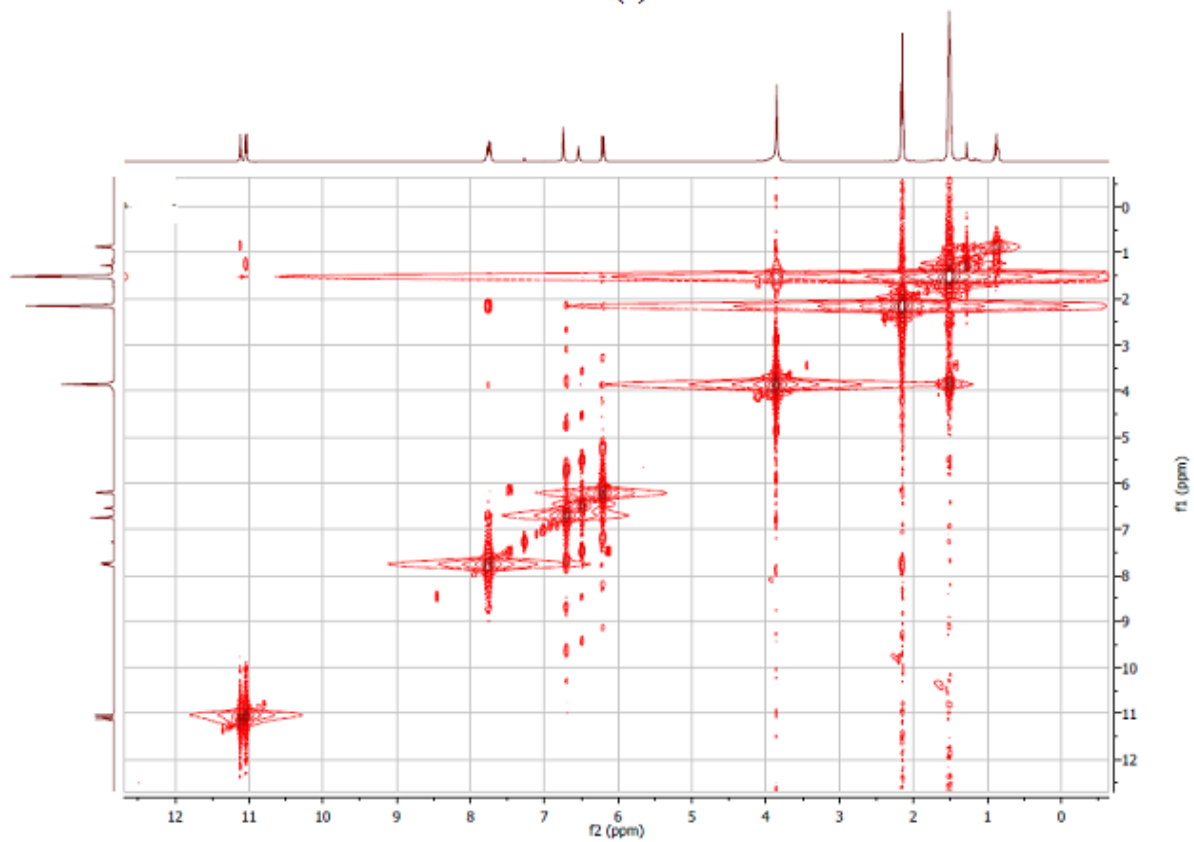
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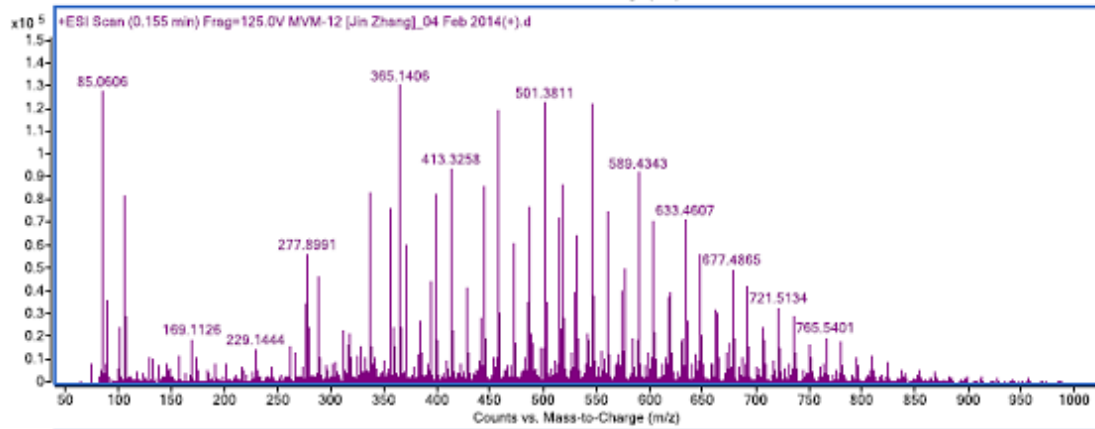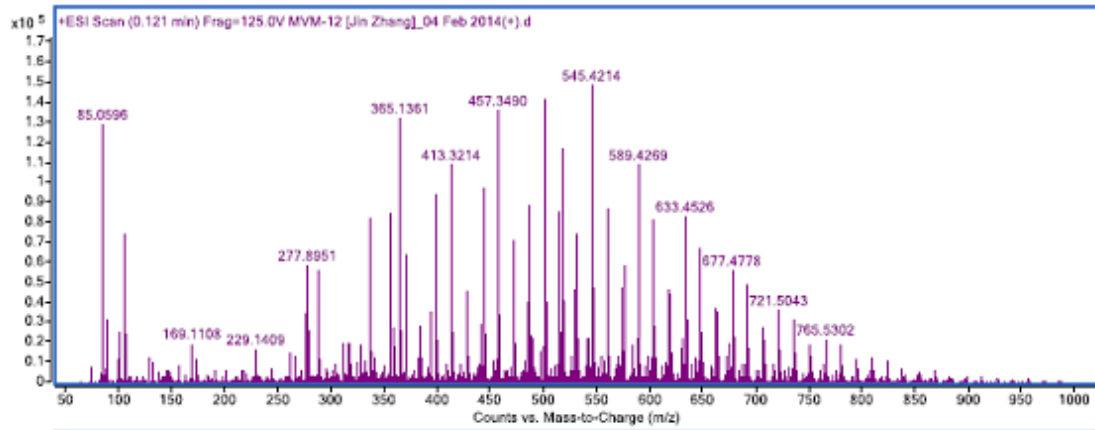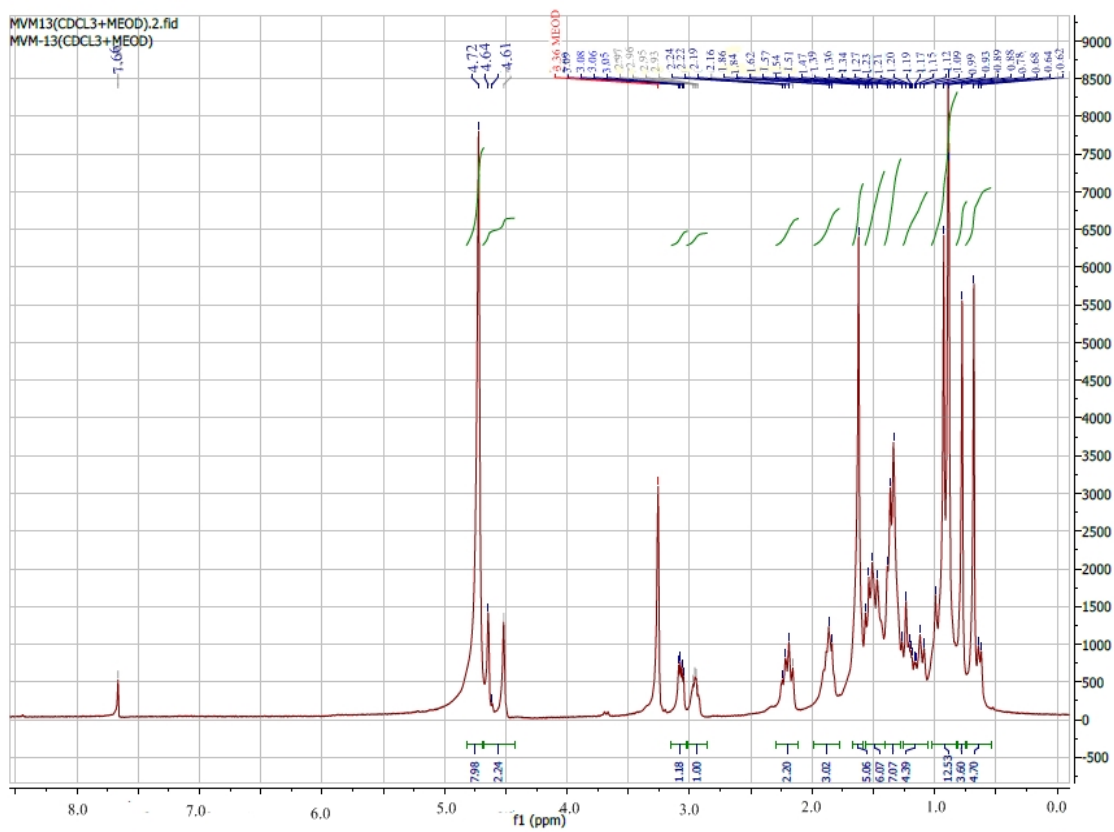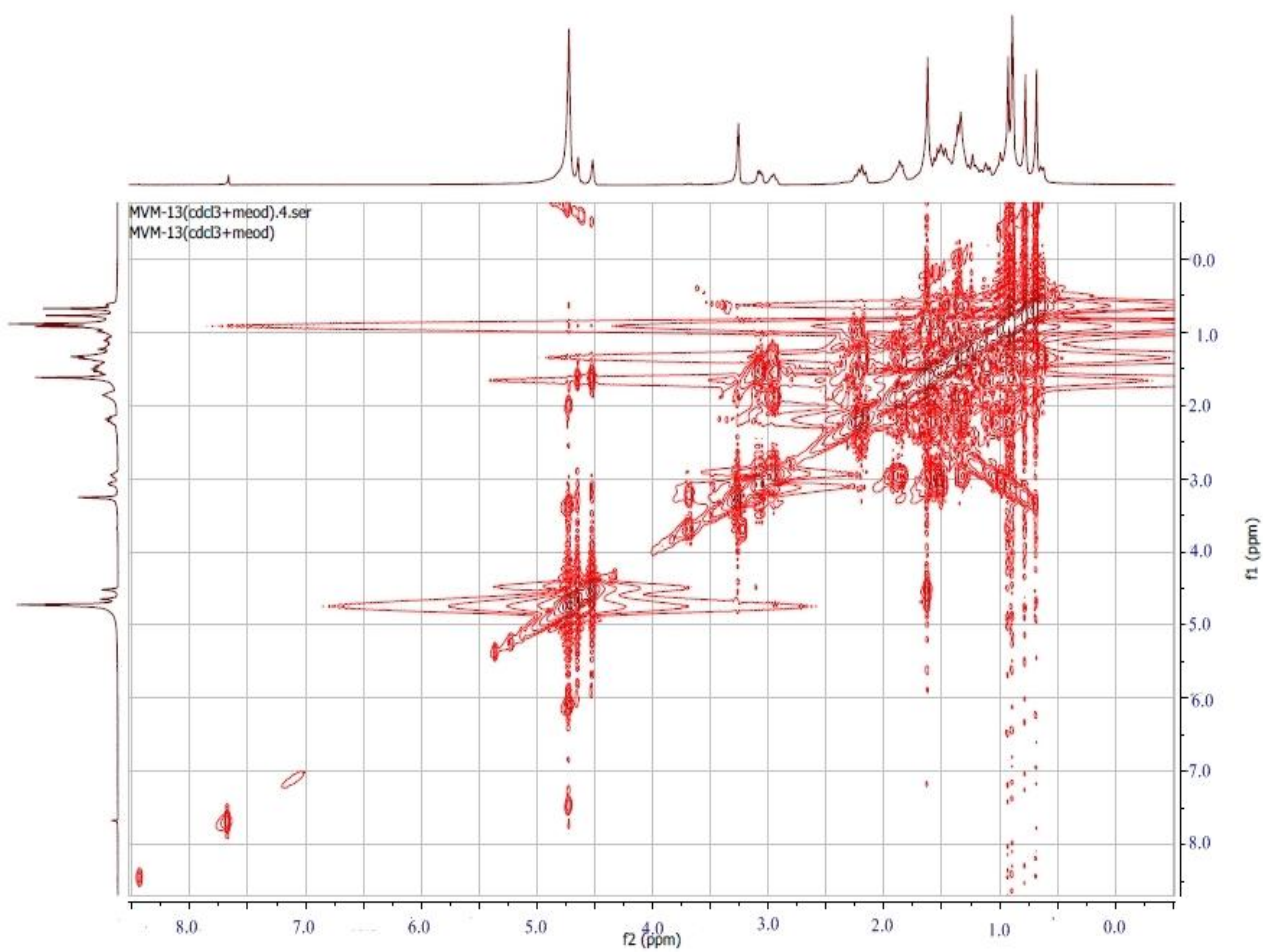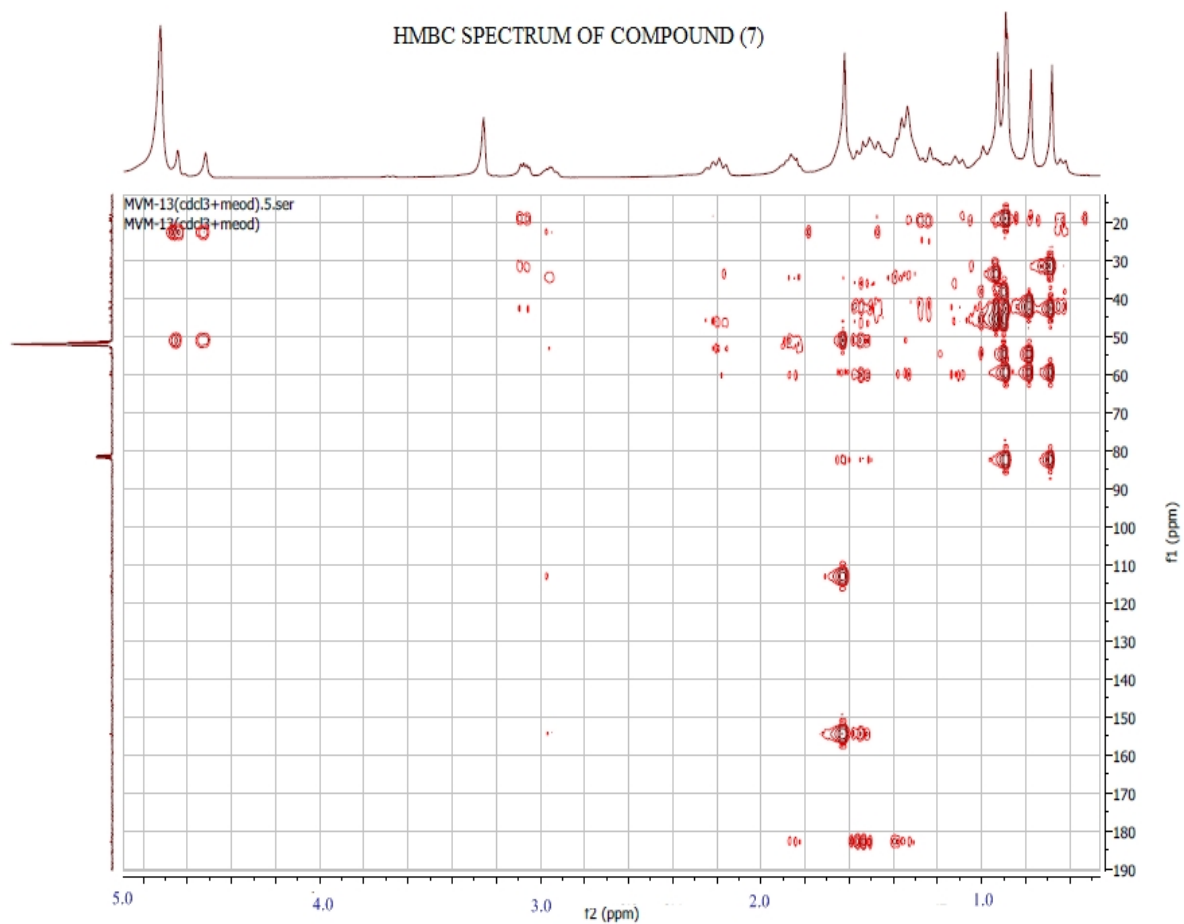
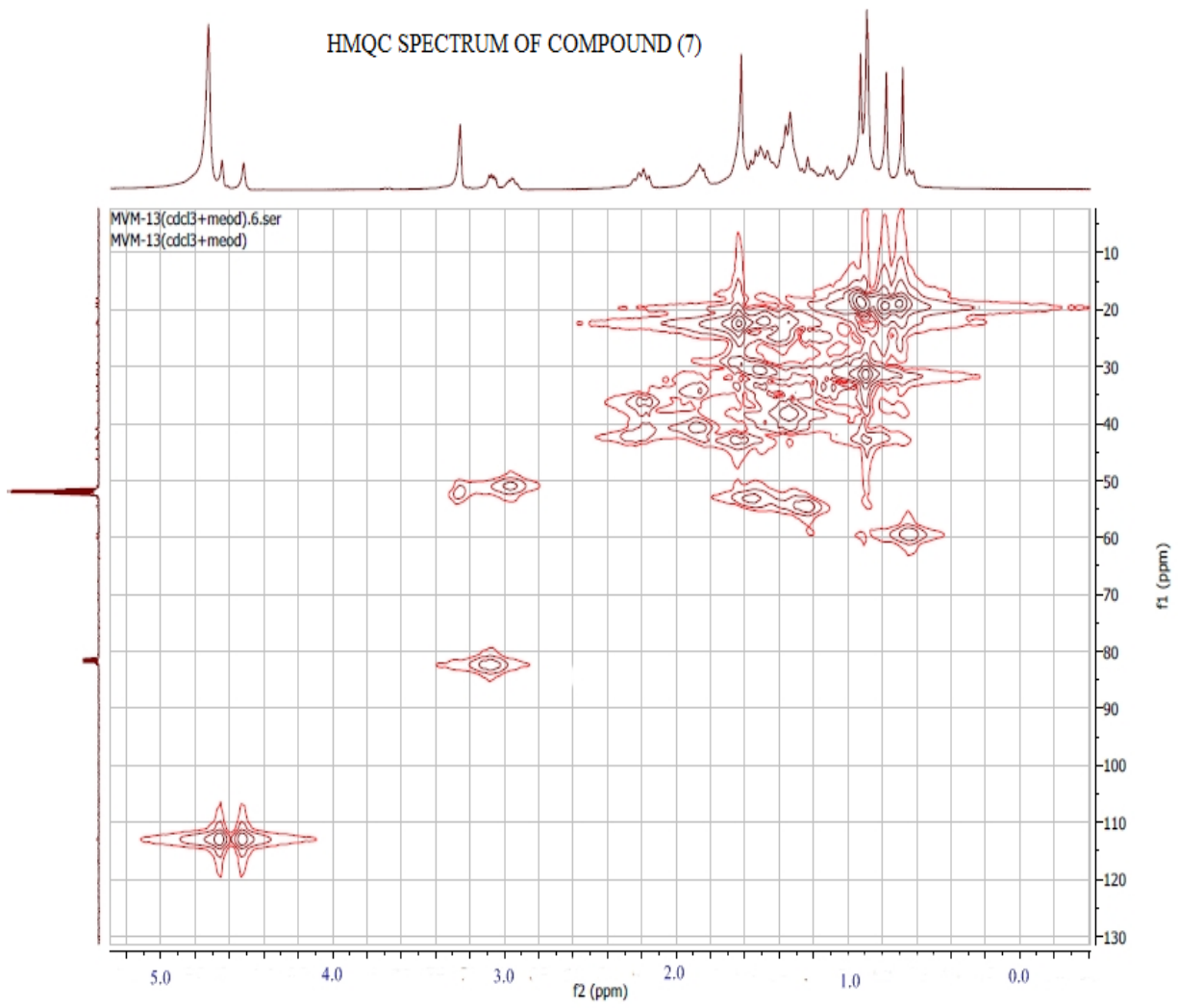^{13}C NMR SPECTRUM OF COMPOUND (5)
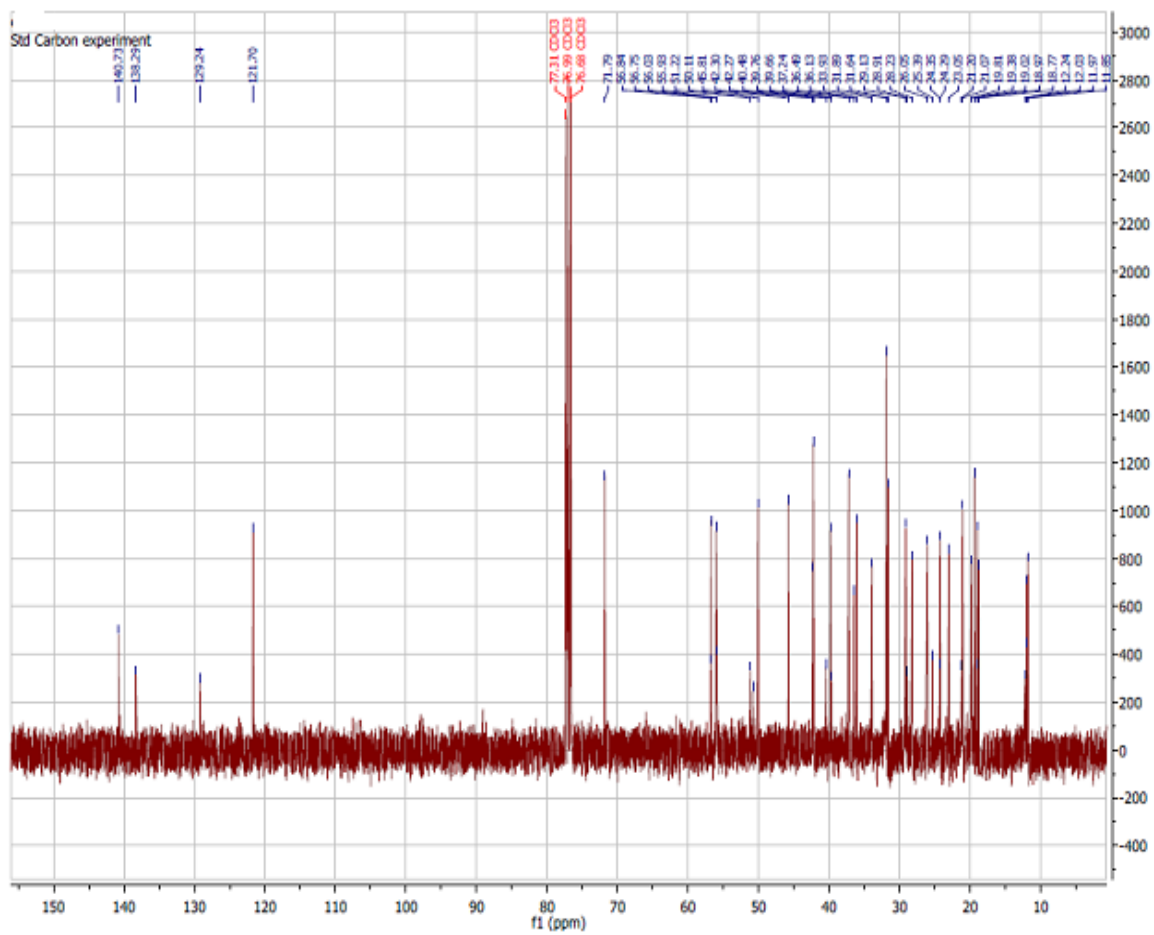
COSY SPECTRUM OF COMPOUND (5)

HMQC SPECTRUM OF COMPOUND (5)

HMBC SPECTRA OF COMPOUND (5)

^{1}H NMR SPECTRUM OF COMPOUND (6)

^{13}C NMR SPECTRUM OF COMPOUND (6)

COSY SPECTRUM OF COMPOUND (6)

HMQC SPECTRA OF COMPOUND (6)

MVM-12(cdcl3+meod).6.ser
MVM-12(cdcl3+meod)

HMBC SPECTRUM OF COMPOUND (6)

MASS SPECTRUM OF COMPOUND (6)

97

H NMR SPECTRUM OF COMPOUND (7)

^{13}C NMR SPECTRUM OF COMPOUND (7)

COSY SPECTRUM OF COMPOUND (7)

MVM-13(cdcl3+meod).4.ser
MVM-13(cdcl3+meod)
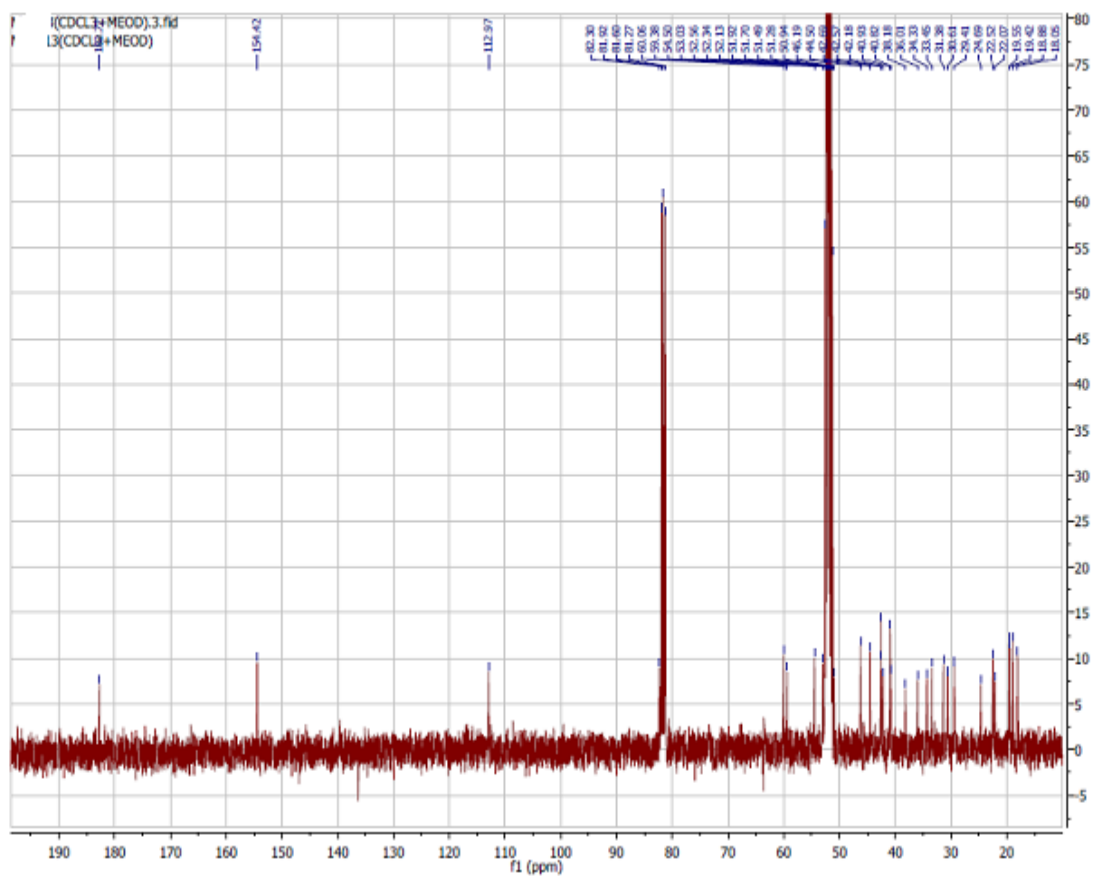
HMBC SPECTRUM OF COMPOUND (7)

HMQC SPECTRUM OF COMPOUND (7)

MVM-13(cdcl3+meod).6.ser
MVM-13(cdcl3+meod)

MASS SPECTRUM OF COMPOUND (7)