ANTIBACTERIAL, ANTIFUNGAL AND PHYTOCHEMICAL SCREENING OF THE PLANT SPECIES *Lannea schweinfurthii* (ENGL.) ENGL.

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DECLARATION

This thesis is my original work and has not been presented for any other degree in any other universities or for any other award.

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We confirm that the work reported in this thesis was carried out by the candidate under our supervision.

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DEDICATION

To my parents, siblings, nieces and nephew for your continued love and support

ACKNOWLEDGEMENTS

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ABBREVIATIONS AND ACRONYMS

AIDS	Acquired Immune Deficiency syndrome
^{13}C NMR	Carbon-13 Nuclear Magnetic Resonance
^{1}H NMR	Proton Nuclear Magnetic Resonance
CC	Column Chromatography
COSY	Correlation Spectroscopy
d	Doublet
DCM	Dichloromethane
dd	Doublets of doublet
DEPT	Distortionless Enhancement by Polarization Transfer
EtOAc	Ethyl acetate
J	Coupling constant
m	Multiplet
MeOH	Methanol
MHA	Mueller Hinton Agar
MIC	Minimum Inhibitory Concentration
NA	Nutrient Agar
NMR	Nuclear Magnetic Resonance
PDA	Potato Dextrose Agar
ppm	Parts per million
PTLC	Preparative Thin Layer Chromatography
R_f	Retention factor
Rt	Retention time
s	Singlet
t	Triplet
TLC	Thin Layer Chromatography
UV	Ultra Violet
VLC	Vacuum Liquid Chromatography
WHO	World Health Organization
δ	Chemical shift
µ	Microgram

ABSTRACT

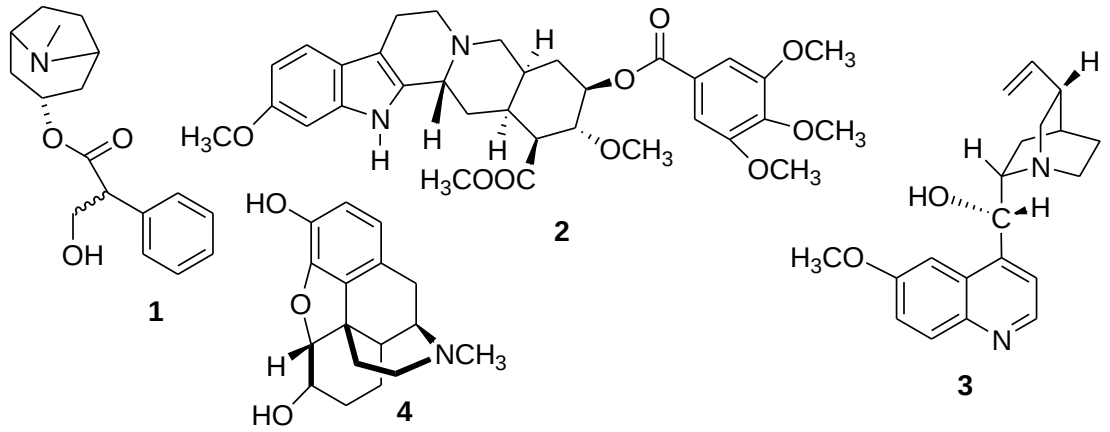
Herbal medicine has been widely used and forms an integral part of primary health care in most countries. Of late, despite emphasis being put in research of synthetic drugs; interest in medicinal plants has been reborn. This is due to the rapidly growing population, the failure of modern medicine to provide effective treatment, increase in chronic diseases and the emergence of the multi-drug resistant pathogens. It is of importance to establish a scientific basis for the use and validation of medicinal plants through biological screening. Phytochemicals are currently receiving more attention due to their effectiveness in the treatment of infectious diseases as well as mitigating many of the side effects caused by conventional antimicrobials. Pharmacological studies of *Lannea schweinfurthii* (Engl.) Engl. has revealed antimicrobial property of the plant but very little has been reported about the active ingredients in the plant. In this study the stem bark of *L. schweinfurthii* (Engl.) Engl. was collected, air-dried and ground into a fine powder. Sequential extraction was done with hexane, dichloromethane (DCM), ethyl acetate (EtOAc) and methanol (MeOH). The crude extracts were subjected to bioassay screening for their antibacterial activity against selected strains of bacteria, including Gram-positive *Staphylococcus aureus* and *Bacillus subtillis* and Gram-negative *Pseudomona aeruginosa* and *Escherichia coli* and antifungal activities against *Candida albicans*. Moderate and high activities, ranging from 11 to 21 mm inhibition zones, were observed for EtOAc and MeOH crude extracts against the microbes used except *P. aeruginosa*. Hexane and DCM crude extracts showed mild activity of 7 mm. Tetracycline and nystatin used as positive controls for bacteria and fungi, respectively had inhibition zones of 18 mm. GC-MS analysis was done on the crude DCM and MeOH extracts to give a preliminary idea of the class of compounds in the plant species. The spectral data obtained from the crude DCM and MeOH extracts indicated presence of phenolic compounds, fatty acids and their derivatives, terpenoids, polyketide derivatives and steroids in the plant. Purification of crude extracts was carried out using solvent partition and chromatography: CC, VLC and PTLC. Structural elucidation and characterization was done using standard spectroscopic methods (^{1}H NMR, ^{13}C NMR, DEPT and COSY). The combined hexane/DCM extract yielded two compounds namely; 3-(10'-tridecenyl) phenol (**LS01**) and di-(2'-ethylhexyl) ester phthalic acid (**LS05**). Ethyl acetate extract gave three compounds namely; lupeol (**LS02**), β-sitosterol (**LS03**) and epicatechin (**LS04**). Bioassay of the isolated compounds was done in which epicatechin (**LS04**) showed high activity against the Gram-positive bacteria *S. aureus* and *B. subtillis* and Gram-negative *E. coli*, with an inhibition zone of 15, 14 and 10 mm, respectively. Further, epicatechin (**LS04**) showed an activity of 14 mm against *C. albicans*. The study has demonstrated that the stem bark of *L .schweinfurthii* has chemical constituents that are bioactive. Further studies such as cytotoxicity tests should be carried out on the crude extracts and isolated compounds to ascertain reported activity so to use bioactive components either as antimicrobials or as templates in drug synthesis. Furthermore, measures should be put in place to conserve the plant species from extinction.

CHAPTER ONE

INTRODUCTION

1.1 Background

Traditional societies in Africa had devised methods of providing every individual in the community with essential healthcare through acceptable and accessible means by the application of indigenous resources such as plants, animals and minerals (Chhabra *et al*., 1987). Besides, herbal medicine takes into account every country's socio-cultural background. Of late, despite emphasis being put in research of synthetic drugs, a certain interest in medicinal plants has been reborn. This is partly due to the fact that many synthetic drugs are potentially toxic and not free of side effects on the host and that the effectiveness of many herbal medicines is now an accepted fact (Thomson, 1978; Geddes, 1985). Further, herbal preparations constitute valuable natural resource from which chemicals of great potential interest for medicine, agriculture, industry and other areas can be identified and isolated. Such valuable drugs such as atropine (**1**), reserphine (**2**), quinine (**3**) and morphine (**4**) were discovered from traditional herbal remedies (Sneader, 1985).



It is estimated that about 25% of the drugs prescribed worldwide are derived from plants and 121 such active compounds are in use (Sahoo *et al*., 2010). Between 2005 and 2007, 13 drugs derived from natural products were approved in the United States. More than 100 natural product-based drugs are in clinical studies (Li and Vederas, 2009), and out of the total 252 drugs in the World Health Organization's (WHO) essential medicine list, 11% are exclusively of plant origin (Sahoo *et al*., 2010).

This has necessitated microbiologists all over the world to search for formulation of new antimicrobial agents and evaluation of the efficacy of natural plant products as a substitute for chemical antimicrobial agents (Pandian *et al*., 2006). Many microorganisms and plants produce compounds that are not related to the basic metabolism of the producing organism called secondary metabolites as their defense mechanism. Many of these products play important roles as therapeutics and stimulants feed additives among others (Hans, 1993). Medicinal plants are well-known natural sources for the treatment of various diseases since antiquity. About 20,000 plant species used for medicinal purposes are reported by the World Health Organization (WHO) (Gullece *et al.*, 2006; Maregesi *et al*., 2008).

Furthermore, natural products, either pure compounds, or as standardized plant extracts, provide unlimited opportunities for new drug leads because of the unmatched availability of chemical diversity (Cos *et al.*, 2006). In the past, the wide range of antimicrobial agents from lower organisms and synthetic drugs sufficed in the treatment or control of infectious diseases, but currently there is a problem of microbial drug

resistance and there is an increase of opportunistic infections especially with acquired immune deficiency syndrome (AIDS) patients and individuals on immunosuppressive chemotherapy. Many antifungal and antiviral drugs are of limited use due to toxicity, while other viral diseases have not yet found a cure. These problems pose a need of searching for more new drug substances.

1.2 Phytomedicine

People all over the world have used plants as medicine from time immemorial. It is estimated by WHO that 80% of the population majority of this in developing countries, still rely on plant-based medicine for primary healthcare (Evans, 1997; Farnsworth, 1998). WHO further estimated that over 80% of the population residing in developing countries depends directly on plants for their primary medical requirements (Czygan, 1993; WHO, 2008). This is attributed to the fact that plant-derived medicines can be easily accessed and are also cheap (Amin and Mousa, 2007; Ramawat and Goyal, 2008; WHO, 2008). The use of some crude extracts whose specific evaluations have not been done could lead to serious complications, overdose and intake of toxic substances. Ineffective herbs could also be used as a matter of belief or tradition (Baker *et al*., 1995).

In most countries, use of herbal medicine continues to coexist with modern pharmacology (Ernest, 2005). The worldwide upsurge in the use of herbal preparations and active ingredients isolated from medicinal plants in healthcare (Jassim and Naji, 2003) is due to increased side effects, lack of curative treatment for several chronic

diseases, high cost of new drugs, microbial resistance and emerging diseases (Humber, 2002). This is indicated by the growing popularity of Traditional Chinese Medicine (TCM) and Ayurvedic medicine of India (Jiang *et al*., 2000; Dubey *et al*., 2004). In Africa, the use of traditional medicine is very high as indicated by the fact that between 70 and 80% of the population depend on herbal preparations for primary healthcare with little or no scientific information on efficacy and side effects (Kokwaro, 1996).

Furthermore, even the people of the developed world are also dependent directly or indirectly on plants for their health care. In the United States, 25% of the prescriptions given from community pharmacies consisted of plant extracts or active ingredients of plant origin (Cragg and Newman, 2005). In Dar es Salaam, Tanzania, 21% of patients who visited public hospitals had consulted a traditional healer before they came to hospital (de Boer *et al.*, 2005). Plant-derived medicines are offered/taken in the form of tinctures, teas, poultices and powders, depending on the knowledge of the use and application method of a particular plant for a given ailment (Fennell *et al*., 2004; Balunas and Kinghorn, 2005).

In Kenya, debate on incorporation of traditional medicine into primary health care has been going on for a while now. There is an attempt to make a law to regulate the practice of traditional medicine by way of legislation. The Traditional Health Practitioners Bill, 2014 was published on the February 2014 and was tabled before the National Assembly by the then Chairperson of the Health Committee Rachael Nyamai. The Bill went through the first reading on 3rd June, 2014 and the Committee further

tabled its report on the deliberations regarding the said bill on 25th April, 2015 but since then there has been no progress. If the Bill is passed into law it seeks to provide for the training, regulation and licensing of traditional practitioners so as to regulate their practice. Further, it seeks to set up traditional health practitioners' council which is a body mandated with regulation. It also seeks to set up a Disciplinary Committee that will discipline practitioners as well as setting offenses and penalties under the said law.

Most of the plants used traditionally for treatment of various ailments locally have not been studied scientifically for their efficacies and side effects (Kokwaro, 1993). Ethno-botanical information is slowly dying out due to lack of vertical transmission to young generation as people holding the information on these plants are fast dying out. There is also imminent loss of some of these plant species due to population pressure and over exploitation without proper conservation policies. Preparations, handling and storage of crude extracts from these plants could lead to decomposition or transformation into ineffective and/or harmful products (Baker *et al*., 1995). This necessitates the evaluation and establishment of a scientific protocol for use of traditional medicinal plants through chemical, pharmacological, toxicological and microbial studies. Kenyan scientists should therefore study the antimicrobial efficacies of these plants.

Data available indicate that, in Africa, medicinal plants can also be used to earn foreign exchange by exporting them to the western world (Busia, 2005). It has been documented that the huge pharmaceutical significance of many tropical medicinal plants could be utilized to generate foreign exchange as well as to create jobs for many

African countries. This shows that the vast field of medicinal plants on the African continent, if harnessed, could rescue the struggling healthcare systems of most such countries (Busia, 2005). This cannot be realized without biological studies of these plants to provide information on their efficacy and safety which is partly covered by this study.

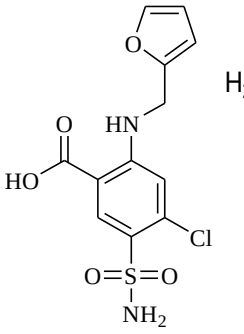
1.3 Antibiotics

With the discovery of microorganisms as the causative agents of infectious diseases, many substances including those of plant origin became recognized as “antiseptics”. These are substances having the ability to inhibit the growth of microorganisms. Later on, this term was changed to “antibiotics” from the term “antibiosis”, which describes antagonism between different species and even between members of the same species in nature. An antibiotic is a biochemical drug, derived from one or more kinds of microorganisms. It may have the ability to inhibit the growth of (bacterial static agent) or to kill (bactericidal agent) a number of other microorganisms. This makes antibiotics be of immense value in the treatment of a number of diseases that result from microbial infection (Amit and Shailendra, 2005).

Antibiotics, also known as antimicrobial drugs are therefore drugs that fight infections caused by bacteria, either by inhibiting the growth of or by destroying these microbes. Their therapeutic success relies on their pharmacokinetic behavior and the contribution that the host’s own defenses are able to make towards clearance of the offending microorganism (Amit and Shailendra, 2005).

1.3.1 Bacteriostatic agents

Bacteriostatic agents are substances that inhibit the growth of other microorganisms by interfering with bacterial protein production, bacterial DNA production and bacterial cellular metabolism. Bacteriostatic antibiotics inhibit growth and reproduction of bacteria without killing them and killing is done by the bactericidal agents. Examples of synthetic bacteriostatic agents include; sulphonamides like furosemide (**5**), trimethoprim (**6**), tetracycline (**7**), chloramophenicol (**8**) and erythromycin (**9**) and their derivatives among others (Sritharan and Sritharan, 2004).



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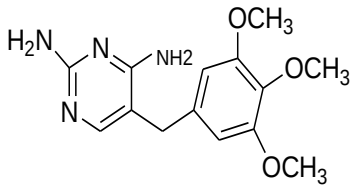

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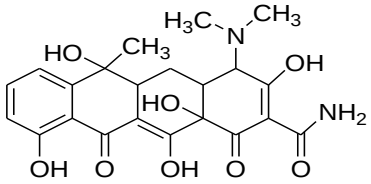

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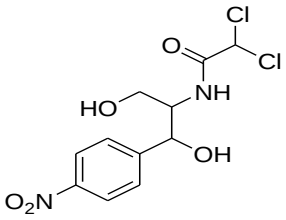

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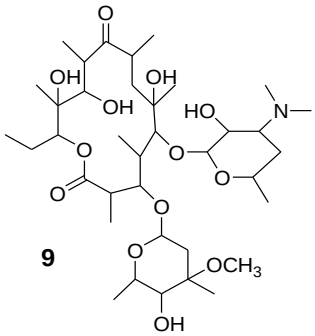

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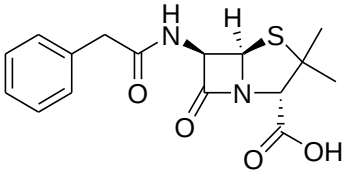
1.3.2 Bactericidal agents

These are substances that kill microorganisms and, preferably, nothing else. Bactericides are disinfectants, antiseptics or antibiotics. Members in this class include;

benzylpenicillin (**10**), cephalosporins like cefalexin (**11**) and amino-glycosides like gentamicin (**12**) (Sritharan and Sritharan, 2004).



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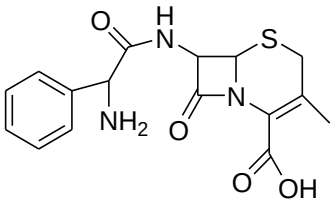

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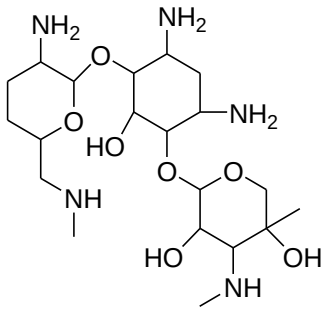

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Effective chemotherapy in the use of antibiotics depends upon selective toxicity, defined as the ability of the drug to inhibit microorganisms at concentrations tolerated by the host (Amit and Shailendra, 2005).

1.4 Active compounds in plant extracts

Plants produce biologically active secondary metabolites often with highly complex chemical structures. These compounds are concerned with survival of the plants and are known to have antimicrobial activity (Waterman, 1992). The compounds vary from plant to plant and are found to be in different concentrations in different parts of the same plant (Lawrence, 2000).

1.5 Statement of the problem

Indigenous communities have for a long time incorporated the use of traditional medicines, mainly from plant sources in the cure or lessening of impact of common ailments. In Kenya, quite a number of plants used in folklore medicine have been

identified and application of their crude extracts documented (Kokwaro, 1993). A lot of medicinal plants are available for the treatment of various diseases especially diarrhoea which is a major problem in the country (Muriithi, 1996). However, little information is known about the phytochemistry of the active ingredients of these plants.

The combined effects of the health-related challenges due to the rapidly growing population, emergence of antimicrobial resistant pathogenic strains to frequently used commercial drugs such as penicillin, increased side effects, the failure of modern medicine to provide effective treatment, high cost of new drugs and emerging diseases, research into natural products with anti-microbial activity is being pursued in earnest. It is of importance to establish a scientific basis for the use and validation of medicinal plants through biological screening. Systematic pharmacological studies of the genus *Lannea* have been done to some extent. However, quite a number of the species, including *Lannea schweinfurthii*, call for further screening to determine the active principles, their efficacy and mechanism of their action. Moreover, little information is available on the phytochemistry of *Lannea schweinfurthii*.

This study sought to isolate, elucidate structures and determine bioactivity of both crude and pure secondary metabolites in the stem bark of *L. schweinfurthii* (Engl.) Engl. plant of the family Anacardiceae.

1.6 Hypotheses

i. Extracts of *L. schweinfurthii* contain compounds that exhibit antibacterial and antifungal activity.
ii. These compounds are stable and can be isolated, purified and their structure elucidated.
iii. The compounds isolated remain biologically active once isolated from the plant.

1.7 Objectives

1.7.1 General objective

The study was aimed at investigating the antibacterial and antifungal components from the stem bark of *L. schweinfurthii*.

1.7.2 Specific objectives

The specific objectives of the study were;

i. To determine antibacterial and antifungal activity of stem bark crude extracts of *L. schweinfurthii* against selected strains of bacteria and fungi by *in vitro* assays.
ii. To determine the structures of compounds isolated from *L. schweinfurthii* by use of spectroscopic methods.
iii. To determine antibacterial and antifungal activity of the isolated compounds by *in vitro* assays.

1.8 Justification and significance of the study

More than 80% of the population living in developing countries depends directly on herbal drugs and traditional medicines for primary healthcare (WHO, 2008). Many of the drugs which are in use today were discovered through their ethno-botanical route (Kareru *et al*., 2007). Moreover, pathogen resistance to existing drugs coupled with increased side effects, lack of curative treatment for several chronic diseases, high cost of drugs and emerging diseases is very common in today's world (Humber, 2002). This makes it necessary to research on medicinal plants in order to obtain more and potent pharmacological agents owing to the fact that natural products and their related moieties have historically been incredible as a source of therapeutic agents (Koehn and Carter, 2005).

Bacterial and fungal infections still pose a problem in Kenya especially in rural areas where medicinal plants are mostly used as part of healthcare system. *Lannea schweinfurthii* species used by communities such as the Luo and Meru in Kenya for traditional therapy of these infections have not been fully investigated phytochemically for antimicrobial agents (Gathirwa *et al*., 2008).

The findings of this study are expected to provide information on the phytochemistry of the main bioactive chemical constituents in the stem bark of *L. schweinfurthii* that can be evaluated for the treatment of antimicrobial diseases and provide templates for synthesis of new drugs.

CHAPTER TWO

LITERATURE REVIEW

2.1 The family Anacardiaceae

The family Anacardiaceae consists of plants usually producing gums, resins or latex whose leaves are alternate and without stipules. Alkylhexenones, tannins and some phenolic compounds are widely distributed in this family (Kapche *et al*., 2007). The family characteristically contains highly hydroxylated compounds like quercetin, kaempferol and their methoxylated derivatives. Tannins are common while saponins are rare but alkaloids and iridoids are absent. Alkylhexenones have been reported in *L. welwittschii* and in *Tapirira,* a genus from the family Anacardiceae (Groweiss *et al*., 1997; Correia *et al*., 2001; Queiroz *et al*., 2003). Several alkyl phenols were isolated from genus *Mangifera, Melanorrhea* and *Panopsis* of the same family (Du *et al*., 1986; Deng *et al*., 1999).

According to the already classical definition of Freudenberg (1932), Anacardiaceae is a family of plant tannins. An example of tannin (**15**) and the other two acids namely; 3-hydroxybenzoic acid (**19**) and 3,5-dihydroxybenzoic acid (**20**) have already been reported in the Genus *Mangifera* and *Rhus* from the Anacardiaceae (Werner *et al*., 2004). Compounds (**14**) and (**15**) were reported in green tea, *Camellia sinensis,* leaf from the family Theaceae (Coxon *et al.,*1972), 3,4,5-trimethoxyphenol (**16**) and its glycoside (**17**) in the genus *Cotoneaster* and *Rosa* in the family Rosaceae (Hiroyuki *et al*., 2006) and compound (**17**) in the genus *Cotoneaster* and *Rosa* of the family Rosaceae. These three families are also known to be the rich source of tannins and

phenolic compounds and the isolation of the four compounds (**14**, **16**, **17** and **18**) in the family Anacardiaceae is particularly interesting since it strengthens the chemotaxonomic relationship of the these four families (Kapche *et al*., 2007).

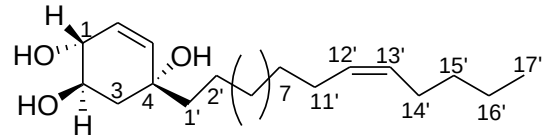
2.2.1 The Genus *Lannea*

Lannea is a genus of trees or shrubs with pinnate leaves often with star-shaped hairs (Agnew and Agnew, 1994). The genus *Lannea* comprises of deciduous plants with tough bark often used for string. In Kenya, there are ten known species of genus *Lannea* namely: *L. alata, L. fulva, L. greenwayi, L. humilis, L. malifolia, L. rivae, L. schimperi, L. schweinfurthii, L. triphylla* and *L. welwitschii.*

2.2.2 Chemical systematics and bioactivity of Genus *Lannea*

The chemical investigation of the Genus *Lannea* has led to the isolation of various secondary metabolites, including alkylphenols, alkylhexenones, flavonoids, tannins and several benzoic acid derivatives (Groweiss *et al*., 1997; Islam and Tahara, 2000; Queiroz *et al*., 2003). The extract from the stem bark of *L. coromandelica* showed significant zoosporicidal activity (Islam *et al.*, 2002) and the bioassay-guided fractionation and chemical characterization of this extract revealed that the active components were angular type polyflavonoid tannins (Islam and Tahara, 2000). The different parts of *L. veluntina* were evaluated for their anti-fungal, larvicidal, molluscidal, anti-oxidant and radical scavenging properties (Diallo *et al*., 2001). In another pytochemical study of *L. nigritana,* in Cameroon, Lanneanol (**13)** 4-(heptadec-

12-enyl) cyclohex-5-ene-1,2,4-triol was reported (Kapche *et al.,* 2007). Compounds (**14 - 20**) were also isolated from *L. nigritana* (Sc. Elliot) Keay (Kapche *et al.,* 2007).



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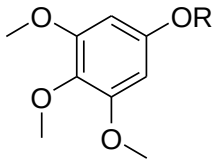
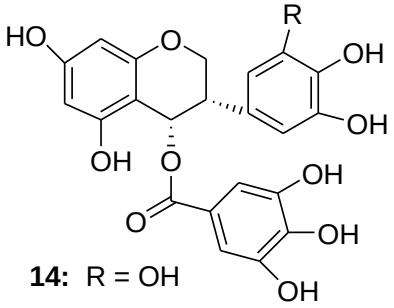
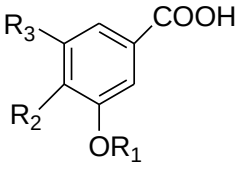


16: R = H
17: R = Glucose



14: R = OH
15: R = H

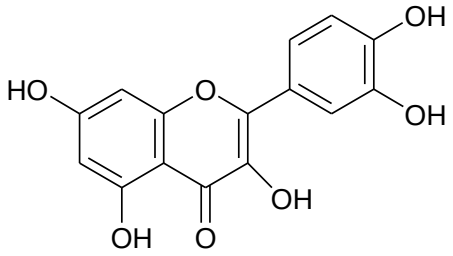


18: $R_1 = CH_3$, $R_2 = H$, $R_3 = H$
19: $R_1 = H$, $R_2 = H$, $R_3 = H$
20: $R_1 = H$, $R_2 = H$, $R_3 = OH$

Other plants with medicinal properties in the genus *Lannea* include *L. microcarpa, L. alata, L. edulis, L. schweinfurthii, L. schimperi L. nigritana and L. coromandelica* among others. Phytochemical studies on *Lannea welwitschi* revealed presence of saponins and anthraquinones in the aqueous bark extracts (Amiram *et al*., 1997). They also showed inhibitory action on gastrointestinal morbidity (Hirschhorn, 1980; Muriithi, 1996; Farthings, 2002).

Further, phytochemical and antimicrobial studies of *L. coromandelica* revealed presence of flavonols (Subramanian and Nair, 1971). 2-(3',4'-dihydroxy phenyl)-3,5,7-trihydroxy-4*H*-chromen-4-one (quercetin) (**21**), (2R,3S)-**(+)**-3',5-dihydroxy-4',7-dimethoxydihydroflavonol (**22**), (2R,3R)-(+)-4',5,7-trimethoxydihydroflavonol (**23**), (2R,3R)-(+)-4',7-di-O-methylhydroxyquercetin (**24**), (2R,3R)-2,3-dihydro-3,5-dihydroxy-7-methoxy-2-(4'-methoxyphenyl)chromen-4-one (**25**) and (2R,3R)-2,3-

dihydro-3,5,7-trihydroxy-2-(3'-hydroxy-4'-methoxyphenyl)chromen-4-one (**26**) were isolated for the first time from the stem bark and their structures elucidated by spectroscopic methods (Islam and Tahara, 2000).



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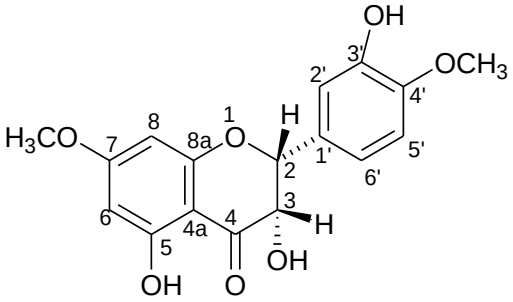

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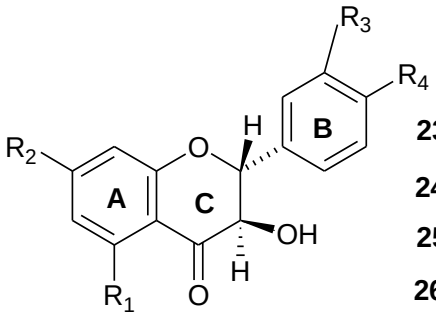


23: $R_1 = OCH_3$, $R_2 = OCH_3$, $R_3 = H$, $R_4 = OCH_3$

24: $R_1 = OH$, $R_2 = OCH_3$, $R_3 = OH$, $R_4 = OCH_3$

25: $R_1 = OH$, $R_2 = OCH_3$, $R_3 = H$, $R_4 = OCH_3$

26: R1 = OH, $R_2 = OH$, $R_3 = OH$, $R_4 = OCH_3$

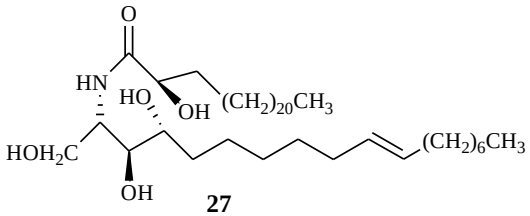
Quercetin (**21**) is a naturally occurring flavonoid found in high concentrations in red wines, onions and green tea. The OH groups in the phenolic compounds such as quercetin are much more reactive and can easily form hydrogen bonds with active sites of enzymes. Leuck (1980) reported that the antimicrobial action of phenolic compounds was due to the inhibition of certain enzyme reactions or enzyme synthesis in the microbial cell or synthesis of important cell constituents. El-Gammal and Mansour (1986) reported that quercetin (**21**), was successful in inhibiting microbial growth of various pathogens used in medicinal and industrial fields such as *Staphylococcus aureus* and various *Bacillus* species. It has been reported that therapy with quercetin (**21**)

provides significant symptomatic improvement in most men with chronic pain syndrome (Shoskes *et al*., 1999). Quercetin (**21**) has demonstrated significant anti-inflammatory activity because of direct inhibition of several initial processes of inflammation. For example, it inhibits both the manufacture and release of histamine and other allergic/inflammatory mediators. In addition, it exerts potent anti-oxidant activity and vitamin C- sparing action. It may have positive effects in combating or helping to prevent cancer, prostatits, heart disease, cataracts, allergies/inflammations and respiratory diseases such as bronchitis and asthma. Quercetin is contraindicated with some antibiotics; it may interact with fluoroquinolones (a type of medicinal antibiotic), as it competitively binds to bacterial Deoxyribonucleic acid (DNA) gyrase (Hilliard, 1995).

The Chagga people of northern Tanzania gargle an extract of the bark of a *Lannea* spp for the relief of throat conditions. The Nyamwezi people of Tanzania apply a paste of *L. stuhlmanii* as a dressing to sores, boils and abscesses. In Tanzania, a decoction of the root bark is taken for dysentery (Watt and Breyer-Brandwijik, 1962). An ethanol extract of *L. coromandelica* bark showed hypertensive activity in anaesthetized dogs and rats (Singh and Singh, 1996). The fruits paste of *L. coromandelica* is therapeutically used for bone fractures by the tribes in eastern Ghat of Andhra Pradesh (Venkata and Venkata, 2008).

Additionally, in order to further explore and make use of Chinese herbal source, a study of the chemical constituents from the barks of *L. coromandelica* was done and

compounds (**27 - 38**) were isolated (Yun *et al*., 2014). The compounds included: (2S,3S,4R,10*E*)-2-[(2'R)-2'-hydroxytetracosanoyl amino]-10-octadecene-1,3,4-triol (**27**), aralia cerebroside (**28**), 5,5'-dibuthoxy-2,2'-bifuran (**29**), β-sitosteryl-3β-glucopyranoside-6'-*O*-palmitate (**30**), β-sitosterol palmitate (**31**), myricadiol (**32**), protocatechuic acid (**33**), *p*-hydroxybenzoic acid ethyl ester (**34**), isovanillin (**35**), *trans*-cinnamic acid (**36**), palmitic acid (**37**), and stearic acid (**38**). Furthermore, compounds **28 – 33** and **35 – 38** had not been reported in any species in *Lannea* (Yun *et al*., 2014).



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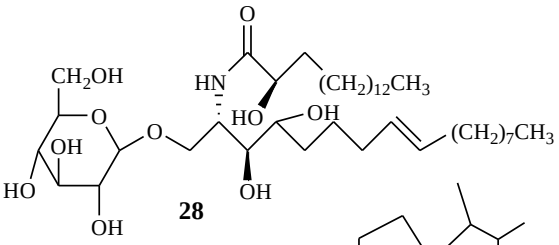

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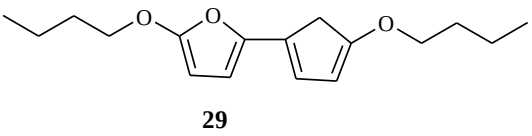

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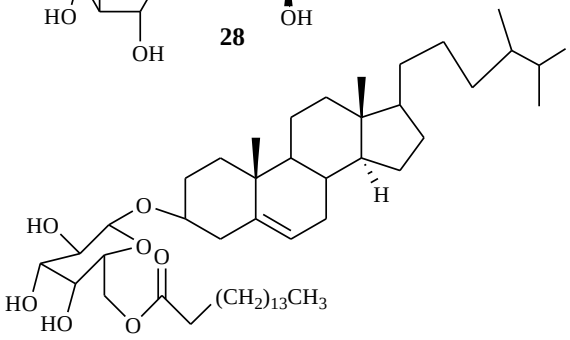

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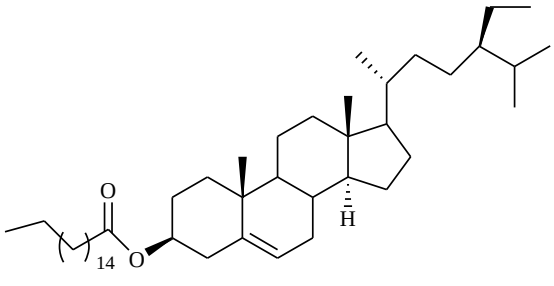

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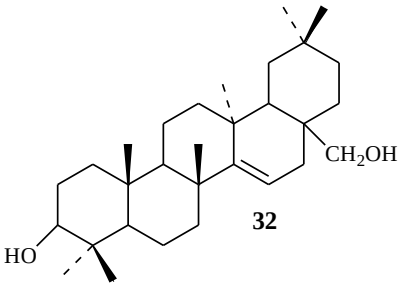

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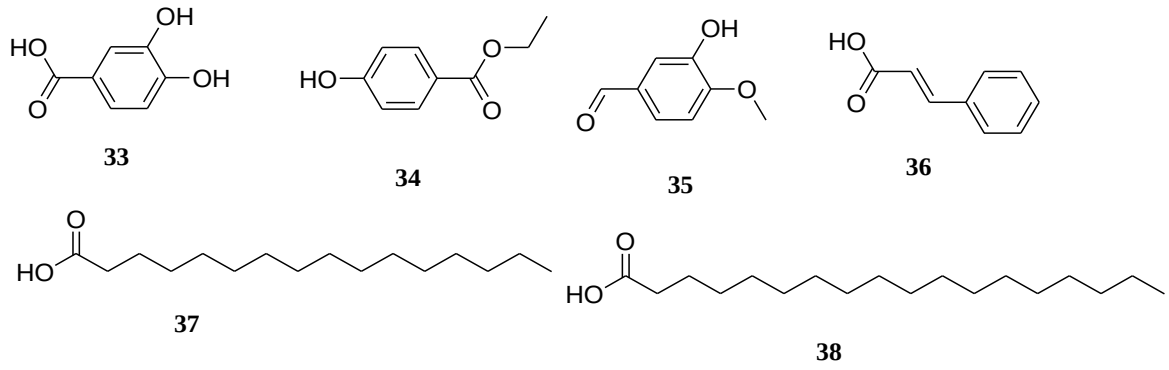


2.3.1 *Lannea schweinfurthii* (Engl.) Engl.

Lannea schweinfurthii (Figure 2.1) is a deciduous shrub or tree of height 3–15m, with spreading crown and drooping branches, growing in wooded grassland, bushed grassland, semi-evergreen bush land, and dry forest woodland (Beentje, 1994). It is known by local names; Muyumbu-Maji (Swahili, Giriama), Ile Tile (Borana), Muraci (Embu), Mwethi Muasi (Kamba), Chepchai, Goinyet (Kipsigis), Bongo, Kuongo (Luo), Oropando (Maasai), Muhandarako (Pokomo), Moino (Pokot), Den (Somali), Rubandi (Taveta), and Mwamo (Tugen) (Beentje, 1994).

Figure 2.1: A photograph of a flowering branch of *Lannea schweinfurthii*

Source: Bingham *et al*. (2016).

2.3.2 Medicinal uses of *Lannea schweinfurthii*

Decoction of the roots is taken as a remedy against stomach ache, fever, dysentery, asthma, with water it is taken against tuberculosis (TB) while with porridge it is taken against feminine sterility (Chhabra *et al*., 1987). Decoction of the stem bark is taken as a remedy against headache, diarrhoea and stomach ache. The bark is also used for making tea that is used as a blood tonic for treating anaemia, to increase hemoglobin (HB) in the patients (Ruffo *et al*., 2002). The leaves are used to relieve abdominal pains and to hasten child birth (Kokwaro, 1976). The plant extract is used to dress sores, boils and carbuncles (Watt and Beyer-Brandwijk, 1962).

In Kenya, the plant extracts have been used traditionally for the treatment of malaria by the Meru community. The plant was tested for *in vitro* anti-plasmodial and *in vivo* anti-malarial activity against *P. falciparum* and *P. berghei*, respectively. Aqueous extracts had the highest anti-plasmodial activity while the methanolic extracts had the highest anti-malarial activity. Combinations of *L. schweinfurthii* and other plant extracts exhibited high malaria parasite suppression and showed good *in vitro* synergistic interactions (Gathirwa *et al*., 2008). Further, the plant extracts have been used by Luo community in the treatment of infectious diarrhoea (Geissler *et al*., 2002) and constipation whereby the extracts exhibited high anti-giardial activity against *Giardia intestnali* (Johns *et al*., 1995). Other biological activities of *L. schweinfurthii* that have been reported include high anti-viral activity (Maregesi *et al*., 2008), HIV-2 activity (Maregesi *et al*., 2010), mild cytotoxicity (Gathirwa *et al*., 2011) and noteworthy radical scavenging activity and good acetylcholinesterase inhibition activity (Adewusi and Steenkamp, 2011).

2.3.3 Other uses of *Lannea schweinfurthii*

The tree (Figure 2.2) supplies a number of commodities that are used by the local people. The fruit is edible; the bark is used for making tea, rope and a red-brown dye. The wood provides timber used to make stools and grain pestles and Embu blacksmiths used to prefer charcoal made from this tree for iron smelting. The plant is an important source of thread for sewing ropes, sacks and bags. It provides fruits and berries and the Kamba of Mwingi use its fruits as food alternative in dry areas (Beentje, 1994).

Figure 2.2: A branch of *L. schweinfurthii* with fruits

Source: Bingham *et al*. (2016).

2.4 Bioassay

2.4.1 Role of bioassay in isolation of pure active compounds from plants

Bioassays are methods used in the estimation of the nature, constitution or potency of a material by means of a reaction that follows its application to living matter. The typical bioassay involves a stimulus (a drug or fungicide) applied to a subject (an animal, a piece of animal tissue or a bacterial culture). The intensity of the stimulus may be varied, generally, in accordance with the wish of the investigator. Bioassays are usually

comparative, the estimate of potency being obtained relative to a standard preparation of the stimulus (Finney, 1964). In research with plants, bioassay is usually done starting with the crude extract to find out if it has the desired effects or not; and if there are more than one fractions, which one shows more activity.

2.4.2 Antimicrobial assays

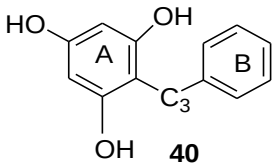
Two principal methods of determining antimicrobial activity (*in vitro*) are diffusion and dilution methods. The diffusion method involves the use of a filter paper disc, a porous cup, or a bottomless cylinder containing measured quantities of drug, which is placed on a solid medium in a petri dish (NCCLS, 2003). After incubation, the diameter of the clear zone of inhibition surrounding the deposit of drug is taken as a measure of the inhibitory power of the drug against the particular test organism (Jawetz *et al*., 1966; Mckane and Kandel, 1996). The disc diffusion method is convenient, technically simple, cheap and reasonably reliable. It was extensively used in this study.

Dilution methods are employed when determining the minimum inhibitory concentration (MIC) of drugs. This is the smallest amount of the drug that inhibits the multiplication of the pathogen. MIC is usually determined by a broth dilution method either in test tubes or in panels of small wells (NCCLS, 2003). A standard inoculum of the pathogen is incubated in a series of tubes or wells containing decreasing concentrations of the drugs or samples being tested. If the drug inhibits the microbe at the concentration in the tube, no growth appears. The organism grows only in concentrations below the one required for inhibition. Therefore, the highest dilution

(lowest concentration) showing no visible growth is the (MIC) (Mckane and Kandel, 1996). The *in vitro* testing of antifungal agents is similar in design to those of antibacterial agents, that is, serial dilutions and agar diffusion tests (Shadomy *et al*., 1985).

2.5 Flavonoids

Flavonoids and their conjugates form a very large group of natural products. They are found in many plant tissues, where they are present inside cells or on the surfaces of different plant organs. Flavonoids are primarily 15-carbon skeleton compounds composed of two benzene rings that are usually phenolic and connected by a 3-carbon unit. The chemical structures of this class of compounds are therefore based on a C_6-C_3-C_6 skeleton (Machocho, 1992). Different substituents can occur on the basic skeleton and thereby contribute to the diversity of flavonoids (Lin *et al*., 2001; López-Lázaro, 2002; Lia *et al*., 2007). A-ring is characteristically of the phloroglucinol (**40**) or resorcinol (**41**) hydroxylation pattern. B-ring is usually 4-, 3,4- or 3,4,5- hydroxylated but may occur unsubstituted (Geissman and Crout, 1969).



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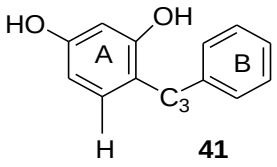

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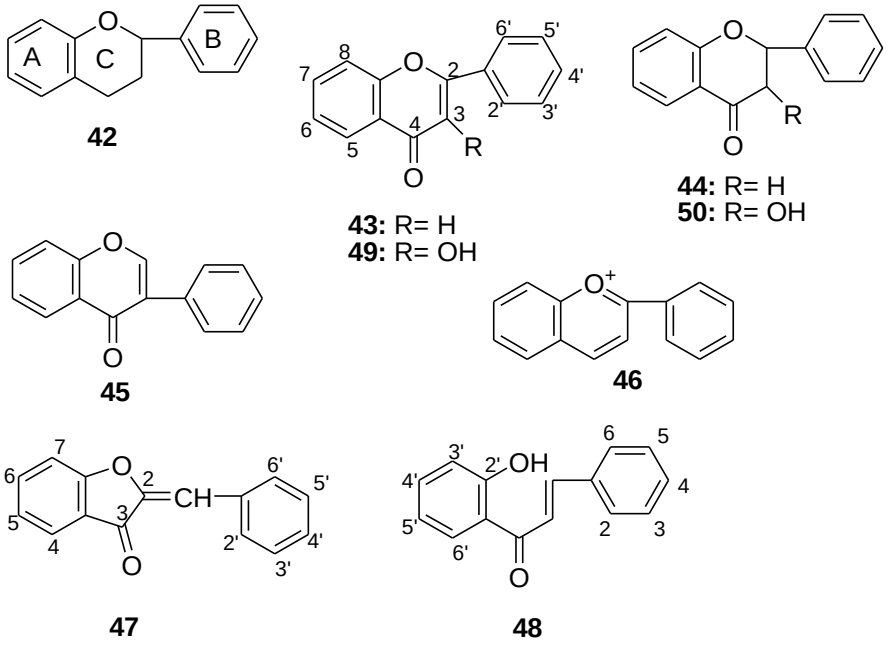
The arrangement and oxidation level of the central three-carbon portion brings about the main classification of flavonoids. A-ring has a hydroxyl group at the *ortho* position that is so situated as to provide the information of the heterocyclic ring in tricyclic

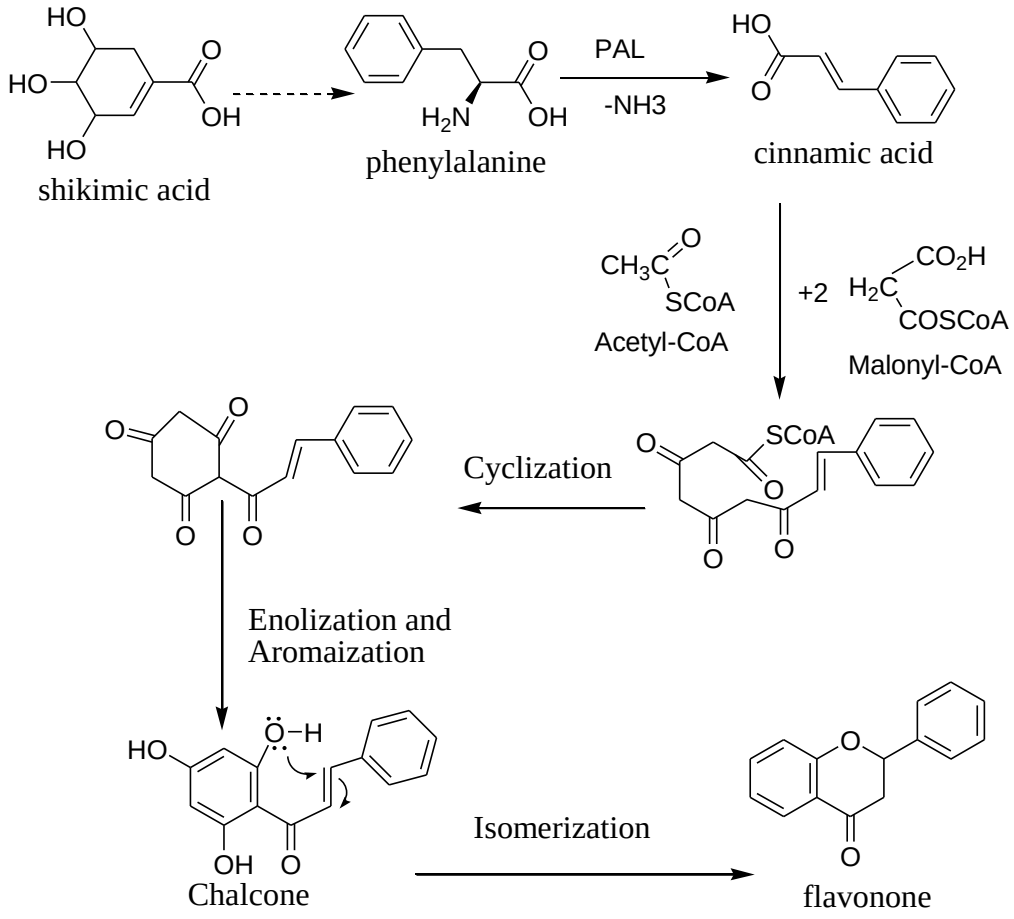
compounds. Flavans (**42**), flavones (**43**), flavanones (**44**), isoflavones (**45**) and anthocyanidines (**46**) have a pryan ring while aurones (**47**) have a furan ring. In chalcones (**48**), the hydroxyl group persists but may be derivatized where the hydrogen of the hydroxyl is replaced with an alkyl group or a sugar molecule (Geissman and Crout, 1969). Flavonols (**49**) and flavononols (**50**) which form classes of their own are the oxidized forms of flavones (**43**) and flavanones (**44**), respectively. In both cases the hydrogen at the carbon-3 of the pyran ring is replaced with a hydroxyl group.



2.5.1 Biosynthesis of flavonoids

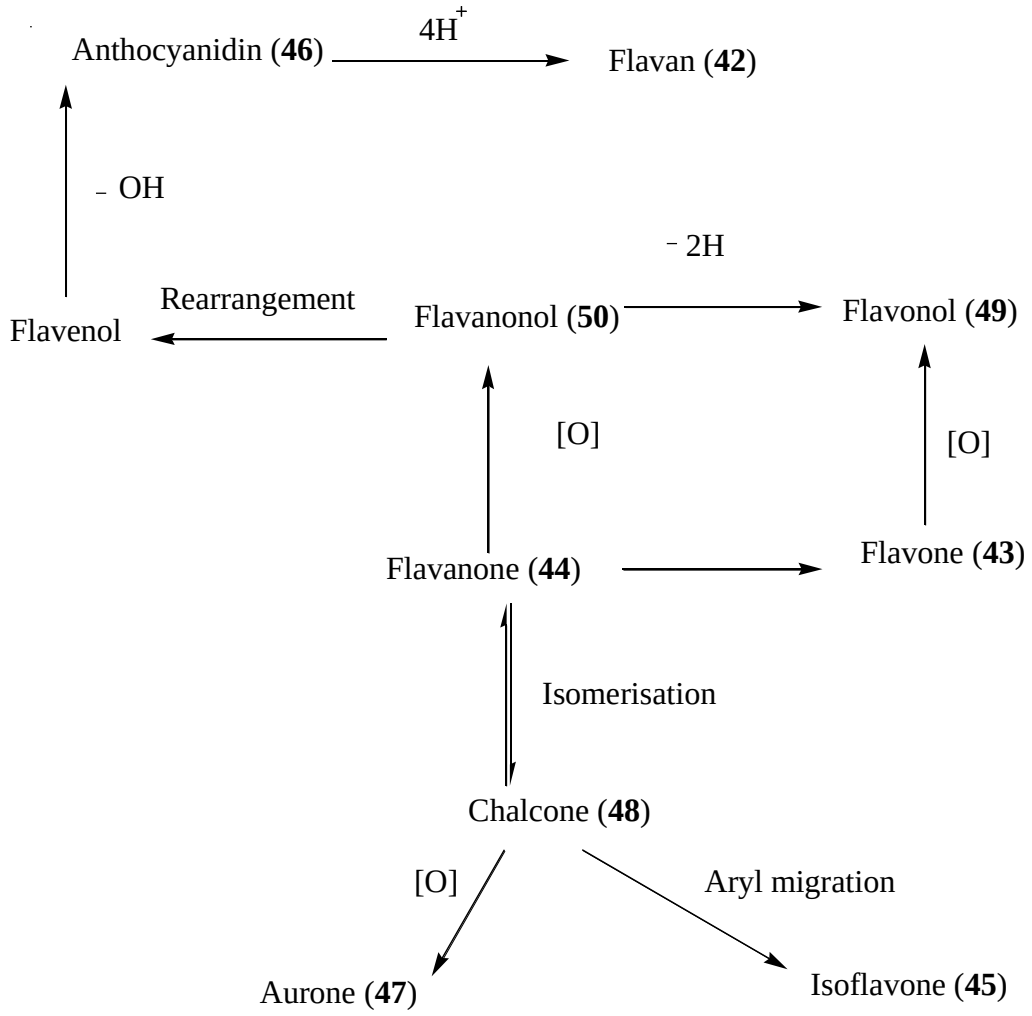
All flavonoids are related by a common biosynthetic pathway. The biosynthesis can be considered in three stages namely: synthesis of the 15-carbon skeleton, modification of the central three-carbon unit and derivatization of each of the flavonoid classes (Harborne, 1967; Ebel and Hahbrock, 1982). Evidence for the biosynthesis routes leading to flavonoids has come from different experimental approaches like genetic

studies and comparison of closely related chemical structures. However, the major contribution has come from tracer studies, where ^{14}C-labelled precursors are fed to plants. This has shown that A-ring of the flavonoids is formed by condensation of acetyl-CoA with two molecules of malonyl-CoA to yield the phlorogucinol structure (Harborne, 1967). The other $C_3 – C_6$ unit is derived from shikimic acid pathway through cinnamic acid (Harborne, 1967) or coumaric acid (Ebel and Hahbrock, 1982). The flavonoid initially formed in biosynthesis is thought to be the chalcone. The other flavonoids encountered in nature can be derived by modifying the chalcone in different ways. Biosynthesis of flavonoids is outlined in scheme 2.1.



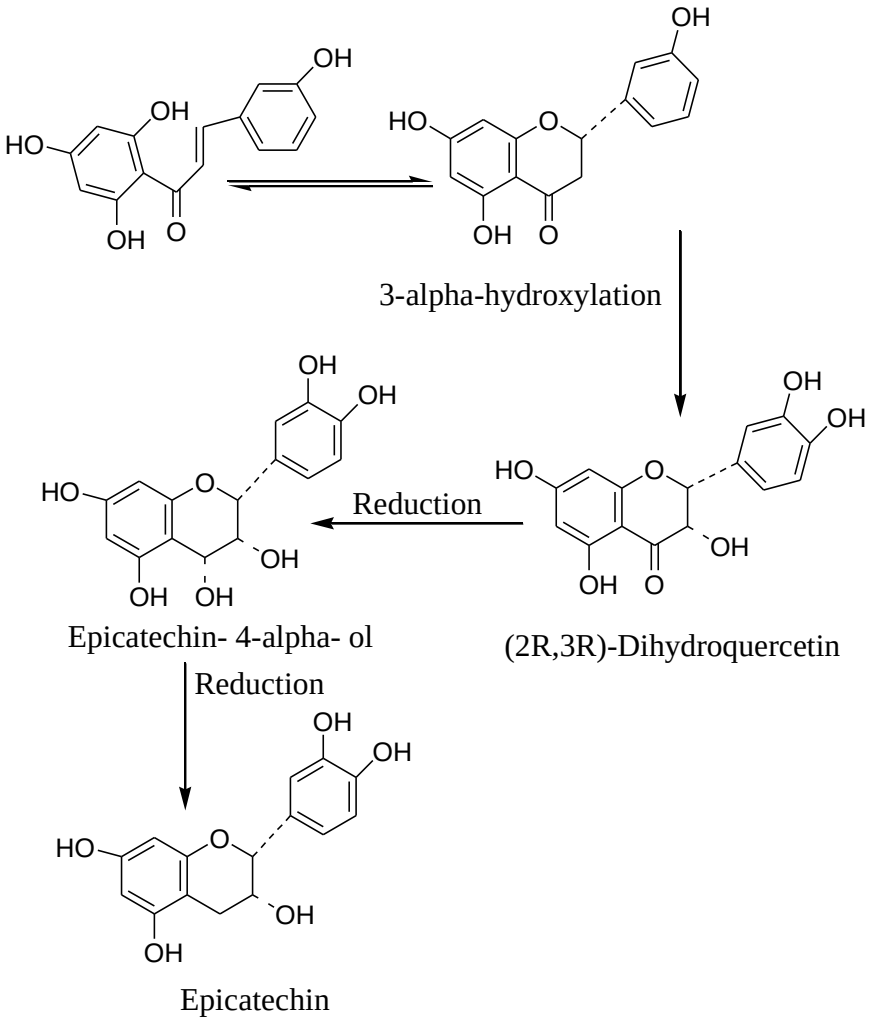
Scheme 2.1: Biosynthesis of 15 – carbon skeleton of flavonoid (Harborne, 1967).

Scheme 2.2 shows the inter conversions of flavonoid compounds. Flavans are formed through flavanones (scheme 2.3).



Scheme 2.2: Flavonoid inter- conversion from chalcone (Harborne, 1967).

Further modification of the flavonoids is by hydroxylation, dehydroxylation, methylation of the hydroxyl groups, alkylation of the flavonoid nucleus and glycosylation to form *o*-glycosides, *c*-glycosides are formed by glycosylation of the flavonoid nucleus. Biosynthesis of epicatechin flavonoid is outlined in scheme 2.3.



Scheme 2.3: Biosynthesis of epicatechin (Harborne, 1986)

2.5.2 Structural elucidation of flavonoids

Once a plant constituent has been isolated and purified, the class of compound to which it belongs is determined from its solubility, R_f values and its UV spectral characteristics. Purity of the compound is first checked by ensuring that it travels as a single spot in several thin layer chromatography (TLC) solvent systems. Complete identification depends on measuring other properties of the compound and then comparing these

properties with those in the literature. These properties include; melting point, optical rotation (for optically active compounds) and spectral characteristics such as ultra-violet (UV), infrared (IR), nuclear magnetic resonance (NMR) and mass spectral (MS) measurements (Harborne, 1998). The most important analytical methods for flavonoids are NMR, MS and UV (Mark and Chari, 1986).

2.5.2.1 Nuclear Magnetic Resonance (NMR)

Both ^{1}H-NMR and ^{13}C-NMR are useful analytical methods. NMR spectral data is always given in support of the structural assignment of flavonoids. ^{1}H-NMR gives information about the structural environment of each proton while ^{13}C-NMR is for the carbon backbone of the molecule. It is complementary to ^{1}H-NMR in the structural analysis of flavonoids.

2.5.2.1.1 2D-NMR

Two dimensional NMR technique may be used to assess the correlations between nuclear in a compound. The experiments include:

i. COSY; COrrelated SpectroscopY. This shows which proton couple with which proton. Thus on each axis there is ^{1}H-NMR spectrum and cross peaks indicate coupling between protons (Aue *et al*., 1976). This method has been used widely for sequencing of sugars.

ii. NOESY; Nuclear Overhauser Enhancement SpectroscopY. This is useful for establishing the stereochemistry and conformation of molecules and give cross peaks due to NOE effects through space and useful location of appended groups

or point of linkage. It is independent of direct bonding and requires about 5 mg of the sample (Bodenhausen *et al*., 1984). It is used in NMR spectral of large molecules (Abraham *et al*., 1995).

iii. HOHAHA; Homonuclear Hartman-Han spectroscopy. This detects complete spin system when long delay time is used and for directly coupled protons if short delay time is taken. It is useful for one dimensional selective spectra and it may require a very small amount of sample (Summers *et al*., 1986).

iv. HETCOR; Heteronuclear correlation 2D-spectroscopy spectrum shows all protons to which carbon atoms are attached. Thus on one axis is the ^{1}H-NMR while the other is the ^{13}C-NMR spectrum. Signals indicate a direct coupling of the protons with the carbon. Geminal protons can easily be recognized and requires about 1mg of the sample (Agrawal, 1992).

v. HMQC; Heteronuclear Multiple Quantum Coherence. This gives essentially the same information as HETCOR but has higher sensitivity. Thus it requires special probe and about 1mg of the sample (Summers *et al*., 1986).

vi. HMBC; Heteronuclear Multiple Bond Connectivity. Like the HETCOR and HMQC, on one axis is ^{1}H-NMR spectrum and on the other ^{13}C-NMR spectrum. Cross peaks are shown due to ^{2}JCH and ^{3}JCH and sometimes ^{4}JCH long range coupling. Useful method to connect different spin systems, location of appended groups in oligosaccharides and establishment of glycosidic linkage (Agrawal, 1992). It has higher sensitivity due to detection through protons instead of carbons and requires about 5 mg of the sample (Summers *et al*., 1986).

vii. COLOC; Correlation spectroscopy via long range Coupling. This can give long range proton-carbon coupling. It has lower sensitivity than HMBC and about 30mg of the sample is required (Kessler *et al*., 1985).

viii. INADEQUATE; Incredible Natural Abundance Double Quantum Transfer Experiment. It has on one axis ^{13}C-NMR while on the other axis the double quantum frequencies. Direct carbon-carbon coupling is indicated by a pair of doublets at certain double quantum frequency. It requires two adjacent ^{13}C nuclei for it to function and about 10mg of the sample is required (Englert, 1985).

ix. J-resolved spectroscopy. This is useful for crowded spectra. The chemical shifts are on one axis while the coupling information is on the other axis. It can be obtained for both ^{13}C-NMR and ^{1}H-NMR using about 10 and 0.5mg of the sample, respectively (Agrawal, 1992).

x. However, for complete structural elucidation of any compound normal ^{1}H-NMR spectra, decoupled ^{13}C-NMR/ DEPT, COSY spectrum, HETCOR or HMBC or HMQC and NOESY spectrum are sufficient.

2.5.2.2 Mass spectroscopy (MS)

The method requires only microgram amounts of material and can provide an accurate molecular weight of the compound and yield a complex fragmentation pattern which is often characteristic of that particular compound (Silverstein *et al*., 1991). The information leads to determination of the overall skeleton of a molecule.

2.5.2.3 Ultra-Violet (UV) spectroscopy

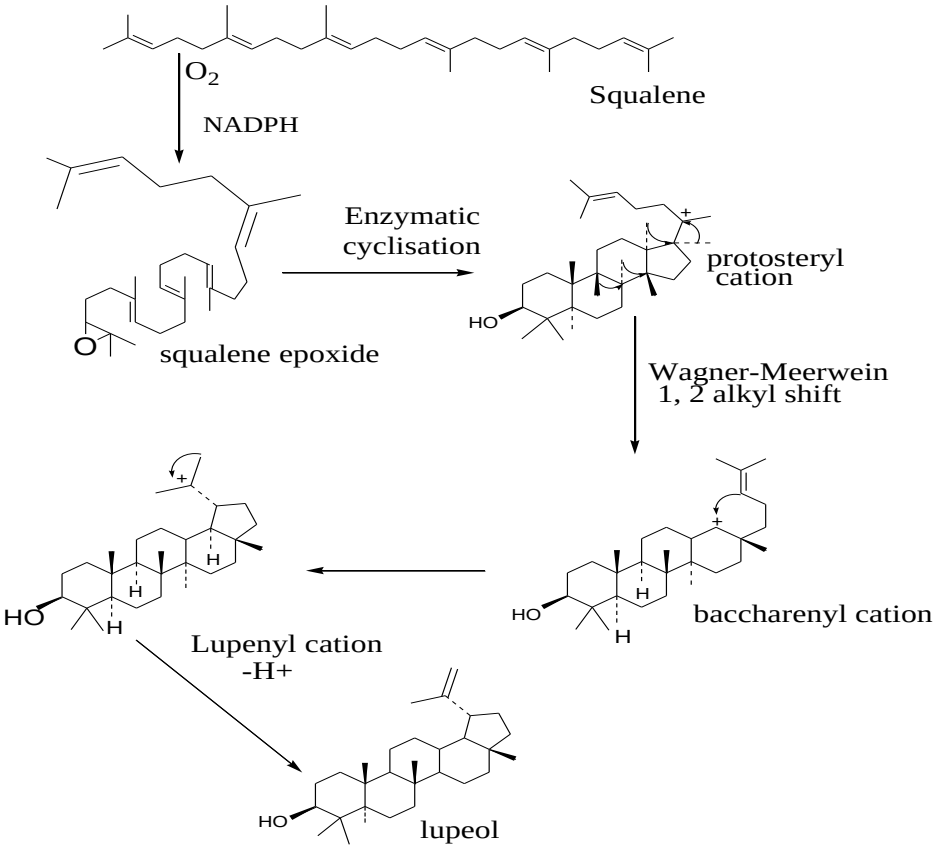
UV spectroscopy centers mainly on the electron structure of a molecule and it gives useful information on the presence or absence of chromophores (multiple bonds, aliphatic and aromatic conjugation) (Silverstein *et al*., 1991).

2.6 Terpenoids

These are hydrocarbons of plant origin with a general formula $(C_3H_8)_n$. Generally the ratio of the number of carbon atoms to that of hydrogen atoms is 5:8, the isoprene unit normally joined head to tail. They may contain 2, 3 or more isoprene units and may be open chains or cyclic structures.

2.6.1 Biosynthesis of lupeol

Lupeol is formed when two molecules of farnesyl pyrophosphate (FPP) are joined tail to tail to form squalene. Squalene undergoes multiple cyclizations because of its six double bonds. Cyclization starts with the formation of an incipient carbocation at the tertiary carbon of the end double bond of squalene. The cation undergoes many transformations mostly by shifting hydride ions and methyl groups before stabilizing through expulsion of a proton (Dewick, 1998). Scheme 2.4 describes the biosynthetic pathway of lupeol (Swartz, 2006).

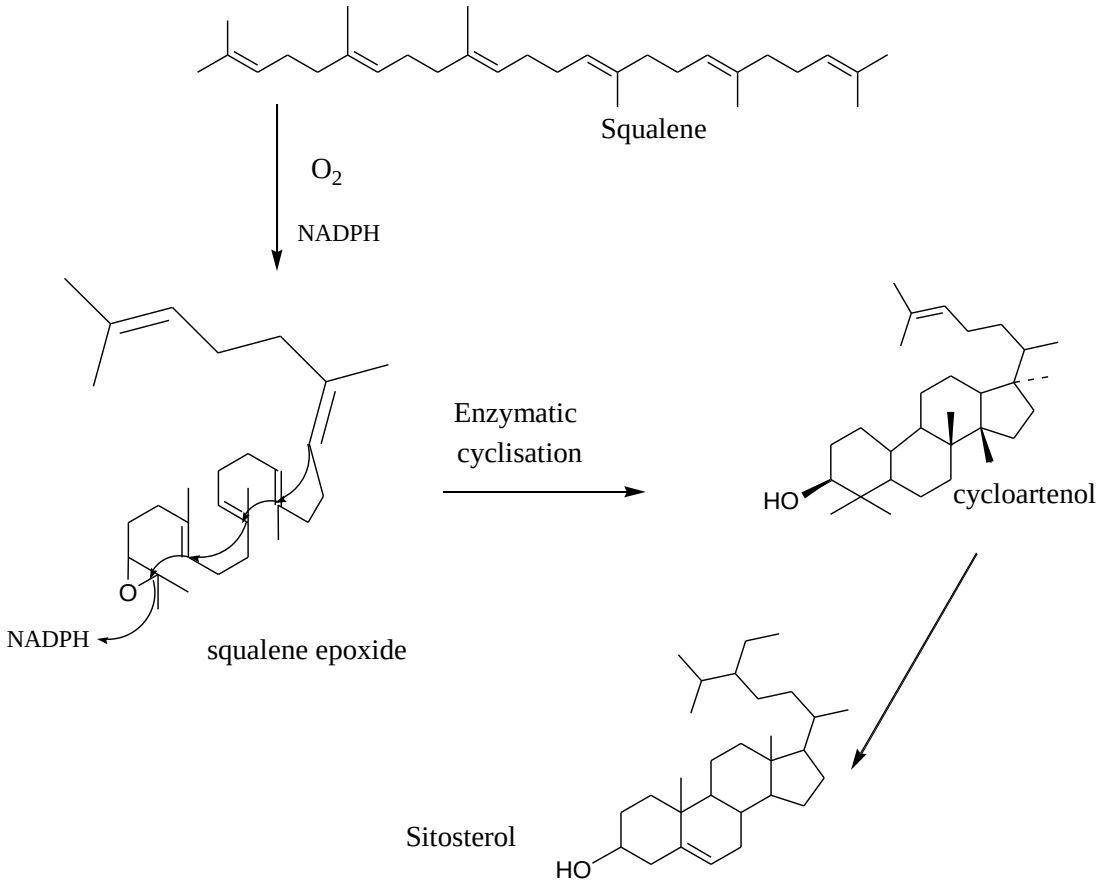


Scheme 2.4: Biosynthesis of lupeol from squalene (Swartz, 2006)

2.6.2 Biosynthesis of β-sitosterol

This is one of several phytosterols (plant sterol) with chemical structures similar to that of cholesterol. Sitosterols are white, waxy powders with a characteristic odor. It is widely distributed in the plant kingdom. Sitosterol is a modified terpenoid in which the modification occurs once the cyclization is complete. The biosynthesis of all natural steroids is believed to proceed from acetic acid to lanosterol or cycloartenol through mevalonic acid and squalene. All animal steroids originate from lanosterol while

cycloartenol is the precursor of plant steroids. The biosynthesis of sitosterol is summarized in scheme 2.5.



Scheme 2.5: Biosynthesis of sitosterol from squalene (Swartz, 2006)

CHAPTER THREE

MATERIALS AND METHODS

3.1 General procedures

Glassware used in the study were cleaned with water and detergent and chemically by soaking them overnight in freshly prepared chromic acid. They were then rinsed with distilled water, followed by acetone and dried at 110 ^{0}C in an electric oven for one hour before use.

3.1.1 Laboratory equipment and instruments used

The plant materials were ground using a motor grinding laboratory mill (Christy and Norris Ltd., Chelmsford, England) and weighed using a top-loading analytical balance (Denver instrument, Colorado, USA). The uncorrected melting points of the pure compounds were determined on a Gallen Kamp melting point apparatus (Sanyo, West Sussex, UK). Ultraviolet lamp, ENF-240 C/F (Supertonics Corporation Westbury, UK) of long and short wave length (365 nm and 254 nm, respectively) was used for visualizing the spots on a developed thin layer chromatography (TLC) plate.

3.1.2 Chromatographic materials and solvents

Analytical TLC pre-coated plastic sheets (polygram Sil G/UV $_{254}$) and aluminum sheets (Alugram Sil G/UV$_{254}$) of 20 by 20 cm (Matcher-Nagel Gmbh and Co. Frankfurt, Germany) were used throughout the study for establishment of optimum solvent systems for separations, complexity of the extracts and purity of isolated compounds. Vacuum liquid chromatography (VLC) was carried out using slurry packing with

Kiesegel silica gel 60 G (0.04 - 0.6 mm Merck, Germany). The column chromatography was carried out using glass columns of internal diameter 3.0 cm and of length 0.8 m. They were packed with Kiesegel silica (Merck, 70 – 230 mesh/ 0.63 – 0.2 mm) using slurry method. Further purification was done using Sephadex LH 20, as sieve material packed using slurry method.

The organic solvents used including hexane, dichloromethane (DCM), chloroform ($CHCl_3$), ethyl acetate (EtOAc), acetone (MeCOMe) and methanol (MeOH) were of general purpose grade (Kobian Kenya Ltd, Nairobi) and as a result, they were freshly distilled before use. Dimethylsulphoxide (DMSO) used was of analar grade (Kobian Kenya Ltd, Nairobi).

3.1.3 Spray reagents

The spray reagents used in detecting and visualizing the isolated compounds on the TLCs included:

i. Anisaldehyde-sulfuric acid prepared by mixing 0.5 ml of *p*-anisaldehyde, 10 ml of acetic acid, 85 ml of MeOH and 5 ml of concentrated sulfuric acid (Krishnaswamy, 2003).
ii. Sulfuric acid/MeOH mixture prepared by mixing 5 ml of concentrated sulfuric acid and 95 ml of distilled MeOH (Krishnaswamy, 2003).
iii. Ammonia vapour for flavonoids (Krishnaswamy, 2003).

3.1.4 Detection of compounds

Spots on the chromatograms were detected by visualizing the plates under UV light at 254 nm and 365 nm for active compounds. This was followed by developing in ammonia vapor (for flavonoids) or by spraying with detecting agents such as *p*-anisaldehyde or 5% solution of sulphuric acid in methanol. Terpenoids would give blue fluorescent in UV 365 nm and turn purple when plates are sprayed with anisaldehyde and then heated at 110 ^{0}C for about 10 minutes. However, most terpenoids did not fluoresce in UV. Flavonoids were visualized using UV light at both 254 nm and 365 nm. Their spots were turning yellow or orange when sprayed with *p*-anisaldehyde and ammonia and then heated at 110 ^{0}C for about 10 minutes.

3.1.5 Nuclear magnetic resonance (NMR) spectroscopy

The NMR spectra were obtained from Varian Gemini 75 and 400 MHz machine. ^{1}H NMR spectra were run in $CDCl_3$ (deuterated chloroform), DMSO (dimethylsulphoxide) or CD_3OD (deuterated methanol) depending on solubility of the isolated compounds. Proton nuclear magnetic resonance (^{1}H–NMR) spectra were recorded in deuterated solvents with tetramethylsilane (TMS) as the internal standard. Chemical shifts were recorded in parts per million (ppm) relative to TMS. The peak multiplicities were indicated by the symbol s (singlet), d (doublet), t (triplet), q (quartet), dd (double of doublet), bd (broad doublet) and m (multiplet). Coupling constants were recorded in hertz (Hz). Carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectra were recorded at between 75 and 100 MHz while ^{1}H-NMR spectra were recorded on the same instrument at 200 and 400 MHz, respectively. Structures of the compounds isolated were

elucidated by interpreting the ^{1}H and ^{13}C-NMR. Complete elucidation was done by use of 2D-NMR experiments which included DEPT and COSY together with comparison with published data.

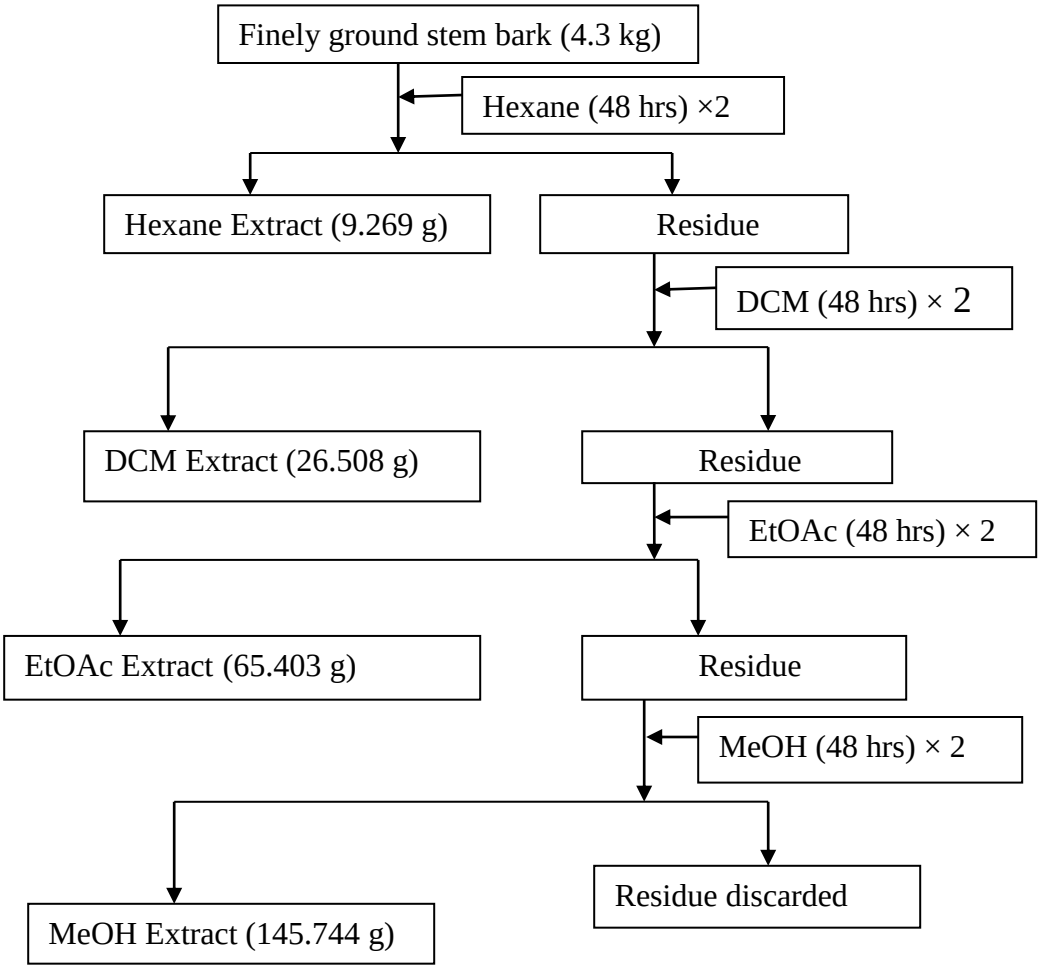
3.2 Plant collection and identification

The plant samples were collected from Bondo, Siaya County with the help of Dr. Amuka of Maseno University. Their botanical identity was determined and authenticated by Mr. Lucas Karimi of the Department of Pharmacy and Complementary/Alternative Medicine, Kenyatta University. Voucher specimen number: CHEM/RK/LS/ SB1 were stored at Kenyatta University Herbarium. The stem bark was separated then air dried under the shade for twenty one days. The dried materials were then ground to a fine texture using the grinding mill (Christy and Norris Ltd., Chelmsford, England). The resulting powdery material was weighed using a top-loading analytical balance (Denver instrument, Colorado, USA).

3.3 Extraction procedure of *Lannea schweinfurthii* stem bark

The powdered plant materials were sequentially extracted (as per scheme 3.1) with solvents of increasing polarity starting with hexane, dichloromethane, ethyl acetate and methanol for 48 hours for each extraction. This was done by soaking the finely ground material in the extracting solvent and allowing it to stay for 48 hours with occasional swirling after which it was filtered under vacuum. The extract was then concentrated using a rotary evaporator (vacuum evaporator) at 45 ^{0}C and low pressure. The extraction was repeated one time for each of the four solvents and the two portions

mixed together to form one extract. The combined concentrated crude hexane, DCM, ethyl acetate and methanol extracts whose quantities are indicated in scheme 3.1 were then carefully sealed, labeled and stored in pre-weighed sample bottles at -20 ^{0}C in a deep freezer. The crude extracts were then assayed for antimicrobial activity.



Scheme 3.1: Schematic presentation of sequential extraction of *Lannea schweinfurthii* stem bark

3.4 Bioassay of the plant extracts

3.4.1 Selected test strains

Antimicrobial assays of the plant species was done in two phases. First, screening of the crude extracts (primary assay) was done to detect the presence or absence of activity. Four genera of clinical isolates of bacteria were used. These included: *Staphylococcus aureus, Pseudomona aeruginosa, Escherichia coli* and *Bacillus subtilis*. *Candida albicans* was used as fungal strain. The pure compounds isolated from the plant species were then subjected to antibacterial and antifungal screening (secondary assay) to determine their potency against the four bacterial strains and two fungal strains which included *Candida albicans* and *Penicillium notatum*. The pure bacterial and fungal strains were obtained from the Department of Microbiology of Kenyatta University (Table 3.1).

Table 3.1: Bacterial and fungal strains used in the bioassay

Name	**ATCC number**	**Type**
Staphylococcus aureus (*Sa*)	25923	Gram positive
Bacillus subtilis (*Bs*)	202638	Gram positive
Escherichia coli (*Ec*)	25922	Gram negative
Pseudomonas aeruginosa (*Pa*)	10622	Gram negative
Candida albicans	10231	Fungi
Penicillium notatum	9478	Fungi

Two standard antimicrobials, obtained from Microbiology Department of Kenyatta University, were used as reference drugs (Table 3.2).

Table 3.2: Standard antimicrobials used as reference drugs

Standard drug name	Abbreviations	Weight µg/discs
Tetracycline	TET	25
Nystatin	NY	10

Key: µg/discs - Amount of the drug in the disc

3.4.2 Antifungal screening tests

The bioassay of the crude extracts and the compounds isolated from *L. schweinfurthii* against the test organisms were carried out using agar-well plate diffusion technique. Two fungal species *C. albicans* and *P. notatum* that affect human beings were obtained from the department of Microbiology, Kenyatta University (Table 3.1). Commercial potato dextrose agar (PDA) powder (39 g, Himedia laboratories, Pvt. Ltd., Bombay) was dissolved in distilled water to make a litre of solution. This was followed by stem sterilization in n autoclave at 121 ^{0}C and 15 psi pressure for twenty minutes. On cooling to 50 ^{0}C, 15 ml portions of this solution were dispensed into sterile petri dishes under sterile conditions then left to solidify. This provided the medium for growing the fungal spores.

3.4.2.1 Preparation of the antifungal drugs

1 mg of each crude extract was weighed and dissolved in DMSO (50 µl) and the solution made up to 1 ml using methanol. This gave a stock solution of 1,000 ppm for initial test. Pure cultures of the two fungus of interest was made on the PDA surface in the petri dishes from the stock cultures and the incubated at 30 ^{0}C for seven days to produce a good crop of spores as described by Clinical and laboratory standards

institute (2013). The fungal inoculum was prepared by harvesting the spores with a bent spore-harvesting needle in sterile environment and transferring them in a sterile tube containing sterile distilled water (Radonović *et al*., 2009). The spore suspension (0.5 ml) was pipetted on to the PDA medium in the petri dishes. The plate was then tilted several times to spread the inoculum and left still for 10 minutes. 4 agar wells were cut out in the inoculated PDA medium using a sterile cork borer (6 mm). The drug (0.1 ml) of known concentration was pipetted into each of the four wells in triplicates. The petri dishes were then covered, sealed and incubated aerobically at 30 ^{0}C for 72 – 96 hours. At the end of the incubation period, the diameter of the incubation zone produced around the agar wells (if any) were measured (in mm) with a transparent laboratory line ruler for another 3 consecutive days, as described by (Chhabra and Uiso, 1991). Sterile distilled water and the solvent mixture in the ratios used to prepare the drugs being screened were used as negative control. Nystatin (10 mg) was used as standard for comparative purpose for fungi (NCCLS, 2003).

3.4.3 Antibacterial screening tests

The pathogenic bacteria that were involved in the investigation included the Gram-negative and Gram-positive bacterial strains listed in table 3.1. *In vitro* antibacterial activity was determined using agar diffusion method. The test organisms were prepared by culturing the required bacterium in nutrient broth medium from stock cultures and later when required, transferred on the nutrient agar in petri dishes (NCCLS, 2003).

3.4.3.1 Preparation of media and growing of bacteria cultures

Nutrient agar (28 g, Oxoid Ltd., Basingstoke, England) was dissolved in distilled water to make 1,000 ml of solution by boiling. It was then autoclaved at 15 psi pressure and at 121 ^{0}C for 15 minutes, before being used (Chhabra and Uiso, 1991). Portions (15 ml) of the sterilized nutrient agar medium were dispensed into 90 mm diameter pre-sterilized petri dishes to yield a uniform depth of 4 mm under septic conditions in a laminar flow. The petri dishes were covered and allowed to cool at room temperature undisturbed until the culture medium had hardened. They were then incubated at 37^0 – 39 ^{0}C for 24 hours in an inverted position to test their sterility. Using a sterile wire loop under septic conditions, bacteria cultures from stock cultures were scooped and spread on the nutrient agar surface with 3 fold dilutions and incubated aerobically at 37^0 – 39 ^{0}C for 24 hours. Using a sterile cotton swab, the suspension was evenly spread on another freshly prepared agar surface (Radovanović *et al*., 2009).

Nutrient broth powder (13 g, Oxford Ltd., Basingstoke, England) was dissolved inn distilled water to make 1,000 ml of solution. Portions (25 ml) of the solution were dispensed into bijou bottles and steam sterilized in an autoclave instrument at 121^0 – 124 ^{0}C and 15 psi pressure for 20 minutes. On cooling, one loopful of the bacterial strain from the 24 hours culture was added to the sterile nutrient broth medium and incubated at 37^0 – 39 ^{0}C for 24 hours in a rotator shaker (NCCLS, 2003). The 24 hours broth bacteria culture (0.1 ml) was pipetted into the nutrient agar media in the petri dishes and spread evenly using a sterilized L-shaped glass rod under sterile conditions.

3.4.3.2 Introduction of the plant extract in the inoculated petri dishes

The disc diffusion method was employed for the determination of antimicrobial activities of the crude extracts as described by NCCLS (1999). A steady air current was used to dry plant extracts for 24 hours in a pre-weighed sample bottle. A solution of each extract was prepared by dissolving 50 mg of the extract in 1 ml of DMSO. 10 µl of the solution were dispensed onto sterile adsorbent filter paper discs (6 mm in diameter) made from Whatman filter No.1 to an approximate concentration of 500 µg/ml per disc.

Other discs were also be soaked in 5% DMSO and sterile normal saline to act as negative control. The impregnated discs were re-sterilized under ultra violet (UV) light for one hour to expel the solvent. Sterile impregnated discs were then carefully placed on the labeled inoculated plates using sterile forceps under sterile conditions. They were then pressed down with slight pressure to ensure complete contact with the inoculated gar surface. The plates were allowed to stand for one hour to allow diffusion to take place, and then they were incubated aerobically at 37 ^{0}C in an inverted position. The inhibition zones (if any) were measured (in mm) after 24 and 48 hours in triplicates. Commonly used antibiotic tetracycline (25 mg) was used as a standard for comparing with the plant extracts by noting their activity against the bacterial strains used (Chhabra and Uiso, 1991).

3.5 Gas chromatography linked with mass spectroscopy Analysis of crude extracts

GC-MS was carried out on both the crude methanol and DCM extracts as a preliminary test to give an idea of the class of compounds present in the plant species. The

composition of the crude extracts of MeOH and DCM was determined using GC system (GC-800 series), with fused capillary column (15 m length, 0.25 mm internal diameter and 0.25 µm film thickness). Static phase methylsilicone (SE-30) was directly coupled to quadruple M/S (Hewlett Packard 5973). Electron Impact Ionization was carried out at energy of 70 eV. Helium was used as a carrier gas. Injector and detector were maintained at 200 and 250 ^{0}C, respectively. The analytical conditions were as follows: oven temperature was 2 minute isothermal at 60 ^{0}C, and then 60 to 240 ^{0}C at a rate of 15^{o}C per min then held isothermal for 6min. The instrument was scanned at a mass range from 60 to 400 atomic mass units (a.m.u).

3.6 Fractionation of the extracts

Separation and purification of the crude plant extracts was carried out using both column chromatography and VLC on silica gel. The VLC column was dry packed with silica gel (Kiesegel 60G, Merck, Germany) and consolidated by applying hexane to the packing and then sucking by use of a vacuum to dry to effect good packing. Care was taken so that the packing was kept uniform and making an evenly flat surface. The crude extracts of hexane (9.269 g) and dichloromethane (26.508 g) showed homogeneity and were constituted into one extract (35.777 g) then mixed with silica gel. The mixture was then applied as dry powder onto the top of the packed VLC. Separation and elution was achieved using 1000 ml portions of different solvent systems in increasing polarity, starting with 100% hexane. Dichloromethane (DCM) was then added in portions of 1000 ml with 5% increase in polarity up to 100% and finally MeOH was introduced until an addition of 10% MeOH in DCM.

Methanol extract was re-extracted with ethyl acetate and the extract obtained (20.500 g) mixed with ethyl acetate extract obtained from sequential extraction (65.403 g). The ethyl acetate (EtOAc) extracts (85.903 g) were separated by packing them in a column chromatography. The column chromatography was packed with silica gel (Kiesegel silica 240G, Merck, Germany). Elution was done in portions of 1000 ml starting with 100% hexane. Polarity of the eluting solvent mixture was gradually increased by introducing 5% of ethyl acetate (EtOAc) up to 100%. Finally, polarity was further increased by adding methanol (MeOH) up to an addition of 10% MeOH in EtOAc.

50 ml fractions were collected for each solvent system. The fractions were further subjected to column chromatography and other chromatographic techniques. Finally, the pure compounds were obtained either through further purification by use of a column packed with Sephadex LH 20 (a sieve gel method) or by use of a series of preparative TLC. The purity was closely monitored at every step by use of TLCs. Specially made glass teat pipettes were used to spot the samples on thin layer chromatography plates. The loaded plates were developed at room temperature in glass jars by the ascending solvent technique. The developed spots were then visualized for detection of compounds. Fractions that showed homogeneity were combined and concentrated together to give pure compounds or partially pure compounds for further purification. Purification and isolation of the five compounds followed the procedures and schemes herein.

3.7 Purification and isolation of compounds

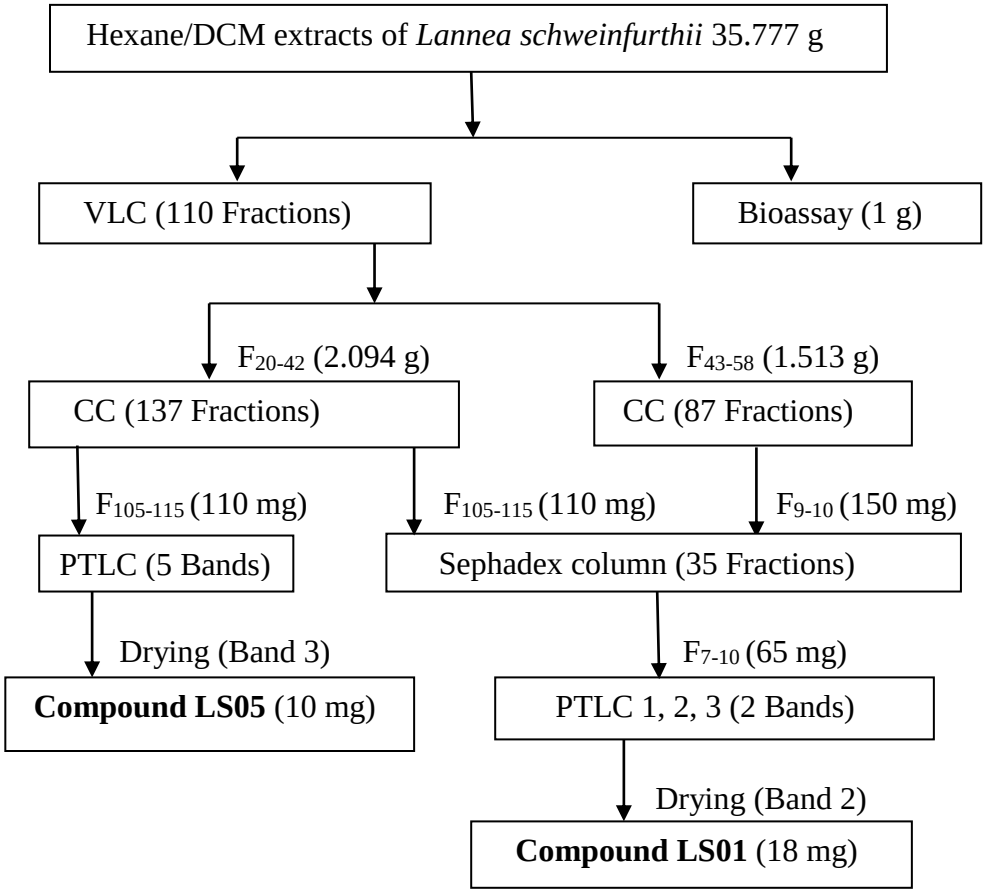
3.7.1 Purification and isolation of compounds from hexane/DCM extract

The hexane (9.269 g) and DCM (26.508 g) crude extracts were found to have overlapping spots on the TLC plates suggesting they were homogeneous. They were combined to form one extract and loaded in a VLC column and eluted with 1000 ml portions of solvent system whose polarity was increased gradually starting with 100% hexane. 5% of DCM was introduced gradually until 100% had been added. Finally, 5% of MeOH was added up to 10% MeOH in DCM. A total of 110 fractions 50 ml each were obtained and they were plotted on TLC plate to establish the optimum solvent system for the separation in which 0.5% MeOH/DCM solvent system was identified.

Fractions F_{20-42} (2.094 g) and F_{43-58} (1.513 g) were loaded in two columns separately and eluted with 500 ml portions of solvent system whose polarity was varied gradually by increasing the more polar solvent by 5%, starting 100% hexane, and then introducing DCM up to 100% and finally MeOH up to 10% MeOH in DCM. The columns gave 137 and 87 fractions, respectively, out of which fractions $F_{105-115}$ (110 mg) and F_{9-10} (150 mg) were found to be homogeneous and were pooled and loaded in a Sephadex column followed by a series of preparative TLCs to yield a compound coded as RK/DCM;Hex/LS/SB/002a. The compound had one spot on TLC chromatogram with an R_f of 0.56 (75% DCM/Hexane) and was an oily liquid serialized as compound **LS01**.

Another portion of fractions $F_{105-115}$ (110 mg) was purified separately using preparative TLC to yield 5 bands out of which the third band coded as RK/DCM;Hex/LS/SB/003

had an R_f of 0.61 in 60% DCM in hexane and was a colorless oily liquid, serialized as compound **LS05**. Scheme 3.2 outlines the purification and isolation of compounds **LS01** and **LS05** from hexane/DCM extract.



Scheme 3.2: Isolation of compounds LS01 and LS05 from hexane/DCM extract

3.7.2 Purification and isolation of compounds from ethyl acetate extract

The ethyl acetate (EtOAc) crude extract (85.903 g) was loaded in a column and eluted with 1000 ml portions of solvent system whose polarity was varied gradually by incremental addition of 5% of EtOAc, starting with 100% hexane. EtOAc was introduced until 100% had been added followed by MeOH which was added up to 10%

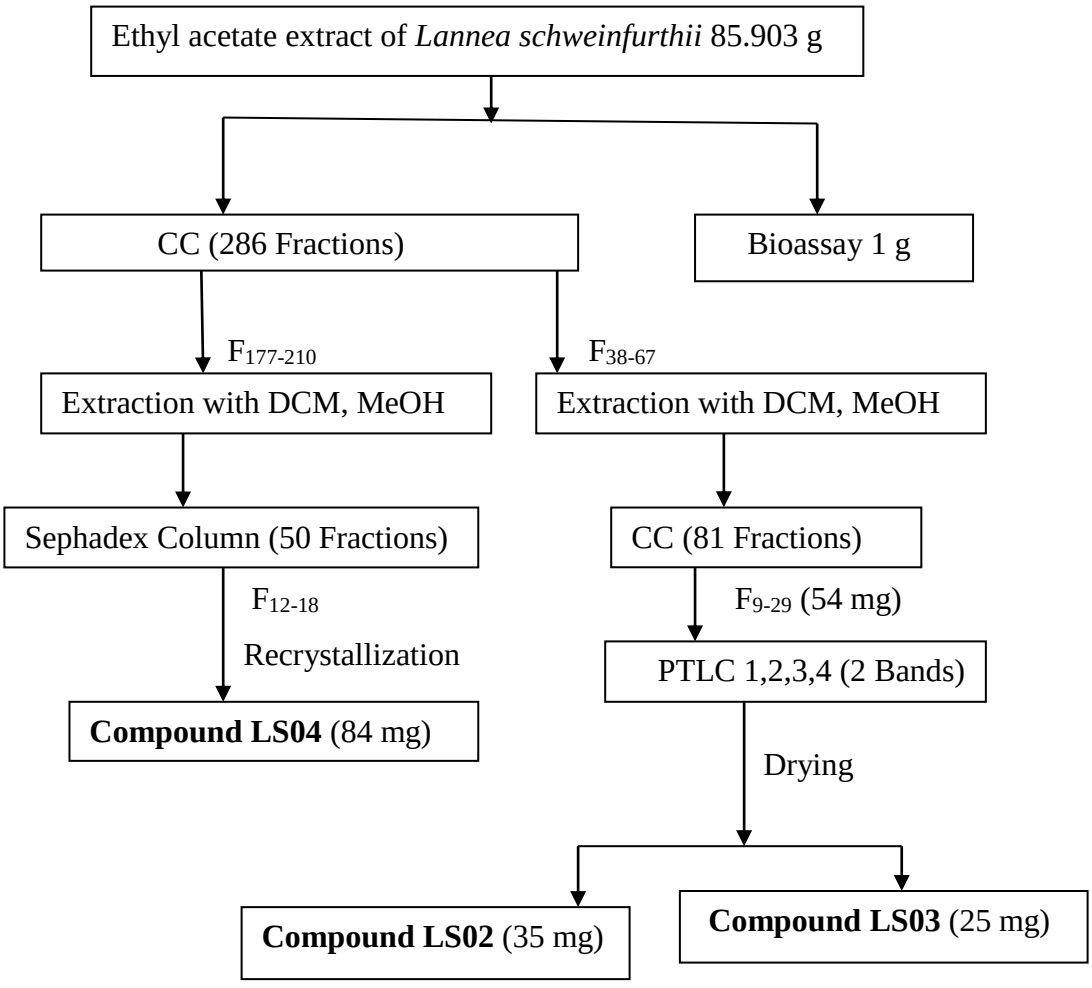
MeOH in EtOAc. A total of 286 fractions, each of 50 ml were obtained and they were plotted on TLC plate to establish the optimum solvent system for the separation in which 0.5% MeOH/EtOAc solvent system was identified.

The fractions $F_{177-210}$ were constituted and extracted with DCM/MeOH in the ratio of 1:1 then loaded into a column and eluted with 60mls of Hexane/EtOAc solvent systems of varying polarity starting with 40:1 ratio then finally adding up to 100% EtOAc. A total of 81 fractions each of 5 ml were collected. Fractions F_{9-29} were found to be homogeneous and were constituted and a series of four preparative TLCs done to yield two bands. The upper band RK/EtOAc/LS/SB/008a had one spot on TLC chromatogram with an R_f of 0.57 in 50% DCM in hexane and was a white needle like solid serialized as compound **LS02**.

The fractions F_{38-67} were constituted and extracted with DCM/MeOH in the ratio of 1:1 then loaded into a column and eluted with 60mls of Hexane/EtOAc solvent systems starting with 40:1 ratio then finally 100% EtOAc. A total of 81 fractions each of 5 ml were collected. Fractions F_{9-29} were constituted and a series of four preparative TLCs done to yield two bands. The lower band RK/EtOAc/LS/SB/006a had one spot on TLC chromatogram with an R_f of 0.56 in 100% DCM and was a white crystalline solid serialized as compound **LS03**.

The fractions $F_{177-210}$ were constituted and extracted with DCM/MeOH in the ratio of 1:1 then loaded into a Sephadex column in which they were eluted with 1000 ml of

DCM/MeOH solvent system in the ratio of 1:1 to give a total of 50 fractions, 20 ml each. Fractions F_{12-18} (84 mg) were homogeneous and they were pooled and re-crystallization done to yield RK/EtOAc/LS/SB/007. The compound was a yellow crystalline solid and had one spot on TLC chromatogram with an R_f of 0.62 in 70% acetone in methanol, which was serialized as compound **LS04**. Scheme 3.3 describes the purification and isolation of compounds **LS02**, **LS03** and **LS04**.



Scheme 3.3: Isolation of compounds LS02, LS03 and LS04 from ethyl acetate extract

The isolated compounds **LS01**, **LS02**, **LS03**, **LS04** and **LS05** were packed in pre-weighed sample bottles then taken for bioassay and NMR analysis.

3.8 Physical and spectroscopic data of the isolated compounds

The physical data obtained for compounds **LS01**, **LS02**, **LS03**, **LS04** and **LS05** included the uncorrected melting point for the solids, the appearance and the retention factor R_f while the spectral data determined included the ^{13}C NMR and ^{1}H NMR spectra. Other spectral data obtained was as described in the GC-MS profile of the crude DCM and methanol extracts (Appendix 6a and 6b).

3.8.1 Compound LS01

Colourless liquid (hexane/DCM); yield (18 mg); R_f of 0.56 (75% DCM in hexane); (^{1}H NMR $CDCl_3$, δ (ppm), 400 MHz) δ 7.13 (1H, t, J=7.7 Hz), 6.75 (1H, bd, J=7.5 Hz), 6.65 (1H, bs), 6.63 (1H, bd, J=7.6 Hz), 5.40 (2H, m), 2.55 (2H, m), 1.98 (m), 1.59 (m), 1.29 (m), 1.25 (m) and 0.96 (3H, t, J=7.2 Hz); ^{13}C-NMR ($CDCl_3$–δ, 100 MHz). δ 14.0 (C-13'), 25.6 (C-12'), 29.2, 29.3, 29.5, 29.5, 29.6, 29.7, 31.3 (C-2'), 32.6 (C-9'), 35.8 (C-1'), 112.4 (C-6), 115.3 (C-2), 121.0 (C-4), 129.4 (C-11'), 129.4 (C-5), 131.9 (C-10'), 145.0 (C-3), 155.4 (C-1).

3.8.2 Compound LS02

White needle like crystals (EtOAc); yield (35 mg); Mp 126-128^{0}C; R_f of 0.57 (50% DCM in hexane); ^{1}H NMR ($CDCl_3$, δ (ppm), 400 MHz) δ (4.67, 4.58, 3.16, 2.30, 1.66, 1.01, 0.95, 0.92, 0.81, 0.77 and 0.74); ^{13}C-NMR ($CDCl_3$ –δ, 100 MHz). δ 14.6 (C-27),

15.4 (C-24), 16.0 (C-26), 16.1 (C-25), 18.0 (C-28), 18.3 (C-6), 19.3 (C-30), 20.9 (C-11), 25.1 (C-12), 27.4 (C-2), 27.4 (C-15), 28.0 (C-23), 29.8 (C-21), 34.3 (C-7), 35.6 (C-16), 37.2 (C-10), 38.1 (C-13), 38.7 (C-1), 38.9 (C-4), 40.0 (C-22), 40.8 (C-8), 42.8 (C-14), 43.1 (C-17), 48.0 (C-19), 48.3 (C-18), 50.4 (C-9), 55.3 (C-5), 79.0 (C-3), 109.3 (C-29), 150.8 (C-20).

3.8.3 Compound LS03

White crystalline solid (EtOAc); yield (25 mg); Mp 128-130 ^{0}C; R_f of 0.56 (100% DCM); ^{1}H NMR ($CDCl_3$, δ, 200 MHz) δ 5.32 (1H, d, J=7.2 Hz), 3.50 (1H, m), 1.03 (3H, s), 0.94 (3H, d, J=8.4 Hz), 0.86 (9H, m) and 0.79 (3H, s); ^{13}C NMR ($CDCl_3$, 75 MHz) 11.9 (C-29), 12.0 (C-18), 18.8 (C-26), 19.0 (C-19), 19.4 (C-21), 19.8 (C-27), 21.1 (C-11), 23.1 (C- 28), 24.3 (C-15), 26.1 (C-23), 28.2 (C-16), 29.2 (C-25), 31.7 (C-7), 31.9 (C-2), 31.9 (C-22), 34.0 (C-8), 36.5 (C-10), 36.5 (C-20), 37.3 (C-1), 39.8 (C-12), 42.3 (C-4), 42.3 (C-13), 45.9 (C-24), 50.2 (C-9), 56.1 (C-17), 56.8 (C-14), 71.8 (C-3), 121.7 (C-6), 140.8 (C-5).

3.8.4 Compound LS04

A yellow crystalline solid (EtOAc); yield (84 mg); Mp 119-221 ^{0}C; R_f of 0.62 (70% Acetone/ MeOH); ^{1}H NMR (DMSO, δ (ppm), 200 MHz) δ 6.87 (1H, s), 6.64 (1H, t, J=8.8 Hz), 5.87 (1H, d, J=1.4 Hz), 5.69 (1H, d, J=1.4 Hz), 4.65 (1H, d, J=4.6 Hz), 3.99 (1H, m), 2.66 (1H, dd, J=16.6, 4.4 Hz) and 2.44 (1H, dd, J=16.6, 3.2 Hz); ^{13}C NMR (DMSO – δ, 75 MHz) δ 28.9 C-4), 65.6 (C-3), 78.7 (C-2), 94.7 (C-6), 95.7 (C-8), 99.2

(C-4a), 115.4 (C-2’), 115.6 (C-5’), 118.6 (C-6’), 131.3 (C-1’), 145.2 (C-4’), 145.1 (C-3’), 156.4 (C-8a), 156.9 (C-7), 157.2 (C-5).

3.8.5 Compound LS05

A colorless oily liquid (hexane/DCM); yield (10 mg); R_f of 0.61 (60% DCM: Hexane); (^{1}H NMR $CDCl_3$, δ (ppm), 400 MHz) δ 7.71 (2H, dd, J=8.7, 5.4 Hz), 7.53 (2H, dd, J=8.7, 5.4 Hz) 4.22 (4H, m), 1.68 (2H, m), 1.42 (m), 1.32 (m), 1.25 (m) and 0.91 (m); ^{13}C NMR ($CDCl_3$ – δ, 100 MHz) δ 11.1, 14.2, 23.1, 23.9, 29.1, 30.5, 38.9, 68.3, 128.9 (C-3, 6), 131.0 (C-1, 2), 132.6 (C-4, 5) and 167.9 (C=O).

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Crude extract yields

Dry powdered stem bark of *Lannea schweinfurthii* which weighed 4.3 kg (4,300 g) was subjected to sequential extraction by soaking the extract in solvents in the order of increasing polarity, starting with hexane, dichloromethane, ethyl acetate, and finally methanol. The amount of crude extracts obtained and percentage yields were recorded and tabulated in table 4.1.

Table 4.1: Masses of sequential extraction of *L. schweinfurthii* stem bark and percentage yields

Extraction solvent	Mass in grams	% Yield
Hexane	9.269	0.216
DCM	26.508	0.616
EtOAc	65.403	1.521
MeOH	145.744	3.389

Methanol extract had the highest percentage yield while hexane extract had the least. The results showed that the percentage yield increased with increase in polarity of the solvent used. This could be attributed to the fact that methanol being more polar than the solvents used dissolved all the other remaining compounds including sugars and other highly polar compounds such as glycosides.

4.2 Antibacterial and antifungal assay of crude extracts

All the four crude extracts were tested against four bacterial strains and one fungus to determine their activity. The pathogens used were *S. aureus* (ATCC 259223) and *B.*

subtilis (ATCC 202638) (Gram-positive bacteria), *P. aeruginosa* (ATCC 10622) and *E. coli* (ATCC 25922) (Gram-negative) and *C. albicans* (ATCC 10231) (fungus). The inhibition zones of bacterial and fungal growth were measured after 48 hours and 7 days, respectively, and in triplicates. The measurements were done in millimeters, from the end of the growth of one side of the disc to the end of growth of the other side including the diameter of the disc as described by Chhabra and Uiso (1991). The results obtained were as tabulated in table 4.2.

Table 4.2: Inhibition zones (in mm) of the crude extracts of *L. schweinfurthii* stem bark

	Staphylococcus aureus	***Bacillus subtilis***	***Pseudomonas aeruginosa***	***Escherichia coli***	***Candida albicans***
Hexane	7.00 ± 0.20^{b}	7.00 ± 0.80^{a}	6.00 ± 0.10^{a}	7.00 ± 0.10^{b}	7.00 ± 0.10^{b}
DCM	7.00 ± 0.50^{b}	7.00 ± 0.60^{a}	6.00 ± 0.30^{a}	7.00 ± 0.10^{b}	7.00 ± 0.10^{b}
EtOAc	20.00 ± 0.70^{e}	19.00 ± 0.70^{c}	6.00 ± 0.20^{a}	16.00 ± 0.20^{c}	11.00 ± 0.20^{c}
MeOH	13.00 ± 0.10^{c}	15.00 ± 0.60^{b}	6.00 ± 0.10^{a}	21.00 ± 0.20^{e}	14.00 ± 0.50^{d}
(-ve) Control	6.00 ± 0.40^{a}	6.00 ± 0.70^{a}	6.00 ± 0.10^{a}	6.00 ± 0.10^{a}	6.00 ± 0.40^{a}
(+ve) Tet.	18.00 ± 0.20^{d}	18.00 ± 0.40^{c}	16.00 ± 0.40^{b}	18.00 ± 0.10^{d}	-
(+ve) Ny.	-	-	-	-	18.00 ± 0.60^{e}
p-value	<0.001	<0.001	<0.001	<0.001	<0.001

Mean values followed by the same small letter within the same column do not differ significantly from one another (One-Way ANOVA,SNK test,α =0.05)
Key: Tet. for Tetracycline (bacteria); Ny. for Nystatin (Fungi); (-ve) Control- DMSO

There was a significant difference between the various extracts and the controls ($p< 0.001$, one-way ANOVA) in all the bacteria and fungi used. Dichloromethane and hexane extracts showed mild activity (inhibition of 7 mm) and their inhibition zones did

not differ significantly ($\alpha = 0.05$). Methanol and ethyl acetate showed high activity (inhibition of 13 mm and above) against the microbes used. The antibiotics used as positive control gave inhibition zones of 18 mm against all microbes except for *P. aeroginosa* which gave an inhibition of 16 mm. The results demonstrate the difficulty of the Gram-negative bacteria to be inhibited by many antibiotics (Tait-Kamradt *et al*., 2000). The inhibition zones for the standard antibiotics were relatively higher compared to those exhibited by crude extracts. However, ethyl acetate crude extract showed significantly higher inhibition zones of 20 and 19 mm against the Gram-positive bacteria *S. aureus* and *B. subtilis*, respectively, compared to other extracts and controls. Methanol extract showed a significantly higher inhibition zone of 21 mm against the Gram-negative *E. coli* bacteria, compared to the standard antibiotics. Further, the highest inhibition zone was shown by ethyl acetate extract which gave an inhibition zone of 21 mm.

The crude extracts had mild and moderate activity (inhibition of between 7 and 14 mm) against *Candida albicans* fungus. The fact that the crude extracts showed activity against both the bacteria and fungi was evidence of their potency. The results support the use of *Lannea schweinfurthii* plant as traditional medicine against bacterial and fungal infections. However, cyctotoxicity tests should be carried out to ascertain the activity observed.

4.3 GC-MS Data for the crude DCM and MeOH extracts

GC-MS was carried out for the crude DCM and MeOH extracts to give a preliminary idea of the class of compounds in *L. schweinfurthii*. The compounds detected were serialized on the basis of their retention time and data obtained was as indicated in appendix 6a and b. The compounds present in the plant species were mainly fatty acids and their derivatives, phenolic compounds, polyketide derivatives, terpenoids and steroids which were tabulated in Table 4.3 and 4.4.

Table 4.3: Number of compounds detected in crude DCM extract of *L. schweinfurthii*

Class of Compounds	Number of compounds
Fatty acids and derivatives	27
Terpenoids	33
Phenolic compounds	6
Polyketide derivatives	2
Steroids	1

Table 4.4: Number of compounds detected in crude MeOH extract of *L. schweinfurthii*

Class of Compounds	Number of compounds
Fatty acids and derivatives	13
Terpenoids	7
Phenolic compounds	6
Polyketide derivatives	4
Steroids	1

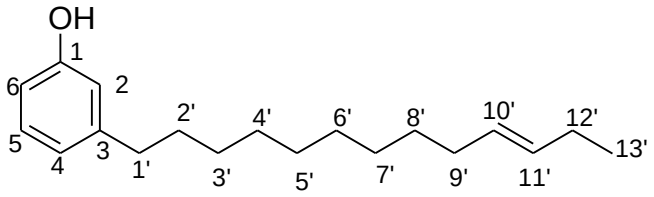
Plant phenolics that were analyzed included shikimic acid derivatives such as coumarates and phenylpropanoids, polyketide derivatives and phenols. Further, terpenoids detected from both DCM and methanol crude extracts included monoterpenenoids, diterpenoids, sequiterpenoids, triterpenoids and their derivatives.

Additionally, there were several fatty acids and their derivatives that were analyzed which included hydrocarbons, acetylenes, alcohols, and isoprene fatty acids while the steroids present in the extracts included sitosterol and androstanone.

4.4 Structure elucidations

4.4.1 Compound LS01

The compound was isolated from hexane/DCM extract as a colorless liquid. On analytical TLC, the compound had an R_f of 0.56 in 75% DCM in hexane. On spraying with anisaldehyde, the compound showed a red spot faded slightly but remained red suggesting a phenolic compound (Dewick, 1998).



LS01

The ^{1}H NMR spectrum (Appendix 1a) showed the presence of aromatic, olefinic and aliphatic protons. The signal at δ 7.13 (1H, t, J=7.7 Hz), 6.75 (1H, bd, J=7.5 Hz), 6.65 (1H, bs), and 6.63 (1H, bd, J=7.6 Hz) were assigned to four aromatic protons in different chemical environments suggesting that the ring was di-substituted with the two groups being at meta-positions with each other. Further analysis of the spectrum indicated a very strong signal at δ 1.29 and a triplet integrating to three protons at δ 0.96, corresponding to a methyl group. This suggested the presence of a long alkyl chain. The signal appearing as a multiplet at δ 5.4 suggested the presence of olefinic protons and thus the existence of a double bond in the side chain.

Moreover, the multiplet at δ 1.98 was characteristic of allylic protons and was assigned to the two methylene protons at position adjacent to the double bond. The position of the double bond in the chain was deduced from ^{1}H - ^{1}H COSY experiment (Appendix 1d) with correlation data given in tale 4.5 , in which correlations were observed between the olefinic proton δ 5.40, the proton multiplet at δ 1.98 and triplet at δ 0.96. The interactions between the multiplet at δ 1.98 of allylic protons and terminal methyl protons at δ 0.96, while at the same time correlating with the multiplet at δ 5.40 of olefinic protons led to the conclusion that the double bond is between the third and the fourth carbons from the tail. Similarly, there were correlations observed between the benzylic protons δ 2.55 (2H, t) and the adjacent methylene protons at δ 1.59 (H-2'). Other signals were assigned to methylene proton pooled at position 3' to 8' for δ 1.29 (m) (Dewick, 1998).

The ^{13}C NMR spectrum (Appendix 1b) there were nineteen peaks which suggested chemical shifts assignable to either aromatic or olefinic carbons between δ 155.4 and 112.4. The other signals appearing between δ 35.8 and 14.0 were assigned to aliphatic carbons. Further, the signal at δ 155.4 was assigned to an aromatic hydroxylated carbon atom, and together with the signal at δ 145.0 (C-3) represented the only two quarternary carbons in the compound as observed in DEPT experiment (Appendix 1c). The signals at δ 131.9 and 129.4 were assignable to the carbon atoms of the double bond. The four protonated aromatic carbon atoms had their signals at δ 129.4 (C-5), 121.0 (C-4), 115.3 (C-2) and 112.4 (C-6).

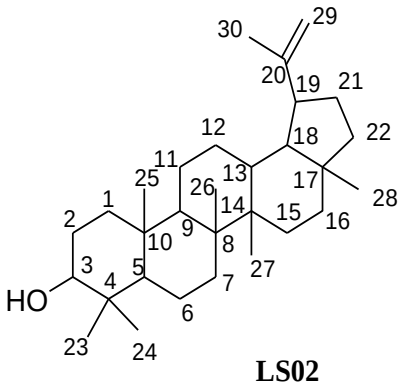
The signal at δ 35.6 was assigned to benzylic carbon (C-1') while that at δ 14.0 was assigned to the terminal methyl carbon (C-13').The remaining signals δ 32.6 to 25.6 were assignable to the methylene carbons in the chain.^{13}C NMR spectrum was instrumental in determining the overall structure of the compound whose name is 3-(10'-tridecenyl) phenol. Okoth (2014) reported extraction of phenolic compounds that are closely related to **LS01** from the plant species. However, the compound **LS01** was isolated for the first time from *Lannea schweinfurthii*. There were no reports of its isolation from the genus, to the best of our knowledge.

Table 4.5: ^{1}H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz) data for compound LS01

Position	**^{1}H NMR (δ)**	**Multiplicity/ Integration/ J values(Hz)**	**COSY ^{1}H- ^{1}H**	**^{13}C NMR (δ)**
1	-	-	-	155.4
2	6.65	bs (1H)	-	115.3
3	-	-	-	145.0
4	6.75	bd (1H) J=7.5	-	121.0
5	7.13	t (1H) J=7.7	-	129.4
6	6.63	bd (1H) J=7.6	-	112.4
1'	2.55	t (2H)	H-2'	35.8
2'	1.59	m (2H)	H-1', 3'-8'	31.3
3'-8'	1.29-1.25	m	H-9'	29.7-29.1
9'	1.98	m	H- 3'-8'	32.6
10'	5.40	m (1H)	H-9'	131.9
11'	5.40	m (1H)	H-12'	129.4
12'	1.98	m	H-13',11'	25.6
13'	0.96	t (3H) J=7.2	H-12'	14.0

4.4.2 Compound LS02- Lupeol

Compound **LS02** was isolated from hexane/DCM extract as needle like crystals with a melting point range of 126-128 ^{0}C. On analytical TLC, the compound had an R_f of 0.57 in 50% DCM in hexane. When the chromatogram was sprayed with anisaldehyde it turned purple suggesting that the compound is a terpenoid. The ^{1}H NMR spectrum (Appendix 2a) displayed protons in three regions namely aliphatic, hydroxylated and allylic, which strongly suggested that the compound was a triterpenoid. Two downfield signals at δ 4.80 and 4.68, appearing as broad singlets, suggested the presence of a terminal double bond (Satomi *et al*., 2002). The signal at δ 3.35 was assigned to the proton of a hydroxylated carbon at position 3. The presence of seven singlets, each integrating to three protons at δ 0.99, 1.00, 1.13, 1.26, 1.27, 1.29 and 1.82 strongly suggested pentacyclic triterpenoids. This was in agreement to the lupeol type structure of a triterpenoid (Satomi *et al*., 2002).



LS02

^{13}C NMR spectrum (Appendix 2b) for compound 5 showed 30 carbons. This further confirms as a pentacyclic structure. The two downfield signals at δ 151.0 and 109.3

represented the olefinic carbons with the downfield signal being that of the quaternary carbon. The signal at δ 79.0 represented the hydroxylated carbon at position 3. The seven methyl carbon atoms appeared at δ 28.1, 16.1, 15.4, 14.6, 16.0, 18.1 and 19.3. They are closely compared to that of lupeol; a pentacyclic triterpenoids reported from various plant sources (Satomi *et al*., 2002).

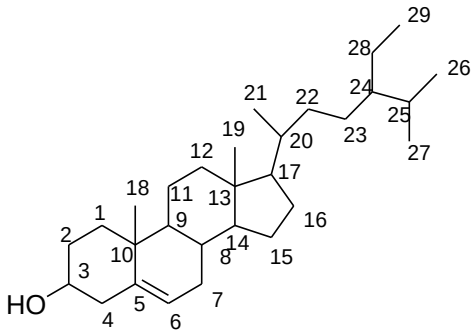
Table 4.6: ^{13}C NMR (100 MHz, $CDCl_3$) data of compound LS02 and lupeol (Satomi *et al*., 2002)

Carbon	Compound LS02	Lupeol		Carbon	Compound LS02	Lupeol
1	40.0	40.3		16	35.6	35.8
2	27.4	27.8		17	43.0	43.2
3	79.0	78.2		18	48.3	48.6
4	39.1	39.6		19	48.0	48.3
5	55.3	55.9		20	151.0	151.1
6	18.3	18.8		21	30.1	30.2
7	34.3	34.6		22	39.0	39.3
8	41.0	41.2		23	28.1	28.7
9	50.5	50.8		24	16.1	16.6
10	37.2	37.5		25	15.4	15.4
11	21.1	21.2		26	14.6	14.8
12	28.0	28.3		27	16.0	16.2
13	38.1	38.3		28	18.1	18.2
14	42.8	43.1		29	109.3	110.0
15	30.1	30.1		30	19.3	19.5

4.4.3 Compound LS03- β-Sitoterol

Compound **LS03** was obtained from ethyl acetate as white crystalline solid with a melting point of 128-130^{0}C. The compound had an R_f of 0.56 in 100% DCM. When the chromatogram was sprayed with anisaldehyde spray, the spot turned purple and later green suggesting that the compound was a terpenoid (Dey and Harborne, 1991). ^{1}H-

NMR spectrum (Appendix 3a) of the compound displayed three regions namely; aliphatic, hydroxylated and allylic region on the spectrum signals and strongly suggested a terpenoid structure. A signal at δ 5.32 (1H, d J=8 Hz) suggested the presence of a double bond at a quaternary carbon atom. A multiplet centered at δ 3.50, characteristic of a proton germinal to a hydroxyl group at C-3 in terpenoids was also observed. Six signals representing the methyl groups were also observed at δ 1.03 (3H, s), 0.94 (3H, d, J=8.4 Hz), 0.86 (9H, m) and 0.70 (3H, s), which is characteristic of a modified terpenoid (Dey and Harborne, 1991).



LS03

^{13}C-NMR spectrum (Appendix 3b) had a total of twenty nine peaks. The signals involving the double bonds were indicated by the peaks at δ 140.8 and 121.7 in which the former represented a quaternary carbon atom. The signal at δ 71.8 represented a hydroxylated carbon atom at C-3. Methyl groups were represented by the signals at δ 11.9, 12.0, 18.8, 19.0, 19.4 and 19.8. The overall spectrum of compound **LS03** compared closely to that of β-sitosterol, a sterol that has been reported from various plant sources (Dey and Harborne, 1991). The ^{13}C-NMR data for compound **LS03**

compared relatively well with that reported by Dey and Harborne (1991) of β- sitosterol represented in table 4.5.

Table 4.7: ^{13}C NMR (100 MHz, $CDCl_3$) data of compound LS03 and β-sitosterol (Dey and Harborne, 1991; Mahato and Kundu, 1994)

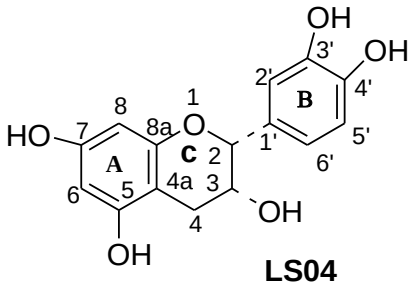
Carbon	Compound LS03	β-sitosterol		Carbon	Compound LS03	β-sitosterol
1	37.3	37.2		16	28.2	28.2
2	31.9	31.9		17	56.1	56.0
3	71.8	72.0		18	12.0	11.8
4	42.3	42.2		19	19.0	19.2
5	140.8	140.7		20	36.5	36.1
6	121.7	121.6		21	19.4	18.9
7	31.7	31.8		22	31.9	32.1
8	34.0	33.8		23	26.1	25.7
9	50.2	50.1		24	45.9	45.8
10	36.5	36.4		25	29.2	29.1
11	21.1	21.4		26	18.8	18.7
12	39.8	39.7		27	19.8	19.7
13	42.3	42.5		28	23.1	23.0
14	56.8	56.7		29	11.9	11.9
15	24.3	24.2				

4.4.4 Compound LS04 - Epicatechin

Compound **LS04** was isolated from a fraction of ethyl acetate as a yellow crystalline solid of melting point 219 - 221 ^{0}C. On analytical TLC, the compound had an R_f of 0.62 in 70% acetone in methanol. When the chromatogram was sprayed with anisaldehyde the spot turned red which later turned yellow suggesting that the compound was a flavonoid (Harborne, 1998).

^{1}H-NMR spectrum (Appendix 4a) displayed chemical shifts values in three different regions of the spectrum; the aromatic, hydroxylated (oxygenated) and aliphatic protons.

The peak at δ 6.87 (1H, s), a doublet of doublets centered at δ 6.64 (1H, J=8.8 Hz) and a doublet at δ 6.64 (1H, J=8 Hz) represented protons of tri-substituted benzene ring. It should be noted however that the peaks at δ 6.64 representing the protons at carbon 5' and 6' were overlapping owing to the insignificant difference in chemical shifts. This resulted in a triplet instead of the usual doublet. Further, two doublets centered at δ 5.87 (1H, J=1.4 Hz) and δ 5.69 (1H, J=1.4 Hz) suggested the existence of another benzene ring that was tetra-substituted. The relatively small coupling constant (J=1.4 Hz) between the two protons indicated a meta configuration with respect to each other. The pattern of the above mentioned protons strongly suggested a flavonoid skeleton with hydroxyl groups at positions 5 and 7 of ring A and 3' and 4' of ring B (Harborne, 1998).



LS04

The doublet at δ 4.65 (1H, J=4.6 Hz) was assigned to the methine proton at position 2 in ring C. Further, the signal at δ 3.99, appearing as a multiplet, was assigned to the proton at position three of ring C of a flavonoid, attached to a carbon atom that is oxygenated, preferably a hydroxyl group. Such a proton appears as doublets of doublets of doublet suggesting that the proton is coupled by three other protons (Harborne, 1998). However, the peak appeared as a doublet possibly due to the spatial orientation of the hydroxyl group. The other two signals appearing as doublets of doublet at δ 2.66 (1H, J=16.6, 4.4 Hz) and δ 2.44 (1H, J=16.6, 3.2 Hz) suggested geminal protons at position 4, and were

assignable to the axial and equatorial protons, respectively (Harborne, 1998). Ring C did not have a carbonyl group and was replaced by the methylene protons represented by the geminal protons described above. This suggested that the compound had a flavan skeleton. In the ^{13}C NMR spectrum (Appendix 4b) there were fifteen signals supporting the flavonoid skeleton. The peaks at δ 157.2, 156.9, 156.4, 145.2 and 145.1 are in agreement with the presence of five oxygen-linked aromatic carbons in the proposed structure. The absence of peaks at δ 170 – 190 showed the absence of carbonyl carbons in the compound supporting the earlier proton assignment. The signal at δ 28.9 represented the methylene carbon assigned position four replacing the carbonyl carbon. This was further supported by DEPT experiment (Appendix 4c) which showed the presence of one methylene carbon at δ 28.9 ppm. The signal at δ 65.7 represented hydroxylated carbon at position three while the signal at δ 78.7 suggested a carbon at position two which appeared more downfield than the one at position three. This is attributed to the carbon being a benzylic one as opposed to oxygenated carbon atom (Harborne, 1998). The signal at 99.2 was assigned to the quaternary at position 4a of ring A. The spectral data for compound **LS04** was in agreement with that published for epicatechin (Table 4.8 and 4.9) (Harborne, 1998). Based on the spectroscopic evidence, compound **LS04** was assigned as epicatechin.

Epicatechin (**LS04**) is known for its antioxidant and anti-carcinogenic activities, it is also an anti-artherogenic and antitumor agent (Xu *et al.*, 2004). The compound was initially called *kakaool* due to the fact that it is found in large quantities in cacao beans (Freudenberg *et al.*, 1932). There are several reports on the isolation of this compound

from many plant species (Rao *et al*., 1997; de Carvalho *et al*., 2008; Reddy *et al*., 2008). Okoth (2014) reported the isolation of epicatechin for the first time from *Lannea schweinfurthii*.

Table 4.8: ^{1}HNMR (200 MHz, DMSO) data for compound LS04 and Epicatechin (Harborne, 1998)

Proton	Chemical shift (δ) forLS04	Multiplicity/ Integration	J values (Hz)	Chemical shift (δ) for Epicatechin
2’	6.87	s (1H)	-	6.90
5’	6.64	t (1H)	8.8	6.66
6’	6.64	t (1H)	8.0	6.66
8	5.87	d (1H)	1.4	5.89
6	5.69	d (1H)	1.4	5.72
2	4.65	d (1H)	4.6	4.74
3	3.99	m (1H)	-	4.01
4 (ax.)	2.66	dd (1H)	4.4, 16.6	2.79
4 (eq.)	2.44	dd (1H)	3.2, 16.6	2.48

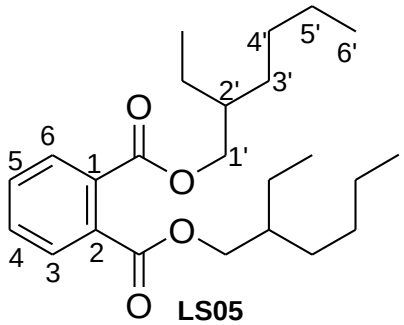
Table 4.9: ^{13}C NMR data (75 MHz, DMSO) for compound LS04 and Epicatechin (Harborne, 1998)

Carbons	Compound LS04 (δ)	DEPT	Epicatechin (δ)
C-4	28.9	CH_2	29.3
C-3	65.6	CH	67.5
C-2	78.7	CH	79.9
C-6	94.7	CH	95.9
C-8	95.7	CH	96.4
C-4a	99.2	C	100.1
C-2’	115.4	CH	115.4
C-5’	115.6	CH	115.9
C-6’	118.6	CH	119.5
C-1’	131.3	C	131.9
C-4’	145.1	C	145.8
C-3’	145.2	C	146.0
C-8a	156.4	C	157.4
C-7	156.9	C	157.7
C-5	157.2	C	158.0

4.4.5 Compound LS05

Compound **LS05** was isolated from a fraction of DCM/ Hexane as a colorless oily liquid. The compound had an R_F of 0.61 in 60% DCM in Hexane. When sprayed with anisaldehyde the spot turned blue suggesting that the compound was a fatty acid derivative (Harborne, 1998).

^{1}H-NMR spectrum (Appendix 5a) displayed chemical shifts values in three different regions of the spectrum; the aromatic, oxygenated and aliphatic protons. The double of doublets centered at δ 7.71 (2H, J=8.7, 5.4 Hz) and at 7.53 (2H, J=8.7, 5.4 Hz) represented protons of di-substituted benzene rings. Further, the splitting patterns in the aromatic region suggested two sets of equivalent protons from an ortho- disubstituted benzene ring. The signal at δ 4.22, appearing as a multiplet, was assigned to the methylene group geminal to the ester alcohol group. In addition, the peaks at δ between 1.68 and 0.91 were assigned to aliphatic protons of the side chains. The downfield signal at 1.68 was assigned to the methine proton of the aliphatic chain suggesting the presence of a branch in the alkyl chain (Rao, *et al*., 2000).



LS05

In the ^{13}C NMR spectrum (Appendix 5b) there were 12 signals representing carbon atoms in three different chemical environments. The peak at δ 167.9 showed the presence of a carbonyl carbon of an ester group supporting the earlier proton assignment. Additionally, the three peaks at δ 132.6, 131.0 and 128.9 were assigned to the three sets of equivalent carbon atoms in the aromatic ring arising from an ortho-substituted benzene ring with similar side chains. The signal at δ 68.3 suggested the presence of oxygen-linked aliphatic carbons and was assigned to the ether carbon in the proposed structure. The signals between δ 38.9 and 11.1 represented the aliphatic carbons in the side chains, with the downfield signal being assigned to methine carbons in the chain (Rao, *et al*., 2000).

The overall assignment of the structure of **LS05** was done by use of GC-MS data (Appendix 6a and 6b). It should be noted that the spectral data of compound **LS05** was closely related to that of di-(2-propylpentnyl) ester phthalic acid (**145**) and dibutyl phthalate (**83**) detected in the crude methanol and DCM extracts of *Lannea schweinfurthii* using GC-MS. It was evident that the compound contained a benzene ring with two similar ester groups that were ortho to each other. Further, ester group contained a branch in the aliphatic chain. The deductions were conclusive in characterizing the compound, the proposed structure of compound **LS05** given and the name assigned di-(2'-ethylhexyl) ester phthalic acid. The compound was isolated for the first time from the plant species.

Table 4.10: ^{1}HNMR (400 MHz, $CDCl_3$) data for compound LS05

Proton	Chemical shift (δ)	Multiplicity/Integration	J–values (Hz)
3, 6	7.71	dd (2H)	5.4, 8.7
4, 5	7.53	dd (2H)	5.4, 8.7
1’	4.22	m (4H)	
2’	1.68	t (2H)	
	1.42	m	
	1.32	m	
	1.25	m	
6’	0.91	m	

Table 4.11: ^{13}C NMR (100 MHz, $CDCl_3$) data for compound LS05

Carbon	Compound LS05 (δ)		Carbon	Compound LS05 (δ)
C=O	167.9		CH_2	29.1
4,5	132.6		CH_2	29.8
1,2	131.0		CH_2	23.9
4,5	128.9		CH_2	23.1
C-O	68.3		CH_3	14.2
CH	38.9		CH_3	11.2
CH_2	30.5			

4.5 Bioassay of isolated compounds

4.5.1 Antibacterial test for the isolated compounds

The compounds **LS01**, **LS02**, **LS03**, **LS04** and **LS05** were subjected to antibacterial test against *S. aureus*, *B. subtilis*, *E. coli*, and *P. aeruginosa*. The results obtained were as tabulated in table 4.12.

Table 4.12: Inhibition zones (in mm) of the isolated compounds after 24hrs; 500 µg/ml per disc

	S.aureus	***B. subtilis***	***E. coli***	***P. aeruginosa***
LS01	8.00±0.10^{c}	7.00±0.30^{b}	6.00±0.50^{a}	6.00±0.40^{a}
LS02	9.00±0.20^{d}	7.00±0.10^{b}	8.00±0.20^{c}	6.00±0.10^{a}
LS03	8.00±0.20^{c}	9.00±0.10^{c}	6.00±0.10^{a}	6.00±0.10^{a}
LS04	15.00±0.50^{e}	14.00±0.20^{d}	10.00±0.50^{d}	9.00±0.40^{b}
LS05	7.00±0.10^{b}	7.00±0.10^{b}	7.00±0.30^{b}	6.00±0.10^{a}
Control (-ve)	6.00±0.20^{a}	6.00±0.40^{a}	6.00±0.20^{a}	6.00 ±0.30^{a}
Control (+ve)	18.00±0.30^{f}	18.00±0.30^{e}	18.00 ±0.20^{e}	16.00±0.40^{c}
P-value	<0.001	<0.001	<0.001	<0.001

Mean values followed by the same small letter within the same column do not differ significantly from one another (One-Way ANOVA,SNK test,α =0.05)
Key: Control –ve - DMSO; +ve Control - Tetracycline

There was a significant difference between the various isolated compounds and the controls ($p< 0.001$, one-way ANOVA) in all the bacteria used. The pure compound extracted from *L.schweinfurthii* stem bark coded **LS01** had mild activities of 8 and 7 mm against the two Gram-positive bacteria, *S. aureus* and *B. subtilis*, respectively. The compound had no activity against the Gram-negative bacteria. An inhibition zone of 9 and 7 mm was shown by lupeol (**LS02**) against the Gram-positive bacteria *S. aureus* and *B. subtilis*, respectively, and 8 mm against the *E. coli* but no activity against the other two Gram-negative bacterial strains. β-sitosterol (**LS03**) showed mild activity against the Gram-positive bacteria but had no activity against the Gram-negative bacteria. The inhibition zones were 8 mm against *S. aureus* and 9 mm against *B. subtilis*. Epicatechin (**LS04**) had a significantly higher inhibition zone against the test strains used compared to the other compounds and the negative control. The inhibition zones of epicatechin (**LS04**) were 10 and 9 mm against *E. coli* and *P. aeruginosa*

respectively, 14 mm against *B. subtilis* and 15 mm against *S. aureus*. All the compounds tested exhibited some activity against the Gram-positive but no activity against the Gram-negative bacteria *P. aeruginosa*, with the exception of epicatechin (**LS04**). The results could explain, to some extent, the usage of *L. schweinfurthii* in many communities as herbal remedies. However, other tests such as cytotoxicity studies should be carried out to ascertain the observed activity.

4.5.2 Antifungal test for the isolated compounds

The isolated compounds were subjected to antifungal assay against two fungi which included *C. albicans* and *P. notatum*. The inhibition zones and the activities of the isolated compounds were as tabulated in table 4.13.

Table 4.13: Inhibition zones (in mm) of isolated compounds in the diffusion method assay after 7 days; 1000 µg/ml per disc

Compound	*C. albicans*	*P. notatum*
LS01	6.00 ± 0.20^{a}	6.00 ± 0.40^{a}
LS02	6.00 ± 0.20^{a}	8.00 ± 0.20^{c}
LS03	6.00 ± 0.10^{a}	9.00 ± 0.10^{d}
LS04	13.00 ± 0.30^{b}	12.00 ± 0.20^{e}
LS05	6.00 ± 0.10^{a}	7.00 ± 0.50^{b}
Control (-ve)	6.00 ± 0.20^{a}	6.00 ± 0.20^{a}
Control (+ve)	18.00 ± 0.40^{c}	18.00 ± 0.50^{f}
P-value	<0.001	<0.001

Mean values followed by the same small letter within the same column do not differ significantly from one another (One-Way ANOVA,SNK test,α =0.05)
Key: Control –ve - DMSO; Control +ve - Nystatin

There was a significant difference between the various isolated compounds and the controls ($p< 0.001$, one-way ANOVA) in the two fungi used. The results further showed

that the activities of the isolated compounds were less than those of the crude extracts from which the compounds were isolated. The activity against fungi was lower than displayed by the compounds against bacteria used. However, some activity was noted from some of the isolated compounds. Epicatechin (**LS04**) had the highest activity against the fungi used. It showed an inhibition zone of 13 mm against *C. albicans* and 12 mm against *P. notatum*. All the other compounds isolated from *Lannea schweinfurthii* had mild activity against *P. notatum* but no activity against *C. albicans*.

The various bacteria strains used in the study are known to cause diseases such as gastro intestinal infections. The fungi used in the study are known to cause ailment such as candidiasis, vaginal thrush, athlete foot and skin abscess (Johns *et al*., 1995). The activity shown by the crude extract as well as isolated compounds from *Lannea schweinfurthii* compares well with the activity reported by Okoth (2014).

CHAPTER FIVE

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

Phytochemicals are useful in the effective control of infectious diseases caused by bacteria and fungi and are currently receiving more attention due to their effectiveness in the treatment of infectious diseases as well as mitigating many of the side effects caused by conventional antimicrobials. This study focused on antibacterial, antifungal and phytochemical screening of *Lannea schweinfurthii*. The following conclusions were made during the study:

i. The percentage yields of the extracts increased from hexane to DCM to EtOAc to MeOH. These results suggested that the stem bark of *L. schweinfurthii* is very rich in both non-polar and polar metabolites.

ii. Hexane/DCM crude extract of *L. schweinfurthii* had mild activity against the Gram-positive, Gram-negative and the fungi used, with an inhibition zone of 7 mm.

iii. The methanol extract had the highest activity towards the Gram-negative *E. coli*, with an inhibition zone of 21 mm.

iv. Ethyl acetate extract had the highest activity against the Gram-positive *S. aureus*, with an inhibition zone of 20 mm.

v. TLC analysis of the crude extracts revealed that the compounds extracted are not the only compounds present in the plant material.

vi. The percentage by mass of the isolated compounds is very low since the plant material contains numerous compounds of almost equal polarity making the isolation process complex.

vii. The study shows that the stem bark of *L. schweinfurthii* contain fatty acids, steroids, terpenoids and plant phenolics among other compounds. Some of these compounds include; 3-(10'-tridecenyl) phenol (**LS01**), lupeol (**LS02**), β-sitosterol (**LS03**), epicatechin (**LS04**) and di-(2'-ethylhexyl) ester phthalic acid (**LS05**). Their structures were elucidated using NMR data.

viii. Three compounds 3-(10'-tridecenyl) phenol (**LS01**), epicatechin (**LS04**) and di-(2'-ethylhexyl) ester phthalic acid (**LS05**) were isolated for the first time from the plant species.

ix. Epicatechin (**LS04**) exhibited high antibacterial activity against the Gram-positive bacteria with inhibition zones of 15 and 14 mm for *S. aureus* and *B. subtilis*, respectively and against the fungus *C. albicans*, with an inhibition zone of 13 mm.

5.2 Recommendations and further studies

i. The study has demonstrated that there is need for further investigation and isolation of other pure compounds from *Lannea schweinfurthii* and other plants in the family Anacardiacae.

ii. Additionally, the crude and pure extracts of *Lannea schweinfurthii* need to be subjected to further tests on other disease causing microbes, both bacteria and fungi.

iii. Further, synergic effects among the crude extracts, isolated compounds and the conventional antibiotics should be carried out.

iv. Moreover, there is need to carry out phytochemical studies of the plant species methanolic extracts and all other fractions that were not analyzed in this study.

v. More work should be done on isolated compounds to determine both pharmacological and biological activities, and on the use of crude blends.

vi. Furthermore, the isolated compounds that showed the highest activity could be subjected to more studies such as cytotoxicity tests in order to be used as antimicrobials or as templates for the synthesis of drugs used in the treatments of diseases caused by bacteria and fungi.

REFERENCES

Abraham, R.J., Fisher, J. and Loftus, P. (1995). Introduction to NMR spectroscopy. John Wiley and Sons, New York, pp 156, 160 – 162, 167 – 168.

Adewusi, E.A. and Steenkamp, V. (2011). *In vitro* screening for acetylcholinesterase inhibition and antioxidant activity of medicinal plants from southern Africa. *Asian Pacific Journal of Tropical Medicine* **4**: 829 – 835.

Agnew, A.D.Q. and Agnew, S. (1994). Upland Kenya Wild flowers: A flora of the fern and herbaceous flowering plants of the Upland Kenya. 2nd Ed. East Africa Natural History Society, Nairobi, pp 114, 164, 195, 202, 241 – 244.

Agrawal, P.K. (1992). NMR spectroscopy in the structural elucidation of oligosaccharides and glucosides. *Journal of Phytochemistry* **31**: 3307 – 3330.

Amin, A. and Mousa, M. (2007). Merits of anti-cancer plants from Arabian Gulf region. *Cancer Therapy* **5**: 55 – 56.

Amiram, G., John, H.C., Lewis, K.P., Duangchan, U., Yoel, K. and Michal, R.B. (1997). Novel cytotoxic: Alkylated hydroquinones from *Lannea welwitschii*. *Journal of Natural Products* **60**: 116 – 121.

Amit, R.N. and Shailendra, S. (2005). Ethno-Medicinal Approach in Biological and Chemical Investigation of Phytochemicals as Antimicrobials. *Pharmainformation* **4**: 2.

Aue, W.P., Berthod, D. and Ernst, R.R. (1976). Two Dimensional Spectroscopy Application to Nuclear Magnetic Resonance. *Journal of Chemical Physics* **64**: 2229 – 2246.

Baker, J., Borris, R.P. and Cartel, B. (1995). Natural drug discovery and development: New perspectives on international collaboration. *Journal of Natural Products* **5**: 1315 – 1325.

Balunas, M.J. and Kinghorn, A.D. (2005). Drug discovery from medicinal plants. *Life Science* **78**: 431 – 441.

Beentje, H.J. (1994). Kenya Trees, Shrubs and Lianas. National Museum of Kenya, Majestic Printing Ltd, Nairobi, pp 425 – 429.

Bingham, M.G., Willemen, A., Wursten, B.T., Ballings, P. and Hyde, M.A. (2016). Flora of Zambia: Species information: individual images: *Lannea schweinfurthii*. http://www.zambiaflora.com/speciesdata/imagedisplay.php?species_id=13650&image_id=2&6, retrieved 31 October 2016.

Bodenhausen, G., Kogler, H. and Ernst, R.R. (1984). Selection of Coherence transfer Pathways in NMR pulse experiments. *Journal of Magnetic Resonance* **58**: 370 – 388.

Busia, K. (2005). Medicinal provision in Africa: past and present. *Phytotherapy Research* **19**: 919 – 923.

Chhabra, S.C., Mahunnah, R.L.A. and Mshiu E.N. (1987). Plants used in traditional medicine in Eastern Tanzania. I. Pteridophytes and angiosperms (Acanthaceae and Canellaceae). *Journal of Ethno-Pharmacology* **21**: 253 – 277.

Chhabra, S.C. and Uiso, F.C. (1991). Antibacterial activity of some Tanzanian plants used in traditional medicine. *Fitoterapia* **62**: 499 – 504.

Clinical and Laboratory Standards Institute, (2013). Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Third Informational Supplement. CLSI document M100-S23.Wayne, **33**(**1**): 100–102.

Correia, S.J., David, J.M., David, J.P., Chai, H.B., Pezzuto, J.M. and Cordell, G.A. (2001). Alkyl phenols and derivatives from *Tapirira obtusa*. *Phytochemistry* **56**: 781 – 784.

Cos, P., Vlietinck, A.J., Berghe, D.V. and Maes, L. (2006). Anti-infective potential of natural products: how to develop a stronger *in vitro* 'proof-of-concept' *Journal of Ethno-Pharmacology* **106** (3): 290 – 302.

Coxon, D.T., Holmes, A., Ollis, W.D., Vora, V.C., Grant, M.S. and Tee, J.L. (1972). Flavanol digallates in green tea leaf. *Tetrahedron* **28**: 2819 – 2826.

Cragg, G.M. and Newman, D.J. (2005). Nature: A vital source of leads for anticancer drug development. *Phytochemistry Review* **8**: 313 – 331.

Czygan, F.C. (1993). Kulturgeschichte and Mystic des Johanniskruates. *Zeitschrift fut Phytotherapie* **5**: 2676 – 2682.

de Boer, J.H., Kool, A., Broberg, A., Mziray, W.R., Hedberg, I. and Levenfors, J.J. (2005). Anti-fungal and anti-bacterial activity of some herbal remedies from Tanzania. *Journal of Ethno-Pharmacology* **96**: 461 – 469.

de Carvalho, M.G., Suzart, L.R., Cavatti, L.C. and Kaplan, M.A.C. (2008). New flavonoids and other constituents from *Ouratea hexasperma* (Ochnaceae). *Journal of Brazilian Chemical Society* **19**: 1423 – 1428.

Deng, J.Z., Starck, S.R. and Hecht, S.M. (1999). Bis-5-alkylresorcinols from *Panopsis rubescens* that inhibit DNA polymerase β. *Journal of Natural Products* **62**: 477 – 480.

Dewick, P. (1998). Medicinal Natural Products, A Biosynthetic Approach. John Wiley and Sons Ltd, United Kingdom, pp 24 – 26.

Dey, P.M. and Harborne, J.B. (1991). Methods in plant Biochemistry. Academic press. New York, London, pp **7**, 370 – 425.

Diallo, D., Marston, A., Terreaux, C., Toure, Y., Paulsen, B.S. and Hostettmann, K. (2001). Screening of Malian Medicinal plants for Antifungal, Larvicidal, molluscicidal, anti – oxidant and radical scavenging activities. *Journal of Phytotherapy Research* **15**: 401 – 406.

Du, Y., Oshima, R., Yamauchi, Y., Kumanotani, J. and Miyakoshi, T. (1986). Long chain phenols from the Burmese Lac tree, *Melanorrhoea usitate*. *Phytochemistry* **25**: 2211 – 2218.

Dubey, N.K., Rajeshkumar, S. and Tripatti, P. (2004). Global promotion of herbal medicine: India's opportunity. *Current Science* **86**: 37 – 41.

Ebel, J. and Hahlbrock, K. (1982). In ''the Flavonoids, Advances in Research'' (Harborne, J.B. and Mabry, T.J., Eds). Chapman and Hall, London, pp 614 – 675.

El–Gammal, A.A. and Mansour, R.M.A. (1986). Antimicrobial activities of some flavonoid compounds. *Zentrablatt fur Mikrobiologie* **141**: 561 – 565.

Englert, G. (1985). NMR of Carotenoids, New Experimental Techniques. *Pure and Applied Chemistry* **57**: 801 – 820.

Ernest, R. (2005). The efficacy of herbal medicine. *Fundamental and Clinical Pharmacology* **19**: 405.

Evans, W.C. (1997). Trees and Evans Pharmacology 14th Edition London W.B., Saunders Company Ltd Fox R. Pharmaceuticals from plants: Great Potential, few funds. *Lancet* **343**: 1513 – 1515.

Farnsworth, N.R. (1998). Human Medicinal Agents from Plants. Kinghorn, A.D. and Balandrin, M.F. (Eds), ACS symposium series. **534**: 2 – 12.

Farthings, M. (2002). Novel Targets for the Control of Secretory Diarrhoea. *Gut* **50**: 15 – 18.

Fennell, C.W., Lindsey, K.L., McGraw, L.J., Spar, S.G., Stafford, G.I., Elgorashi, E.E., Grace, O.M. and van Staden, J. (2004). Assessing African medicinal plants for efficacy and safety: Pharmacological screening and toxicology. *Journal of Ethno-Pharmacology* **94**: 205 – 217.

Finney, D.J. (1964). Statistical method in biological assay. 2nd ed. Charles Griffin & Co. Ltd., London. 1 – 5, 21, 58.

Freudenberg, K., Cox, R.F.B. and Braun, E. (1932). The catechin of cacao bean. *Journal of American Chemical Society* **54**: 1913 – 1917.

Gathirwa, J.W., Rukunga, G.M., Njagi, E.N., Omar, S.A., Mwitari, P.G., Guantai, A.N., Tolo, F.M., Kimani, C.W., Muthawa, C.N., Kirira, P.G., Ndunda, T.N., Amalemba, G., Mungai, G.M. and Ndiege, I.O. (2008). The *in vitro* anti-plasmodial and *in vivo* anti-malarial efficacy of Combinations of some Medicinal Plants used traditionally for the treatment of Malaria by the Meru Community in Kenya. *Journal of Ethno-Pharmacology* **115**: 223 - 231.

Gathirwa, J.W., Rukunga, G.M., Mwitari, P.G., Mwikwabe, N.M., Kimani, C.W., Muthaura, C.N., Kiboi, D.M., Nyangacha, R.M. and Omar, S.A. (2011). Traditional herbal antimalarial therapy in Kilifi district, Kenya. *Journal of Ethno-Pharmacology* 134: 434 – 442.

Geddes, A.M. (1985). Prescribers' needs for developed and third world. In: Greenwood, F.O.O.' Grady (Ed.), The Scientific Basis of Antimicrobial Chemotherapy, vol. 1, Cambridge University Press, Cambridge, pp 1 - 12.

Geissler, P.W., Harris, S.A., Prince, R.J., Oslen, A., Achieng'-Odhiambo, R., Oketch-Rabah, H., Madiega, P.A., Andersen, A. and Mølgaard, P.A. (2002). Medicinal plants used by Luo mothers and children in Bondo district, Kenya. *Journal of Ethno-Phamarcology* **83**: 39 – 54.

Geissman, T.A. and Crout, D.H.G. (1969). Organic Chemistry of Secondary Plants Metabolism. Free Cooper Company, California pp 185 – 230.

Groweiss, A., Cardellina, J.H., Pannell, L.K., Uyakul, D., Kashman, Y. and Boyd, M.R. (1997). Novel Cytotoxic, Alkylated Hydroquinones from *Lannea welwitschii*. *Journal of Natural Products* **60**: 116 – 121.

Gullece, M., Aslan, A., Sokmen, M., Sahin, F., Adiguzel, A., Agar, G. and Sokmen, A. (2006). Screening of the antioxidant and antimicrobial properties of the lichens *Parmelia saxatilis*, *Platismatia glauca*, *Ramalina pollinaria*, *Ramalina polymorpha* and *Umbilicaria nylanderian*. *Phytomedicine* **13**: 515 – 521.

Hans, G.S. (1993). General Microbiology (7th Ed). Cambridge University Press, New York pp 69 – 377.

Harborne, J.B. (1967). Comparative Biochemistry of the Flavonoids. Academic press, London, pp 267 – 279.

Harborne, J.B. (1998). Phytochemical Methods: A guide to modern technique of plant analysis. Chapman and Hall, London.

Hilliard, J.J., Krause, H.M. and Bernstein, J.I. (1995). A Comparison of Active Site Binding for Quinolones and Novel Flavone Gyrase Inhibitor to DNA Gyrase. *Advanced Medical Biology* **390:** 59 – 69.

Hiroyuki, N., Yoshahisa, T., Naonobu, T., Koichiro, T., Hirofumi, S. and Tomihiko, H. (2006). *Journal of Natural Products* **69**: 1177.

Hirschhorn, N. (1980). Treatment of acute diarrhoea in children: A historical and physiological perspective. *American Journal of Clinical Nutrition* **33**: 637 – 663.

Humber, J.M. (2002). The role of complementary and alternative medicine: accommodating pluralism. *Journal of American Medical Association* **288**: 1655 – 1656.

Islam, T. and Tahara, S. (2000). Dihydroflavonols from *Lannea coromandelica*. *Phytochemistry* **54**: 901 – 907.

Islam, T., Ito, T., Sakasai, M., and Tahara, S. (2002). Zoosporidal Activity of Polyflavonoid tannin Identified in *Lannea coromandelica* stem bark against phytopathogenic Oomycete A*shanomyces cochlioides*. *Journal of Agricultural and Food Chemistry* **50**: 6697 – 6703.

Jassim, S.A.A. and Naji, M.A. (2003). Novel antiviral agents: a medicinal plant perspective. *Journal of Applied Microbiology* **95**: 412.

Jawetz, E., Melnick, J.L. and Adelberg, E.A. (1966). Review of medical Microbiology. 7th ed. Lange Medical Publications, London. pp 115 – 134.

Jiang, Y., Wang, Y. and Yan, X. (2000). Chinese pharmaceautical Companies: an emerging industry. *Drug Discovery Today* **6**: 610 – 612.

Johns, T., Faubert, G.M., Kokwaro, J.O., Mahunnah, R.L.A. and Kimanani, E.K. (1995). Anti-giardial activity of gastrointestinal remedies of the Luo of East-Africa. *Journal of Ethno-Pharmacology* **46**: 17-23.

Kapche, G.D.W.F., Laatsch, H., Fotso, S., Kouam, S.F., Wafo, P., Nyadjui, B.T. and Abegaz, B.M. (2007). Lanneanol: A new cytotoxic dihydroalkylcyclohexenol and Phenolic Compounds from *Lannea nigritana* (Sc. Ell.) Keay. *Biochemical Systematics and Ecology* **35**: 539 – 543.

Kareru, P.G., Kenji, G.M., Gachanja, A.N., Keriko, J.M. and Mungai, G. (2007). Traditional medicines among the Mbeere people of Kenya. *African Journal of Traditional, Complementary and Alternative Medicines* **4** (1): 75 – 86.

Kessler, H., Bermel, W. and Griesinger, C. (1985). Recognition of NMR spin systems of cyclosporine A via heteronuclear proton-carbon long range coupling. *Journal of American Chemical Society* **107**: 1083 – 1084.

Koehn, F.E. and Carter, G.T. (2005). The evolving role of natural products in drug discovery. *Nature Reviews Drug Discovery* **4**: 206 – 220.

Kokwaro, J.O. (1993). Medicinal plants of East Africa (2nd Ed). Kenya Literature Bureau, Nairobi, Kenya.

Kokwaro, J.O. (1996). An ethnobotanical study of East African Medicinal plants and Traditional Medicine. Science Symposium. Baltimore, Maryland, pp 23 – 24.

Krishnaswamy, N.R. (2003). Chemistry of natural products: A Laboratory Handbook. Orient Blackswan publisher. Hyderabad, India. pp 75 – 78.

Lawrence, B.M. (2000). Essential Oils: agriculture to chemistry. *Introduction Journal of Aromather* **10**: 82 – 98.

Li, J. and Vederas, J.C. (2009). Drug discovery and natural products: End of an era or an endless frontier? *Science* **325**: 161 – 165.

Lia, Y., Fang, H. and Xu, W. (2007). Recent advance in the research of flavonoids as anticancer agents. *Minimum Review Medical Chemistry* **7**: 663 – 678.

Lin, Y.M., Anderson, H., Flavin, M.T., Pai, Y.H.S., Mata-Greenwood, E., Pengsuparp, T., Pezzuto, J.M., Schinazi, R.F., Hughes, S.H. and Chen, F.C. (2001). *In vitro* anti-HIV activity of Biflavonoids isolated from *Rhus succedanea* and *Garcinia multiflora*. *Journal of Natural Products* **60**: 884 – 888.

López-Lázaro, M. (2002). Flavonoids as anticancer agents: structure-activity relationship study. *Current Medicinal Chemistry- Anti-Cancer Agents* **2** (6): 691 - 714.

Leuck, E. (1980). Antimicrobial food additives. Springer – Verlag. Berlin pp 233 – 234.

Machocho, A.K. (1992). Flavonoids from the roots of *Tephrosia emoroides* and their antifeeding effects on the Larvae of the spotted stalk borer, *Chilo partellus*, M.Sc., Thesis, Kenyatta University.

Mahato, S.B. and Kundu, A. (1994). ^{13}C NMR spectra of pentacyclic triterpenoids – A compilation and some salient features. *Journal of Phytochemistry* **37** (6): 1517-1575.

Maregesi, S., Van Miert, S., Pannecouque, C., Haddad, M.H.F., Hermans, N., Wright, C.W., Vlietinck, A.J., Apers, S. and Pieters, L. (2010). Screening of some

Tanzanian medicinal plants against *Plasmodium falciparum* and human immunodeficiency virus. *Planta Medica* **76** (2): 195 – 201.

Maregesi, S.M., Pieters, L., Ngassapa, O.D., Apers, S., Vingerhoets, R., Cos, P., Berghe, D.A.V. and Vlietinck, A.J. (2008). Screening of some Tanzanian medicinal plants from Bunda district for antibacterial, antifungal and antiviral activities. *Journal of Ethno-Pharmacology* **119**: 56 – 66.

Mark, K.R. and Chari, V.M. (1986). In "Flavonoids, Advances in Research" (Harborne, J.B. Eds). Chapman and Hall, London, pp 23 – 53.

Mckane, L. and Kandel, J. (1996). Microbiology: Essentials and applications. 2nd Ed. McGraw-Hill Inc., New York. 375 – 406.

Muriithi, A.W. (1996). Control of diarrhoea disease programmes, The Kenyan situation. *African Medical Journal* **281**: 189 – 195.

National Committee for Clinical Laboratory standards (NCCLS) (1999). Performance Standards Antimicrobial susceptibility testing. 9th International Supplement. M100 S9, Wayne PA.

National Committee for Clinical Laboratory standards (NCCLS) (2003). Standardization of antimicrobial susceptibility testing by disc diffusion: Standard approved, 8th Ed. p 23.

Okoth, D.A. (2014). Phytochemistry and bioactive natural products from *Lannea alata,Lannea rivae, Lannea schimperi* and *Lannea schweinfurthii* (Anacardiaceae), Phd., Thesis, KwaZulu-Natal University.

Pandian, M.R., Banu, G.S. and Kumar, G. (2006). A study of antimicrobial activity of *Alangium salviifolium*. *Indian Journal of Pharmacology* **38**: 203 – 204.

Queiroz, E.F., Kuhl, C., Terreax, C., Mavi, S. and Hostettmann, K. (2003). New Dihydroalkylhexenones from *Lannea edulis. Journal of Natural Products* **66**: 578 – 580.

Radovanović, A., Radovanović, B.B. and Jovančićević, B. (2009). Free radical scavenging and antibacterial activities of southern Serbian red wines. *Journal of Food Chemistry* **117**: 326-331.

Ramawat, K.G. and Goyal, S. (2008). Bioactive Molecules and Medicinal Plants. Springer, Heidelberg, New York.

Rao, G.N., Kumar, P.M., Dhandapani, V.S., Krishna, T.R. and Hayashi, T. (2000). Constituents of *Cassia auriculata*. *Fitoterapia* **71**: 82 – 83.

Rao, K.V., Sreeramulu, K., Venkata, R.C., Gunasekar, D., Martin, M.T. and Bodo, B. (1997). Two new biflavonoids from *Ochna obtusata*. *Journal of Natural Products* **60**: 632 – 634.

Reddy, B.A.K., Reddy, N.P. Gunasekar, D., Blond, A. and Bodo, B. (2008). Biflavonoids from *Ochna lanceolata*. *Phytochemistry* **1**: 27 – 30.

Ruffo, C.K., Birnie, A. and Tengnas, B. (2002). Edible wild plants of Tanzania. Regional Land Management Unit, Nairobi.

Sahoo, N., Manchikanti, P., and Dey, S. (2010). Herbal drugs: Standards and regulation. *Fitoterapia* 81 (**6**): 462 – 471.

Satomi, F., Naomichi, T., Masateru, O., Alaa, M. N., Toshihiro, N., Hiroyuki, S., Shima, D. and Hideo, Y. (2002). Two novel long chain alkanoic acid esters of lupeol from *Alecrim propolis*. *Chemical and Pharmaceutical Bulletin* **50**: 439 – 440.

Shadomy, S., Espinel-Ingroff, A. and Cartwright, R. (1985). Laboratory studies with antifungal agents; Susceptibility tests and bioassays. In: ‘‘Manual of clinical Microbiology, 4th ed.’’ (Lennette, E.A., Balows, A., Hausler, W.J.Jr. and Shadomy, H.J., Eds), McGraw-Hill Inc., New York, pp 991 – 999.

Shoskes, D.A., Zeitklin, S.I., Shared, A. and Rajfer, J. (1999). Quercetin in men with category (III) chronic prostatitis. A preliminary prospective, double-blind, placebo-controlled trial. *Urology* **54**: 960 – 963.

Silverstein, R., Bassler, G. and Morrill, T. (1991). Spectroscopic identification of Organic Compounds, John Wiley and Sons, New York, pp 119 – 228.

Singh, S. and Singh G.B. (1996). Hypotensive activity of *Lannea coromandelica* bark extract. *Phytotherapy Research* **10**: 429 – 430.

Sneader, W. (1985). Drug discovery: The evolution of modern medicine, John Wiley and Sons, New York, pp 1 – 14.

Sritharan, M. and Sritharan, V. (2004). Emerging problems in the management of Infectious diseases. The Biofilm. *Indian Journal of Medical Microbiology* **22**: 140 – 142.

Subramanian, S.S. and Nair, A.G.R. (1971). Polyphenols of *Lannea coromandelica*. *Journal of Phytochemistry* **10**: 1939 – 1940.

Summers, M.F., Marzili, L.G. and Bax, A. (1986). Complete ^{1}H and ^{13}C assignment of Co-enzyme B_{12} through the use of new two-dimensional NMR Experiment. *Journal of American Chemical Society* **108**: 4285 – 4294.

Swartz, G.V. (2006). Phytochemical studies of *Herichrysum patulum*. M.Sc., Thesis, University of the Western Cape Town.

Tait-Kamradt, A., Davies, T., Appelbaum, P.C. and Depardieu, F. (2000). Two new mechanisims of macrolide resistance in clinical strain of *Streptococcus pneumoniae* from Eastern Europe and North America. *Journal of Antimicrobial Agents* **44**: 337 – 340.

Thomson, W.A.R. (1978). Healing Plants. McGrawhill Book Co. Ltd, London.

Venkata, R.K. and Venkata, R.R. (2008). Traditional medicine used by the Adivasis of the eastern Ghat, Andhra Pradesh- for bone fractures. *Ethnobotnical Leaflets* **12**: 19 – 22.

Waterman, P.G. (1992). Role of secondary metabolites in plants in: Chadwik, D.J. and Whelman, J. (Eds). Secondary metabolites, their function and evolution. Ciba Foundation Symposium 171. Chichester: Wiley, 225 – 275.

Watt, J.M. and Beyer-Brandwijk, M.G. (1962). Medicinal and poisonous plants of Southern and Eastern Africa, (2nd Ed), E. and S. Livingstone Ltd, Edinburg, pp 46, 205 – 297.

Werner, R.A., Rossmann, A., Schuarz, C., Bacher, A., Schmidt, H.L. and Eiseneich, W. (2004). Biosynthesis of gallic acid in *Rhys typhina*: discrimination between alternative pathways from natural oxygen isotope abundance. *Phytochemistry* **65**: 2809 – 2813.

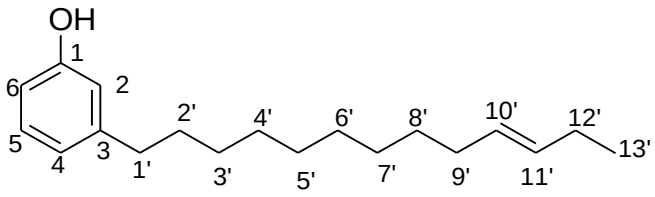
WHO (2008). Traditional medicine. Fact sheet No. 134.

Xu, J.Z., Yeung, S.Y.V., Chang, Q., Huang, Y. and Chen, Z. (2004). Comparison of antioxidant activity and bioactivity of tea epicatechins with their respective epimers. *British Journal of Nutrition* **91**: 873 – 881.

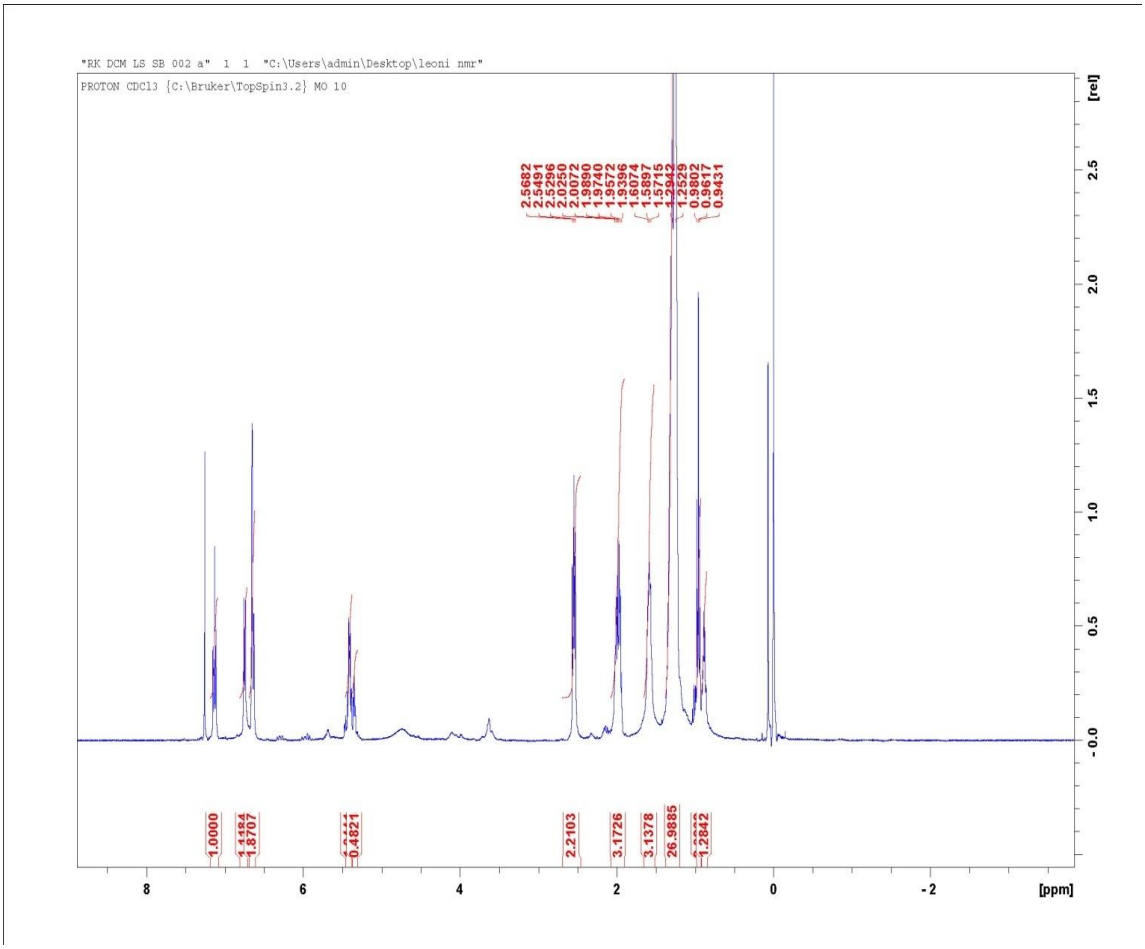
Yun, X.J., Shu, H.M., Chen, G.Y., Ji, M.H. and Ding, J.Y. (2014). Chemical constituents from barks of *Lannea coromandelica*. *Chinese Herbal Medicines* **6** (1): 65 – 69.

APPENDICES

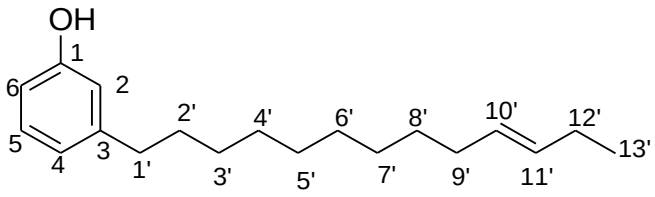
Appendix 1a: ^{1}H NMR for LS01



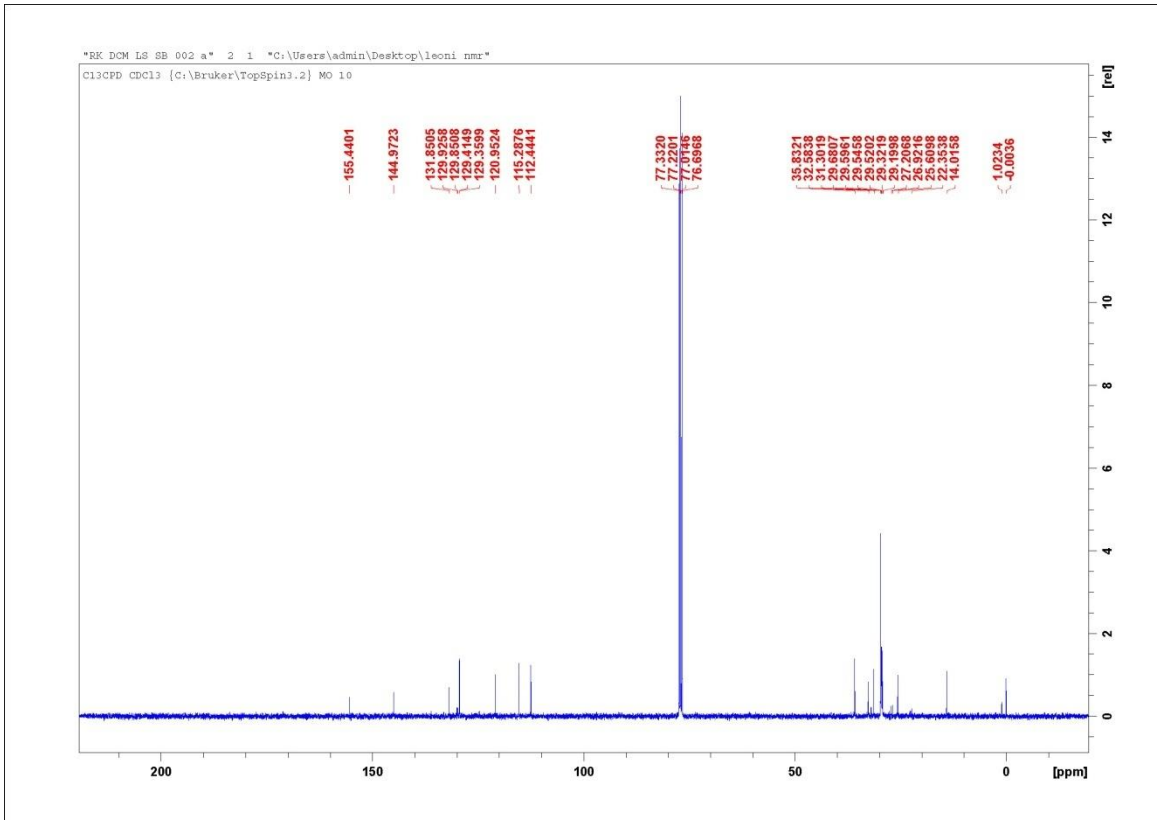
LS01



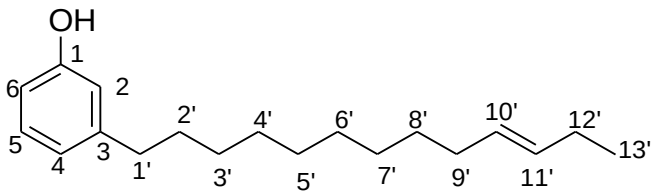
Appendix 1b: ^{13}C NMR for LS01



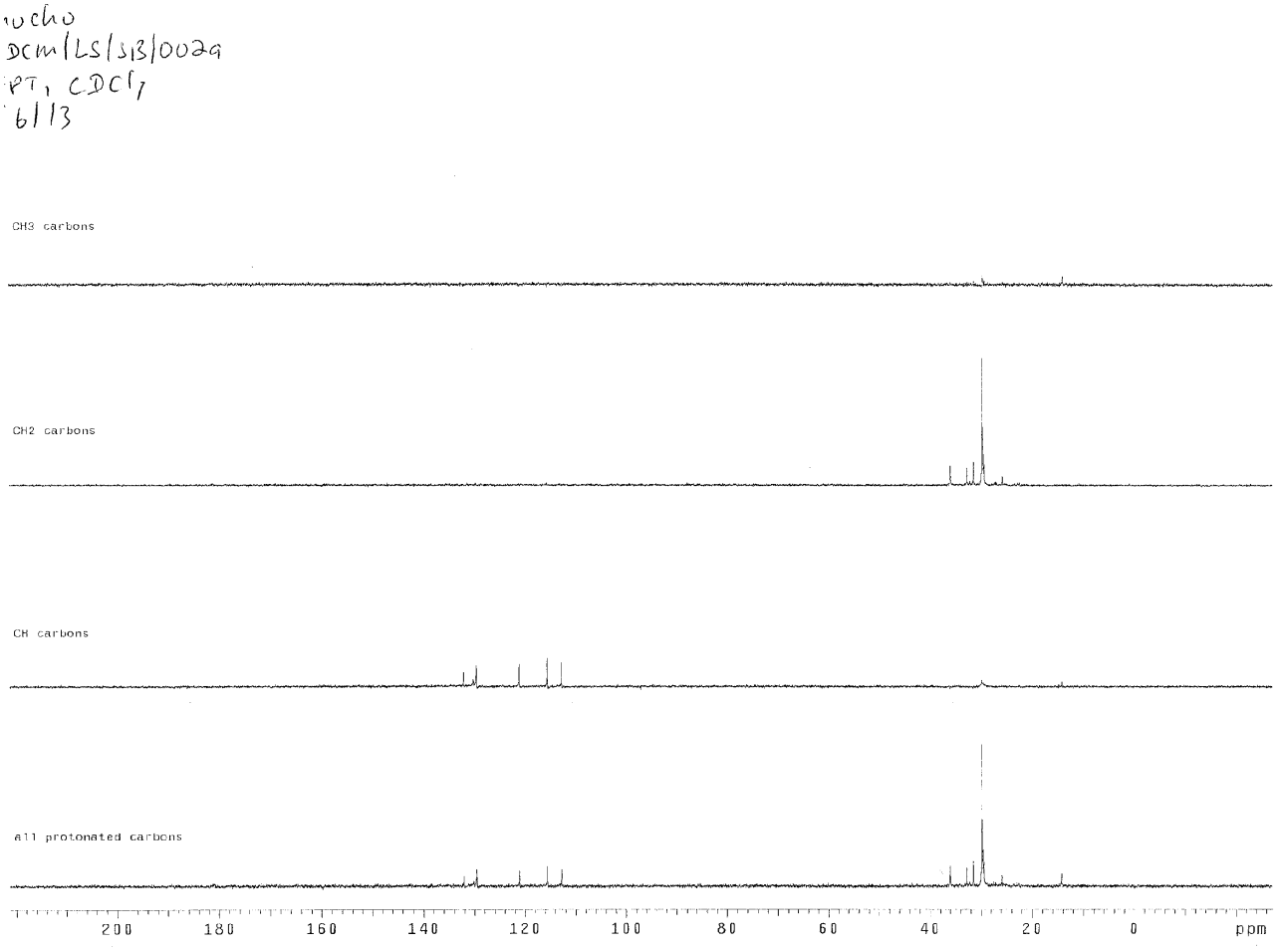
LS01



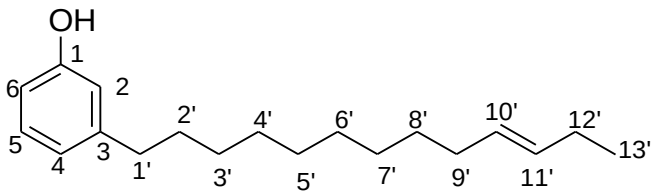
Appendix 1c: DEPT for LS01



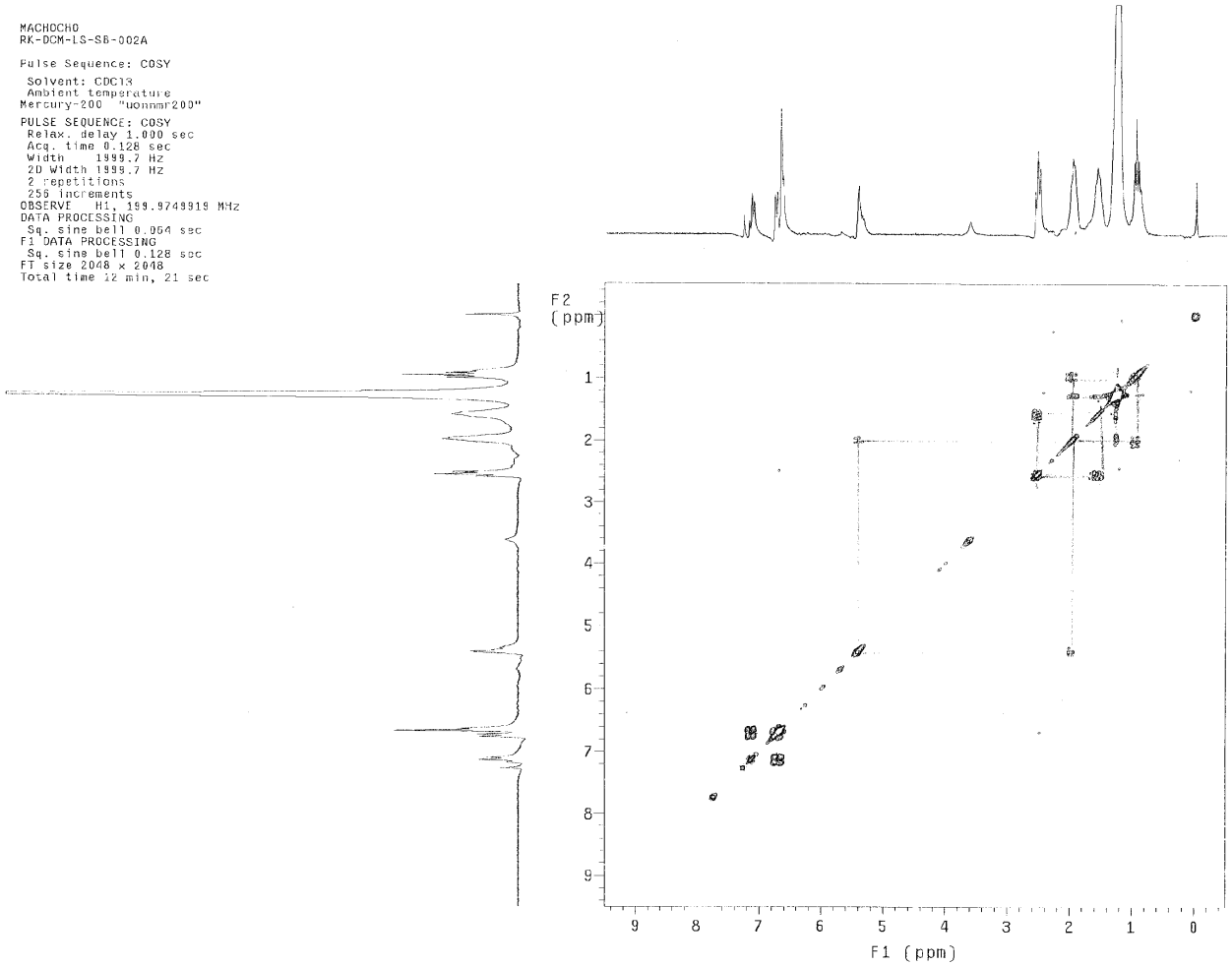
LS01



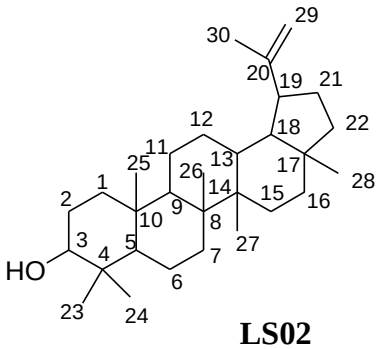
Appendix 1d: COSY for LS01



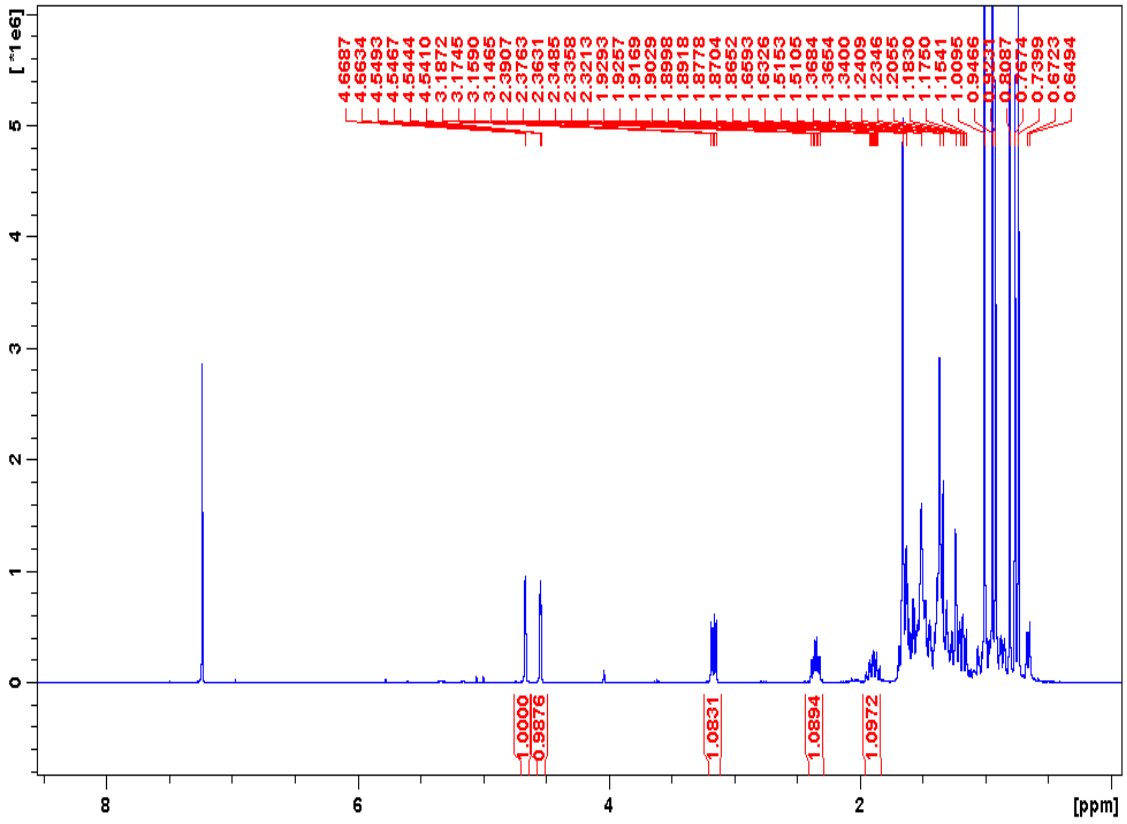
LS01



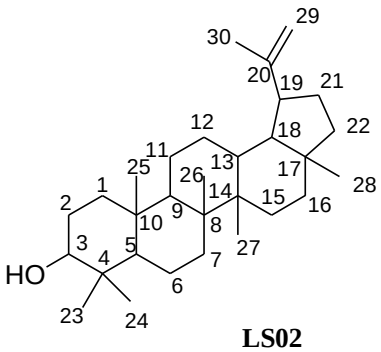
Appendix 2a: ^{1}H NMR for compound LS02



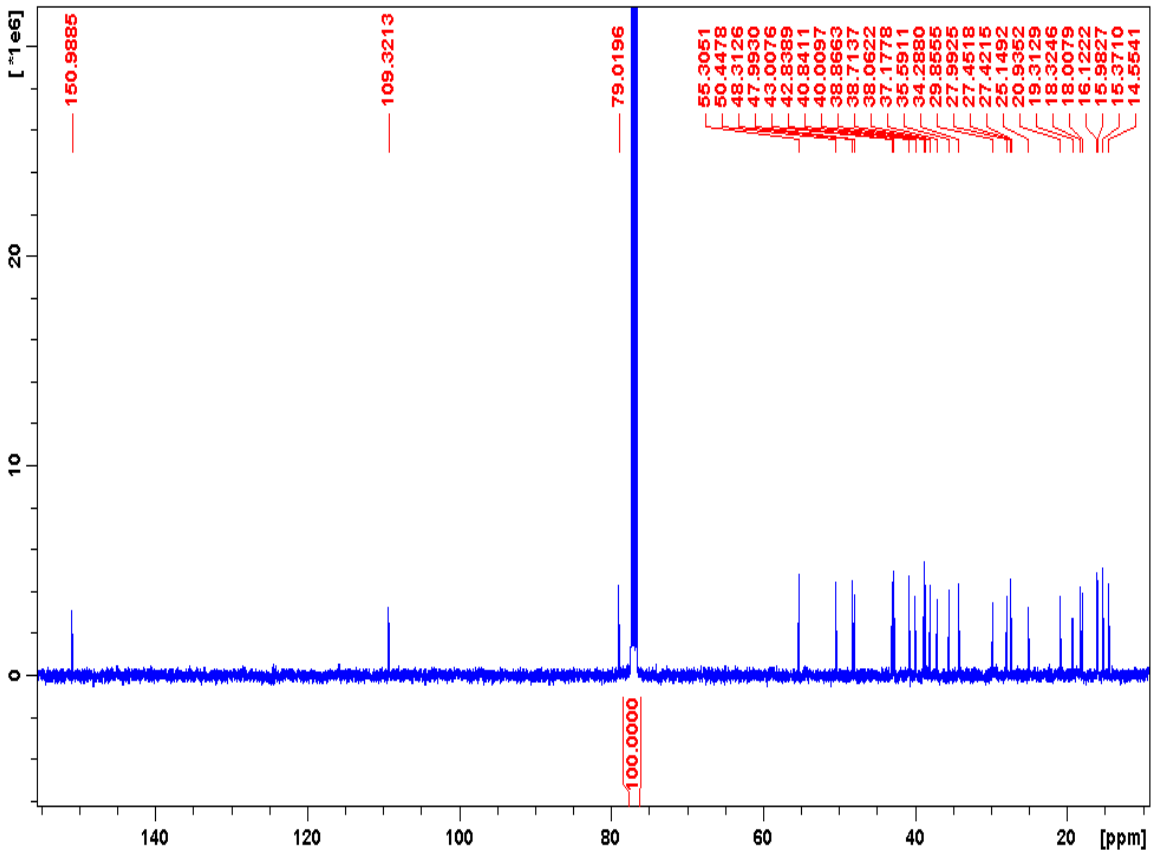
LS02



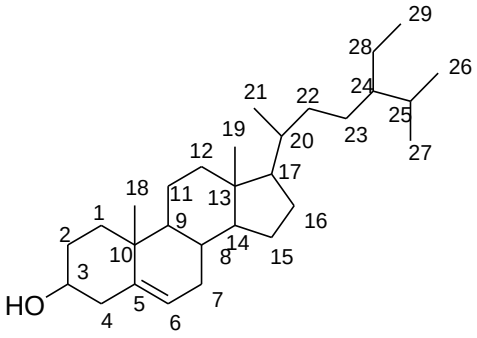
Appendix 2b: ^{13}C NMR for compound LS02



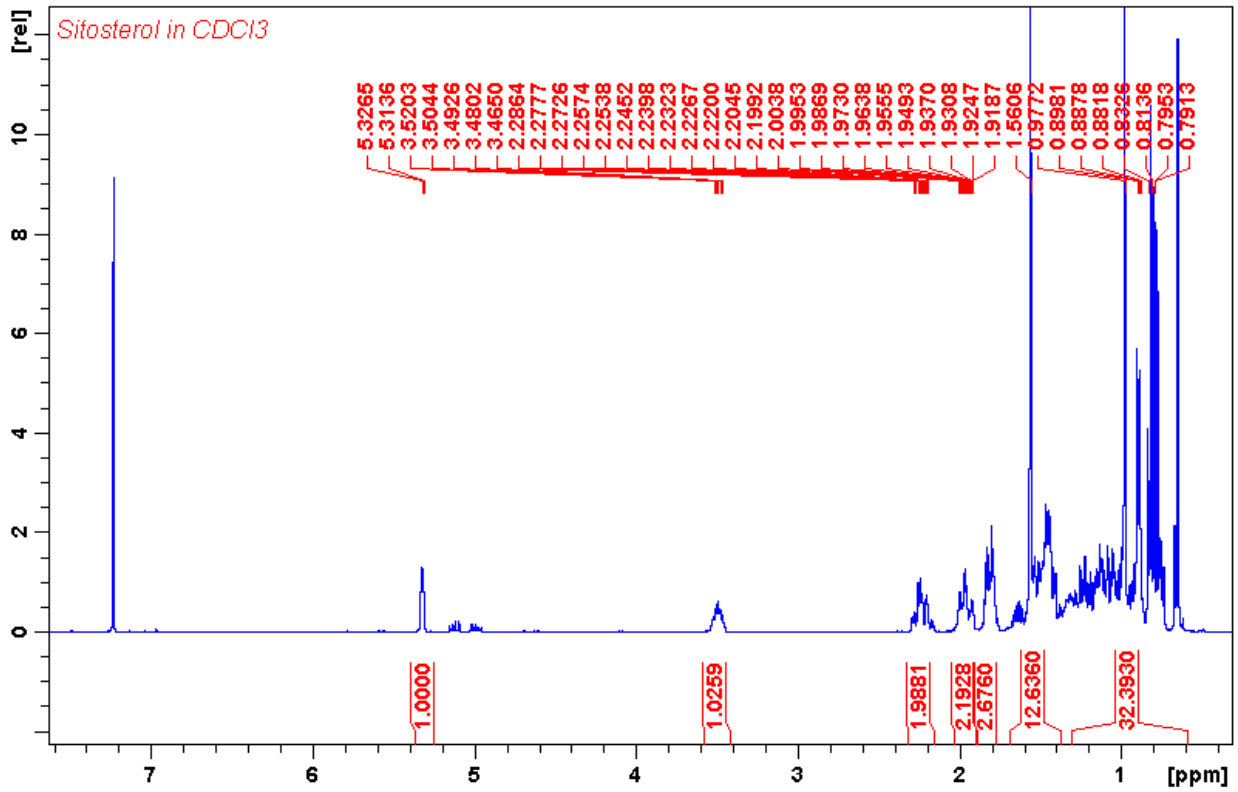
LS02



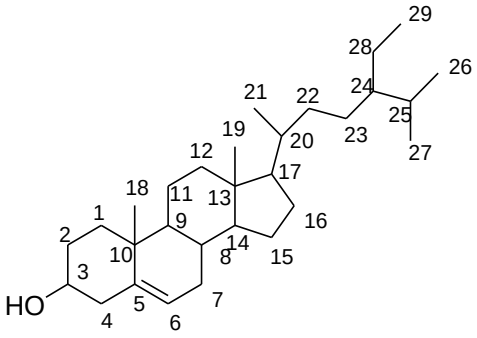
Appendix 3a: ^{1}H NMR for compound LS03



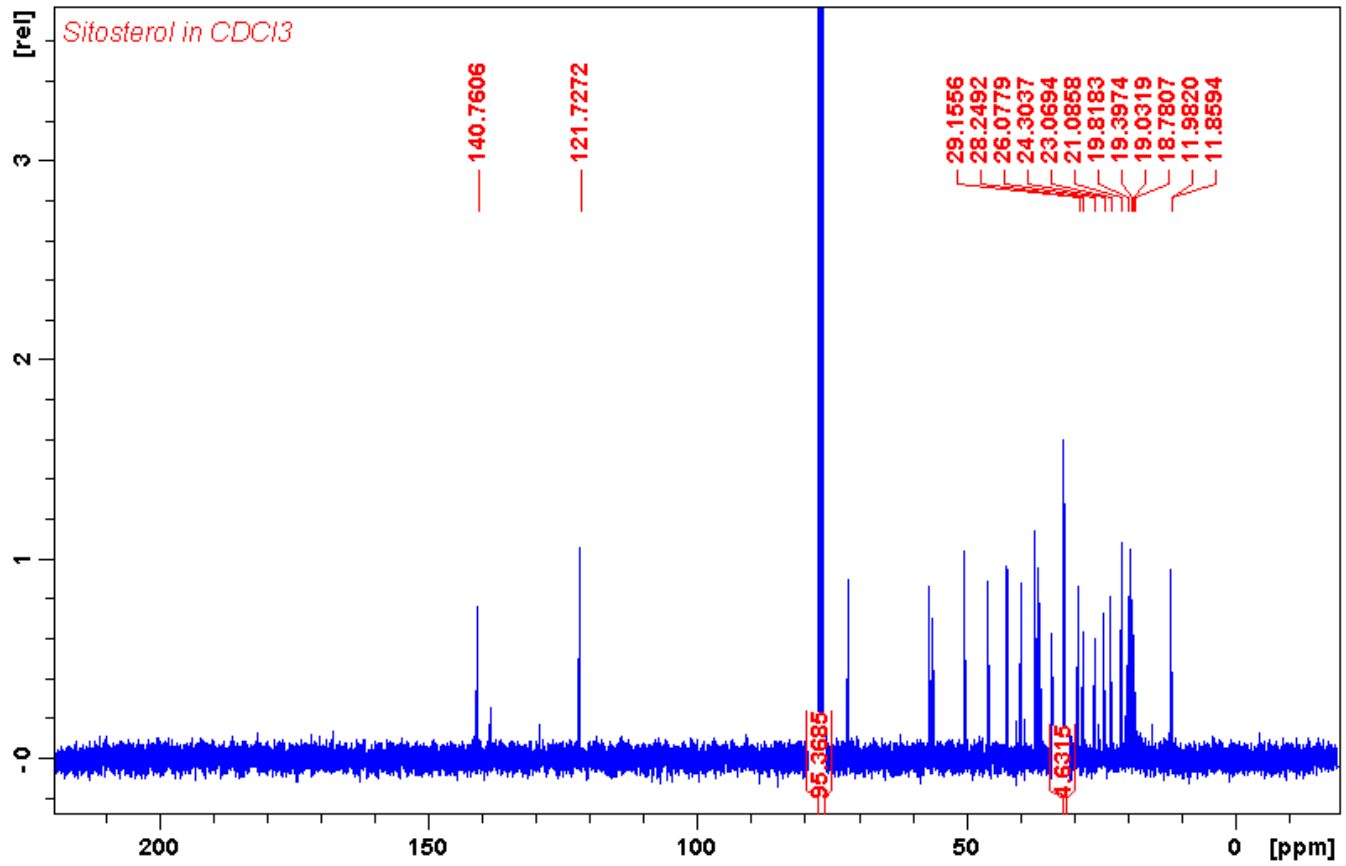
LS03



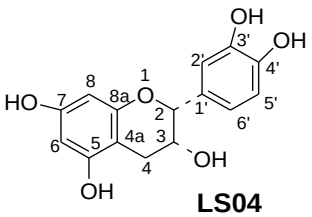
Appendix 3b: ^{13}C NMR for compound LS03



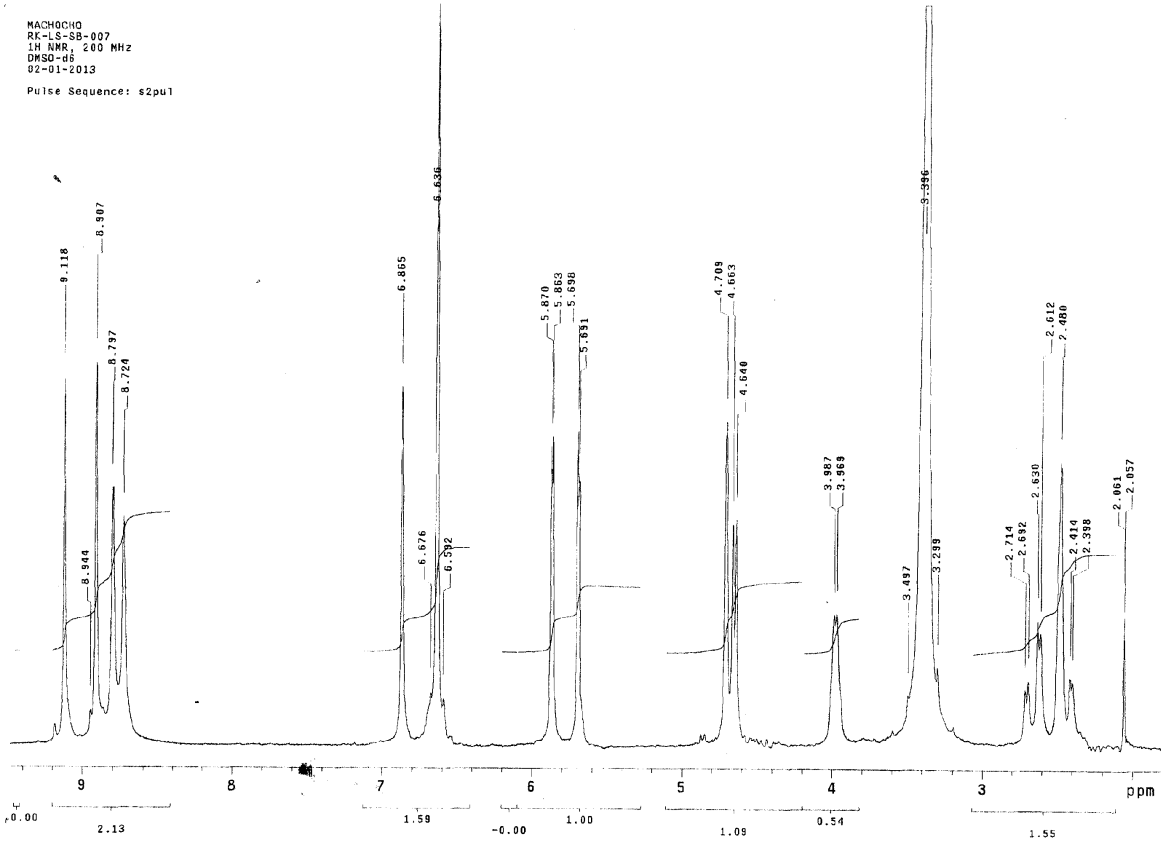
LS03



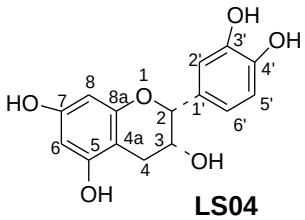
Appendix 4a: ^{1}H NMR for LS04



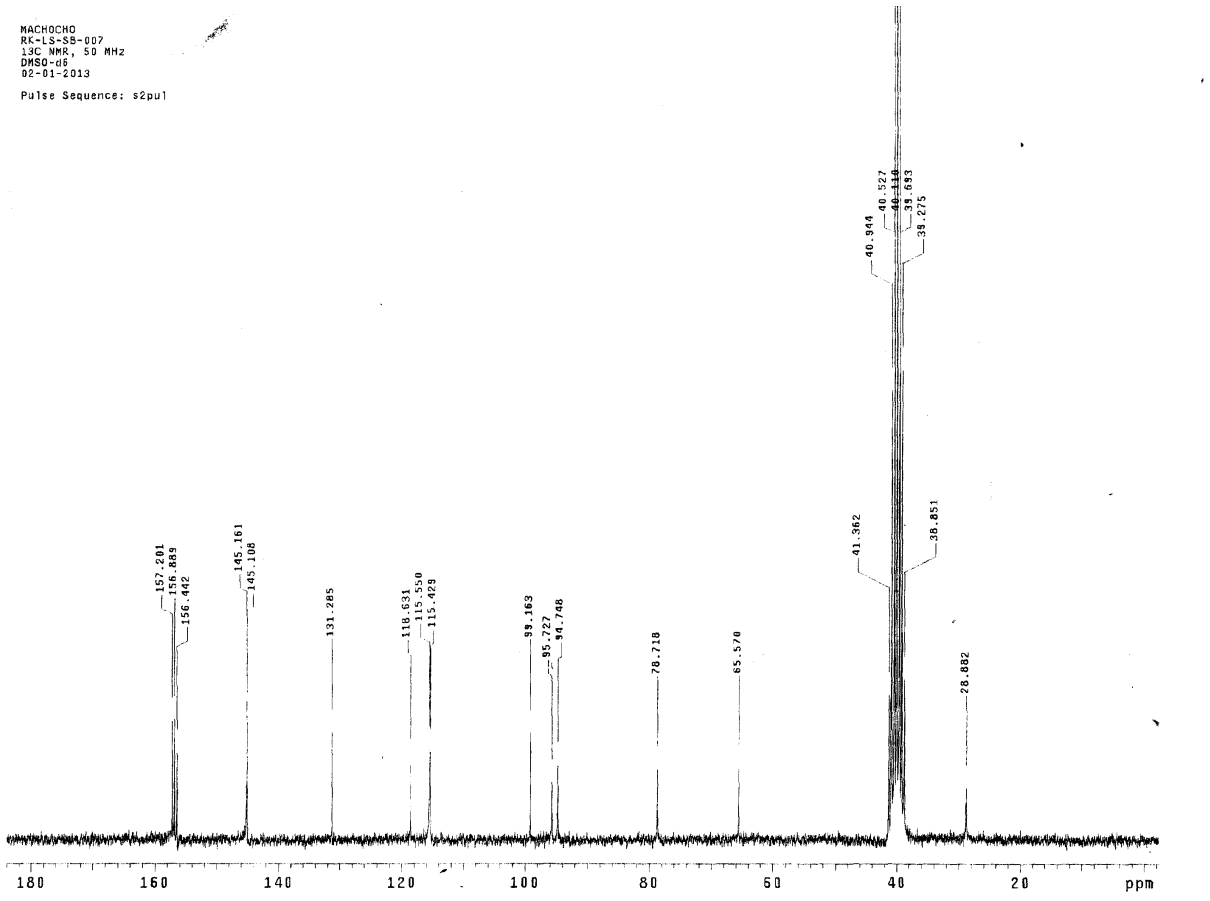
LS04



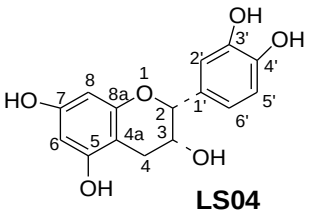
Appendix 4b: ^{13}C NMR for LS04



LS04

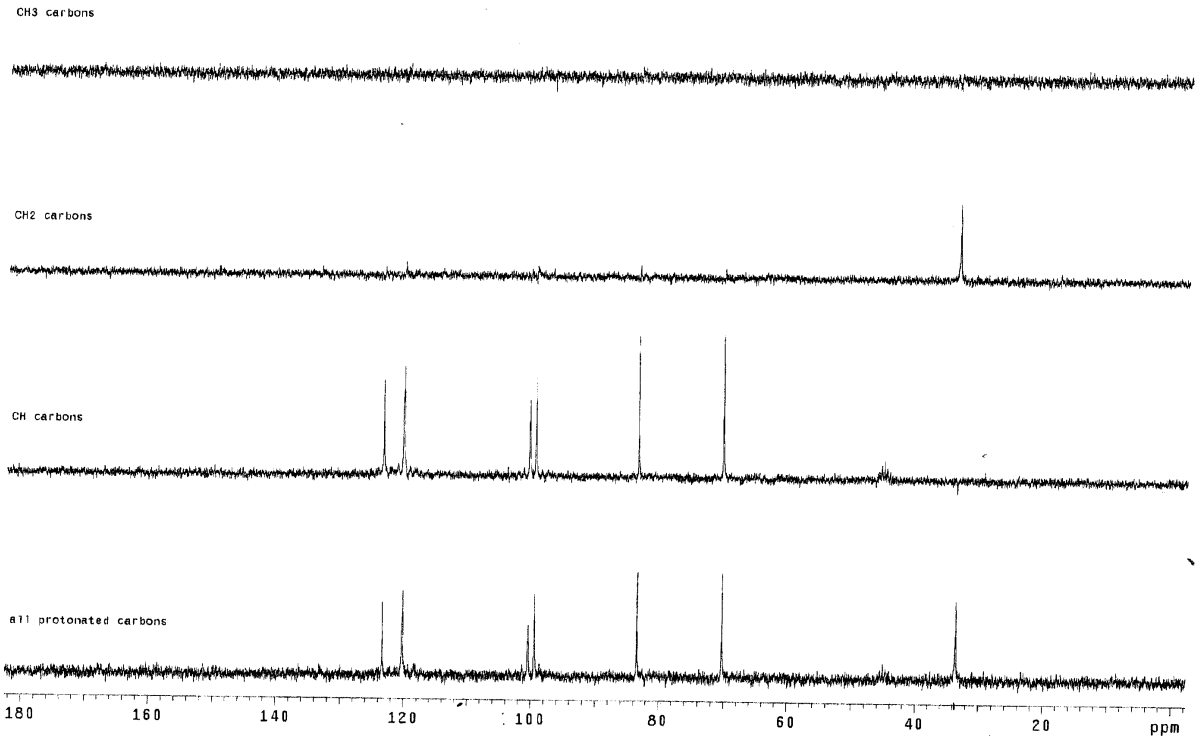


Appendix 4c: DEPT of LS04

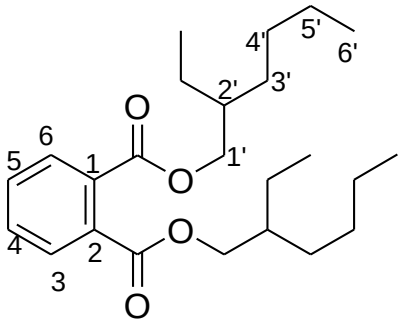


LS04

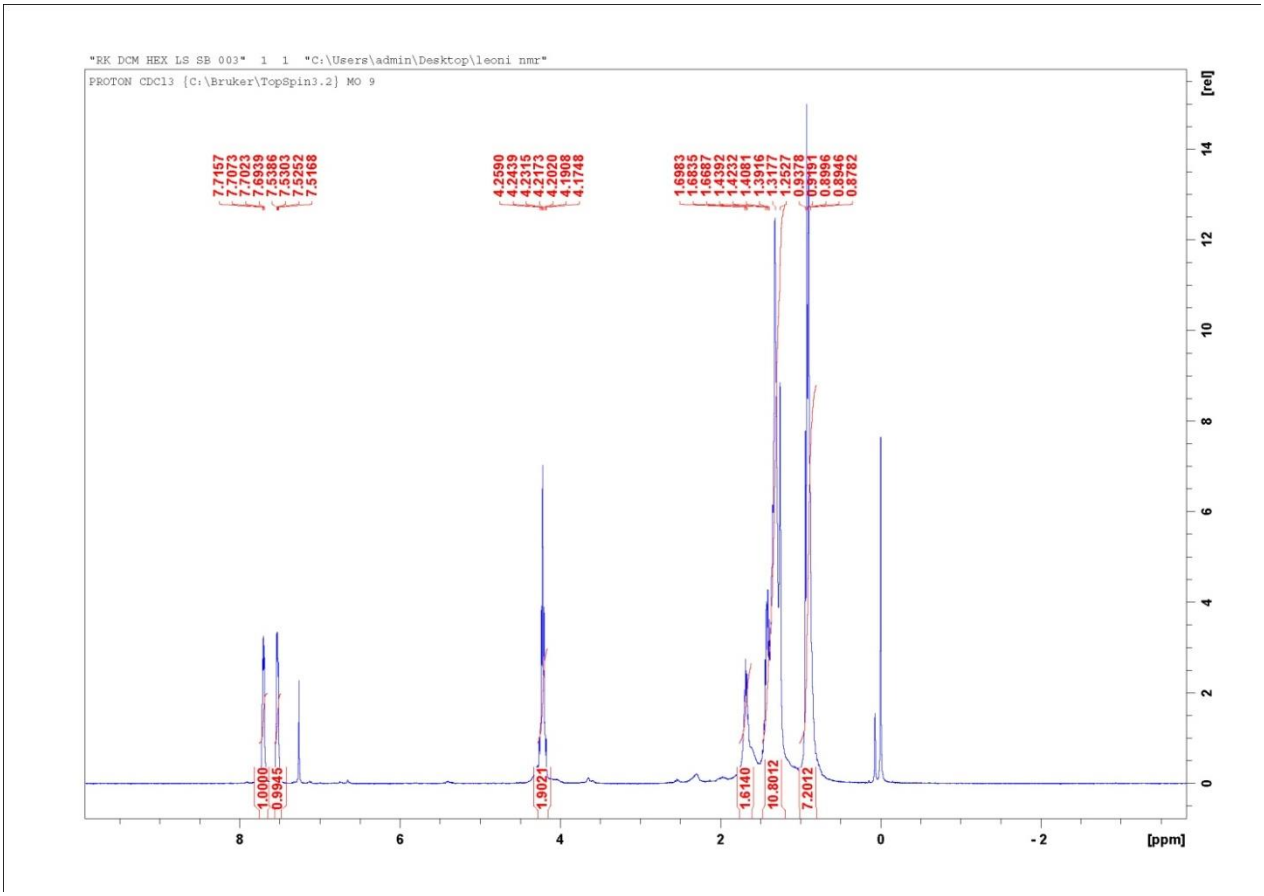
LS/SB/007
EPT, DMSO-d6
13/01/2018



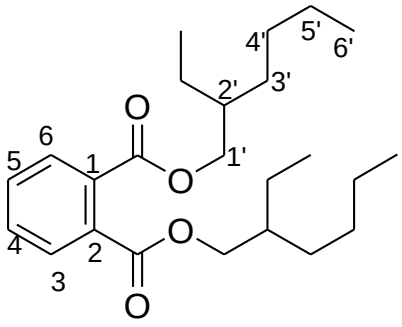
Appendix 5a: ^{1}H NMR for LS05



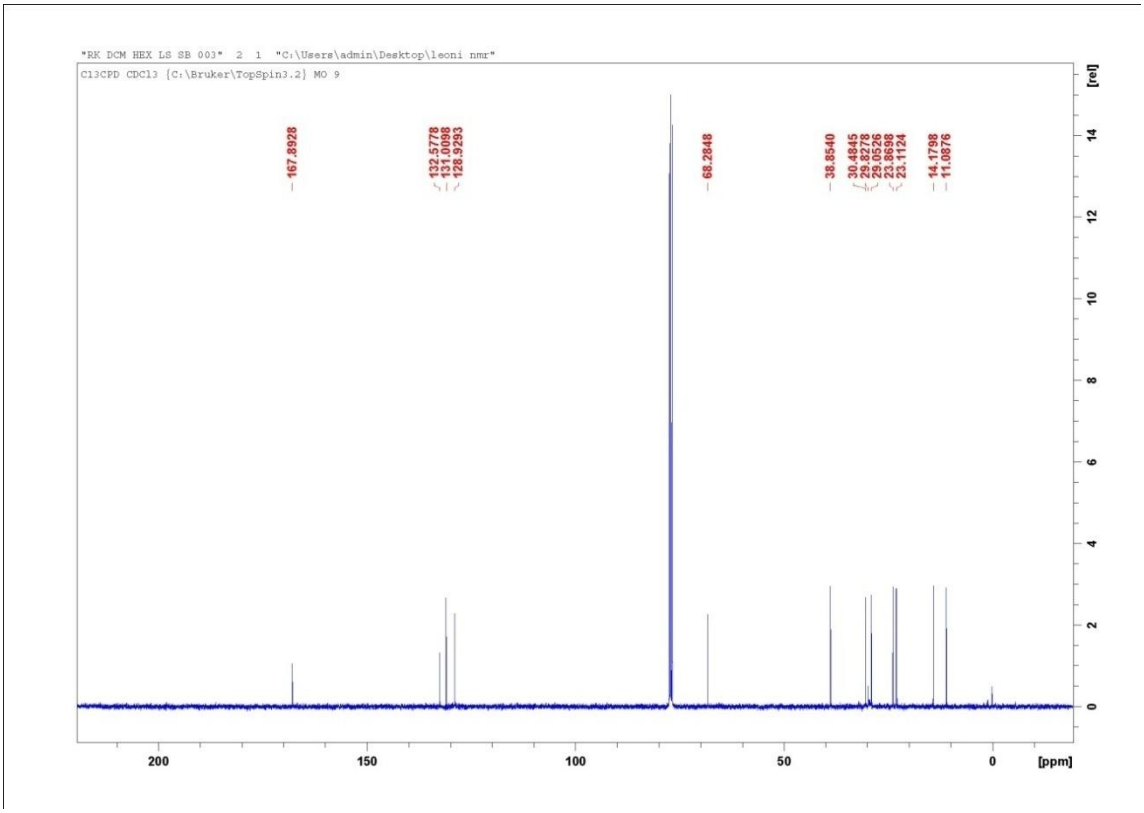
LS05



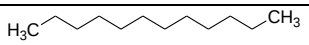
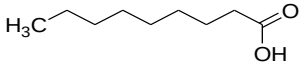
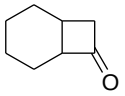
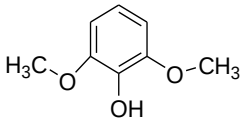
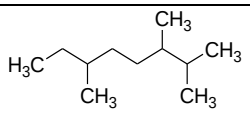
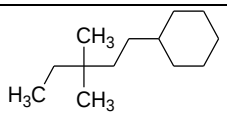
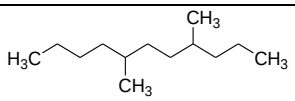
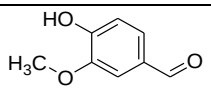
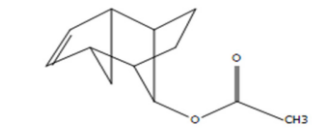
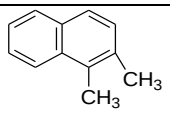
Appendix 5b: ^{13}C NMR for LS05

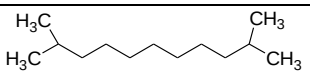
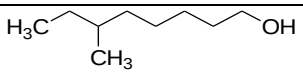
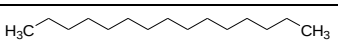
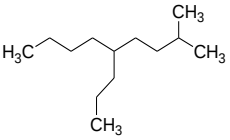
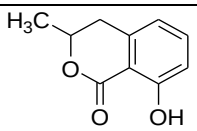
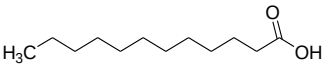
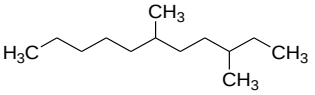
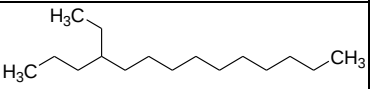
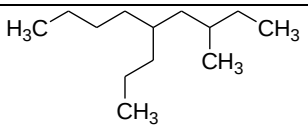
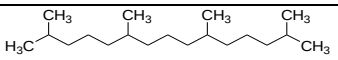


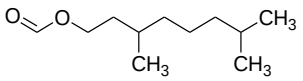
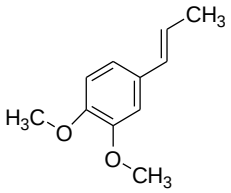
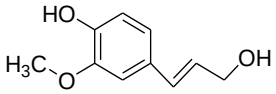
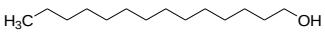
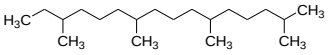
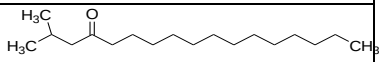
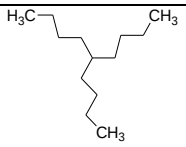
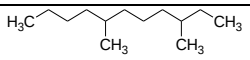
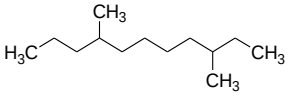
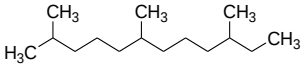
LS05

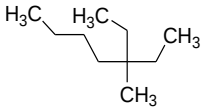
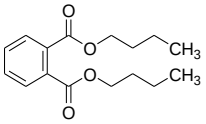
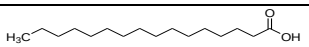
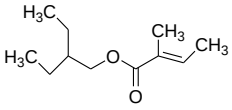
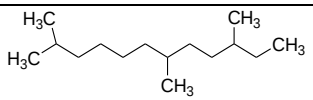
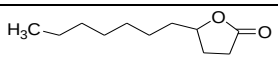
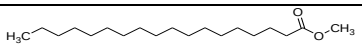
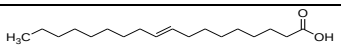
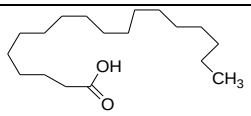
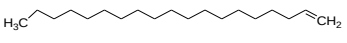
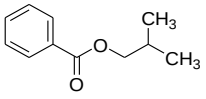


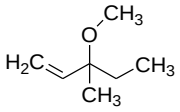
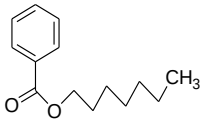
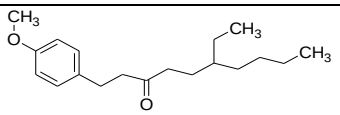
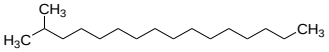
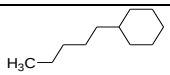
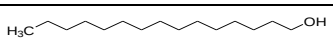
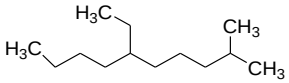
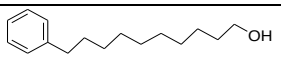
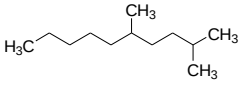
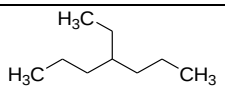
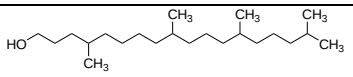
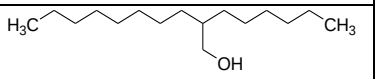
Appendix 6a: Compounds detected by GC-MS in crude DCM extract

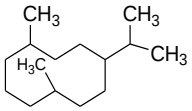
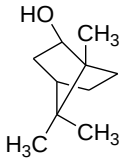
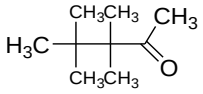
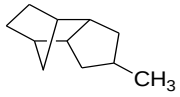
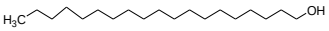
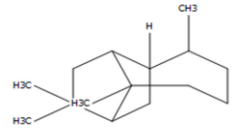
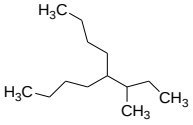
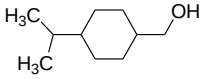
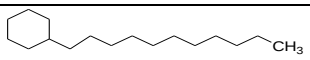
No.	RT	Molecular formula	Name	Class	Structure
51	4.1	$C_{12}H_{26}$	Dodecane	Modified fatty acid	
52	5.0	$C_9H_{18}O_2$	Nonanoic acid	Fatty acid	
53	5.4	$C_8H_{12}O$	bicyclo[4.2.0]octan-7-one	Modified fatty acid	
54	6.1	$C_8H_{10}O$	2,6-dimethoxyphenol	Phenolic (shikimate)	
55	6.4	$C_{11}H_{24}$	2,3,6-trimethyloctane	Modified sesquiterpenoid	
56	6.6	$C_{13}H_{26}$	(3,3-dimethylpentyl)-cyclohexane	Modified sesquiterpenoid	
57	6.7	$C_{13}H_{28}$	4,7-dimethylundecane	Modified sesquiterpenoid	
58	6.8	$C_8H_8O_3$	Vanillin	Polyketide derivative	
59	7.1	$C_{12}H_{16}O_2$	Acetate tricycle[4.2.1.1(2,5)]dec-3en-9-ol	Modified fatty acid	
60	7.1	$C_{12}H_{12}$	1,2-dimethylnaphthalene	Modified sesquiterpenoid	

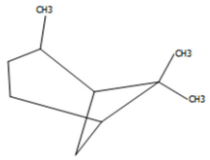
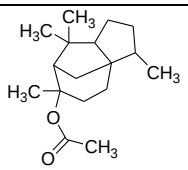
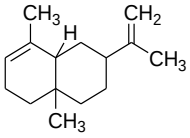
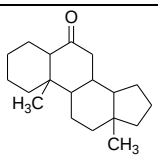
61	7.4	$C_{13}H_{28}$	2,10-dimethylundecane	Modified sesquiterpenoid	
62	7.6	$C_9H_{20}O$	(S)-(+)-6-methyl-1-octanol	Modified monoterpenoid	
63	7.9	$C_{15}H_{32}$	Pentadecane	Modified fatty acid	
64	8.5	$C_{13}H_{28}$	2-methyl-5-propylnonane	Modified sesquiterpenoid	
65	8.5	$C_{10}H_{10}O_3$	3,4-dihydro-8-hydroxy-3-methyl-1H-2-benzopyran-1-one	Phenolic (polyketide derivative)	
66	8.7	$C_{12}H_{24}O_2$	Dodecanoic acid	Fatty acid	
67	9.1	$C_{16}H_{34}$	Hexadecane	Modified fatty acid	
68	9.7	$C_{13}H_{28}$	3,6-dimethylundecane	Modified sesquiterpenoid	
69	9.8	$C_{16}H_{34}$	4-ethyltetradecane	Modified sesquiterpenoid	
70	10.3	$C_{13}H_{28}$	3-methyl-5-propylnonane	Modified sesquiterpenoid	
71	10.3	$C_{19}H_{40}$	2,6,10,14-tetramethylpentadecane	Modified diterpenoid	

72	10.4	$C_{11}H_{20}O_2$	3,7-dimethyl-, formate; 6-octen-1-ol	Monoterpenoid	
73	10.8	$C_{11}H_{14}O_2$	1,2-dimethoxy-4-(1-propenyl)-benzene	Phenolic (coumarate)	
74	10.8	$C_{10}H_{12}O_3$	4-((1E)-3-hydroxy-1-propenyl)-2-methoxyphenol	Phenolic (DOPA)	
75	11.3	$C_{14}H_{30}O$	1-tetradecanol	Fatty acid	
76	11.5	$C_{20}H_{42}$	2,6,10,14-tetramethylhexadecane	Diterpenoid	
77	11.7	$C_{17}H_{34}O_2$	Isopropyl myristate	Fatty acid derivative	
78	11.9	$C_{13}H_{28}$	5-butylnonane	Modified sesquiterpenoid	
79	11.9	$C_{13}H_{28}$	3,7-dimethylundecane	Modified sesquiterpenoid	
80	12.2	$C_{13}H_{28}$	3,8-dimethylundecane	Modified sesquiterpenoid	
81	12.6	$C_{15}H_{32}$	2,6,10-	sesquiterpenoid	

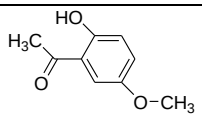
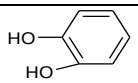
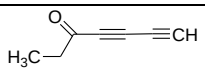
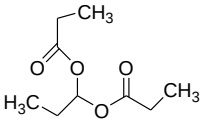
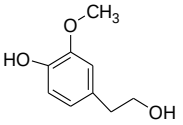
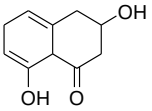
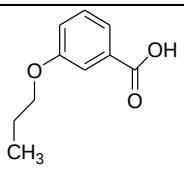
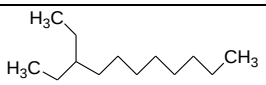
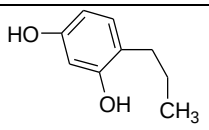
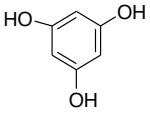
			trimethyldodecane		
82	13.3	$C_{10}H_{22}$	3-ethyl-3-methylheptane	Modified monoterpenoid	
83	13.6	$C_{16}H_{22}O_4$	dibutyl phthalate	Ester of fatty acid	
84	13.8	$C_{16}H_{32}O_2$	n-hexadecanoic acid	Fatty acid	
85	14.6	$C_{24}H_{50}$	Tetracosane	Modified fatty acid	
86	14.7	$C_{11}H_{20}O_2$	2-ethylbutyl(E)-methylbutyl-2-enoate	Fatty acid derivative	
87	15.4	$C_{15}H_{32}$	2,7,10-trimethyldodecane	sesquiterpenoid	
88	15.6	$C_{11}H_{20}O_2$	5-heptyldihydro-2(3H)-furanone	Fatty acid derivative	
89	15.9	$C_{19}H_{38}O_2$	methyl stearate	Ester of fatty acid	
90	16.4	$C_{18}H_{34}O_2$	(E)-9-octadecenoic acid	Fatty acid	
91	16.6	$C_{18}H_{36}O_2$	Octadecanoic acid	Fatty acid	
92	16.9	$C_{19}H_{38}$	Nonadecene	Modified fatty acid	
93	17.0	$C_{11}H_{14}O_2$	2-methylpropyl ester benzoic acid	Shikimic acid derivative	

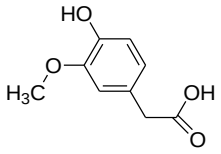
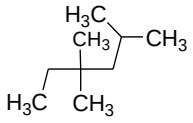
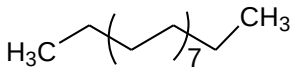
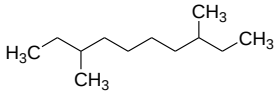
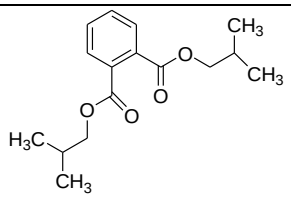
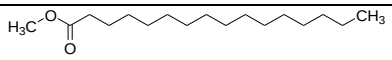
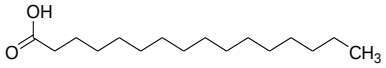
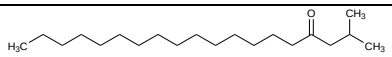
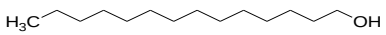
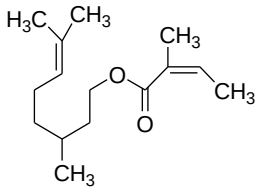
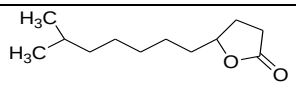
94	17.9	$C_7H_{14}O$	3-methoxy-3-methyl-1-pentene	Modified isoprene	
95	18.6	$C_{14}H_{20}O_2$	heptyl ester benzoic acid	Shikimic acid derivative	
96	18.8	$C_{18}H_{26}O_3$	2-ethylhexyl trans-4-methoxycinnamate	Coumarate and fatty acid	
97	19.0	$C_{17}H_{36}$	2-methylhexadecane	Modified fatty acid	
98	19.7	$C_{11}H_{22}$	pentyl cyclohexane	Modified fatty acid	
99	19.9	$C_{15}H_{32}O$	n-pentadecanol	Modified fatty acid	
100	22.4	$C_{13}H_{28}$	6-ethyl-2-methyldecne	Modified sesquiterpenoid	
101	23.1	$C_{13}H_{26}O$	10-phenyl-n-decanol	Shikimate and fatty acid	
102	23.4	$C_{12}H_{26}$	2,5-dimethyldecane	Modified sesquiterpenoid	
103	24.6	C_9H_{20}	4-ethylheptane	Modified monoterpenoid	
104	26.1	$C_{22}H_{38}O$	4,9,13,17-tetramethyl-4,8,12,16-octadecatetraen-1-ol	Modified diterpenoid	
105	27.1	$C_{16}H_{34}O$	2-hexyl-1-decanol	Fatty acid derivative	

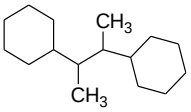
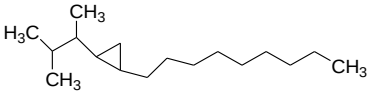
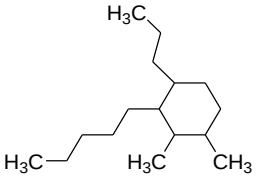
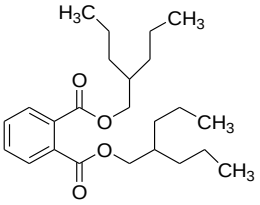
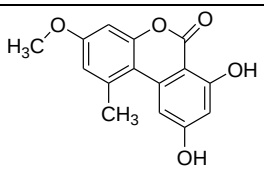
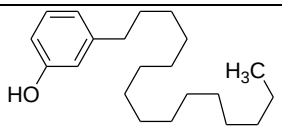
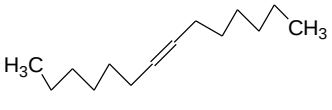
106	28.4	$C_{15}H_{30}$	1,7-dimethyl-4-(1-methylethyl) cyclodecane	sesquiterpenoid	
107	28.7	$C_{10}H_{18}O$	endo-borneol	Monoterpenoid	
108	28.8	$C_9H_{18}O$	3,3,4,4-tetramethyl-2-pentanone	Modified monoterpenoid	
109	29.4	$C_{17}H_{32}O$	(R)-(-)-14-methyl-8-hexadecyn-1ol	Fatty acid derivative	
110	29.8	$C_{11}H_{18}$	cis-4-methyl-exo-tricyclo[5.2.1.0(2.6)]decane	Modified monoterpenoid	
111	30.0	$C_{19}H_{40}O$	n-noandecanol-1	Fatty acid derivative	
112	30.6	$C_{15}H_{26}$	dihydro-(-)-neoclovene-(II)	sesquiterpenoid	
113	31.6	$C_{13}H_{28}$	5-(1-methylpropyl)-nonane	Modified sesquiterpenoid	
114	32.1	$C_{10}H_{20}O$	4-(1-methylethyl)-, trans-cyclohexanemethanol	Monoterpenoid	
115	32.6	$C_{17}H_{34}$	undecyl-cyclohexane	Fatty acid derivative	

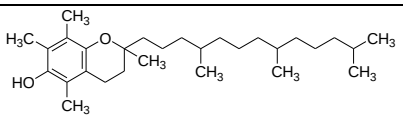
116	33.2	$C_{10}H_{18}$	2,6,6-trimethyl-bicyclo[3.1.1]heptane	Monoterpenoid	
117	34.5	$C_{17}H_{28}O_2$	[3R-3.alpha.,3a.beta.,6.alpha.,7.beta.,8a.alpha.)]-1H-3a,7-methanoazulen-6-ol,octahydro-3,6,8,8-tetramethyl,acetate	Modified sesquiterpenoid	
118	36.3	$C_{15}H_{24}$	2-isopropenyl-4a,8-dimethyl-1,2,3,4a,5,6,8a-octahydronaphthalene	sesquiterpenoid	
119	36.7	$C_{19}H_{30}O$	(5.alpha.)-androstan-6-one	Steroid	

Appendix 6b: Compounds detected in crude methanol extract using GC-MS

No.	RT	Molecular formula	Name	Class	Structure
120	4.4	$C_9H_{10}O_3$	1-(2-hydroxy-5-methoxyphenyl) -ethanone	Polyketide derivative	
121	4.5	$C_6H_6O_2$	Catechol	Phenolic	
122	6.5	C_7H_6O	4,6-Heptadiyn-3-ene	Fatty acid derivative	
123	7.3	$C_9H_{16}O_4$	Propane-1,1-diol dipropanoate	Fatty acid derivative	
124	8.5	$C_9H_{12}O_3$	Homovanillyl alcohol	Shikimate	
125	8.7	$C_{10}H_{10}O_3$	3,8-dihydroxy-3,4-dihydronaphthlen-1(2H)-one	Polyketide derivative	
126	8.8	$C_{10}H_{12}O_3$	3-propoxy-benzoic acid	Shikimic acid derivative	
127	9.1	$C_{13}H_{28}$	3-ethylundecane	Modified sesquiterpenoid	
128	9.6	$C_9H_{12}O_2$	4-propyl-1,3-benzenediol	Shikimate	
129	9.7	$C_6H_6O_3$	1,3,5-benzenetriol	Polyketide derivative	

130	9.8	$C_9H_{10}O_4$	Homovanillic acid	Coumarate	
131	10.2	C_9H_{20}	2,4,4-trimethylhexane	Modified monoterpe noid	
132	11.3	$C_{18}H_{36}$	(E)-3-Octadecene	Modified fatty acid	
133	11.3	$C_{12}H_{26}$	3,8-dimethyldecane	Modified monoterpe noid	
134	12.3	$C_{16}H_{22}O_4$	bis(2-methylpropyl) ester 1,2-benzenedicarboxylic acid	Shikimate	
135	13.0	$C_{17}H_{34}O_2$	methyl ester hexadecanoic acid	Ester of fatty acid	
136	13.4	$C_{16}H_{32}O_2$	n-Hexadecanoic acid	Fatty acid	
137	14.3	$C_{19}H_{38}O_2$	Isopropyl palmitate	Fatty acid derivative	
138	15.2	$C_{14}H3_0O$	1-teradecanol	Fatty acid derivative	
139	15.4	$C_{15}H_{26}O_2$	Citronellyl tiglate	Modified monoterpe noid and isoprene	
140	15.5	$C_{12}H_{22}O_2$	10-methylundecan-	Isoprene fatty acid	

			4-olide		
141	15.9	C_8H_{14}	cis-bicyclo[4.2.0] octane	Modified monoterpe noid	
142	16.0	$C_{16}H_{30}$	1,1'-(1,2-dimethyl-1-thanediyl)bis-cyclohexane	Modified sesquiterpe noid	
143	16.8	$C_{18}H_{36}$	1-(1,2-dimethylpropyl) -1-methyl-2-nonyl-cyclopropane	Cycloprope noid and isoprene fatty acid	
144	19.9	$C_{16}H_{32}$	1,2-dimethyl-3-pentyl-4-propyl cyclohexane	Isoprene fatty acid	
145	22.1	$C_{24}H_{38}O_4$	di(2-propylpentyl) ester phthalic acid	Ester of fatty acid	
146	23.7	$C_{15}H_{12}O_5$	7,9-dihydroxy-3-methoxy-1-methyl-6H-dibenzo[b,d]pyr an-6-one	Polyketide derivative	
147	27.7	$C_{21}H_{36}O$	3-pentadecylpheno l	Phenol and Fatty acid	
148	29.1	$C_{14}H_{26}$	7-tetradecyne	Modified fatty acid	

149	30.4	$C_{29}H_{50}O_2$	Vitamin E	Modified triterpenoid	
150	33.9	$C_{29}H_{50}O$	Gamma - sitosterol	Steroid