PHYTOCHEMICAL AND ANTIMICROBIAL STUDIES ON THE STEM-BARK OF *COMMIPHORA MOLLIS (Oliv.) Engl.* (BURSERACAEA)

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PHYTOCHEMICAL AND ANTIMICROBIAL ACTIVITIES ON THE STEM-BARK OF *COMMIPHORA MOLLIS* (*Oliv*.) Engl. (BURSERACEAE)

BY

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FACULTY OF PHARMACEUTICAL SCIENCES

AHMADU BELLO UNIVERSITY, ZARIA - NIGERIA.

SEPTEMBER, 2015.

DECLARATION

I declare that the work in this dissertation entitled **PHYTOCHEMICAL AND ANTIMICROBIAL STUDIES ON THE STEM-BARK OF *COMMIPHORA MOLLIS* (BURSERACAEA)** has been carried out by me in the Department of Pharmaceutical and Medicinal Chemistry, the information derived from the literature has been duly acknowledged in the text and a list references provided. No part of this dissertation was previously presented for another degree at this or any other institution.

Jamilu Hussaini Kura _____ _____

Name of Student Signature Date

CERTIFICATION

This dissertation entitled "**PHYTOCHEMICAL AND ANTIMICROBIAL STUDIES ON THE STEM-BARK OF *COMMIPHORA MOLLIS* (BURSERACAEA)"** by Jamilu Hussaini KURA meets the regulation governing the award of the degree of Masters of Science in Pharmaceutical and Medicinal Chemistry of the Ahmadu Bello University Zaria, and is approved for its contribution to knowledge and literary presentation.

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ABSTRACT

Commiphora mollis is traditionally used in the treatment of fever (malaria and typhoid) wound healing, cancer, ulcer and rheumatic condition. The plant grows in Nigeria and across Africa. Phytochemical analysis of methanol extract of the stem-bark of *Commiphora mollis* showed the presence of flavonoids, saponins, tannins, terpenoids and alkaloids. Extensive Phytochemical studies of ethylacetate water soluble fraction of methanol extract resulted in isolation (55 fractions, 100ml each were collected and pooled together based on similarities in their TLC profile to gave 8 major fractions and repeated gel filtration chromatography of Fraction 5 on Sephadex LH-20 packed column then preparative gave 7.5 mg of compound X1) and characterization using ^{1}HNMR (9 proton signal 5 aromatic proton δ 7.0- 5.94 ppm, 2 oxymithine protons at 4.8 and 4.2 ppm and 2 methylene protons at 2.8 and 2.75 ppm), ^{13}CNMR (15 carbon signals 12 in aromatic region, 2 oxymethine carbon and 1 methylene carbon.), DEPT (7 methine 1 methylene 1 quatenery), ^{1}H ^{1}H COSY (correlation between protons at 7.0, H2' // 6.83, H6' 7.0, H2' // 4.8, H2 6.83, H6' // 4.8, H2 4.8, H2 // 4.2, H3 4.8, H2 // 2.8, H4 4.2 , H3 // 2.8, H4), HSQC (correlation between proton and carbon at 7.0, H2' // 114, C2' 6.83, H6' // 118.10, C6' 6.8, H5' // 114.60, C5' 5.8, H6 // 94, C6 4.82, H2 // 78.48, C2 4.21, H3 // 66.10, C3 2.8, H4 and 2.7, H4 // 27.84, C4), HMBC and NOESY. The antimicrobial studies of the crude methanol extract, Chloroform and ethylacetate fractions were carried out using disc diffusion and broth agar dilution methods on clinical isolates of *Corynebacterium ulcerans, Salmonella typhi, Proteus mirabilis, Bacillus sabtilis, Staphylococcus aureus, Streptococcus pyogens, Escherichia coli, Candida brusei, Shigella dysenteriae* and *Candida albicans.* The crude methanol extract, chloroform and ethylacetate fractions showed strong inhibitory activity against all tested microoganisms with exception of C*orynebacterium ulcerans, Salmonella typhi* and *Proteus mirabilis.* Crude methanol extract was found to have MIC at 10 mg/ml for all organisms and

variable value of MBC/MFC and that of chloroform and ethylacetate fractions have variable value for both MIC and MBC. The stem-bark of *Commiphora mollis* was rich in bioactive phytochemicals which have antimicrobial activity and could serve as a potential source of compounds effective against disease causing micro- organisms.

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LIST OF ABBREVIATIONS

C	Carbon
CC	Column Chromatography
CD_3OD	deuteriated methanol
^{13}C-NMR	^{13}Carbon Nuclear Magnetic Resonance
COSY	Correlation Spectroscopy
2D	Two Dimensional
d	doublet
Fig.	Figure
H	proton
HMBC	Hetronuclear Multiple Bond Correlation
HMQC	Hetronuclear Multiple Quantum Correlation
^{1}H-NMR	Proton Nuclear Magnetic Resonance
Hz	Hertz
J	Coupling constant
NMR	Nuclear Magnetic Resonance
NOESY	Nuclear Overhauser Enhancement Spectroscopy
prep.	preparative
ppm	parts per million
PTLC	Preparative Thin Layer Chromatography
s	singlet
TLC	Thin Layer Chromatography
CME	Crude Methanol Extract
°C	Degree Celsius

CHAPTER ONE

1.0 INTRODUCTION

Natural products are the chemical compounds found in nature that usually have a pharmacological or biological activity for use in pharmaceutical drug discovery and drug design (Samuel, 1999). According to the World Health Organization (WHO), about 80 % of the world's population relies on traditional medicine for their primary health care need (WHO, 2002). For thousands of years natural products have played a very important role in health care and prevention of diseases. The ancient civilizations of the Chinese, Indians and North Africans provide written evidence for the use of natural sources for curing various diseases (Phillipson, 2001).

Secondary metabolites are chemical compounds derived from living organisms. The study of natural products involves isolation in a pure form of these compounds and investigation of their structure. Secondary metabolites appear to function primarily in defense against predators and pathogens and in providing reproductive advantage as intraspecific and interspecific attractants. They may also act to create competitive advantage as poisons of rival species (Croteau, *et al.,* 2000). Many plant terpenoids are toxins and feeding deterrents to herbivores or are attractants, and many possess pharmacological activity. Tannins, lignans, flavonoids, and some simple phenolic compounds serve as defenses against herbivores and pathogens, also many flavonoid pigments are important attractants for pollinators and seed dispersers.

The study of natural products has had a number of rewards. It has led to the discovery of a variety of useful drugs for the treatment of diverse ailments and contributed to the development of separation science and technology, spectroscopic methods of structure elucidation and synthetic methodologies that now make up the basics of analytical organic

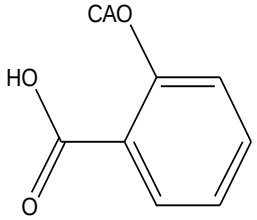
chemistry. One of the most important areas of application of natural products is in the treatment of human and veterinary ailments (Newman *et al.*, 2000).

Although the use of natural products as medicinal agents presumably predates the first recorded history as the earliest humans used various, but specific plants to treat illness, the treatment of diseases with pure pharmaceutical agents is a relatively modern phenomenon.

For thousands of years medicine and natural products have been closely linked through the use of traditional medicines and natural poisons (Butler, 2004). Clinical, pharmacological, and chemical studies of these traditional medicines, which were derived predominantly from plants, were the basis of most early medicines such as aspirin (I), morphine (II), digitoxin (III) and pilocarpine (IV) (Butler, 2004).

The discovery of antibacterial filtrate "penicillin" by Fleming in 1928, re-isolation and clinical studies by Chain, Florey, and co-workers in the early 1940s, and commercialization of synthetic penicillins evolutionized drug discovery research (Butler, 2004). Following the success of penicillin, drug companies and research groups soon assembled large microorganism culture collections in order to discover new antibiotics. The output from the early years of this antibiotic research was prolific and included examples such as streptomycin (V), chloramphenicol (VI), chlortetracycline (VII), cephalosporin C (VIII), erythromycin (IX), and vancomycin (X) (Butler, 2004).

All of these compounds, or derivatives thereof, are still in use as drugs today.



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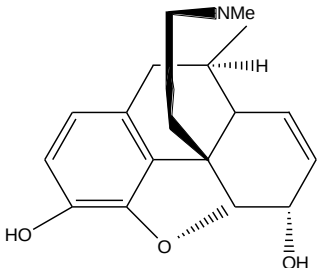

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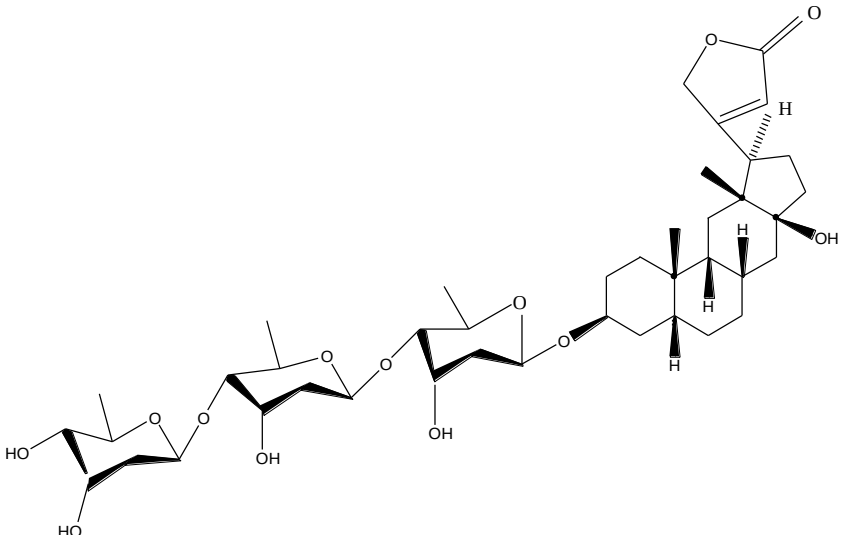

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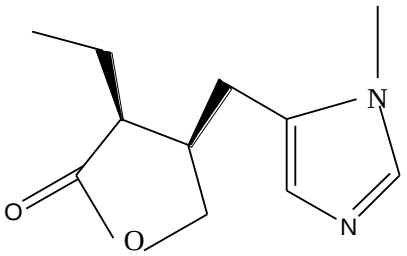

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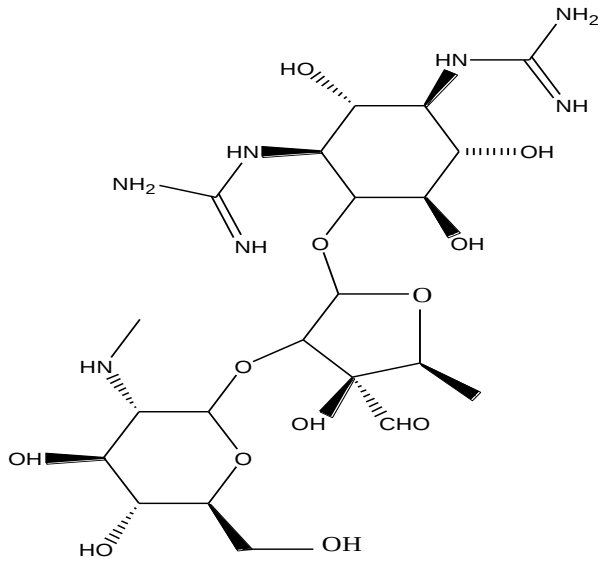

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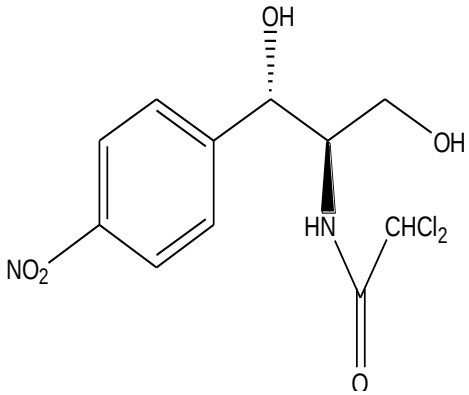

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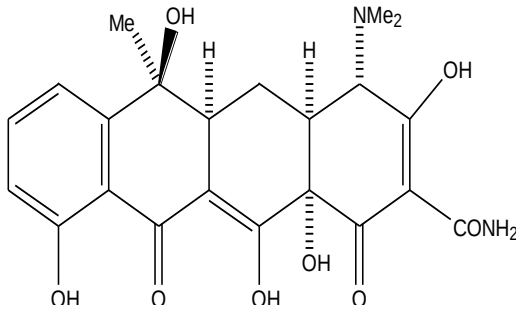
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CONH2
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OH
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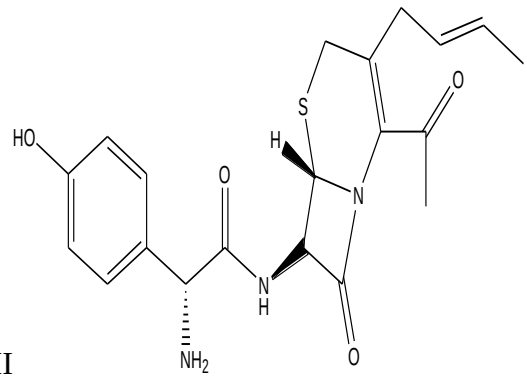
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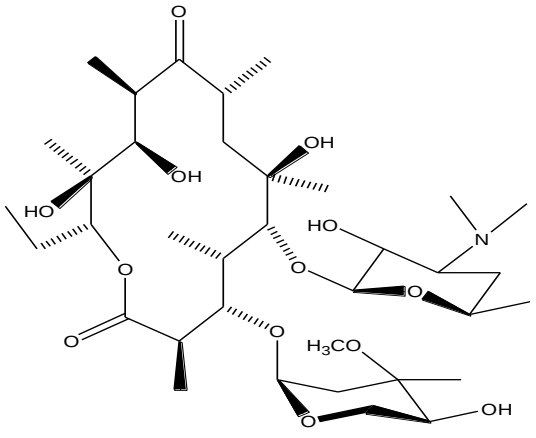
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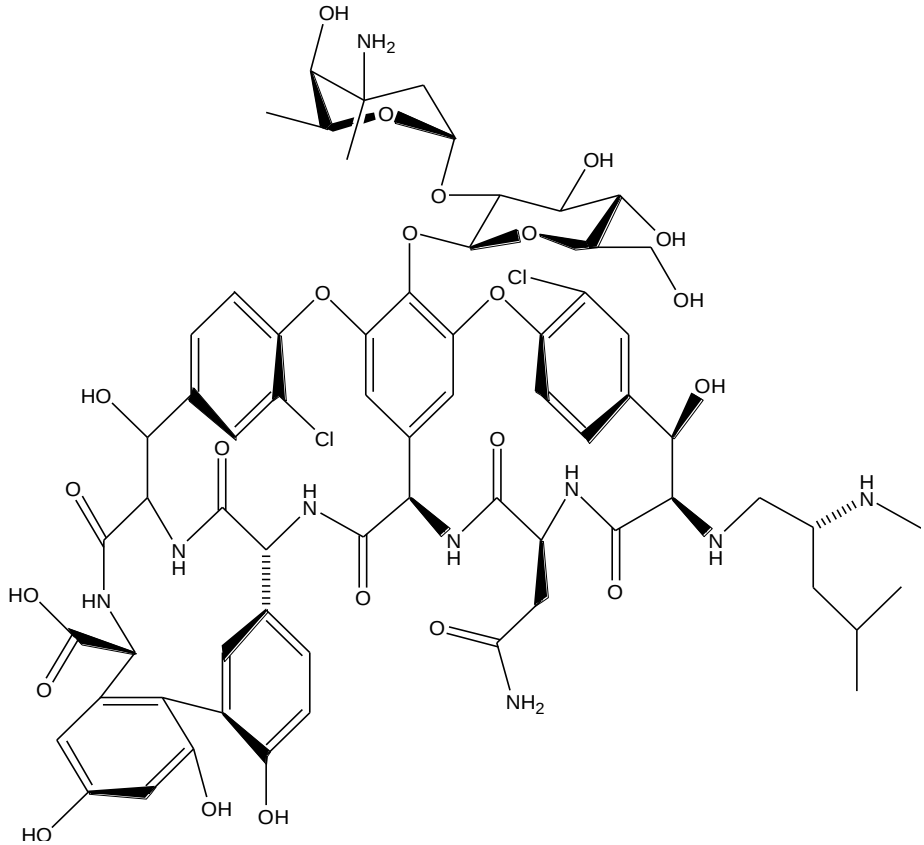
IX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X

1.1 Traditional medicine (TM):

Traditional medicine is the sum total of the knowledge, skill, and practices based on the theories, beliefs, and experiences indigenous to different cultures, whether explicable or not, used in the maintenance of health as well as in the prevention, diagnosis, improvement or treatment of physical and mental illness (WHO, 2013). It is widely practiced, especially in developing countries. This is a result of primary health care facilities being unable to manage the number of patients requiring aid, the high cost of Western pharmaceuticals and health care, as well as the fact that traditional health care is highly sought after in terms of certain cultural elements in the lives of these individuals within these societies (Taylor *et al*., 2001).

The World Health Organization estimates that 80% of the populations of Asia, Africa and Latin America use traditional medicine to meet their primary health care needs. For many people in these countries, particularly those living in rural areas, this is the only available, accessible and affordable source of health care (WHO, 2010).

Traditional medicine has also maintained its popularity in all regions of the developing world and its use is rapidly spreading in the industrialized countries. In China, for example, traditional herbal preparations account for 30%-50% of the total medicinal consumption (Bannerman *et al.*, 1993). In Ghana, Mali, Nigeria and Zambia, the first line of treatment for 60% of children with high fever resulting from malaria is the use of herbal medicines at home (Bannerman *et al.*, 1993).

Practices of traditional medicine vary greatly from country to country, and from region to region, as they are influenced by factors such as culture, history, personal attitudes and philosophy. In many cases, their theory and application are quite different from those of conventional medicine. Long historical use of many practices of traditional medicine, including experience passed on from generation to generation, has demonstrated the safety

and efficacy of traditional medicine. However, scientific research is needed to provide additional evidence of its safety and efficacy (WHO, 2000).

In many parts of the world, policy-makers, health professionals and the public are wrestling with issues regarding the safety, effectiveness, quality, availability, preservation and regulation of traditional medicines. At the same time, interest in traditional medicine is expanding beyond products to focus on practices and practitioners (WHO, 2013).

This vast usage of and great dependence on traditional plants as the preferred form of health care is aided by the fact that most of these plants are widely available and affordable, and additionally encompasses practices based on the socio-cultural norms and religious beliefs. It is evident that, even though scientific advances have been made in our quest to understand the physiology of the body, biotechnology and the treatment of disease, natural products remain a crucial component of the comprehensive health care strategy for the future (Patwardhan, 2005). The Greek physician Dioscorides (AD 70) compiled an extensive listing of medicinal herbs and their virtues. This was originally written in Greek, and later translated into Latin as *De Materia Medica,* and remained the authority in medicinal plants for over 1500 years (Mendonça-Filho, 2006). Another Greek physician, Galen (AD 129-200), devised the pharmacopoeia describing the appearance, properties and use of many plants of his time. It was the discovery of medicines that sparked an interest in the study of plants as medicinal agents; with the isolation of morphine from opium by Serturner (1805) being the start of natural product chemistry (Patwardhan *et al*., 2004).

Currently It is clear, however, that there is a need to validate the information through an organised research for it to be used as an effective therapeutic means, either in conjunction with existing therapies, or as a tool in novel drug discovery. Traditional medicine utilises biological resources and the indigenous knowledge of traditional plant groups, the latter

being conveyed verbally from generation to generation. This is closely linked to the conservation of biodiversity and the related intellectual property rights of indigenous people (Timmermans, 2003). Although it is these traditional medicines that provided the link between medicine and natural products, it was not until the 19th century that active compounds were isolated and principles of medicinal plants identified (Phillipson, 2001).

1.2 Anti-microbial Agents

Anti-microbial agents are substances that kill micro organisms or inhibit their growth. They are widely employed to cure bacterial diseases. Antimicrobial agents that reversibly inhibit growth of bacteria are called bacteriostatic whereas those with irreversible lethal action on bacteria are known as bactericidal (Rajesh and Rattan, 2008).

Ideally, antimicrobial agents disrupt microbial processes or structures that differ from those of the host. They may damage pathogens by hampering cell wall synthesis, inhibiting microbial protein and nucleic acid synthesis, disrupting microbial membrane structure and function, or blocking metabolic pathways through inhibition of key enzymes (Willey *et al*., 2008). Haslam *et al.* (1989) reported that plant extracts and their products are used in many parts of the world as the active principles in herb remedies. They are used locally in the treatment of infections, many centuries before scientific studies were discovered. Before an antimicrobial agent is accepted for use in human beings it must demonstrate most, if not all, of the following properties: selective toxicity (it should act on bacteria without damaging the host tissues); it should be bactericidal rather than bacteriostatic; it should be effective against a broad range of bacteria; it should not be allergic; it should remain active in plasma, body fluids etc.; it should be stable and preferably water soluble; desired levels should be reached rapidly and maintained for adequate period of time; it should not give

rise to resistance in bacteria; it should have long shelf life; it should not be expensive (Rajesh and Rattan, 2008).

1.3 Statement of Reseach Problem

It has been reported that most bacteria are resistant to currently used antimicrobial agents (Truiti *et al*., 2003). Also the severe side effects of most antimicrobial agents have necessitated the search for new antimicrobial with little or no side effect. This phenomenon of increased drug resistance, combined with the multiplicity of side effects caused by existing agents and the emergence of diseases for which no treatment yet exists, makes the search for new antimicrobial agents a highly relevant and important subject for research. For centuries, plants have been used in the traditional treatment of microbial infections. This assembly of knowledge by indigenous peoples about plants and their products continue to play an essential role in health care of a great proportion of the population (Iwu *et al*., 1999). Traditionally *Commiphora mollis* has been used in the treatment of fever infection, malaria, typhoid and cancer, oxidant, ulcer, rheumatic condition. The resin is applied topically to aid wound healing. For this reason it was concluded to screen and chemically characterized the stem-bark of this important medicinal plant.

1.4 Justification

Traditionally *Commiphora mollis* has been claimed to have medicinal properties which include, treatment of fever (malaria and typhoid), wound healing, cancer, ulcer and rheumatic condition.

Despite this, literature survey showed that no work has been carried out on the phytochemical analysis and pharmacological activities of the *Commiphora mollis*. For this purpose, such studies were initiated as a basis for scientific verification regarding the traditional use of *Commiphora mollis.*

1.5 Aim of the Study

The aim of this reseach work is to carry out phytochemical studies of the stem bark of *Commiphora mollis* and justify the ethnomedicinal claims of the use of the plant in the treatment of antimicrobial infection.

1.6 Objective of the Study

The Objectives of this study is to:

- Carry out pyhtochemicals screening of the stem-bark of *Commiphora mollis*
- Isolate some of the bioactive compounds present in the stem-bark of *Commiphora mollis*
- Elucidate the structure of the compounds isolated from the stem-bark of *Commiphora mollis*
- Validate the medicinal use of the stem-bark of the plant to treat infectious diseases.

1.7 Research Hypothesis

The stem-bark of *Commiphora mollis* contains phytochemical constituents with antimicrobial activities.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Morphorlogy of *Commiphora mollis*

Commiphora mollis belongs to the family *burseraceae*, common name "corkwood*"* *hausa* language - *dashi*. The name *Commiphora* originates from the greek word *kommi (*meaning gum) and *phero* (meaning to bear*), mollis* (meaning soft). The majority of the species produce fragrant gum resin following damage to the bark (Steyn, 2003*).*

Commiphora mollis is a tree which is not spiny and the bark does not peel. The bark differs in structural appearance; it may be wr/inkled, smooth, or fragmented and is silvery when burnt by the sun. The trunks are sometimes knobbly or angular. The young branchlets are sparsely pilose to densely pubescent (van der Walt, 1986). The leaves are compound, pinnate, with 3 – 7 leaflets present. They are greyish-green dorsally and a paler green ventrally, and are densely covered or scattered with velvety hairs. The flowers are small and are found in groups on long, red slender stalks. The flowers are unisexual, maroon-red in colour and velvet (Steyn, 2003). The fruit is round and red in colour when ripe and a distinctive red pseudo-aril with four arms is present when the fruit is halved.

Of the more than 200 species of *Commiphora* native to the seasonally dry tropics of Africa, Arabia and India, about 40 species occur in southern Africa (Steyn, 2003).

Fig. 2.1: *Commiphora mollis* growing on its natural habitat

2.2 Ethonomedicinal Uses of *Commiphora mollis* and Other Species of the Genus

Traditionally *commiphora mollis* has been used in the treatment of fever (malaria and typhoid), wound healing, cancer, ulcer and rheumatic condition. Traditionally, *myrrh* is used for the common cold, to relieve nasal congestion and coughing. It is also used for the treatment of wounds and ulcers, especially infections of the mouth, gums and throat (van Wyk and Wink, 2002).

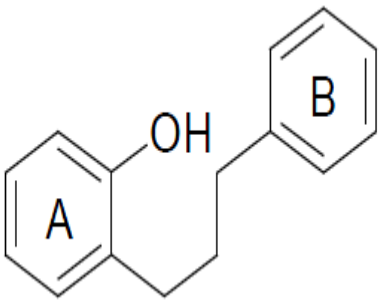
The stem bark of *C. africana* was used for colds, fever, malaria and snake bite (Kokwaro, 1976 ; Hutchings *et al.,* 1996). The root for typhoid, the resinous exudates for wound healing and antiseptic, the fumes of burnt resin serves as antiseptic, migraine and insecticide (Kokwaro, 1976). The bark of *C. pyracanthoides* was reported to used for diseases of the gall bladder (Steyn, 2003), *C.* Serrata roots for chest ailments (Steyn, 2003), C. *viminea* resin for skin ailments (Steyn, 2003) and the root of *C. zanzibarica* is also used for ulcers (Steyn, 2003).

2.3 Flavonoids

The flavonoids are distributed in a major part of plant material. They have not been found only in algae and a few varieties of plants. The flavonoids are one of the most structurally diverse groups of compounds. For example, about thirty flavonoid types have been identified in Asteraceae (Bruneten, 1995).

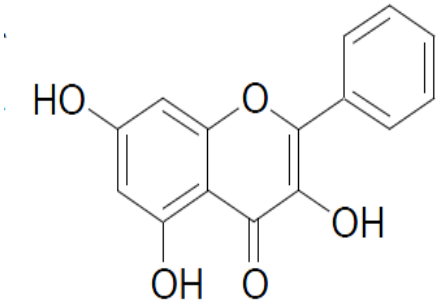
2.3.1 Structure diversity of flavonoids

The basic flavonoid structure is derived from a diphenylpropane system C_6-C_3-C_6 (XI).

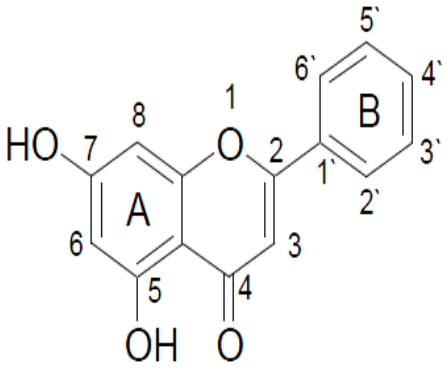


(XI)

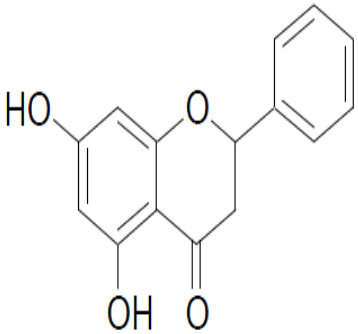
The flavonoids can be classified into nine general structures figure: flavone (XIII**)**, flavonol (XIV), flavanone (XV), dihydroflavonol (XVI), flavan-3-ol **(**XVII), flavan-3,4-diol (XVIII), chalcone (a structure with one opening ring), aurone, and anthocyaninidine (with a positive charge on oxygen O-1), except these nine basic structures, the flavonoid exist in biflavonoid and the glycosidic form (Smejkal *et al*., 2007).



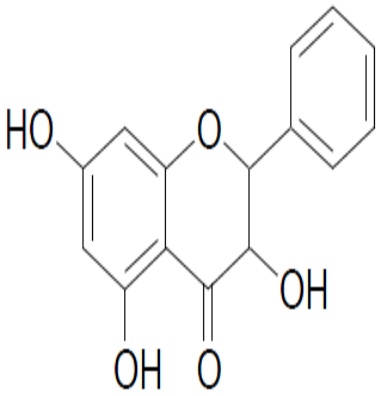
XII



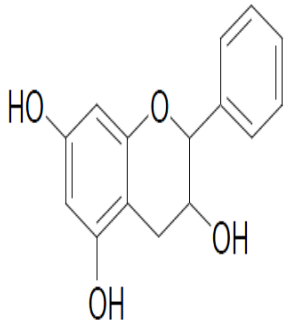
XIII



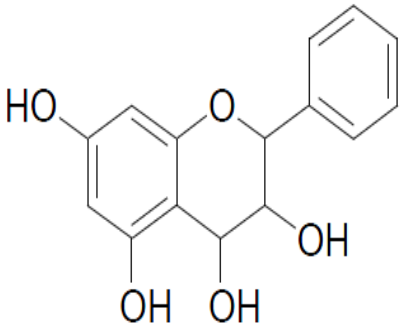
XIV



XV



XVI



XVI

The prefix “iso” is used for flavonoids characterized with a ring B bonded at position C-3 the isoflavonoids.

Flavonoids can be bonded together through their hydroxy groups at position C-6 and C-8, the resulting dimeric structure is known as a biflavonoid and has an oxygen bridge between these positions. The other alternative is a bond between C-3` and C-8. The hydroxyl groups of the flavonoid structure can be free or methylated. A substituent side chain at position C-6 is typical for flavonoids (Grycova, 2007).

The sugar part of glycosylflavonoids may be a chain of mono-, di-, or trisaccharides, such as D-glucose, D-galactose, or D-allose, some pentoses, D-glucuronic acid or D-galacturonic acid. This sugar side chain is bonded to the flavonoid structure through a hydroxyl group at position C-3 or C-5. The C-glycosylflavonoids with a side chain at C-6 or C-8 and without an oxygen bridge form a group of special case. Mono-C-glucosylflavones are the most common C-glycosylflasvonoids (Grycova, 2007).

2.3.2 Therapeutic potential of flavanoids

The establishment of an inverse correlation between the intake of fruits and vegetables and the occurrence of diseases such as inflammation, age-related disorders, cancer and cardiovascular disease is derived from clinical trials and epidemiological studies (Middleton *et al.,* 2000). Polyphenolic compounds are effective in the prevention of

oxidative stress related diseases. Flavonoids are a group of polyphenolic compounds with diverse characteristics and chemical structures. The therapeutic potential of these flavonoids has been determined and are known to have a number of pharmacological and biochemical properties, including antibacterial, antiviral, anti-allergic, vasodilatory and antiinflammatory, exhibiting activity against the enzymes cyclo-oxygenase and lipoxygenase (Paraskeva, 2011).

Flavonoids are also effective anti-oxidants, free radical scavengers and are chelators of divalent cations (Cook and Samman, 1996). exert the As discussed elsewhere, it is the excessive generation of the free radicals, reactive oxygen species (ROS), such as superoxide anions, hydroxyl radicals and hydrogen peroxide, that contribute to the causes of various diseases such as cancer, rheumatoid arthritis, various neurodegenaritive diseases, tissue damage and also ageing, especially if their production exceeds the capacity of tissues to remove them. Flavonoids have been shown to be effective scavengers of ROS (Middleton *et al.,* 2000).

Anti-oxidant properties elicited by plant species therefore have a full range of applications in human healthcare, as they protect against these radicals. Synthetic anti-oxidants, such as butylated hydroxyanisole and butylated hydroxytoluene, have been developed, but their uses are limited due to their toxicity. In search for sources of novel anti-oxidants with low toxicity, medicinal plants have over the past few years been studied extensively for their radical scavenging activity (Molyneux, 2004). As plants produce a large number of anti-oxidants to control the oxidative stress caused by sunbeams and oxygen, it is clear that plants may represent a source of new compounds with antioxidant activity (Scartezzini and Speroni, 2000).

2.4 Phytochemistry of C*ommiphora mollis* and Other Species in the Genus

No previous phytochemical study was reported on *Commiphora mollis*, but a wide range of organic molecules have been isolated from other species of the genus. Identification of chemical compounds isolated from *Commiphora myrrha* include D-galactose, L-arabinose, and 4-methyl D glucuronic acid, acidic oligosaccharides and aldobiuronic acids.

Furanosesquiterpenoids terpene and terpenoid with the diterpenoids and triterpenoids, steroids and sterols. It is reported that flavonoids quercetin and derivatives (quercetin-3-O-α-L-arabinoside, quercetin-3-O-β-D-galactoside, quercetin-3-O-α-L-rhamnoside, and quercetin-3-O-β-D-glucuronide) isolated from the flower of *Commiphora mukul.* (El-Ashry *et al.*, 2003; Hanuš *et al.*, 2005).

Galactose, arabinose, 4-O-methyl-glucuronic acid, arabino-3,6-galactan protein fractions and protein, 3 new furanogermacrenes isolated $C_{16}H_{22}O_3$, $C_{18}H_{24}O_3$, $C_{16}H_{20}O_3$, Sesquiterpene from *Commiphora molmol.* It was also reported that Dihydroflavonol glucoside – phellamurin was isolated from *Commiphora africana*. Condensed tannins found in powdered bark of *Commiphora agolensis.* Seven dammarene triterpenes from the stem bark, lupeol and β-myrin isolated from *Commiphora dalzeilli*. Pentacyclic triterpene, 2α,3β,23-rihydroxylean-12-ene was isolated from *Commiphora merkeri* (Hanuš *et al.*, 2005)

Other Studies carried out on a few *Commiphora* species to identify several of the constituents of the species. research groups identified the following chemical constituents: dammarene triterpenes (Dekebo *et al*., 2002a; Dekebo *et al*., 2002b; Manguro *et al*., 2003), triterpenes (Provan and Waterman, 1985), ferulates (Zhu *et al*., 2001), furanosesquiterpenes (Manguro *et al*., 1996) guggultetrols (Kumar and Dev, 1987), guggulsterones (Swaminathan *et al*., 1987), lignans (Provan and Waterman, 1985; Dekebo *et al.*, 2002c), flavanones (Fatope *et al.*, 2003), sesquiterpenes (Andersonn *et al*., 1997) and steroids (Bajaj and Dev, 1982)

2.5 Biological Action of *Commiphora mollis* and Other Species in the Genus

i. Antimicrobial activity

The antibacterial activity of some constituents of *C. mukul* oleo-gum-resin essential oil, chloroform extract have been evaluated. A wide range of inhibitory activity against Gram-positive and Gram-negative bacteria was observed (Saeed and Sabir, 2004). The isolation and identification of muscanone from *C. wightii* by Fatope *et al*. (2003), was found to be active against *Candida albicans*. *Commiphora* has been used in combination with other plant species in the development of a pharmaceutical formulation. One of the formulas of "The Jerusalem Balsam", found in a manuscript form in the archive of the monastery contains four plants: olibanum (*Boswellia* spp.), myrrh (*Commiphora* spp.), aloe (*Aloe* spp.) and mastic (*Pistacia lentiscus* L.). Pharmacological assays conducted on this formulation indicated antiseptic properties (Moussaieff *et al*., 2005).

ii. Antimycobacterial activity

Commiphora mukul, used traditionally for the treatment of tuberculosis, was assayed for antimycobacterial activity (Newton *et al.,* 2002). The crude methanolic resin extract displayed significant antimycobacterial activity (Paraskeva, 2011).

iii. Antitumour activity

Recently the cytotoxic and antitumor activity of myrrh has proved to be substantially significant (Paraskeva, 2011).

iv. Anti-oxidant activity

Antioxidant effects are a possible mediator in the protection against myocardial necrosis, inhibition of platelet aggregation, as well as increased fibrinolysis by guggulipid, the extract from the myrrh resin (Paraskeva, 2011).

v. Anti-inflammatory activity

A number of studies suggest that *guggul* elicits significant anti-inflammatory activity (El Ashry *et al.,* 2003).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Solvents/reagents

Solvents used were of general purpose grade and were distilled twice before used. These include methanol, chloroform, n-hexane, ethylacetate and n-butanol.

3.2 Collection, identification and preparation of plant materials

The sample of the plant material was collected from Basawa, Zaria and was authenticated at the Herbarium unit of Biological Science Department Ahamadu Bello University, Zaria Nigeria where a voucher specimen (No. 0335) has been deposited. The bark was separated from branches and air dried under shade. It was then made into powder using pestle and mortar and stored in an air-tight container.

3.3 Preparation of extract

The powdered bark (1200 g) was extracted using cold merceration with occassional shaking using methanol for 21 days. The extract was filterated and concentrated in vacuo to yield a residue referred to as the crude methanol extract (CME) with a yield of 71.56 g. It was suspended in distilled water and filtered using a filter paper to obtain water soluble and water insoluble fraction. The water soluble fraction was partitioned in a separation funnel with n-hexane, chloroform, ethylacetate, and n-butanol to give n-hexane, chloroform, ethylacetate and n-butanol fraction respectively.

3.4 Preliminary Phytochemical Screening

3.4.1 Test for sterols/terpenes

(a) Leiberman-Buchard test:

To methanol extract (0.2 g), 2 ml of acetic acid was added; the solution was cooled well in ice followed by the addition of conc. H_2SO_4 carefully. The solution was observed for blue ,green, red, or orange colour that changes with time (Sofowora, 1993.)

(b) Salkowski test

A little quantity of the extract was dissolved in 1ml chloroform and to it 1 ml of concentrated sulfuric acid was added down the test tube to form two phases. Formation of red or yellow coloration was taken as an indication for the presence of sterols (Silva *et al*., 1998).

3.4.2 Test for flavonoids

(a) Ferric chloride test

Abaut 0.5 g of the extract was boiled with distilled water and then filtered. To 2 ml of the filtrate, few drop of 10 % ferric chloride solution were then added. A green-blue or violet coloration indicated the presence of a phenolic group (Trease and Evans, 2002).

(b) Shinoda's test:

About 0.5 g of the extract ware dissolved in ethanol, warmed and then filtered .three pieces of magnesium chips ware then added to the filtrate followed by few drops of conc. HCl. A pink, orange, or red to purple coloration indicates the presence of flavonoids (Trease and Evans, 2002).

(c) sulphuric acid test: the sample was dissolved in concentrated sulphuric acid and the colour change was observed (Silva *et al.*, 1998).

(d) Sodium hydroxide test:

Little quantity the extract was dissolved in water and filtered ;to this , 2 ml of the 10 % aqueous sodium hydroxide was later added to produce a yellow colouration. A change in colour from yellow to colourless on addition of dilute hydrochloric acid was in indication of the presence of flavonoids (Trease and Evans, 2002).

3.4.3 Test for Alkanoids

Few quantity of extract was stirred with 5 ml of 1 %aqueous HCl on water bath and then filtered. of the filtrate,1 ml was taken individually into 2 test tubes. To the first portion, few drops of Dragendorff's reagent was added and observed for orange-red precipitate. To the second 1 ml, mayer's reagent was added and observed for buff coloured precipitate (Sofowora,1993).

3.4.4 Test for Tannins

About 0.5 g the extract was stirred with abaut 10 ml of distilled water and then filtered. Few drops of 1 % ferric chloride solution were added to 2 ml of the filtrate filtered. Few drops of 1 % ferric chloride solution were added to 2 ml of the filtrate occurrence of a blue-black, green precipitate indicates the presence of tannins (Trease and Evans, 2002).

3.4.5 Test for saponins

About 0.5 g of the extract was shaken with water in a test tube. Frothing which persisted for 15 minutes indicates the presence of saponins (Silva *et al.*, 1998).

3.4.6 Test for anthraquinones

(a) Free anthraquinones

The extract was shaken with 10 ml of benzene, the content was filtered, and 5 ml of 10 % ammonia solution was added to the filtrate, the mixture. Presence of a pink, red or violet colour in the ammonical layer (Lower phase) indicates the presence of free anthraquinone (Trease and Evans, 2002.)

(b) Combined anthraquinones

The extract was boiled with 10 ml of aqueous sulphuric acid and filtered hot. The filtrate was shaken with 5 ml benzene, the benzene layer was separated and half of its own volume, 10 % NH_4OH was added. A pink, red or violet colouration in the ammonia phase (Lower phase) indicates the presence of combined anthraquinone or anthraquinone derivatives (Trease and Evans, 2002).

3.5 Chromatography Procedure

3.5.1 Thin layer chromatography (TLC)

Thin layer chromatography was carried out on TLC aluminium silica gel 60 PF_{254} precoated with thickness of 0.2 mm.

Techinique: one way ascending

Spotting and development: spot was applied manually using capillary tube; plates were dried using air blower and developed at room temperature using a Shandon chromatotank.

Solvent system: various solvent systems were used depending on the material, and they include:

a. Chloroform 100%

b. Chloroform: Ethylacetate 9:1

c. Chloroform: Ethylacetate 8:2

d. Chloroform: Ethylacetate 7:3

e. Chloroform: Ethylacetate 6:4

Detection: spots on TLC plates were visualised with eyes and spraying with 10% sulphuric acid, followed by heating at 111 °C for 5-10 min.

3.5.2 Column chromatography

The following column conditions were employed in running the column chromatography:

a. *Technique* - Gradient elution.

b. *Column*- Glass column with sintered disc at the bottom of dimension 70 x 3.5.

c. *Stationary phase:* Silica gel, 60-120 mesh size.

d. *Column parking*- Wet slurry method.

e. *Sample loading*- The sample was applied using dry load method (Cannell, 1998). The sample was dissolved in small amount of ethylacetate, mixed with a small quantity of silica gel, dried, triturated and loaded on top of the column.

f. *Solvent for elution*- Various solvents system were used.

g. Elution was carried out using one or mixture of ethylacetate and chloroform.

3.6 Chromatographic Separation

3.6.1 Thin layer chromatography of the methanol extract of *Commiphora mollis*

The methanol extract of *Commiphora mollis* was subjected to thin layer chromatography using precoated TLC aluminium plate. Various solvent -chloroform:ethylacetate.

3.6.2 Column chromatography of ethylactate fraction

The water soluble ethylacetate fraction (6.5 g) of the methanol extract was subjected to colunm chromatography. The column was packed using wet slurry method, cotton-wool was used to partially block the tap end of the column and 100 ml of hexane was poured into the empty column to saturate the cotton wool. Slurry of silica gel was packed into column and allowed to settle tightly, a second cotton-wool was placed on the top of the packed silica gel. The sample was previously adsorbed on silica gel by dissolving it in a minimum amount of ethylacetate, small quantity of silica gel was added to form a paste. The paste was dried, triturated and loaded on the previously packed column the column was eluted continuously using chloroform (100%), Chloroform: Ethylacetate (9:1), Chloroform: Ethylacetate (8:2), Chloroform: Ethylacetate (7:3) Chloroform: Ethylacetate (6:4) and finally eluted with 100 % methanol as solvent system. Fifty five (55) fractions, 100 ml each were collected. The fraction were pooled together based on the similarities on their TLC profile to give eight major fraction (D_1 - D_8) . The fraction D_5 was subjected to sphedex LH - 20 and preparative chromatography. The ethylacetate soluble was syringed and transferred into an empty clean beaker. The solution was concentrated to give white amorphous compound coded X1. TLC profile of X1 using Ch:EA 4:6 revealed a single spot. It was then subjected to spectroscopic analysis to elucidate its chemical structure

3.6.3 Spectral analysis

Proton and Carbon -13 NMR spectra

NMR spectrum was obtained on (600 MHz , CD_3OD for ^{1}H and 125 MHz, CD_3OD for ^{13}C) spectrophotometer, using the residual solvent peaks as internal standard. Chemical

shift values (δ) were reported in parts per million (ppm) relative to appropriate internal solvent standard. The NMR solvents used for these measurements are deutrated methanol.

3.7 The Antimicrobial Screening

3.7.1 Susceptibility test

The antimicrobial activities of crude methanol extract, Chloroform and Ethyl-acetate fraction were investigated using some pathogenic microbes obtained from the department of medical microbiology A.B.U. Teaching Hospital, Zaria. All the microorganisms were checked for purity and maintained in slants agar. Preliminary antimicrobial activities of the extract were carried out using disc diffusion methods. 0.4 g of the extract was weighed and dissolved in 10 ml of distilled water to obtain a concentration of 40 mg/ml as the initial concentration of the extract used to check the antimicrobial activities of the plant.

Mueller Hinton agar was used as the growth medium and was prepared according to the manufacturer instruction, boiled to dissolve and was sterilized at 121 °C for 15 mins, the medium was cooled to 45 °C and 40 ml of the sterile medium was poured in to sterilezed petridishes seeded with 0.1 ml standard inoculum of the test microbe. The inoculum was spread evenly on the surface of medium by the use of the sterilized swab over the surface of the medium. The seeded plates were allowed to dry in an incubator at 37 °C for 30 mins. A standard cork borer of 6 mm in diameters was used to cut cups (well) at the centre of each inoculated medium and 0.1 ml of the solution of the extract was introduced into each well on the medium , the plates were incubated at 37 °C for 24 hours after which each plate of the medium was observed for the zone of inhibition of growth. The zone was measured with transparent ruler and the result recorded in millimeters.

3.7.2 Minimum inhibition concentration (MIC)

The minimum inhibition concentration of the extracts was determined using broth dilution method (Vollekova *et al.,* 2001) as modified by Usman *et al*. (2007). Nutrient broth was prepared according to manufacturer's instruction. 10 ml of the medium was dispensed into test tubes and sterilized at 121 °C for 15 mins, the broth was allowed to cool. Mc-Farland's Standard turbidity scale no. 0.5 was prepared to give turbid solution. Normal saline was inoculated with each of the test micro-organism and incubated at 37 °C for 6 hrs to make turbid suspension of the micro-organism. After incubations, dilution of the micro-organism was done until the turbid matched that of Mc-Farland scale by visual comparison. Two- fold serial dilution of the extract in the sterile broth was done to obtain the concentrations of 40 mg/ml, 20 mg/ml, 5 mg/ml and 2.5 mg/ml. The initial concentration was obtained by dissolving 0.4 g of the extract in 10 ml of the sterile broth. 0.l ml of the test micro-organism in the normal saline was then inoculated into the different concentrations of the extract in the test tubes. The tubes were then incubated at 37 °C for 24 hrs, after which each test tube of the broth, were observed for turbidity (growth). The lowest concentration of the extract in the broth which shows no turbidity was recorded as the minimum inhibition concentration.

3.7.3 Minimum Bacteriacidal Concentration/Minimum Fungicidal Concentration (MBC/MFC)

The contents of the MIC tubes in the seriel dilution were then sub cultured onto the prepared medium, by dipping steriled wire loop into each test tubes and streaking the surface of the labelled agar plates. They were then incubated at 37 °C for 24 hrs, after hich they were observed for colony growth. The MBC/MFC were the plates with lowest concentrations of the extract without colony growth.

CHAPTER FOUR

4.0 RESULTS

4.1 Preliminary Phytochemical studies

The result of phytochemical screening revealed the presence saponins, steroids, flavonoids, tannins and alkaloid (Table 4.1)

Table 4.1: Result of phytochemical screening of crude methanol extract of *Commiphora mollis*

Constituents	Test	Observation	Inference
Flavonoids	Shinoda	Formation of red pink colour	+
	Sodium Hydroxide	Colour change from allow to colourless	+
	Sulphuric acid	Red solution formed	+
Saponins	Frothing	Frothing persisted for more than 15 minutes	+
Tannins	Lead acetate	White precipitate formed	+
Ferric chloride		Green –black colour formed	+
Steroids/Terpenes	Salwoski	Red ring was formed at the interphase	+
Liberman- Burchard		Brown ring with brown supernatant was formed	+
Alkaloids	Dragendoff	Formation of dense precipitate	+
	Wagner	Formation of dense precipitate	+

Key : + = Present - = Absent

4.2 Result of Column Chromatographic Separation

Fifty five fractions resulted from silica gel coloum chromatographic separation of the ethylacetate fraction of *Commiphora mollis* , which were pooled together based on their TLC profiles . This gave eight major fraction D_1 - D_8 (Table 4.3).

Table 4.3: Result of column chromatograpic seperation

Collection	No. of major spots	Solvent system	Fraction
1-10	-	Chloroform100%	
11-13	3	CF: E.A 90:10	D1
15-19	3	CF: E.A 80:20	D2
20-29	3	CF: E.A 70:30	D3
30-32	5	CF: E.A 70:30	D4
33-34	2	CF: E.A 70:30	D5
35-46	3	CF: E.A 70:30	D6
47-53	3	CF: E.A 70:30	D7
54-55	3	CF: E.A 60:40	D8

TLC Profile of Ethylacetate fraction using Chloroform:Ethylacetate of various ratio sprayed with 10 % sulphuric acid

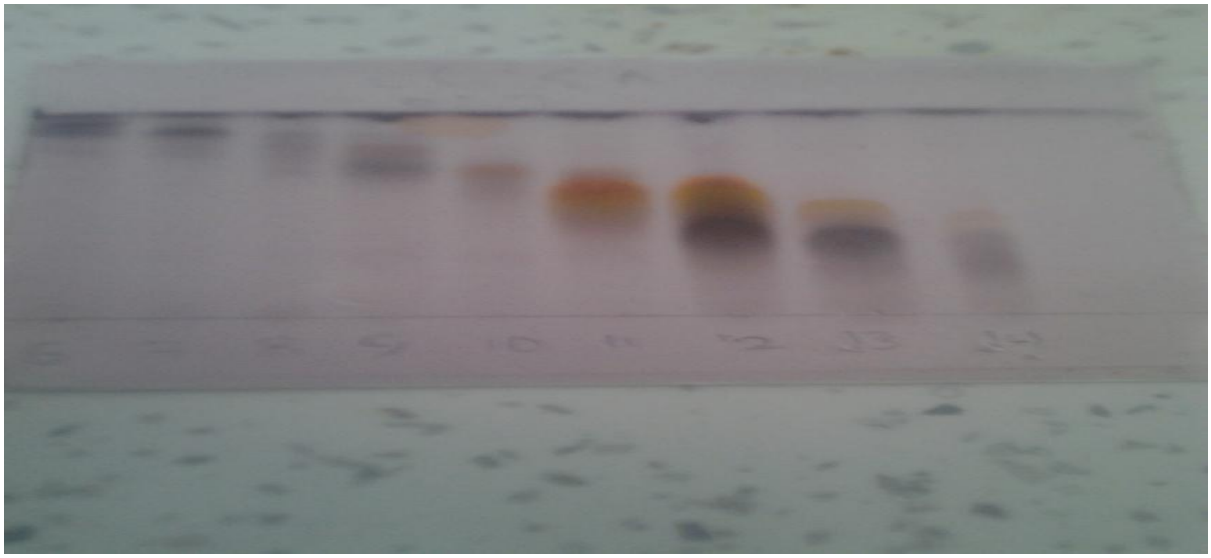


Plate 1: TLC profile of fraction 6-14 (Chloroform:Ethylacetate 8:2)

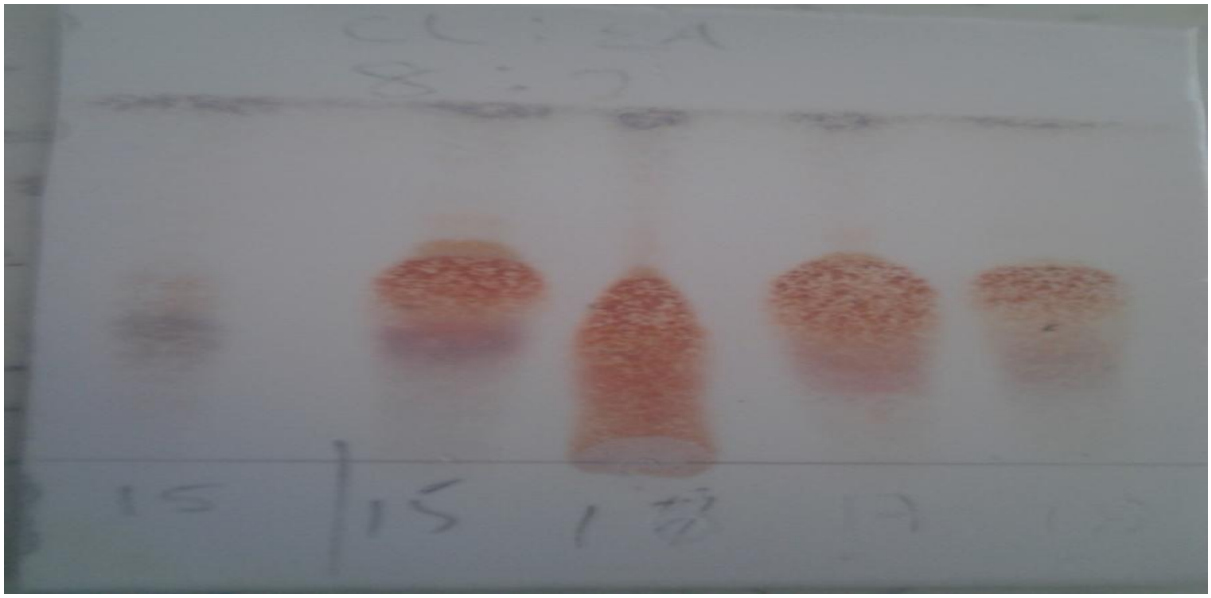


Plate 2: TLC profile of fraction 15 - 18 (Chloroform:Ethylacetate 8 : 2)

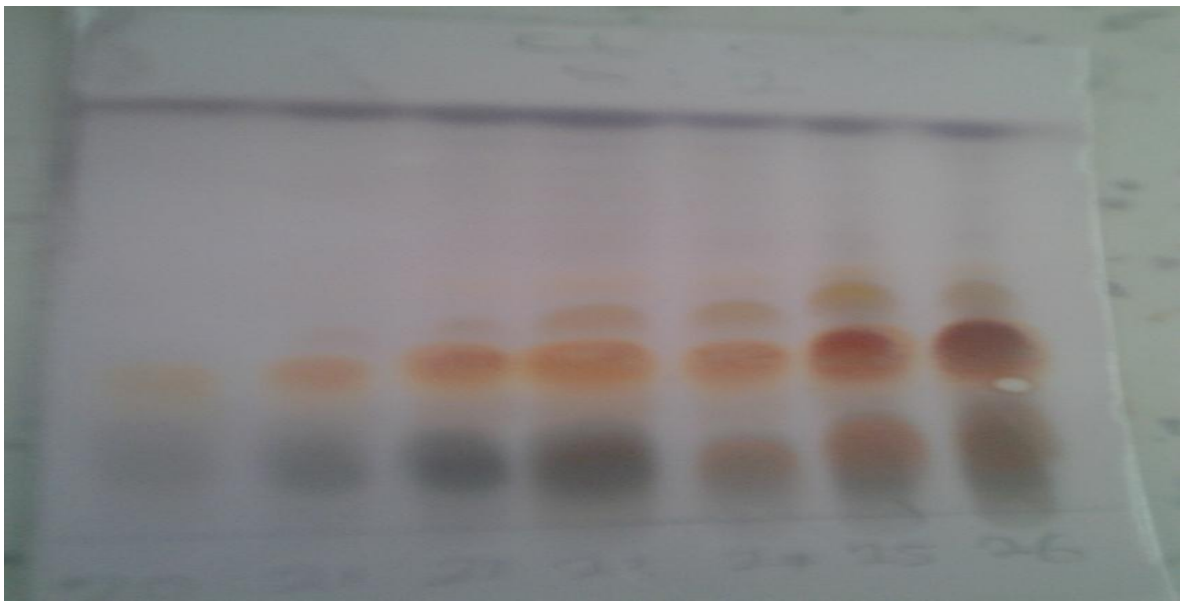


Plate 3: TLC profile of fraction 20 - 21 (Chloroform:Ethylacetate 8 : 2)

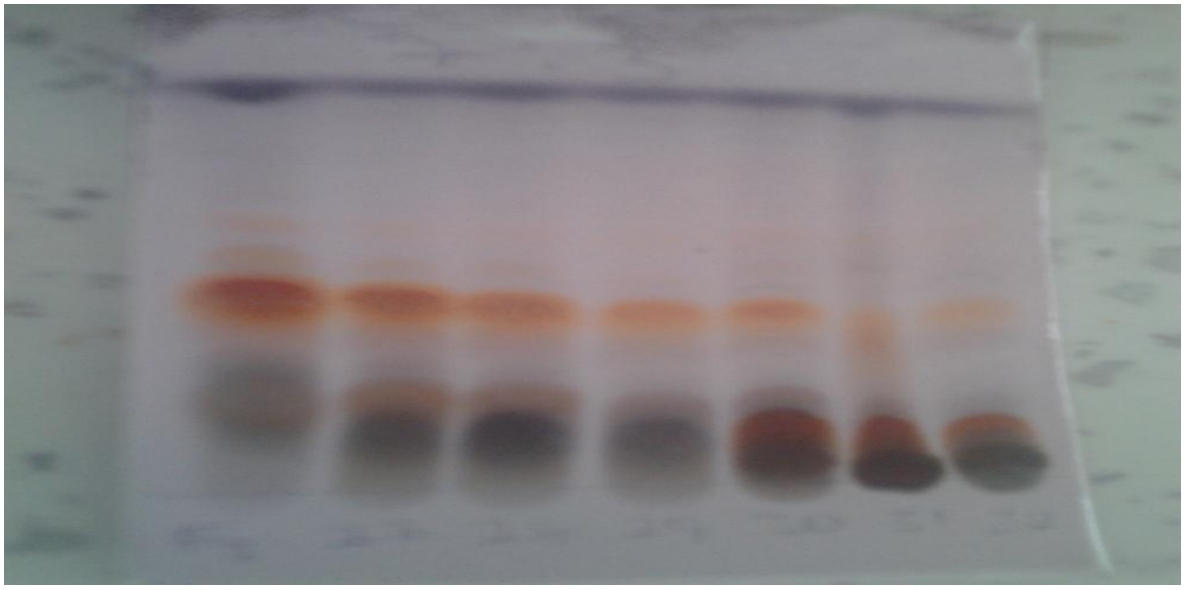


Plate 4: TLC profile of fraction 27 - 32 (Chloroform:Ethylacetate 7 : 3)

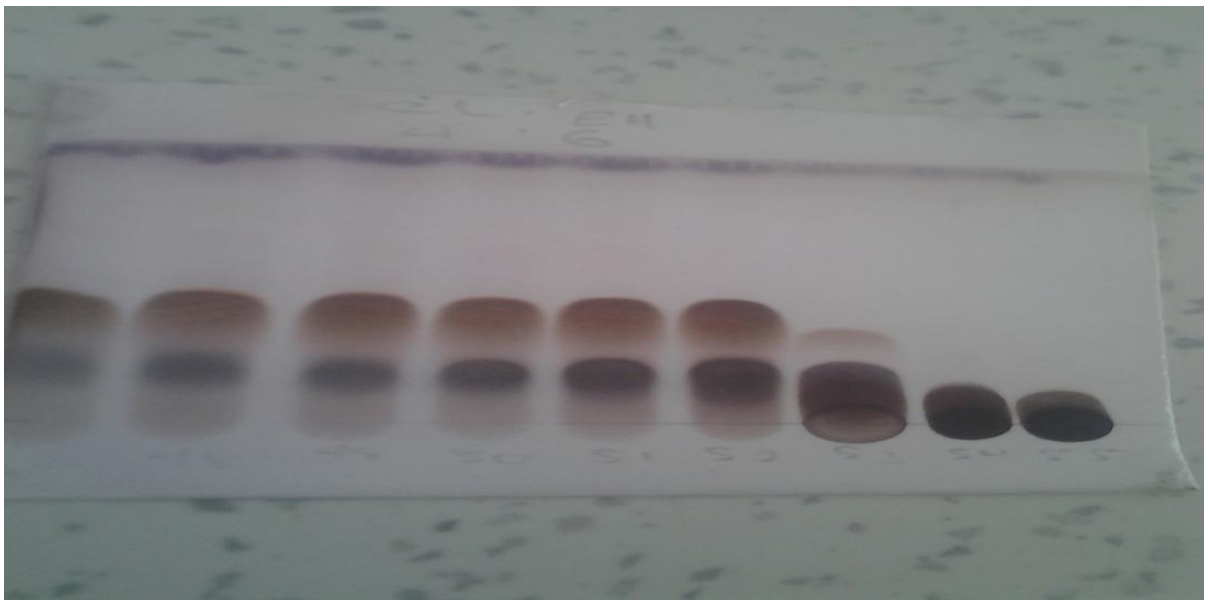


Plate 5: TLC profile of fraction47 - 55 (Chloroform:Ethylacetate 4 : 6)

4.2.1 Gel- Filteration Chromatography of Fraction D_5

Repeated gel filtration chromatography of fraction D_5 led to the isolation of compound X1 (7.5 mg). Compound X1 was isolated as a yellow amorphous solid, it gave a single spot on TLC using Chl:E.A, 4:6 as solvent system with R_f value of 0.9.

Melting point: the melting point of the isolated compound was found to be between 222 ^{0}C - 224 ^{0}C

4.3 Thin layer chromatographic profile of X1

TLC analysis of X1 using Chloroform:Ethylacetate 4:6 revealed a single spot

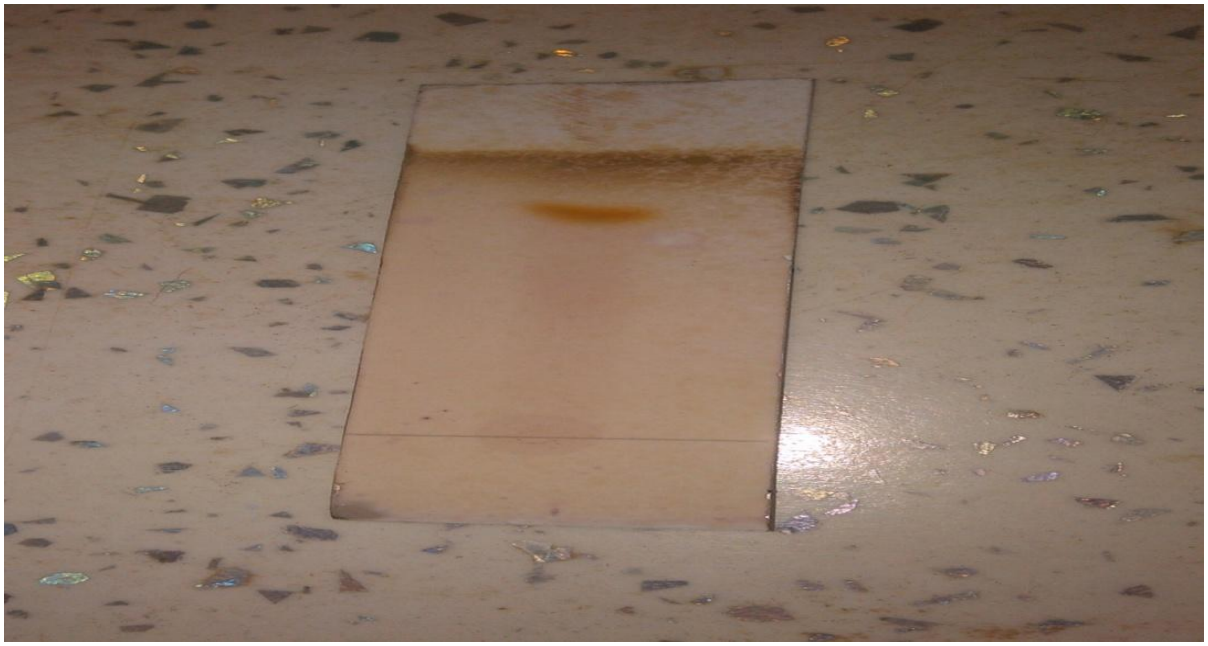


Plate 6: TLC profile of X1 (Chloroform : Ethylacetate 4:6)

4.4 SPECTRAL ANALYSIS OF X1

4.4.1 ^{1}H NMR of X1 (δppm, 600 MHz, CD_3OD)

The proton NMR spectrum of X1 revealed the total of 9 proton of which 5 aromatic proton resonances, 2 oxymethine and 2 methylene proton signal. Fig.4.1

4.4.2 ^{13}C NMR of X1 (δppm, 125 MHz, CD_3OD)

The ^{13}C NMR spectrum of X1 revealed the total of 15 carbon signal with 12 unsaturated 2 oxymethine, and a methylene signal. Fig 4.2

4.4.3 DEPT experiment of X1

The DEPT spectrum of X1 revealed 7 methine carbon signals, a quarternary carbon signal and a methylene carbon signal. Fig. 4.3

4.4.4 ^{1}H-^{1}H COSY correlation of X1

The ^{1}H COSY spectrum of X1 showed correlations between the protons at δ_H 7.0 ppm and 6.8 ppm and also between protons at δ_H 4.8 ppm , 4.2 ppm, 2.8 ppm and 2.7 ppm. Fig 4.4

4.4.5 HSQC experiment of X1

The HSQC spectrum of X1 showed the following H//C correlations; 7.0//114, 6.82//118.10, 6.8//114.60, 5.8//94, 4.82//78.48, 4.21//66.10, 2.8//27.84 and 2.75//27.84. Fig. 4.5

4.4.6 HMBC experiment of X1

The HMBC spectrum of X1 showed the following major H//C correlations. Fig. 4.6

2.77// 156.5, 98.7, 78.5, 66.1

4.21// 130.9, 98.7, 78.5

4.82// 130.9, 114.62, 78.5

5.98// 156.2, 98.7, 96.1

6.82// 144.37, 130.9, 114.62, 78.5

7.10// 144.53, 130.9, 144.37, 78.5

Table 4.3: NMR Spectra data for X compound (CD_3OD, 600 MHz) for ^{1}H and (CD_3OD, 125 MHz) ^{13}C

Position	δ_H mult., J (Hz)	δ_c mult.,	HMBQ
1	-	-	-
2	4.8 s	78.48 (CH)	1', 5', 2
3	4.2 s	66.10 (CH)	1', 10, 2
4	2.8 (dd, *J* = 16.68 Hz, 4.56 Hz	27.84 (CH_2)	9, 10, 3,
	2.7 (dd, *J* = 16.68 Hz, 2.76 Hz)		9, 10, 2, 4, 3
5	-	156.2 (CH)	-
6	5.95 (d, *J* = 2.16 Hz)	94.60 (CH)	5, 10, 6
7	-	155.9	-
8	5.98 (d, *J* = 1.9 Hz)	98.10 (CH)	-
9	-	156.5	-
10	-	98.7	-
1'	-	130.9	-
2'	7.0 (d, *J* = 1.3 Hz)	114.0 (CH)	4', 1', 3', 2
3'	-	144.37	-
4'	-	144.53	-
5'	6.8 (d, *J* = 8.2 Hz)	114.62 (C	3', 1', 2
6'	6.83 (d, *J* = 8.2 Hz)	118.10 (CH)	4', 1',3',

Key s = single, d = double, dd = double doublet

^{1}H-NMR

The ^{1}H-NMR spectra (600 MHz CD_3OD) of X1 showed five aromatic protons signals appeared as doublets at δ 7.0 (1H, d, J=1.3Hz, H-2'), δ 6.83 (1H, d, J=8.2Hz, H-6'), δ 6.8 (1H, d, J=8.2Hz, H-5'), δ 5.98 (1H, d, J=1.9 Hz, H-8) and δ 5.95 (1H, d, J=2.16 Hz, H-6). Also observed are two oxymethine protons signals appeared as singlets at δ 4.8 (1 H, s, H-2) and δ 4.2 (1H, s, H-3). The methylene protons signal appeared as double-doulet at δ 2.8 (1H, dd, J=16.68 Hz, H-4) and 2.7 (1H, dd, J=16.68 Hz, H-4). Fig. 4.1

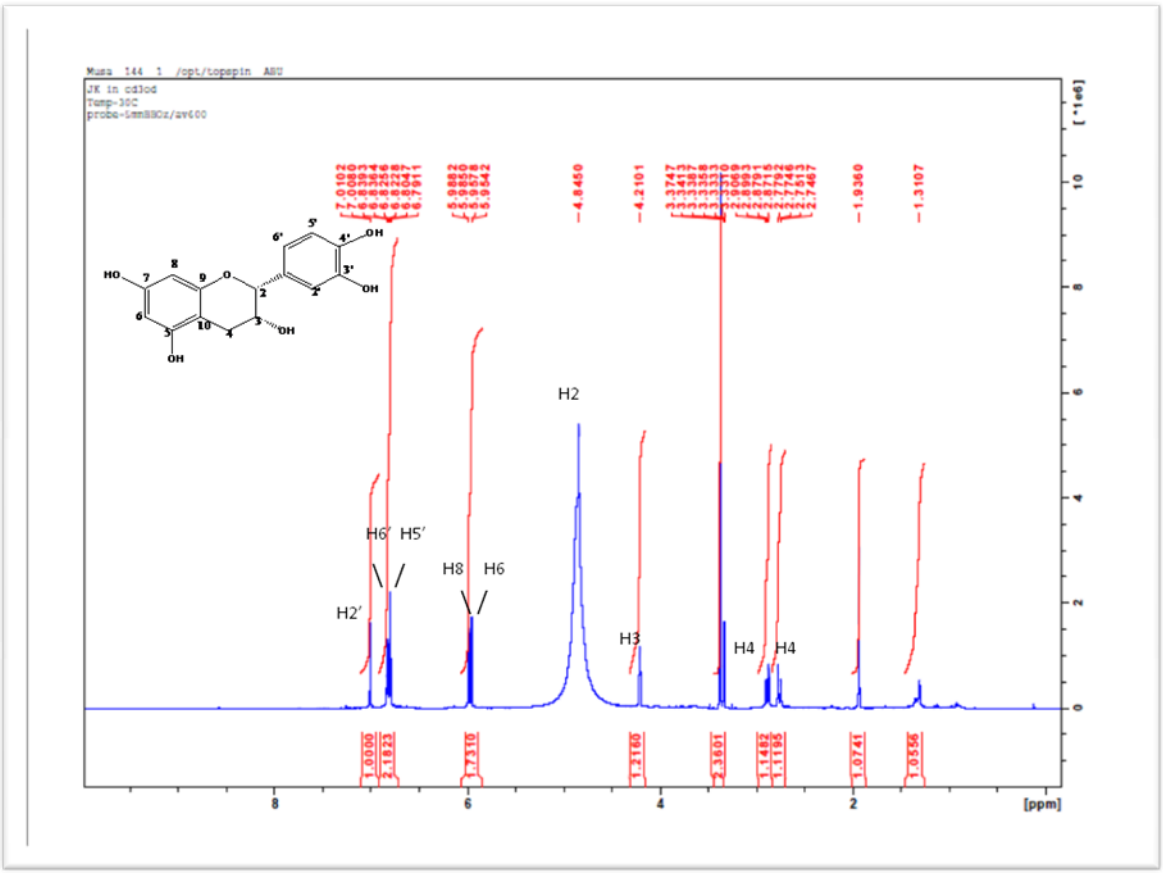


Fig. 4.1: ^{1}H NMR spectra of X1

^{13}C NMR Spectral Analysis of X1

The ^{13}C NMR (125 MHz CD_3OD) of X1 revealed 15 C- signal with the 12 aromatic carbon signals appeared at δ 156.5 (C-9), δ 156.2 (C-5), δ 155.9 (C-7), δ 144.53 (C-4'), δ 144.37 (C-3'), δ 130.9 (C-1'), δ 118.10 (C-6'), δ 114.62 (C-5'), δ 114.0 (C-2'), δ 98,7 (C-10), δ 98.10 (C-8) and δ 94.6 (C-6). Oxymethine carbon atoms signals appeared at 78.48(C-2) and 66.10 ppm (C-3). A Methylene carbon signal appeared at δ 27.84 (C-4). Fig. 4.2

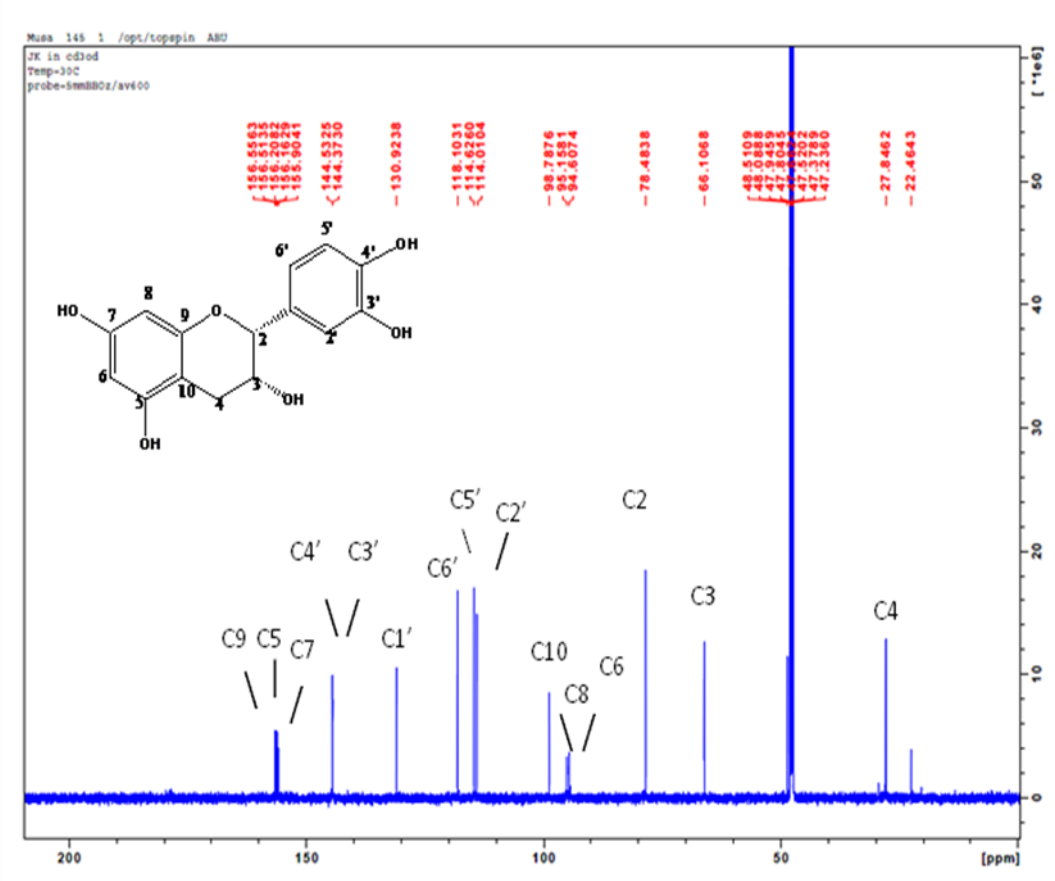


Fig. 4.2: ^{13}C NMR spectra of X1

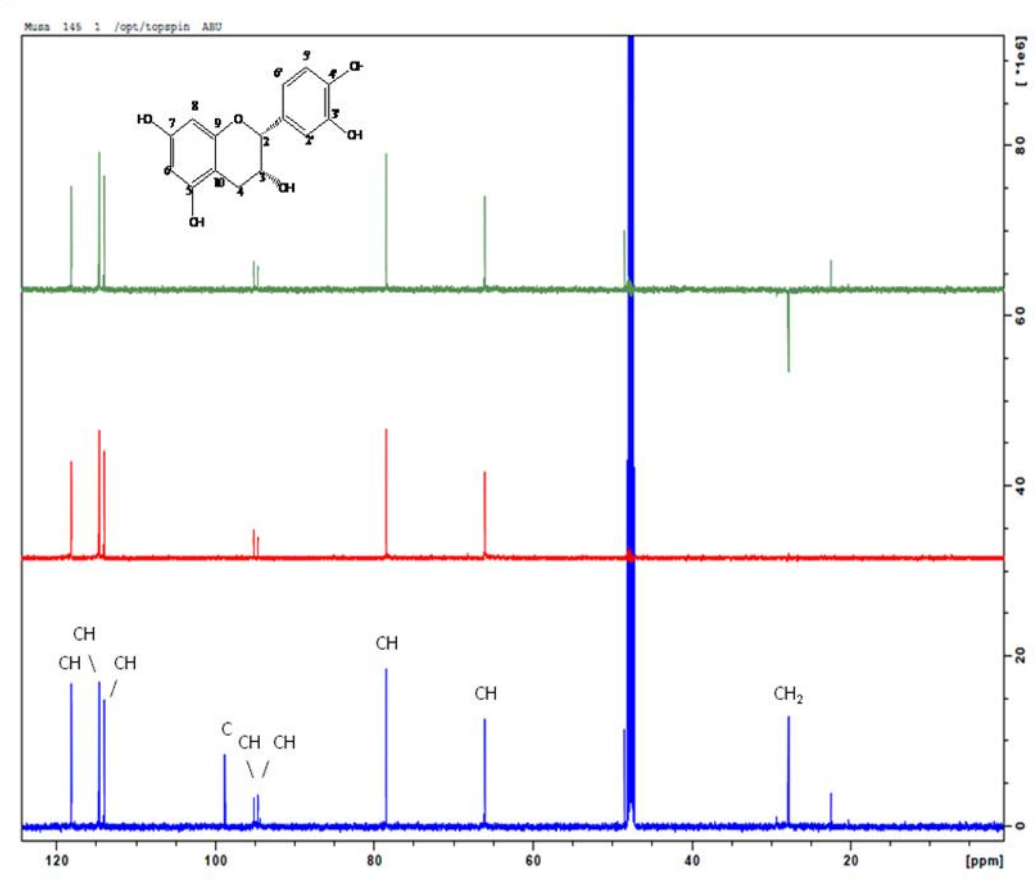


Fig. 4.3: DEPT experiment of X1

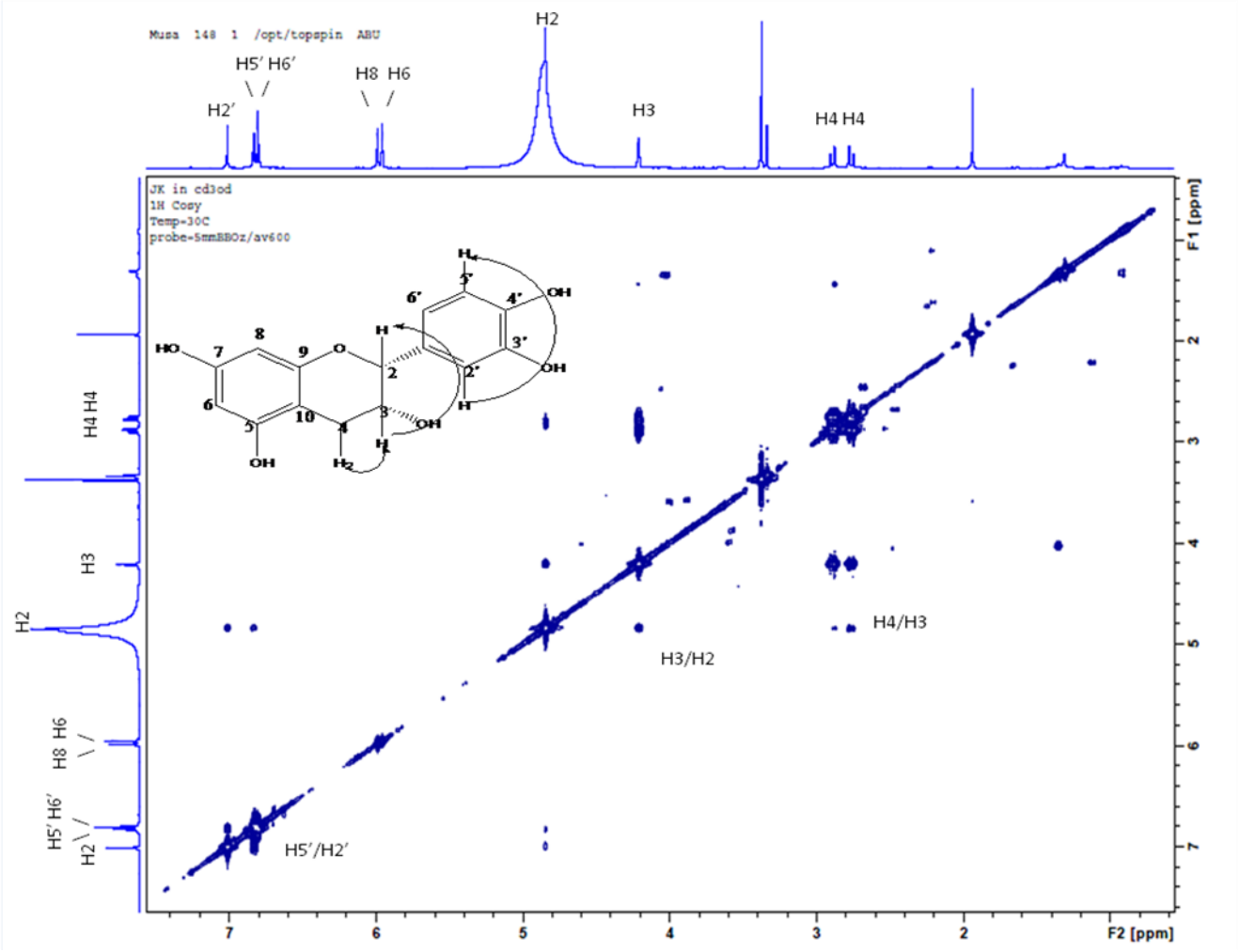


Fig. 4.4: COSY of X1

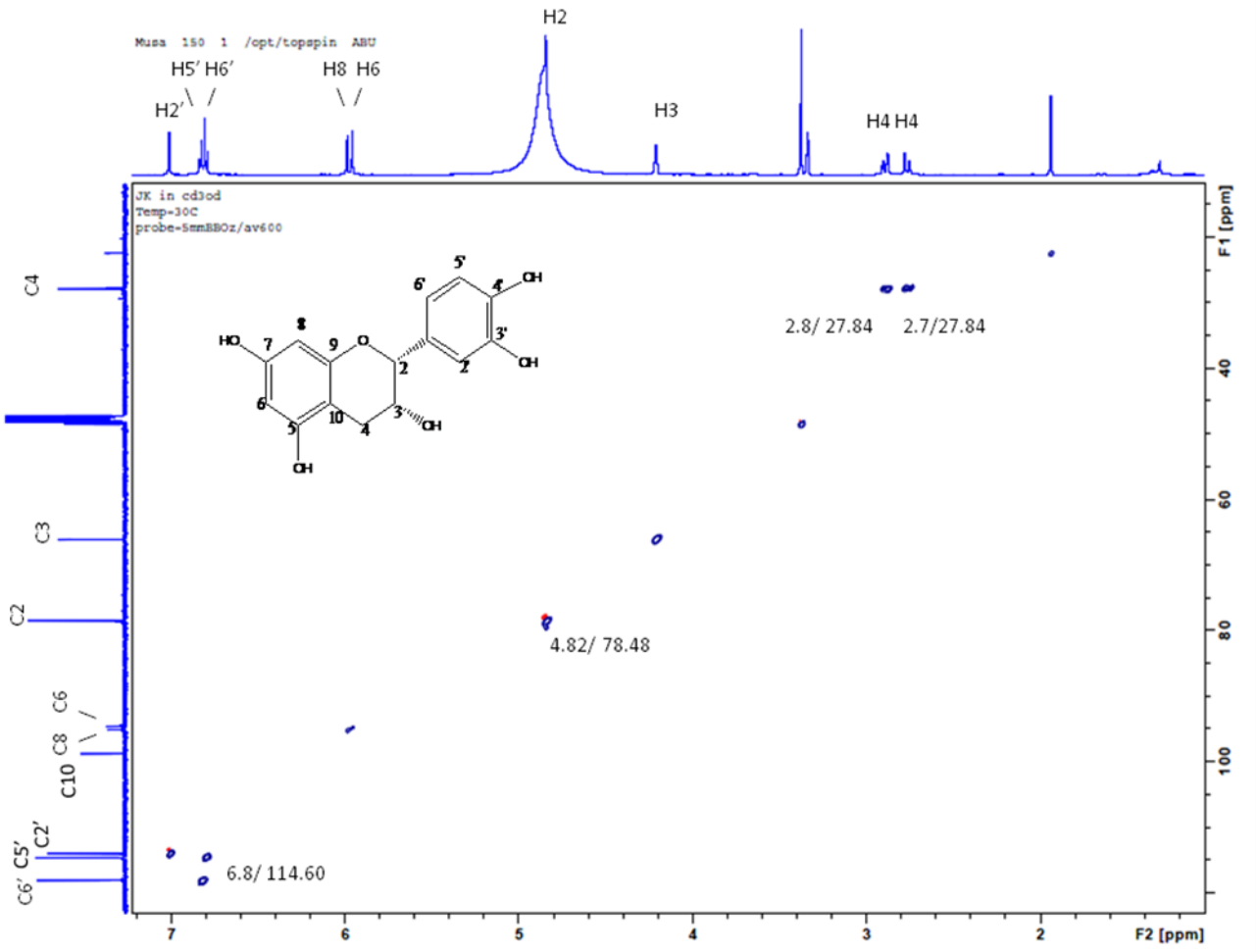


Fig. 4.5: HSQC of X1

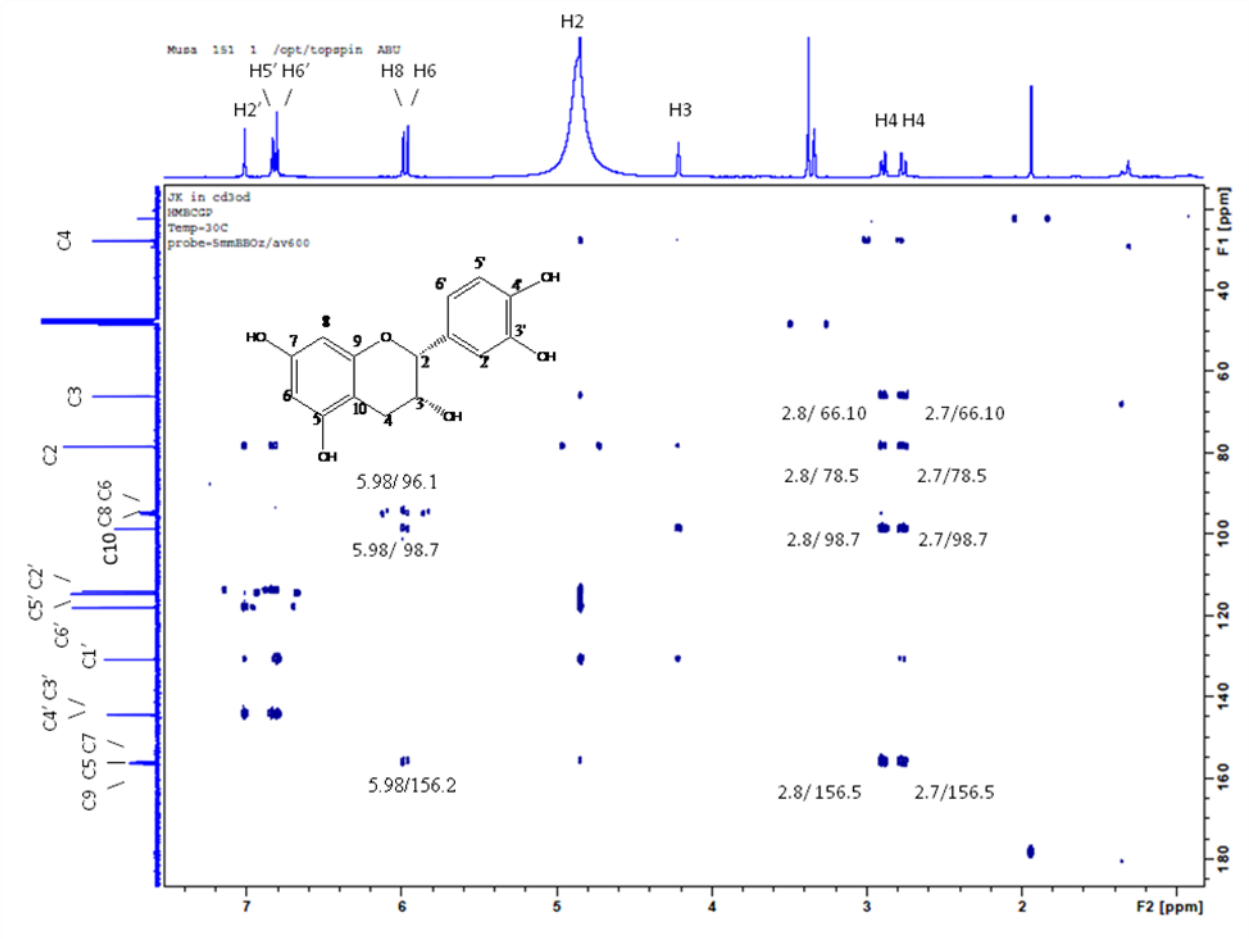


Fig. 4.6: HMBC of X1

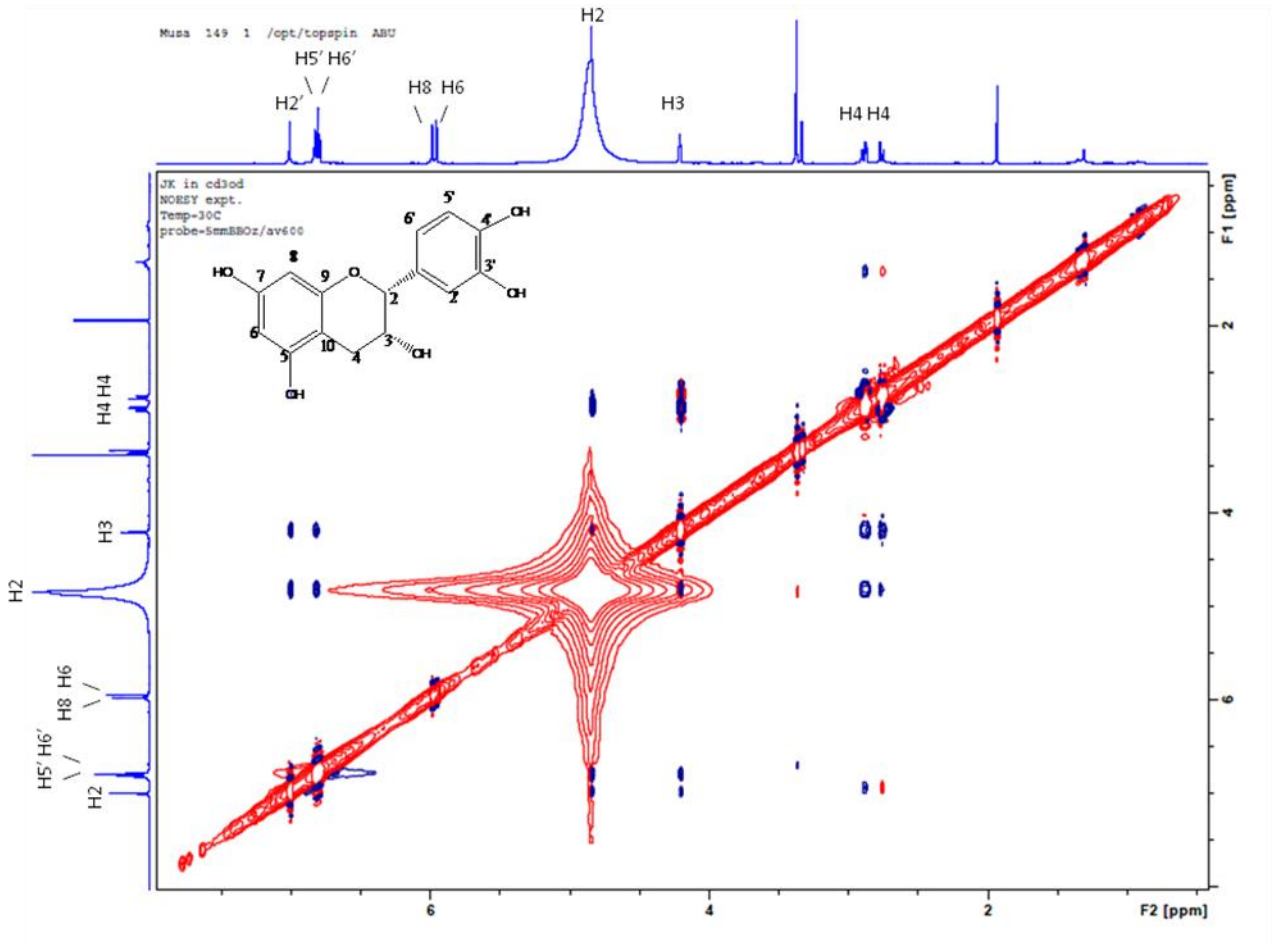


Fig. 4.7: NOESY of X1

Table 4.4: Zone of inhibition of the crude methanol extract, chloroform, ethylacetate fraction and standard drugs against the test organism (mm).

Test organism	Crude methanol extract	Chloroform	Ethytacetate	Ciprofloxacin	Fluconazole
Staphylococcus aureus	22	24	26	37	NT
Streptococcus pyogenes	22	23	27	35	NT
Corynebacterium ulcerans	0	0	0	37	NT
Bacillus subtilis	25	27	31	42	NT
Escherichia coli	21	26	30	35	NT
Salmonella typhi	0	0	0	40	NT
Shigella dysenteriae	20	28	30	41	NT
Proteus mirabilis	0	0	0	34	NT
Candida albicans	21	22	24	NT	35
Candida brusei	20	20	25	NT	37

NT= not tested Drug concentration 5 μg/ml

Table 4.5: Minimum Inhibition/Bacteriacidal Concentration of the Crude and fractions against the Test Microbes.

Test organism	MIC (mg/ml)			MBC (mg/ml)		
	CME	CFF	EA F	CME	CFF	EAF
Staphylococcus aureus	10	10	10	40	20	20
Streptococcus pyrogenes	10	10	5	40	20	20
Bacillus subtalis	10	5	5	20	20	10
Escherichia coli	10	10	5	40	20	10
Shigella dysenteriae	10	5	5	40	10	10
Candida albicans	10	10	10	40	40	20
Candida brusei	10	10	10	40	40	20

CME= Crude methanol extract, CFF= Chloroform fraction, EAF= Ethylacetate fraction

CHAPTER FIVE

5.0 DISCUSSION

5.1 PRELIMINARY PHYTOCHEMISTRY.

Flavanoids have reported to have anti-microbial activities (Joshi and Gayathri, 2004). Flavanoid also have reported to have anti-inflammatory, (Ko, 2004 and Gerritsen, 1995), antispasmotic, (Capasso, 1991), antidiarrhoea, (Carlo, 1993) vasorelaxant, (Zhang, 2000) and antiproliferative (Comalada, 2006 ; Kim, 2005), as well as an alleopathic activity. Similarly (Cowan, 1999) reported that flavonoids and tannins to have antimicrobial activities.

The antimicrobial activity of flavonoids is due to their ability to complex with extracellular and soluble protein and to complex with bacterial cell wall while that of tannins may related to their ability to inactivate microbial adhension of enzymes and cell envelop proteins (Cowan, 1999). Therefore the antimicrobial activity of *Commiphora mollis* might be associated with the presence of these phytochemical consitituents.

5.2 STRUCTURE ELUCIDATION OF COMPOUND X1

^{1}H NMR Analysis

The ^{1}H NMR spectrum exhibited ortho-coupled doublets at δ_H 6.8 (1 H d, J= 8.2 Hz , H-5') and 6.83 (1 H, d, J= 8.2 Hz, H-6') as well as meta-coupled doublets at δ_H 6.83 (1 H, d,J= 8.2, H-6′) and 7.0 (1 H, d, J=1.3 Hz, H-2′), which were consistent with a 3′,4′-disubstituted B ring of flavon- 3 – ol, as reported (Martin *et al*., 2000). Two meta-coupled doublets at δ_H 5.98 (1 H, d, J=1.9 Hz, H-8) and 5.95 (1 H, d, J=2.16 Hz, H-6) were consistent with a 5,7-

dioxygenated A ring of flavon- 3 -ol. These resonances together with δ_H 2.7 (1 H,dd, J=16.68, 2.78 Hz, H-4), 2.8 (1 H, dd, J=16.68, 4.0 Hz, H-4), 4.20 (1 H, H-3) and 4.8 (1 H,

H-2) and their corresponding carbon signals in HMQC spectrum revealed that X1 was 3,5,7,3′,4′-pentahydroxyflavon-3-ol (Xiaobin *et al.*,2011). The following spectroscopic analysis made the absolute configurations at C-2 and C-3 assignable. The small coupling constant of H-2 and H-3, which was consistent with $J_{2,3}$ of epicatechin and disagreed with large value of catechin (6.7 Hz) (Cai *et al.*, 1991).

^{13}C NMR Analysis

Inspection of this spectrum and comparison\ with the published data (Czochanska *et al*., 1980) show typical signals due X1. The strong signal centered at δ_c 144.37 ppm, with a small shoulder at 144.53 ppm, is attributed to C3' and C4' in the B-ring of the flavan-3-ol (catechin/epi- catechin). Other specific aromatic resonances show the δ_c 114.0 (112.5 - 117.5) ppm for the C2' and the C5'; δ_c 118.10 (117.5 - 120.5) ppm for the C6'; δ_c 90 - 110 ppm for the C8, the C6. It was re- ported (Lorenz *et al*., 2002) that the region between δ_c 70 and 90 ppm is sensitive to the stereochemistry of the flavonoid C-ring and that the signals centered at δ_c = 78.48 (75-78) ppm could be assigned to the C2 of cis (epicatechin) stereoisomers, while signals at δ_c = 82 - 84 ppm could be assigned to the C2 of trans (catechin) stereoisomers. The stronger intensity of the signal centered at δ_c 78.48 ppm clearly indicates that the cis stereoisomer dominates, which means that was constituted mainly of the epicatechin (Papa *et al*., 2013).

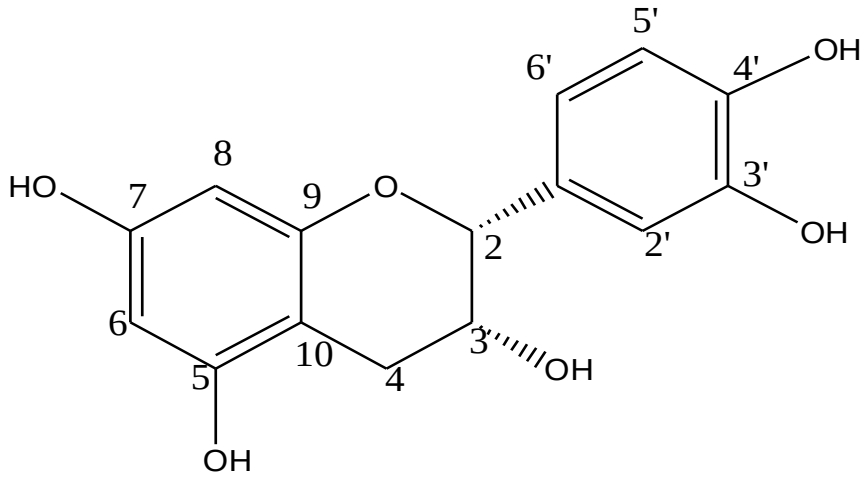


Fig. 5.1: Proposed structure: Epicatechin.

Comparison of the ^{1}H NMR and ^{13}C NMR with those previously reported for Epicatechin (Martin *et al*., 2000) revealed them to be in fully agreement as shown in Table 5.1.

Table 5.1 comparison of the ^{1}H NMR and ^{13}C NMR data of Epicatechin and compound X1 (CD_3OD)

Position	H^a	C^a	H^b	C^b
1	-	-	-	-
2	4.8	78.48	4.88 (t, *J* = 0.7 Hz)	79.3
3	4.2	66.10	4.19 (m)	66.8
4	2.8 (dd, *J* = 16.68 Hz, 4.56 Hz)	27.84	2.87 (dd, *J* = 16.6, 4.6 Hz)	29.0
	2.7 (dd, *J* = 16.68 Hz, 2,76 Hz)		2.74 (dd, *J* = 16.6, 2.9 Hz)	
5	-	156.2	-	157.5
6	5.95(d, *J* = 2.16 Hz)	94.60	5.92 (d, *J* = 2.2 Hz)	96.1
7	-	155.9	-	156.7
8	5.98 (d, *J* = 1.9 Hz)	98.10	6.02 (d, *J* = 2.2 Hz)	95.6
9	-	156.5	-	157.0

10	-	98.7		99.7
1’	-	130.9		132.1
2’	7.0 (d, *J* = 1.3 Hz)	114.0	7.05 (d, *J* = 1.95 Hz)	115.1
3’	-	144.37	-	145.3
4’	-	144.53		145.2
5’	6.8 (d, *J* = 8.2 Hz)	114.62	6.78 (d, *J* = 8.1 Hz)	119.2
6’	6.83 (d, *J* = 8.2 Hz)	118.10	6.83 (d, *J* = 8.1 Hz)	115.4

5.3 THE ANTIMICROBIAL PROFILE

The result of susceptibility tests on crude methanolic extract, chloroform and ethylacetate fraction showed strong inhibitory activity against all tested microoganisms (*Bacillus subtilis, Staphylococcus aureus, Streptococcus pyogens, Escherichia coli, Candida brusei, Shigella dysenteriae* and *Candida albicans*) with exception of *Corynebacterium ulcerans, Salmonella typhi and Proteus mirabilis* with zone of inhibition ranging from 20-31 mm. The inhibitory activity of crude methanol extract was between 20–24 mm, chloroform fraction range from 20–28 mm and ethylacetate from 24–32 mm. These concluded that among the three ethylacetate fraction has the highest activity against all the organism tested but compared to standard drugs Ciprofloxacin showed strongest inhibition activity against all the organism tested with exception of *Candida albicans and Candida brusei* while all the organism tested were resistant to Fluconazole with exception of *Candida albicans and Candida brusei.* Crude methanolic extract was found to have equal value MIC of 10 mg/ml and variable MBC with *Bacillus subtilis* having least value at 20 mg/ml

while *Staphylococcus aureus*,*Streptococcus pyogens, Escherichia coli, Candida brusei, Shigella dysenteriae* and *Candida albicans* have aqual value of 40 mg/ml.

Chloroform fraction have variably MIC value with *Bacillus subtilis* and *Shigella dysenteriae* having least MIC value of 10 mg/ml while S*taphylococcus aureus, Streptococcus pyogens, Escherichia coli, Candida brusei,* and *Candida albicans* have aqual value of 20 mg/ml, while the MBC result indicated that *Candida albicans* and *Candiida brusei* have MBC value of 40 mg/ml, *Bacillus subtilis, Staphylococcus aureus, Streptococcus pyogens, Escherichia coli* of 20 mg/ml and S*higella dysenteriae* of 10 mg/ml.

The ethylacetate fraction was found to have MIC value of 10 mg/ml for *Staphylococcus aureus, Candida albicans* and *Candida brusei* and that of *Streptococcus pyogens, Escherichia coli, Shigella dysenteriae, Bacillus subtIlis* having equal MIC value of 5 mg/ml, and the MBC was also found to have variable MBC value with *Escherichia coli, Shigella dysenteriae, Bacillus subtIlis* having least value of 10 mg/ml and that *Staphylococcus aureus, Candida albicans***,** *Candida brusei and Streptococcus pyogens* having equal value of 20 mg/ml.

These concluded that averagely, the extract was more susceptible against the gram positive bacteria than the gram negative bacteria which agree with the report that gram positive bacteria are more susceptible to plant extract than gram negative bacteria (Vlietinck *et al*., 1995; Rabe and Staden, 1997).

The isolation and identification of muscanone from *C. wightii,* by Fatope *et al*. (2003), was found to be active against *Candida albicans* while this research shows no activity to the

afore mention organism. These diffirences might be due to diffirence in climate, soil condition, age and period of plant collection.

The strong activity of the crude methanol extract, chloroform fraction and ethylacetate fraction against *Staphylococcus aureus* which is known to play a role in skin diseases (Srinivazsan *et al.,* 2001), shows that the plant *Commiphora mollis* can be a source of compound that may be effective in the treatment of skin infection. The MIC value of crude methanol extract, chloroform and ethylacetate fraction were all lower than MBC value for all the micro-organisms. This suggest that both crude, chloroform and ethylacetate fraction were bacteristatic at lower concentration and bactericidal at higher concentration. It has also reported that bactericidal effect of phenolic compounds viz tannin and flavonoids in plants are known to form complexes with peptidoglycans, sterols and other cell wall

components resulting in cell leakage (T'Hart *et al.,* 1988; T'Hart *et al.,* 1989). Thus since crude methanol extract contains flavonoids, saponin, tannin and terpenes, these may accout for its antibacterial activity.

CHAPTER SIX

6.0 CONCLUSSION AND RECOMMENDATIONS

6.1 CONCLUSION

It could be concluded from this study the presence of secondary metabolites in the stem-bark of *Commiphora mollis* namely flavonoids, saponins, tannins, steroids/terpenes could have been responsible for the antimicrobial activities against *Bacillus subtilis, Staphylococcus aureus ,Streptococcus pyogens, Escherichia coli, Candida brusei, Shigella dysentae* and C*andida albicans.* To the best of our knowledge this is first report of the isolation of Epicatechin from this plant *Commiphora mollis.* However, its isolation was reported from different plants such as *Pterocarpus marsupium* (Gairola *et al.,* 2010), *amao-mo tsako* (Martin *et al.,* 2000).

It has further confirmed that the plant extracts could be used for the treatment of various infections. The result validates the traditional use of this plant in treating microbial infections and suggests that .*Commiphora mollis* could be exploited for new potent antibiotics.

6.2 RECOMMENDATION

It is recommended that phytochemical studies be carried out on the stem-bark of plant to isolate tannins and saponins present in the more polar region of the extract.

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