HAWASSA

COLLEGE OF NATURAL AND COMPUTATIONAL SCIENCES

CHEMISTRY DEPARTMENT

Phytochemical and Antibacterial activity study of the root extract of *Ehretia cymosa*

A THESIS SUBMITTED TO THE DEPARTMENT OF CHEMISTRY OF HAWASSA UNIVERSITY IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN CHEMISTRY

BY:-

SITOTA ASAMINEW

ADVISOR: Dr SALAH HAMZA

CO-ADVISOR: FILIPPO TAMIRU (MSc)

MAY, 2024

HAWASSA, ETHIOPIA

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DECLARATION

I, undersigned, declare that this thesis is my original work and has not been presented for a degree or diploma in any other universities and that all sources of materials used for this thesis have been duly acknowledged.

Name: Sitota Asaminew

Signature: _____

This thesis has been submitted for examination with my approval as university

Advisor and Co-advisor

Name of advisor: Dr. Salah Hamza

Signature: _____

Name of Co-advisor: Filippo Tamiru (Msc)

Signature: - _____

ADVISOR'S APPROVAL SHEET-1

This is to certify that the thesis entitled "Phytochemical and Antibacterial activity study of the root extract of Ehretia *cymosa"* submitted in partial fulfillment of the requirements for the degree Master of Science in Chemistry with specialization in Organic Chemistry of the graduate program of the Department of Chemistry, Hawassa university, and is a record of original research carried out by **Sitota Asaminew Rekiso (ID, No PGOrgCR/0006/14),** under my supervision, and no part of the thesis has been submitted for any other degree or diploma.

The assistance and help received during the course of this investigation have been duly acknowledged. Therefore, I recommend that it is accepted as fulfilling the thesis requirements.

Sahala hamza (PhD) _____ _____

Name of advisor Signature Date

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DEPARTMENT OF CHEMISTRY

EXAMINER 'S APPROVAL SHEET-2

We, the undersigned, members of the Board of Examiners of the final open defense by Sitota Asaminew Rekiso, have read and evaluated his thesis entitled" Phytochemical and Antibacterial activity study of the root extract of *Ehretia cymosa*," and examined the candidate. This is, therefore, to certify that the thesis has been accepted in partial fulfillment of the requirements for the degree.

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LIST OF ABBREVIATIONS AND SYMBOLS

CC	Column Chromatography
IR	Infra Red
Rf	Retention factor
TLC	Thin Layer Chromatography
Uv	Ultraviolet
WHO	World Health Organization
CLSI	Clinical and laboratory standards institute
DCM	Dichloromethanol
EC	Ehretia cymosa
DMSO	Dimethyl Sulphoxide

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ABSTRACT

Ehretia cymosa is a medicinal plant that is used for traditional medicine in many African countries including Ethiopia. The different plant parts of *E. cymosa* are used in traditional medicine for the treatment of several health problems such as Toothache, tetanus, dysentery, gastric ulcers, wound healing, and skin diseases. The aim of this study was to investigate chemical constituents, partially characterize isolated compounds of root extract of *E. cymosa* and testing antibacterial activities of crude extracts and isolated compounds. The successive extraction of the roots 500g powder was carried out using solvent systems n-hexane, dichloromethane, dichloromethane: methanol (50:50 % by volume), and methanol that afforded 2.5 g (0.5%), 3.5 g (0.7%), 15.5 g 3.1%) and 5 g (1%) of crude extracts respectively. Phytochemical screening that was carried out by employing standard procedures revealed the presence of secondary metabolites such as alkaloids, tannins, saponins, steroids, flavonoids, phenols, glycosides, and terpenoids. Thus, dichloromethane: methanol (50:50 % by volume) extract was subsequently subjected to chromatographic separation which led to the isolation of three compounds EC-1, EC-2, and EC-3. Crude extract of dichloromethane/methanol (1:1) was subjected to column chromatography and afforded three compounds partially characterized using spectroscopic UV-Vis and IR analysis. The in vitro antibacterial activity evaluation of the crude extracts and isolated compounds was done on four different bacterial strains *S. auras*, *E. coli*, *P. aeruginosa*, and *S. pyogen* using the gar diffusion method. Among the bacteria strains used in the study, *S. pyogene* showed the widest zone of inhibition for methanol extract even though other extracts were antibacterial active enough. The observed antibacterial activities of the crude extracts and isolated compounds justified the traditional use of plants for the treatment of different bacterial infections. Thus, further testing is recommended on a large number of bacterial strains to decide their potential as candidates in the development of antibacterial agents.

Key words: Antibacterial activity, crude extraction, *Ehretia cymosa*, Antibacterial activity, Phytochemicalscreening.

CHAPTER ONE

1. INTRODUCTION

1.1 Background of the study

A natural product is a chemical compound or a substance produced by a living organism, found in nature and having pharmacological or biological activity for use in pharmaceutical drug design [1]. Sources of natural products may be plants, marine organisms, animals, or microorganisms. A crude extract from any one of these sources typically contains novel, structurally diverse chemical compounds. The characteristic features of natural products responsible for their biological activity are chiral centers, architectural complexity [2]. The natural compound that has some form of biological activity is known as the active principle and may lead to the discovery of a new drug. Many of today's medicines are obtained directly from natural sources[3].

The medicinal value of plants lies in some chemical substances that produce a definite physiological action on the human body. Contrary to synthetic drugs, antimicrobials of plant origin are not associated with many side effects and have an enormous therapeutic potential to heal many infectious diseases[4]. Nearly all cultures and civilizations from ancient times to the present day have used herbal medicines as antimicrobial sources to cure infections[5].

Plant-based antimicrobials represent a vast untapped source of medicines and are effective in the treatment of infectious diseases while simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials. Herbs are also an invaluable source of modern drugs. More than 25% of modern drugs are derived from plants[6].

Herbal drugs play a key role in health, especially in developing countries such as Ethiopia. Currently, herbal medicines are used to treat common diseases such as typhoid, pneumonia, and malaria because they are cheaper, easily available, and are believed to have fewer side effects.

Herbal medicines have been used by billions of people around the world for thousands of years to treat various diseases [7]. According to WHO, traditional medicine can be defined as including diverse health practices, approaches, knowledge, and beliefs incorporating plant,

animal, and/or mineral-based medicines, spiritual therapies, manual techniques, and exercises applied alone or in combination to maintain well-being, as well as to treat, diagnose or prevent illnesses. Herbal drugs are unprocessed parts of the plant such as flowers, leaves, fruit, seeds, stem, wood, roots, or other plant parts which may be entire, fragmented, or powdered [8]. More than 80% of the population within developing countries relies on the use of herbal medicines for their primary health care due to their lower cost and accessibility in nature[9].

Medicinal plants are those plants that are used to cure human and animal diseases. They have a long history of use in most communities throughout the world. Around 80% of the world's population, especially in developing countries, is people who still use medicinal plants for their health problems. Many of these plants are readily available in rural areas and this has made them relatively cheaper than modern medicine [10]. Their medicinal uses can be attributed to the presence of phytochemical constituents [11].

Phytochemical constituents are naturally occurring molecules in leaves, fruits, vegetables, and roots of these plants. They are used as a defense mechanism and protect the plants from various diseases. They could be categorized as primary and secondary metabolites. Chlorophyll, proteins, and common sugars are included in primary constituents whereas classes of compounds such as terpenoids, alkaloids, flavonoids, saponins, tannins, steroids, anthraquinones, phenolic, and so on are considered secondary metabolites [12].

1.2. Statement of the problem

Even though, the different plant parts of E. cymosa are used in traditional medicine for the treatment of several health problems (Toothache, tetanus, dysentery, gastric ulcers, wound healing and skin diseases). No Phytochemical studies have been carried out on the root so far to identify its constituents. Therefore, the present study aimed to identify the chemical constituents (by taking its root) of E. cymosa to assess its chemical composition and perhaps substantiate the traditional use of the plant-based on scientific study.

1.3. Objectives

1.3.1 General objective

- The main objective of this research is to investigate chemical constituents of root of *Ehretia cymosa* and evaluate the antibacterial activity of the extracts and isolated compounds.

1.3.2 Specific objectives

The specific objectives of the present study were to:

- To carry out the extraction of the root of *Ehretia cymosa* using organic solvents
- To carry out phytochemical screening tests on the crude extract.
- To isolate compounds from the crude extract using column chromatographic technique.
- To evaluate the antibacterial activities of the crude extracts and isolated compounds using different bacterial strains and disc diffusion methods.
- To partially characterize structures of isolated compounds by spectroscopic techniques UV and IR.

1.4. Significance of the Study

The results of this study would:-

- Provide information about the nature of the chemical constituents of the root of E. cymosa.
- The findings will be used to validate the traditional use of the plant against bacterial infections. They will also be an output to substantiate the existing conventional use with the observed screening results against pathogenic microorganisms.
- Serve as a baseline for other researchers from related field who are interested to carry out further studies on the plant in search of new drugs.

CHAPTER TWO

2. LITERETURE RIVIEW

In this section, Genus of *Ehretia*, Ethnomedicinal uses, phytochemical studies, biological activity study reports and isolation of compounds from different parts of some *Ehretia* species are discussed briefly.

2.1 Genus Ehretia

The *Ehretia* genus has around 150 species belonging to the family Boraginaceae. Numerous species are substantially distributed in tropical Asia, Africa, Australia, Europe, and Northern America. All species of *Ehretia* are trees and shrubs [13]. It is a genus of flowering plants in the borage family, Boraginaceae. It contains the generic name honors German botanical illustrator George Dionysius Ehret (1708-1770). Deciduous shrub or small to medium-sized tree up to 20–25 m tall; low branching and crooked, up to 30 cm in diameter; bark surface grey to pale brown, with prominent lenticels, inner bark soft, white, spotted with orange-brown, quickly turning brown upon exposure; crown spreading, often with drooping branches; twigs short-hairy but soon becoming glabrous[14].

Ehretia cymosa is a deciduous shrub or small tree, only growing up to 7 m tall in the western parts of its range, but can sometimes reach heights of 20-25 m recorded from Guinea to Southern Nigeria. It is a glabrous shrub with ovate leaves and white flowers. The fruit is black, ovoid to globose drupe, 2-6 mm long. The greyish-brown wood has alternate darker and lighter bands. The tree has many uses, supplying medicines, food, and wood for the local population [15].

These species are used in many herbal and traditional medicines in India and China because of their good response in many biological activities. Ehretia genus has reported the presence of phenolic acids, lignans, flavonoids, nitrile glycosides, quinonoids, steroids, triterpenoids, and pyrrolizidine alkaloid [16, 17].

Genus Ehretia distributed in different parts of Africa, some in bush land and others in riverine forest. It is a popular hedge plant in Ethiopia which is traditionally used as febrifuge, laxative, pain-killer and cure for toothache. It is also used against diarrhea, skin wounds, stomach pain, paralysis, epilepsy, tonsillitis, typhoid, malaria, asthma, convulsions and to regulate menstrual

cycle [18-20]. In Ethiopia, stomach complaints are managed by crushing the roots in water and taking this infusion orally [21].

Figure 1. Aerial parts of *E. Cymosa* (Gidincho) photo taken by Sitota Asaminew, from sara'ra kebele, sidama region, Eth, Oct 28, 2023 G.C

2.2 Ethnomedicinal uses of genus *Ehretia*

Ethnomedicine broadly refers to the traditional medical practices concerned with the cultural interpretation of health, diseases, and illness that addresses the healthcare process and healing practices [22]. Ehretia species were used as ethnopharmaceuticals in folklore for the treatment of various ailments such as inflammation, cough, itches, swellings, pain, diarrhea, dysentery, fever, cachexia, and syphilis [23].

In Zimbabwe, different parts of Ehretia obtusifolia are used for treating sore throat, teething pains in infants, menstrual pain, abdominal pains, and infertility in women [24]. In China, the species Ehretia thyrsiflora has been used to make kudingcha, a bitter tea [25]. In India, Ehretia laevis is used to treat headaches and ulcers, it also possesses potent anthelmintic, diuretic, demulcent, expectorant, and astringent properties. The inner bark of E. laevis is used as food [26].

Ethnobotanical studies in various districts of Ethiopia reported that Ehretia cymosa (E. cymosa) is used for the management of headaches, abdominal pain, arthritis, and rheumatism [27-29]. It is

a popular hedge plant in Ethiopia that is traditionally used as a febrifuge, laxative, pain-killer, and cure for toothache [30, 31].

The bark and leaf juice of *E. cymosa* are used to treat wounds because they are considered to be astringent. The bark and roots are boiled and are used traditionally in the treatment of epilepsy and menstruation problems. The bark decoction is applied externally in treating skin diseases. The roots and leaf extract are known to be poisonous, but with apparent inconsistency they have a reputation as an aphrodisiac [32]. The Maasai people use the roots to treat brucellosis while in Ethiopia crushed roots soaked in water are used to treat stomach complaints and the root juice is applied to wounds [21]. Ehretia acuminata is a wild medicinal plant found in Asia, America, Australia, and Africa. Many traditional claims are reported as medical treatments for various diseases like some kind of fever, dysentery and other health ailments. The bark of E. acuminata is useful in sores on the tongue, ripe fruit is edible and unripe fruit is used as a pickle by tribal[1] communities. Ethno-botanical surveys on genus Ehretia also suggest snake antivenom activity and anti-tubercular activity of different parts of different species [33]. The bark, fruit, leaf, root, root bark, stem and stem bark decoction or infusion of *E. amoena* are mainly used as anthelmintic and traditional medicine for fever, typhoid, sleeping sickness, wounds, menstrual problems, abdominal pains, sexually transmitted infections, skin diseases, vomiting, pain, muscle pain and gastro-intestinal problems and use of leaf infusion against hypertension [34].

2.3 Phytochemistry of some selected species of *Ehretia*

In this section the phytochemical studies of *E.rigida, E. amoena, E. laevis, E. longiflora, E. microphylla and E. cymosa* is reviewed.

2.3.1 *Ehretia rigida*

In South Africa various researchers identified chemical compounds from the bark and leaves of *E. rigida*. Chemical compounds identified from *E. rigida* include α-amyrin(**1**), β-amyrin(**2**), β-sitosterol (**3**), 1-triacontanol(**5**), and allantoin(**4**) from bark and leaves of *E. rigida* [35-38].

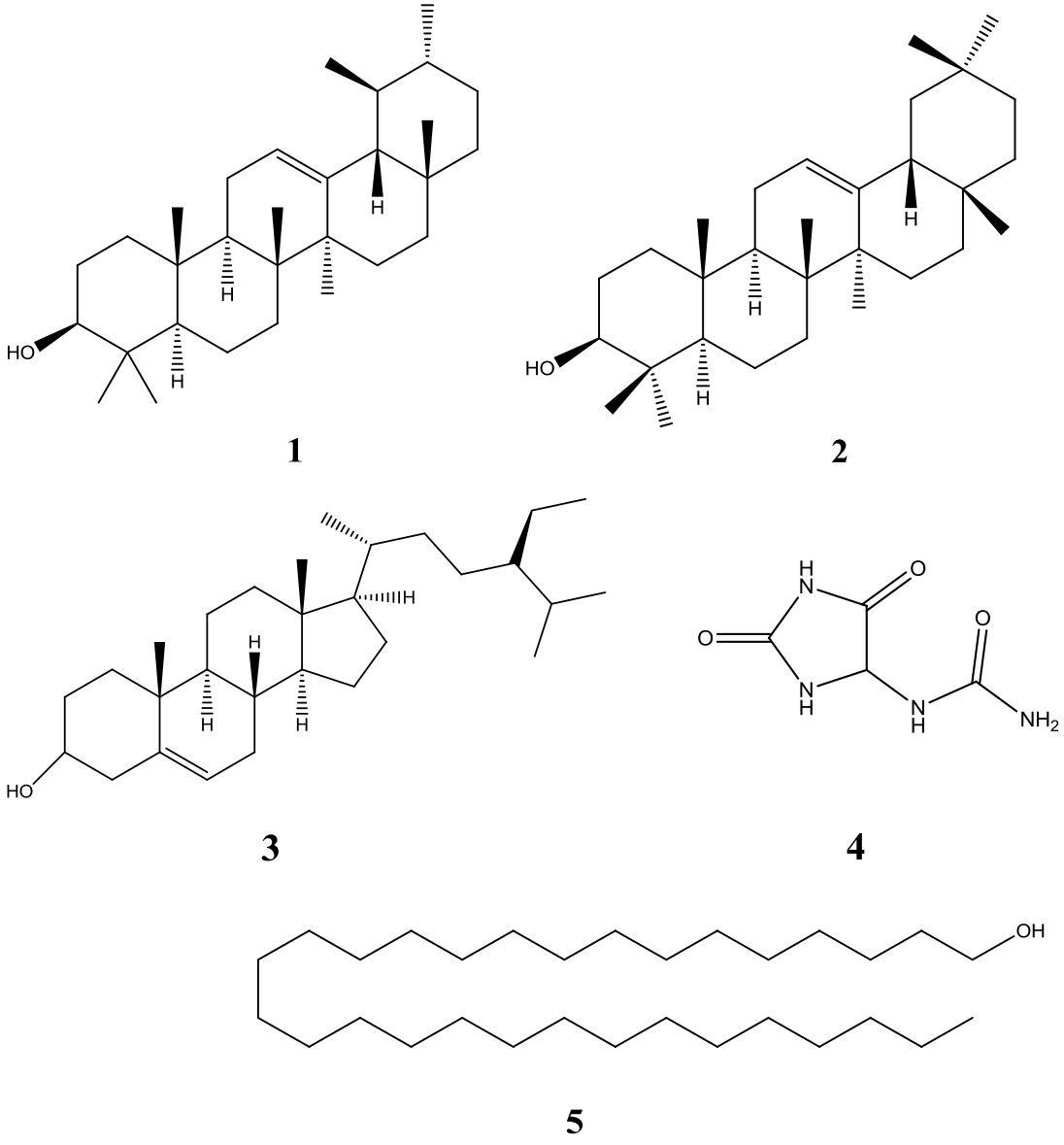
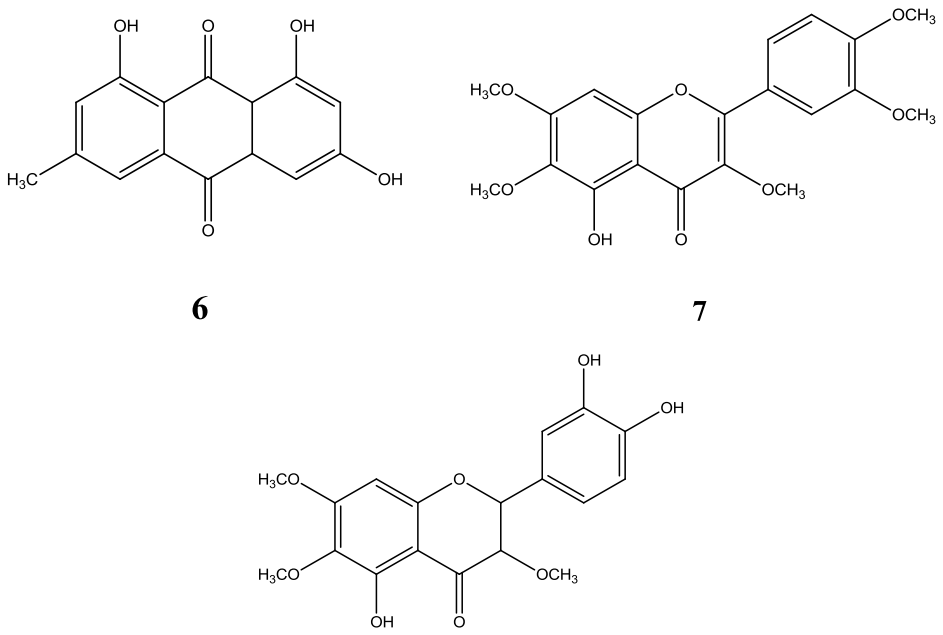


Figure 2. The chemical structure of compounds (**1-5**) isolated from bark (**5**) and leaves (**1-4**) of *E. rigida*.

2.3.2 *Ehretia amoena*

Researchers such as Chhabra et al and Räz et al. Identified phytochemical compounds such as Emodins (**6**) chrysosplenetin (**7**), chrysosplenol D (**8**), isolated from methanol extracts of the root and leves *E. amoena* [39-41].



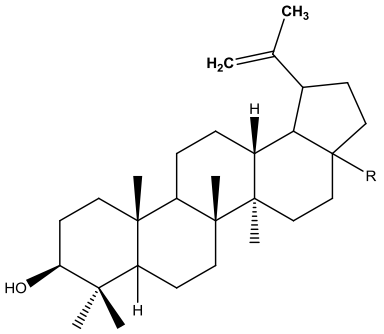
6 **7**

8

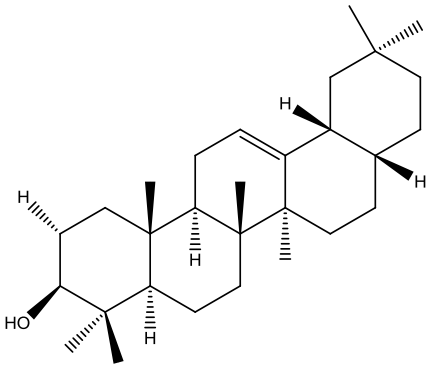
Figure 3. The chemical structure of compounds root (**6**) and leaves (**7, 8**) isolated from of *E. amoena*

2.3.3 *Ehretia laevis*

Joshi and Wagh, reported a GC-MS analysis to isolate the triterpenoids such as lupane (**9**), olenane (**13**), ursane (**14**), betulinic acid (**10**), betulin (**11**), lupeol (**12**), ursolic acid (**15**) α-amyrin (**1**), β-amyrin (**2**), bauerenol (**16**), bauerenol acetate (**17**) and β-sitosterol (**3**) from petroleum ether, chloroform and methanolic extracts of its barks and leaves of *E. laevis*[42-44].



9. R=H
10. R=COOH
11. R=CH2OH
12. R=CH3



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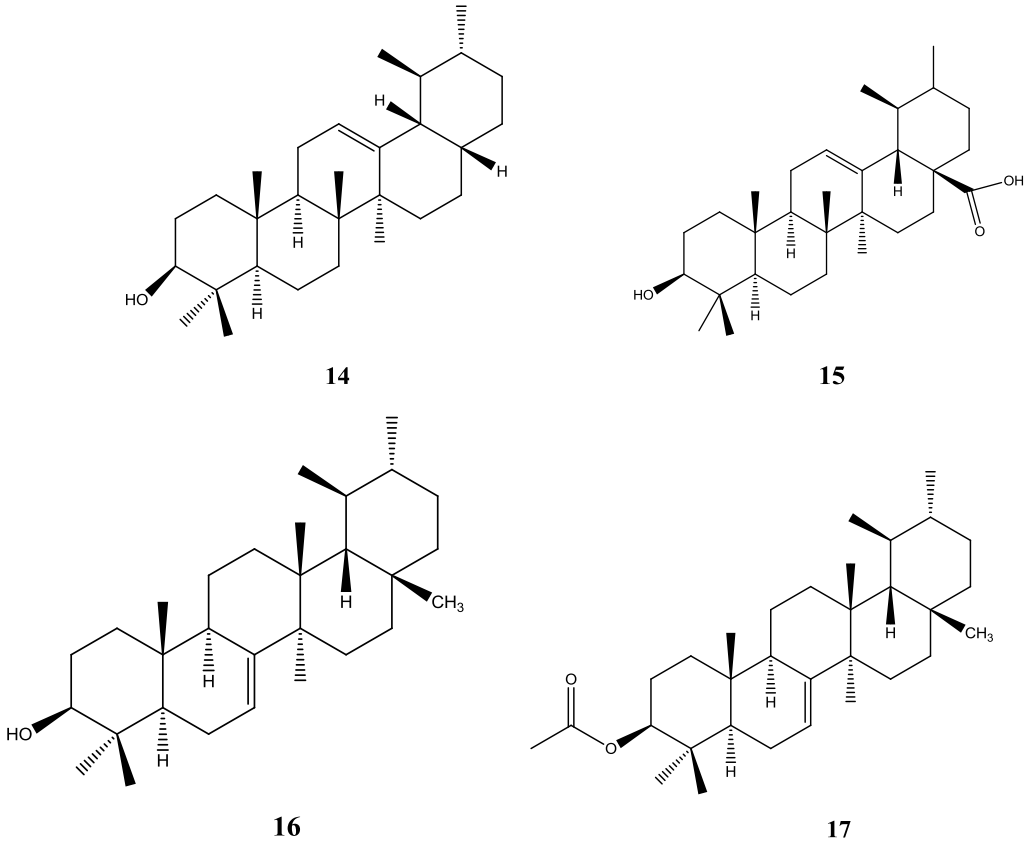
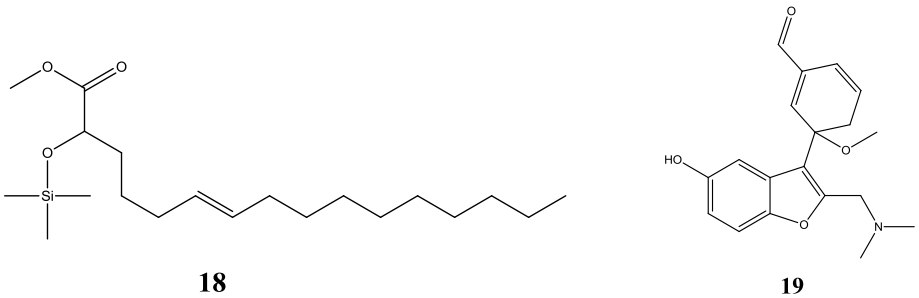


Figure 4. Structures of compounds (**1, 3, 9, 12, 16**, and **17**) bark and (**10, 11, 13,** and **15**) leaves isolated from the extract of *E. laevis*.

The structures of isolated compounds are methyl ester of 2-trimethylsiloxy-6- hexadecenoate (**18**), trimethylsilyl-eicosa-5,8,11,14-tetranoate (**20**) and 4-(dimethylaminomethyl5-hydroxybenzofuran-3-yl)(4-methoxyphenyl) methanone (**19**), Methyl-14-methylhexadecanoate (**21**), Methyl-4,7,10,13,16,19-docosahexaenoate (**22**), Phthalic acid, butyl oct-3-yl ester (**25**), Z,Z4,16-octadecadien-1-ol acetate (**23**) and 9-(ethoxymethyl)-heptadeca-2,15-diene (**24**), (Figure 5) have also been isolated from methanolic extract from the bark of *E. laevis* [42].



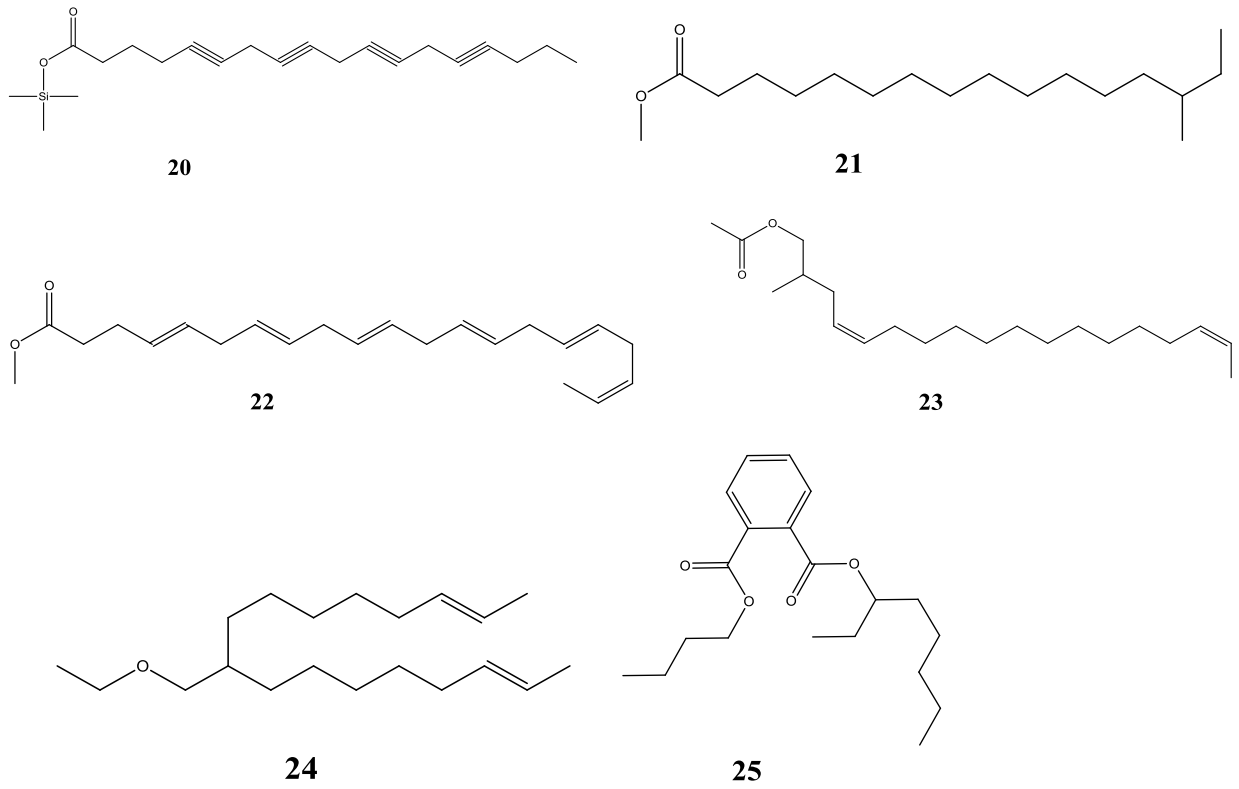
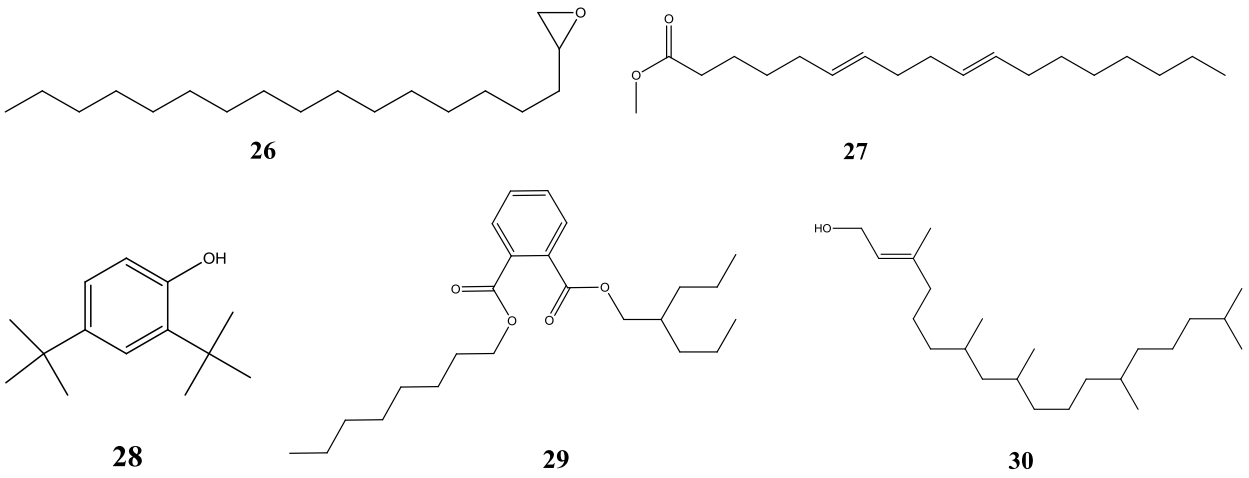


Figure 5. The chemical structure of compounds (**18-25**) isolated from bark *E. laevis Roxb*

Joshi and Wagh identified compounds, including hexadecyl-oxirane (**26**), phthalic acid, octyl 2-propylpentyl ester (**29**), methyl-6,10-octadecadienoate (**27**), 3,7,11,15-tetramethyl-2-hexadecen-1-ol (**30**), 2,4-bis-(1,1-dimethylethyl)phenol (**28**), 1,2-dichloro-2-methyl-propane (**32**), 1-chloro-2-ethoxy-2-methoxy-propane (**33**), octyl ester of 1,2-benzenedicarboxylic acid (**31**), 4-chloro-2,4-dimethylhexane (**34**) (figure 6) from chloroform extract of bark of the plant [42, 45].



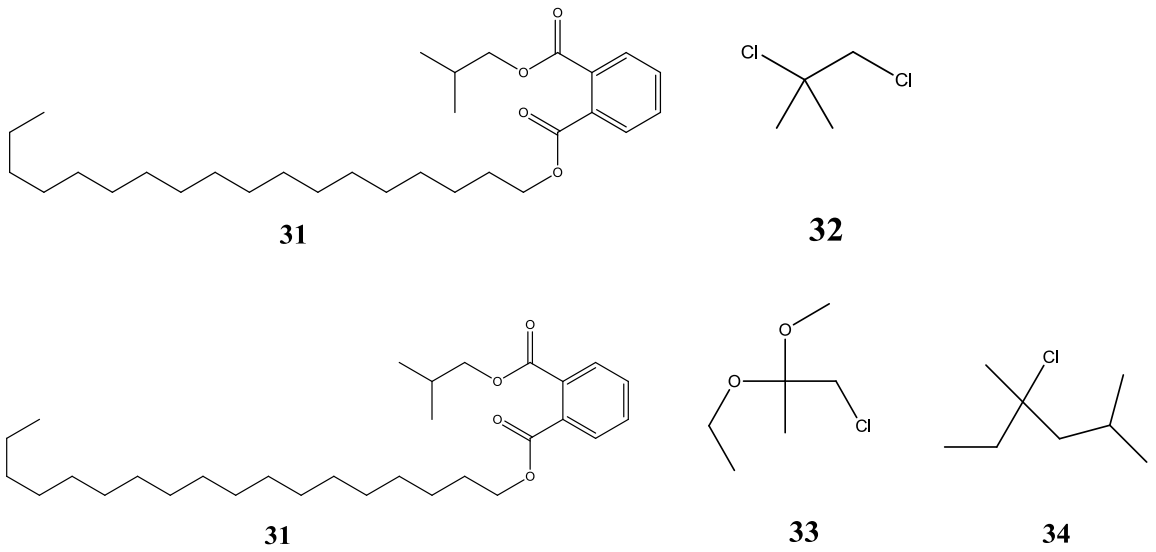
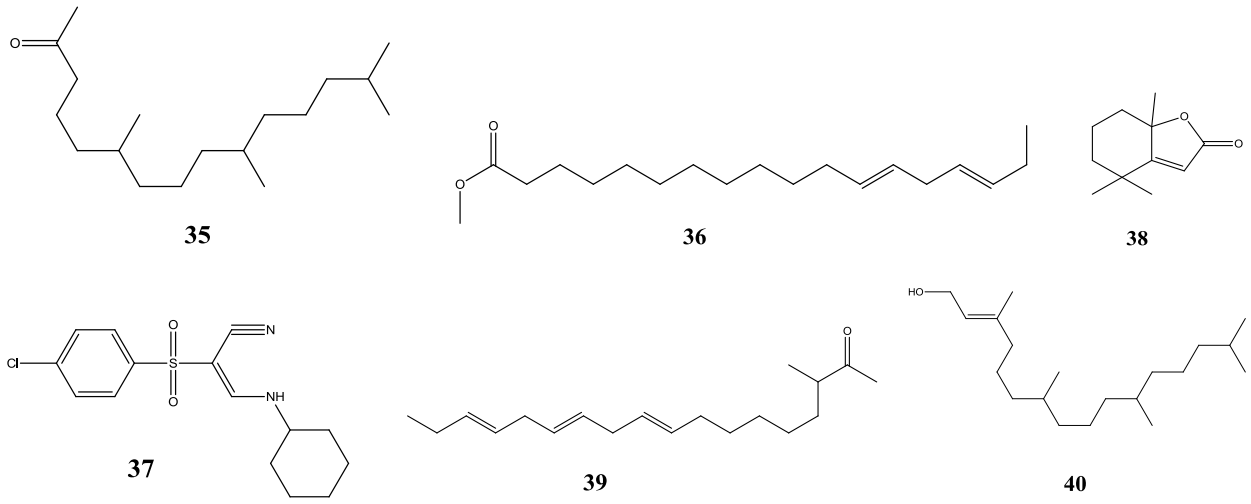


Figure 6. The chemical structure of compounds (**26-34**) isolated from bark *E. laevis Roxb*

From petroleum ether extract of bark of the plant. These include 6,10,14-trimethyl-2-pentadecanone(**35**),(12*E*,15*E*)-methyl octadeca-12,15-dienoate (**36**), 2-(4-chlorophenylsulfonyl)-3-cyclohexylamino-propenenitrile(**37**),5,6,7,7a-tetrahydro-4,4,7a-trimethyl-2(4H)-benzofuranone(**38**), methyl-8,11,14-heptadecatrienoate (**39**), 3,7,11,15-tetramethyl-2-hexadecen-1-ol (**40**), methyl octadeca-8,11-dienoate (**41**), 6,10,14-trimethyl-2-pentadecanone (**42**), 15-methylhexadecanoate (**43**) and 2-hydroxy-octadeca-9,12,15-trienoate (**44**) [46, 47].



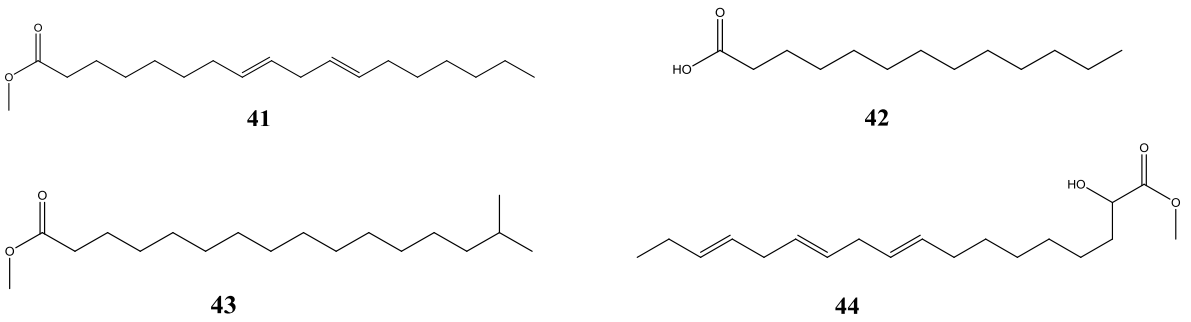


Figure 7. The chemical structure of compounds (**35-44**) isolated from bark *E. laevis Roxb*.

Torane et al. [1] reported that the leaves of *Ehretia laevis* contains chemical constituents extracted by hexane and isolated dominantly hydrocarbon compounds such as docosane (**45**), 1-tridecene (**46**) and tetradecene **(47**) [48, 49].

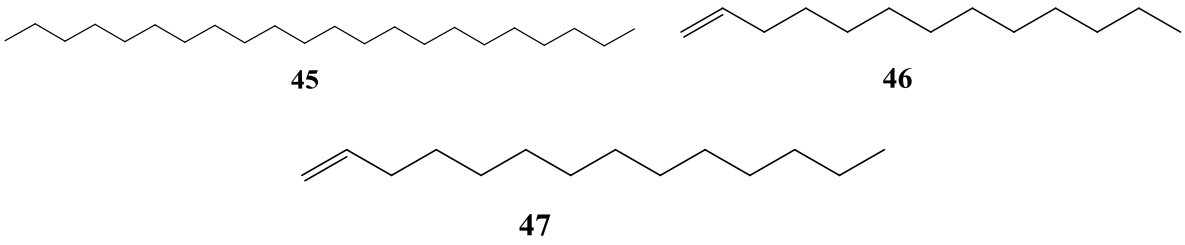


Figure 8. The chemical structure of compounds (**45-47**) isolated from leaves *E. laevis Roxb.*

2.3.4 *Ehretia microphylla*

Isolation of several compounds has been reported from its parts [50, 51]. The isolated compounds have been such as, 2-Propanol,1,1'-oxybis-(**48**), Benzenemethanol, alpha,-ethyl (4**9**), Benzene,1,3-bis(1,1-dimethylethyl) (**50**), 1-(4-Ethoxyphenyl)propan-1-ol (**51**), Benzoicacid,4-ethoxy-,ethyl ester(**52**), Tetradecanoic acid(**53**), Neophytadiene (**54**), Phytol (**55**), Hexadecanoicacid,2-hydroxy-1-(hydroxymethyl) ethyl ester (**56**), gamma-Sitosterol (**57**), 13 – Docosenamide (**58**) From Chloroform, Hexane, Ethyl acetate, Methanol (1:1:1:1) extract of leaves part of *E. micropphylla* [50, 51].

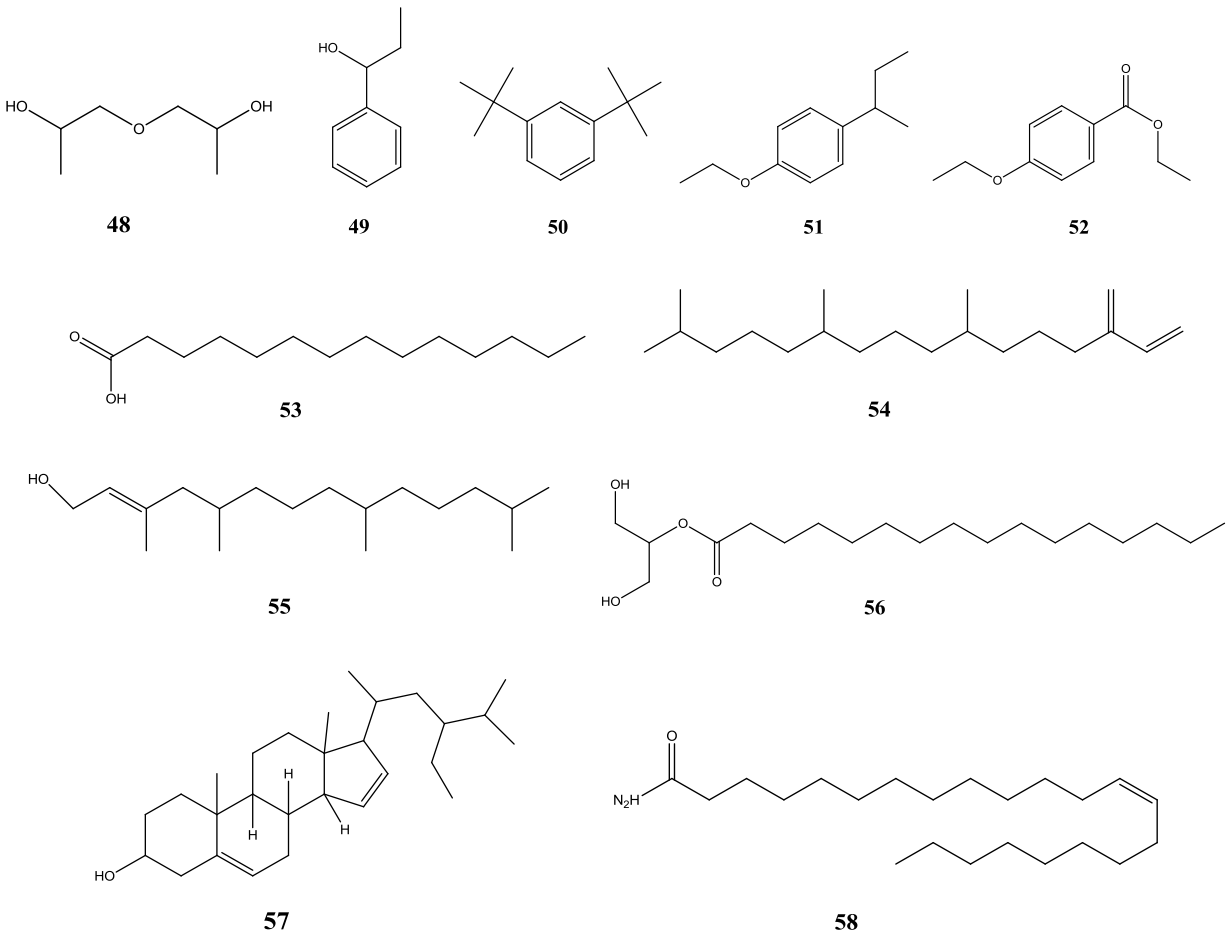


Figure 9. The chemical structure of compounds (**48**-**58**) isolated from the leaves part of *E. microphylla.*

Reports have been shown that compounds such as, microphyllone(**59**), dehydromicrophyllone (**60**), hydroxymicrophyllone (**61**), cyclomicrophyllone (**62**), allomicrophyllone (**63**) have been isolated from methanol extract of the aerial parts of *E. microphylla* [52, 13].

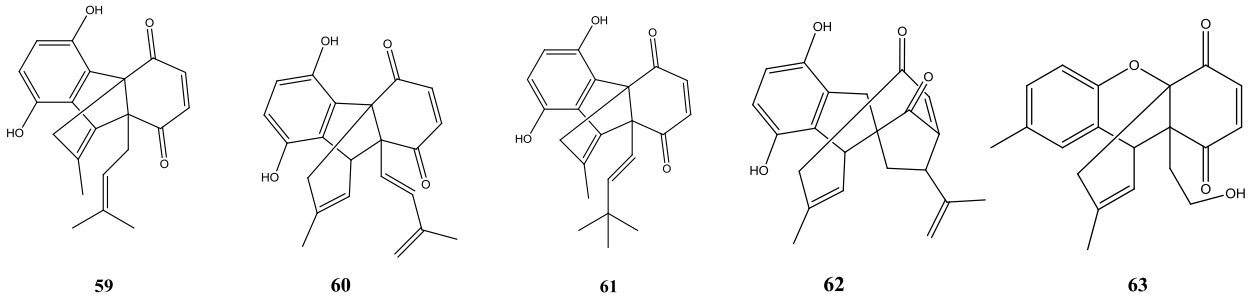
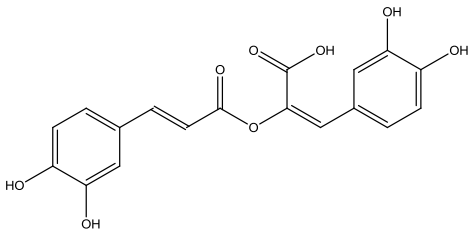


Figure 10. The chemical structure of compounds (**59-63**) isolated from the aerial part of *E. microphylla*

Charisse et al. documented, the plant is recognized to contain a variety of phytoconstituents such as rosmarinic acid (**89**), bauerenol (**10**), α-amyrin (**1**), β-amyrin (**2**), kaempferol-3-O-rutinoside (**64**) and kaempferol-3-O-glucoside (**65**) from methanol extract of the leaves of *E. microphylla* [53].



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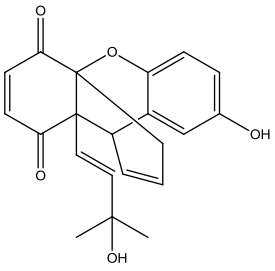
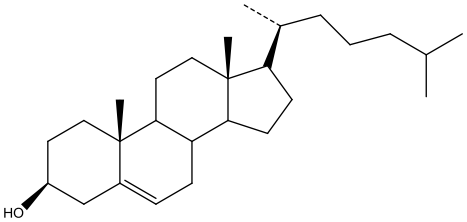


65

Figure 11. The chemical structure of compounds (**64, 65**) isolated from the leaves of *E. microphyll.*

In another reports isolated compounds such as, Ehretianone (**70**), β-sitosterol (**3**), stigmasterol (**69**), α-spinasterol (**68**), campesterol (**67**), cholesterol (**66**) from methanol extract of the root bark of E. microphylla and Microphyllone (**59**) ethanol extract of the leaves of *E. microphylla* [54, 55].



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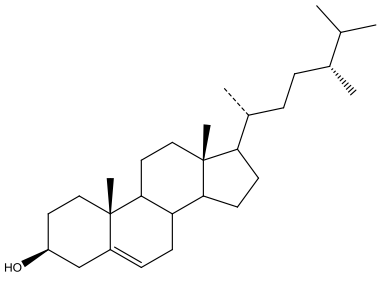

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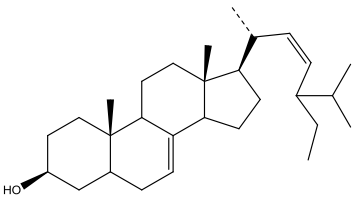

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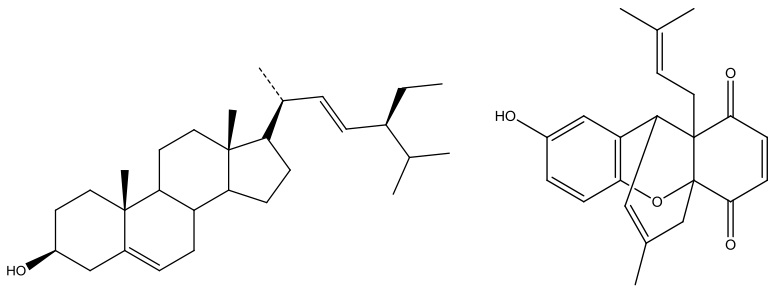
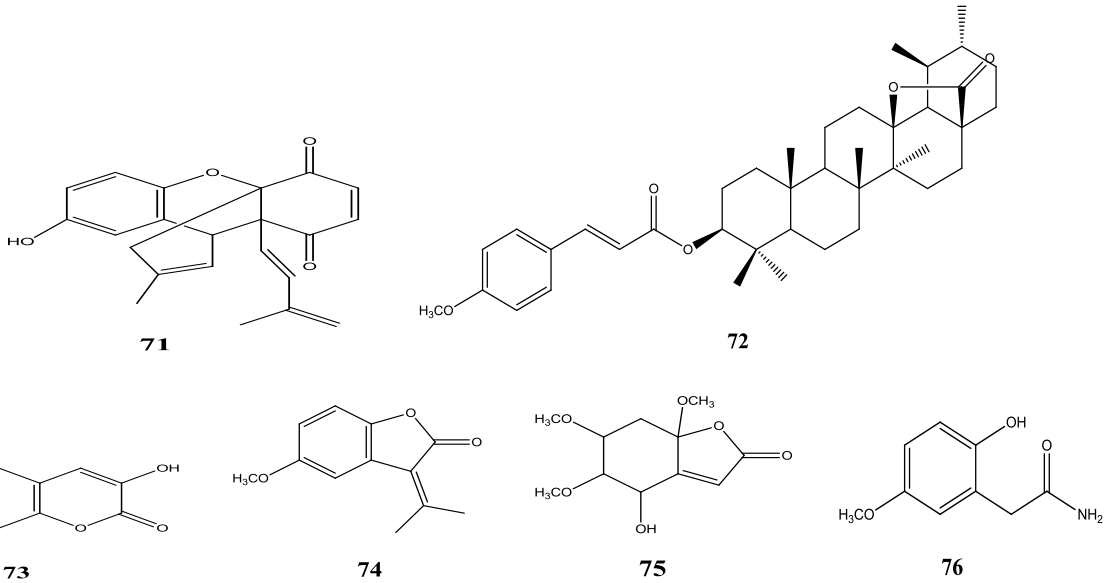


69 **70**

Figure 12. The chemical structure of compounds (**3, 59, and 66-70**) isolated from the leaves of *E. microphylla*.

2.3.5 *Ehretia longiflora*

The methanolic extract of the root of Ehretia longiflora (Boraginaceae) afforded Eight compounds, ehretiquinone (**71**), ehretiolide (7**2**), ehreticoumarin (7**3**), ehretilactone A (7**4**), ehretilactone B (**75**), ehretiamide **(76**), ehretine (**77**), and ehretiate (7**8**), (+)-(2E,6E)-methyl-10,11-dihydroxy-3,7,11-trimethyl-2,6-dodecadienoate (7**9**), methyl linoleate (**80**) [56]. oleanolic acid (**81**), O-acetyloleanolic acid (**82**), 3-methylbut-2-enoic acid (**83**) [57], prenylhydroquinone (**84**), 4-hydroxy-3-prenylbenzoic acid (**85**), proglobeflowery acid (**86**) [58], syringaldehyde (**87**), 2,3-dihydroxypropyl palmitate (**88**), from methanol extract of the root of *E. longiflora* [59].



71 **72**

73 **74** **75** **76**

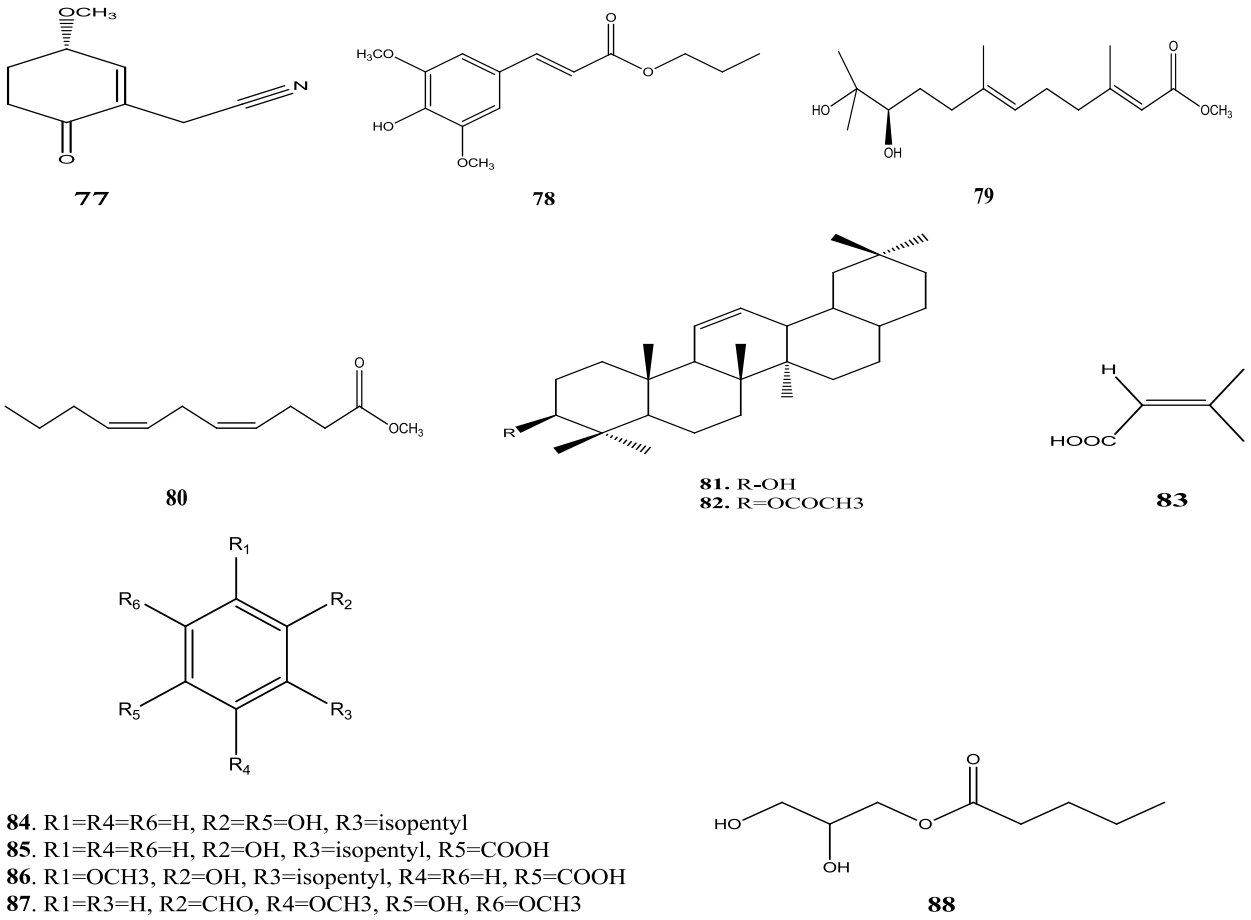


Figure 13. The chemical structure of compounds (**71-88**) isolated from the root of *E.longiflora*.

Another Isolated compounds such as, menisdaurin(**1**), rosmarinic acid(**89**), sitosterol(**67**), campestero l(**67**), stigmasterol(**69**), and ursolic acid (**7**) have been isolated from the stem bark of *E. longiflora* [60].

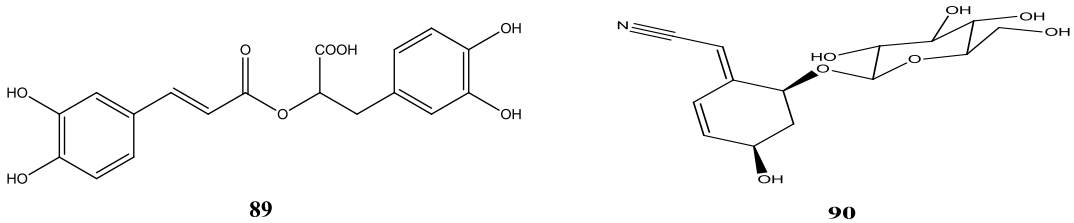


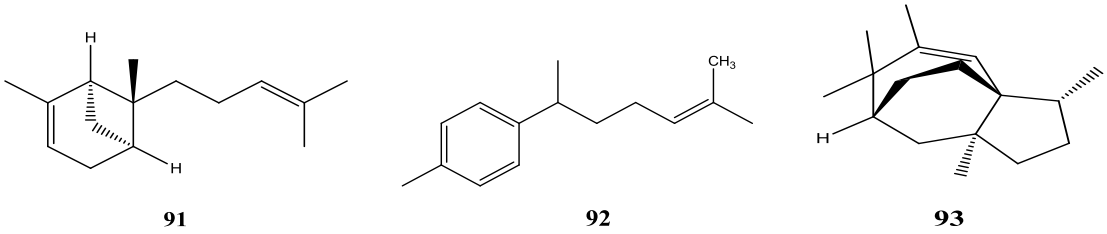
Figure 14. The chemical structure of compounds (**89, 90**) isolated from the stem bark of *E.longiflora*.

2.3.6 *Ehretia cymosa*

Three terpeniods compounds such as, α-amyrin (**1**), β-amyrin (**2**) and bauerenol (**10**) have been isolated from n-haxane extract of leaves of *E. cymosa* [61]. And ethyl acetate extract of the leaves of *E. cymosa* furnished two isomeric triterpenes identified as α-amyrin and β-amyrin [62].

Tole, Feso and Adane, reports of three triterpenoids identified as α-amyrin (**1**), β-amyrin (**2**), and bauerenol (**3**) isolated from dichloromethane: methanol ratio extract of root *E. cymosa* (*H. verdcourtii*) in Ethiopia [63].

In another Akintayo et al. reported as the chemical constituents of essential oils of the leaves of *E. cymosa* was obtained by hydro distillation. The leaves oil of *E. cymosa* comprised mainly of sesquiterpene hydrocarbon compounds represented mainly by trans-α-bergamotene (15.2%) (**103**), α-curcumene (14.5%) (**104**), and β-cedrene (14.0%) were isolated from the hexane extract of the leaves of *E. cymosa* [64].



91 **92** **93**

Figure 15. Structures of compounds (**91-93**) isolated from the leaves extract of *E. cymosa*.

2.4 Biological activities of genus *Ehretia*

The practice of plant based traditional medicine is predicated on hundreds of years of belief and observation. Today's modern medicine was mostly derived from ancient herbal traditions [52]. In rural Africa and Asia, medicinal plants have been prescribed by traditional healers, and because of their affordability and availability they have become fundamental in the African health care system [52, 65]. Genus *Ehretia* Plants contain abundant natural products that exhibit different biological activities [66]. In the genus *Ehretia*, literature has reported a wide variety of biological activities from extracts and isolated compounds [67].

From methanol extract of the leaves of *E. microphylla*, rosmarinic acid (**89**) was isolated as one of the active inhibitory constituents [68]. Anti-allergic effects the ethyl acetate fraction of *E.*

microphylla showed inhibitory activity on exocytosis in antigen-stimulated rat basophils. The bioassay-guided separation afforded five benzoquinones. Microphyllone(**59**) and allomicrophyllone(**63**) showed strong activities (IC50 values were 33 and 36 μmol/L, respectively). Dehydromicrophyllone(**60**) and cyclomicrophyllone(**62**) had weaker activity (IC50 values were 62 and 106 μmol/L, respectively). Hydroxymicrophyllone (**61**) was inactive [52]. A study of the antioxidant activity of leaf extract of *E. laevis* showed that it exhibited significant antioxidant activity with a minimal inhibitory concentration (MIC) value of 0.02 mg/mL [69]. Another study investigated the antioxidant capability of the leaves and stems of *E. laevis*, in which the ethanolic extracts of the leaves and stem were subjected to DPPH scavenging activity [70]. Two compounds ehretiolide(**72**) and prenylhydroquinone(**84**) have extracted from root of *Ehretia longiflora* are responsible for antitubercular activity[59]. While ehretianone(**72**), obtained from the root bark of *E. buxifolia* has antisnake venom properties [71].

CHAPTER THREE

3. Materials and methods

3.1 Materials

3.1.1 Chemicals

n- hexane, dichloromethane and methanol, are used for gradient extraction, on the other hand ethyl acetate, and n-hexane for column elution, pre-coated TLC (silica gel, Uv 254nm and 365nm) for chromatographic analyses and to determine phytochemicals, reagents like Dragendroff's, dilute sodium hydroxide, ferric chloride, concentrated hydrochloric acid, concentrated sulphuric acid glacial acetic acid, acetic anhydride and chloroform was used. The chemicals used were purchased from Ran chem. Co. Ltd. Agents in Addis Ababa, Ethiopia.

3.1.2 Instruments

Rotary evaporator (Heidolph, UK) for concentration of crude extracts, Grant (GLS 400) thermostatic bath shaker were used. Oven N50L, GENLAB, WIDNES, ENGLAND), Analytical Balance ADAM (AFP-110L), Iodine chamber, UV chamber (Uvitec). Infrared (IR) spectra were obtained from a Perkin Elmer BX infrared spectrometer (400 - 4000cm-1). All spectroscopic analyses were carried out at Hawassa University, Department of Chemistry.

3.1.3 Plant materials collection

The root of *E. cymosa* was collected on October 28, 2023, from Daye woreda, eastern sidama zone sidama region around Sagara kebele. The place is found in between Daye city and kinkamo kebele. It is 420 km far from Addis Ababa, the Capital city of Ethiopia, and 145 km from Hawassa University.

3.2 Methods

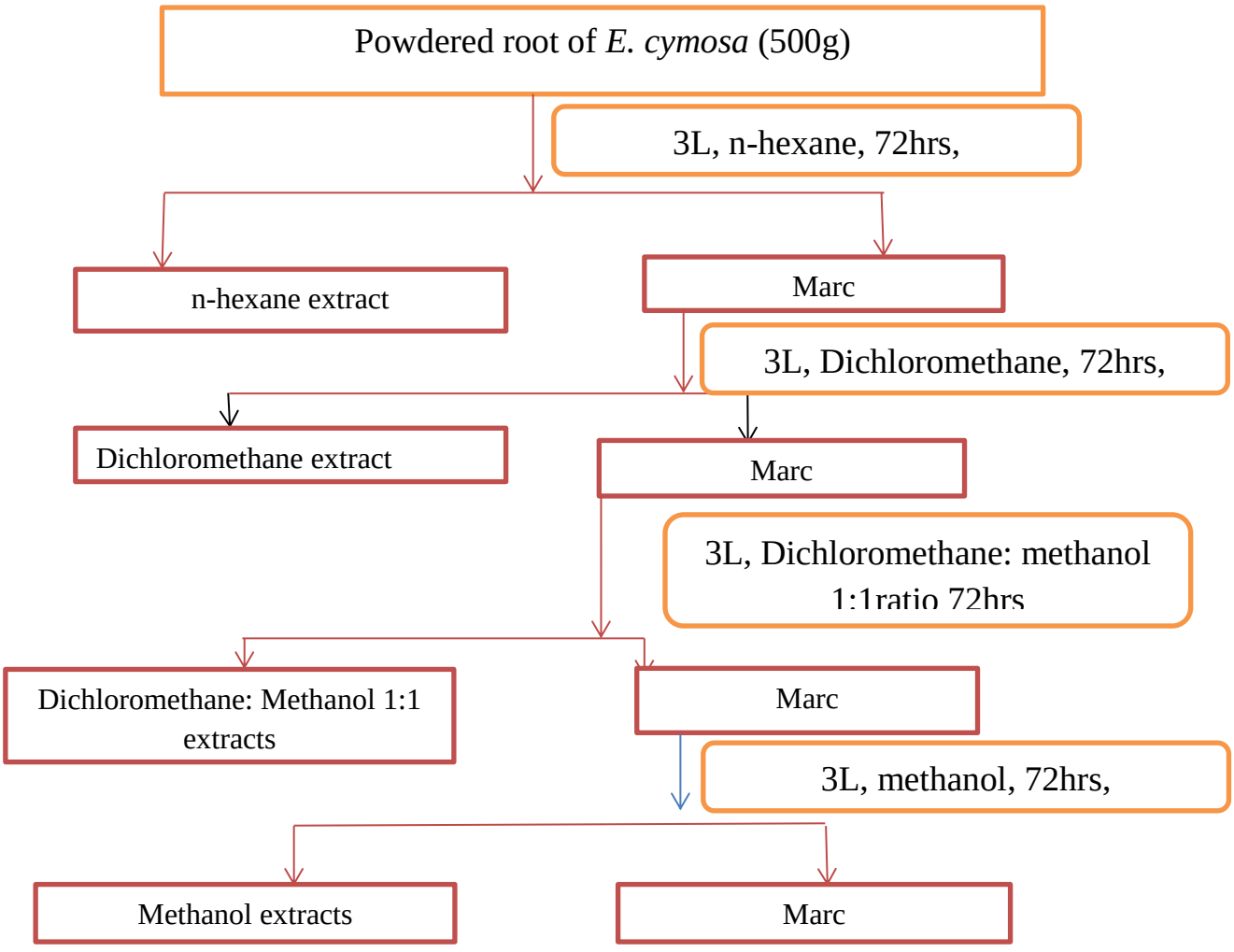
Preparation of plant specimen

The collected plant morphological parts of the root were chopped into small pieces, air dried under shade on a plastic material for one month, and milled to a suitable size with a grinding machine to facilitate the extraction process.

Extraction

The root of E. *cymosa* 500g was sequentially extracted with n-hexane, dichloromethane, dichloromethane/methanol (1:1), and methanol, using maceration techniques for 72 hrs with continuous shaking. The extracted matter was filtered using Whatmann No.1 filter paper, and the marc solvent in each gradient extract was removed using a Rotary evaporator under reduced pressure. The crude extracts of each solvent were stored in a hood until it became dry to determine the weight and safety for further analysis.General schematic flow chart indicates general extraction techniques (scheme 1).

Scheme 1. The general outline of the procedures used in the extraction processes.



Phytochemical screening

The phytochemical screening of various medicinal plants was carried out to analyze the presence of various secondary metabolites. The extracts were analyzed for the presence of various secondary metabolites (phytochemical constituents) such as alkaloids, saponnis, cholesterol, flavonoids, phenols, terpenoids, steroids and tannins [71].

Test for alkaloids

Extracts were dissolved individually in dilute hydrochloric acid and filtered using filter paper. Dragendroff's Test: Filtrates were treated with Dragendroff's reagent (a solution of Potassium Bismuth Iodide). The formation of a red precipitate indicates the presence of alkaloids.

Tests for flavonoids

About 0.2 g of each solvent crude extract was taken in different clean test tube and treated with few drops of sodium hydroxide solution. The formation of an intense yellow color, which becomes colorless with the addition of a dilute sulphuric acid, confirms the presence of flavonoids [72].

Tests for phenols

About 0.2 g of plant extract was weighed separately for each solvent extract and treated with a few drops of 5% ferric chloride and observed for the formation of a deep blue colour which indicates the presence of phenol [73].

Tests for terpenoids

When 0.3 g of plant extract was taken in a separate clean test tubes and 2 mL of chloroform was added and vigorously shaken, with evaporation for dryness. Then 3 mL of sulphuric acid was carefully added to form a layer and observed a reddish brown color interface to show the positive results for the presence of terpenoids [74].

Tests for tannins

Each solvent crude extract plus 4 mL of distilled water and drops of ferric chloride were mixed, and the immediate green precipitate formation was observed as an indication for the presence of tannin [74].

Tests for saponins

Froth test: Extracts were diluted with distilled water to 20 ml and this was shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicates the presence of saponins.

Tests for steroids

The crude extract was dissolved in 10 mL of chloroform and 2 mL of concentrated sulphuric acid was added by the sides of the test tube. The upper layer turns red and sulphuric acid layer showed yellow with green fluorescence which indicates the presence of steroids [72].

Tests for cholesterol

To 0.2 g of extract and 2 mL of chloroform was added in clean dry test tube. Then 10 drops of acetic anhydride and 2 to 3 drops of concentrated sulphuric acid was added. A red rose color changed to a blue-green color which confirms the presence of cholesterol [73].

Test for glycosides

The extract was hydrolyzed with dil. HCl, and then subjected to test for glycosides. Modified Borntrager's Test: The extracts was treated with Ferric Chloride solution and immersed in boiling water for about 5 minutes. The mixture was cooled and extracted with equal volumes of benzene. The benzene layer was separated and treated with ammonia solution. Formation of rosepink colour in the ammonical layer confirms the presence of anthranol glycosides [61].

Solvent selection for Column Chromatography

The TLC profiles of the crude extracts were analyzed in various solvent systems by increasing gradient of n-hexane in ethyl acetate with various proportions (90:10, 80:20, 70:30, 60:40, and 50:50% by volume). Accordingly, this solvent mixture was found to be an appropriate solvent to

use in chromatographic separation with increasing gradient. The yield of n-hexane and dichloromethane extracts was too small to check the TLC profile in various solvent systems.

The TLC profile analysis was carried out for dichloromethane: methanol and methanol extracts in different solvent systems to identify the appropriate solvent system for column chromatography. The TLC profile of dichloromethane: Methanol crude extract was conducted in n-hexane/ethyl acetate, ethyl acetate /chloroform, ethyl acetate: methanol, chloroform: methanol and many more combination by varying combination ratio. n-hexane: Ethyl acetate showed relatively good separation of components of the crude extracts.

Compound isolation and partial characterization

About 15.5 g of dichloromethane/methanol (1:1) dried crude extract was adsorbed onto 20g of silica gel and subjected to column chromatographic isolation (180 g silica gel 60-120 mesh size). The column was eluted with an increasing gradient of n-hexane in the ethyl acetate solvent system. The elution process was started by n-hexane and progressed through various ratios of an increasing portion of ethyl acetate in n-hexane. A total of 87 fractions were collected and each fraction was checked by TLC. The spots on the TLC plates were visualized using UV light (at 254nm and 365nm). The collected fractions were concentrated using a rotary evaporator. Fractions were tested using TLC and those with the same TLC profiles were combined. Then, the structural elucidations of the compounds were carried out based obtained from the partial characterization of spectroscopic (UV-vis and IR). The elution process was started by pure n-hexane (100%) and followed by n-hexane/Ethyl acetate mixture in different ratios. This successive elution was repeated for many times and a total of 87 fractions were collected as shown in Table 3.

Table 1, Column chromatography fractionation of the crude using n-hexane/ethyl acetate solvent system

Fraction No	Solvent system	Solvent ratio v/v (%)	Volume
1	n-hexane/ ethyl acetate	100:0	30
2	n-hexane/ ethyl acetate	95:5	30
3	n-hexane/ ethyl acetate	90:10	30
4	n-hexane/ ethyl acetate	85:25	30
5	n-hexane/ ethyl acetate	80:20	30
6	n-hexane/ ethyl acetate	75:25	30
7	n-hexane/ ethyl acetate	70:30	30
8	n-hexane/ ethyl acetate	65:35	30
9	n-hexane/ ethyl acetate	60:40	30
10	n-hexane/ ethyl acetate	40:60	30
11	n-hexane/ ethyl acetate	35:65	30
12	n-hexane/ ethyl acetate	30:70	30
13	n-hexane/ ethyl acetate	25:75	30
14	n-hexane/ ethyl acetate	20:80	30
15	n-hexane/ ethyl acetate	10:90	30
16	n-hexane/ ethyl acetate	0:100	30
17	Methanol in ethyl acetate	10:90	30
18	Methanol in ethyl acetate	20:80	30
19	Methanol in ethyl acetate	30:70	30
20	Methanol in ethyl acetate	40:60	30
21	Methanol	100%	30

3.2.5 Antibacterial activity tests

The activity of the plant extracts was tested against four American Type Culture Collection (ATCC) bacterial strains: namely *S. auraes*, *E. coli* and *S. pyogenes*, and *P. aeruginosa* were used to perform the antibacterial activities of the agents. Strains obtained from Adama Science and Technology University, Department of Applied Biology School of Applied Natural Sciense,

was inoculated on nutrient agar plate using sterile loop. The bacterial strains were selected on the basis of the diseases against which *E. cymosa* is used. Testing of the plant extracts for their antibacterial activity was done by the modified agar disc diffusion method reported in literature [75, 76].

In vitro antibacterial susceptibility test was determined by disc diffusion method [77]. The medium was prepared by dissolving 38 g of Mueller Hinton agar medium in 1000 mL of distilled water and the autoclaved at 121 °C for 15 min. The autoclaved medium was poured into sterile plates (20-25 mL/plate) and the plates were allowed to solidify under sterile condition at room temperature. After solidification, the plates were seeded with overnight grown culture approximately 1.5 X 10^8 CFU/mL by swabbing evenly on to the surface of the medium with a sterile cotton swab.

The isolated compounds at the concentrations of 150 mg/mL and 100mg/ml were dissolved in DMSO solvent. Whatman filter paper no. 1 was used to prepare discs approximately 6 mm in diameter. The sterile discs were infused with the synthesized compounds and position on the surface of the medium with sterile forceps and gently pressed down to ensure contact with the MHA. Tetracycline was used as standard antibiotic and then plates were inverted and then incubated at 37 ℃ for 24 hrs. After incubation the inhibition zone produced by the synthesized compounds were evaluated by measuring the diameter (mm) of the clear zone around the disc against the test organisms using a ruler [78].

CHAPTER FOUR

4. Results and Discussion

In this chapter, yield of extracts, phytochemical tests, partial characterization of isolated compounds, and antibacterial activity test are discussed.

4.1 Yields of extracts

Dry powdered root of *E. cymosa* weighing 500 gm were soaked in the four solvents systems, in order of increasing polarity starting from hexane, dichloromethane, dichloromethane: methanol 1:1 and finally methanol. The amount of extract obtained was recorded and tabulated as in (table 1).

Percent yield $\frac{mass\ of\ extract}{mass\ of\ the\ plant\ material\ used\ for\ extraction} \times 100$

Table 2. Wight of crude extracts

solvent	Mass of Crude Extract	Yield (%)
n-hexane	2.5g	0.5%
Dichloromethane	3.5g	0.7%
Dichloromethane : Methanol	15.5g	3.1%
Methanol	5g	1%

The dichloromethane: methanol (1:1) extracts had the highest percentage yield (3.1%) than the rest while the dichloromethane had the least (0.7%).

4.2 Phytochemical screening

Phytochemical screening tests was carried out on all the crude extracts of (n-hexane, dichloromethane, dichloromethane: methanol (1:1 v/v) and methanol) following standard procedures. The focus was made on testing presence or absence of secondary metabolites such as alkaloids, flavonoids, phenols, glycosides, terpenoids, tannins, saponnins, Cholesterol, and steroids. The result of the n-hexane, dichloromethane, and dichloromethane: methanol 1:1 ratio

and methanol extracts with their phytochemical analysis of the root of *E. cymosa* were presented below (Table. 2)

Table 3. Phytochemical screening on the root of *E. cymosa*

No	Constituents	n-hexane	Dichloromethane	Dichloromethane:Methanol	Methanol
1	Alkaloids	+	+	+	+
2	Flavanoids	-	+	+	+
3	Terpinoids	+	+	+	+
4	Phenols	-	+	+	+
5	Glycosides	-	+	+	-
6	Tannins	-	+	+	+
7	Saponnins	-	+	+	+
8	Steriodes	+	+	+	+
9	Cholesterol	+	+	+	-

Note: (+): presence of constituents; (-): absence of constituents

According to the results in the above table, (Table 2) n-hexane crude extracts indicate the presence of alkaloids, steroids, cholesterol, and terpenoids. Whereas, Flavanoids, phenols, saponins, Glycoside, and tannins are absent. Crude extract of dichloromethane and dichloromethane/methanol (1:1) indicates the presence of alkaloids, saponins, flavonoids, phenols, steroids, glycosides, terpenoids, cholesterol, and tannins. On the other hand crude extract of methanol indicates glycosides absent and others are presence of secondary metabolites listed in the above paragraph.

Plants of the genus Ehretia are rich in bioactive constituents such as phenolic acids, lignans, flavonoids, nitrile glycosides, quinonoids, steroids, triterpenoids, and pyrrolizidine alkaloid [79, 80]. The previous report on phytochemical screening of the leaf extracts of E. cymosa shows the presence of alkaloids, saponins, glycosides, terpenoids, anthraquinones, phenolics, and flavonoids [81].

The classes of secondary metabolites found in the plant have the following biological activities. Flavonoids are known by antioxidative, free radical scavenging, coronary heart disease

prevention, hepatoprotective, anti-inflammatory, anticancer and antiviral activities [82]. Saponins are known for their biological activities such as antimicrobial, antifungal, anti-inflammatory, antiviral, antioxidant, anticancer, and immunomodulatory effects [83].

Glycosides are known to possess remarkable therapeutic potential and pharmacological activities. Analgesic, anti-inflammatory, cardiotonic, antibacterial, antifungal, antiviral, and anticancer effects are some of the pharmacological activities [84, 85].

Terpenoids have antimicrobial and antidiarrheal activities [85]. Phenolic constituents exhibit antibiotic, antimicrobial, and antidiarrheal activities [85, 86]. Alkaloids exhibit a wide range of activities. They are therefore constituents that have high prospective in medicine, plant defense, veterinary, or toxicology. The presence of such classes of secondary metabolites supports the ethnomedicinal use of the species.

4.3 Isolated compounds

Fractions 1-13 were colorless and did not show any spots; and discarded them. Fractions 14-28 were discarded due to absence of any spots to be observed on the TLC under different solvent systems.

Fraction 29-31 gave a yellowish crystalline compound (coded as **EC-1**) with a mass of 350 mg and an R_f value of 0.95 in n-hexane: ethyl acetate (80:20). A White crystalline solid compound was obtained from combined fractions from 39-41 and revealed single spot with few impurities having the R_f value 0.6 in (80:20) n-hexane/ethyl acetate solvent system. Before combining them together they are further purified through washing with n-hexane repeatedly for several times and checked by TLC coded by **EC-2** with mass of 280 mg.

Fractions 50-61 were yellow, and were combined based on their TLC. The combined fractions were concentrated to obtain a yellow precipitate with R_f 0.18 in (80:20) n-hexane/ethyl acetate solvent system and coded **EC-3** with a mass 320mg.

A total of three compounds (**EC-1, EC-2,** and **EC-3**) were isolated and submitted for UV-Vis and FTIR spectral data generation and structures were partially elucidated.

4.4. Partially structure elucidation of Isolated Compounds

All isolated compounds are from the dichloromethane/methanol (1:1) extract of root of *E. cymosa*. The UV and IR spectra were recorded for compound-EC-1, compound EC-2, and compound EC-3. The data were interpreted systematically as per the theoretical knowledge gained through spectroscopic courses.

4.4.1. Compound EC-1

Compound EC-1 was isolated as yellowish crystal from dichloromethane: methanol (1:1 ratio) extract. Structural elucidation of this compound was done based on the spectroscopic data obtained from UV-Vis and IR.

Compound (EC-1) (Figure 8) was isolated as yellowish crystal (350mg) and a melting point of 214 ºC and R_f value 0.95 in n-hexane/ethyl acetate (80:20) solvent system. The structural elucidation was done using the data obtained from UV-Vis and IR. Compound EC-1 was isolated as yellowish crystal exhibiting the UV absorption maxima at 236 nm.

Table 4. UV-Vis spectrum for **EC-1**

Absorption peak (nm)	Functional group	Electronic transition	Ranges of absorption	Region
236	C=O (Carbonyl)	n-π*	190-380 nm	(Near) ultraviolet

The IR spectral of Compound **EC-1** is shown in (Appendix 2) analysis showed stretching frequency at δ 3480 cm^{-1} due to the presence of hydroxyl group. The band at δ 2931 cm^{-1} suggests the presence of C-H stretching of methyl group. Also observed was a band at δ 2848 and 1454 cm^{-1} due to C-H stretching of methylene, and C=C stretching absorption, respectively. The presence of carbon C=O double bond is evident at 1734 cm^{-1} [87].

Table 5. Typical Infrared (IR) absorption frequencies of the compound EC-1

Observed frequency (cm-1)	Possible Frequency Range (cm-1) Literature reported data	Assignment
3460	3441	O-H
2923, 2848	2930	asym and sym aliphatic C-H stretching
1734	1725	Carbonyl stretching (C=O)
1645	1640	Alkene C=C
1456	1454	methy
214 °C	214-215 °C	Melting point

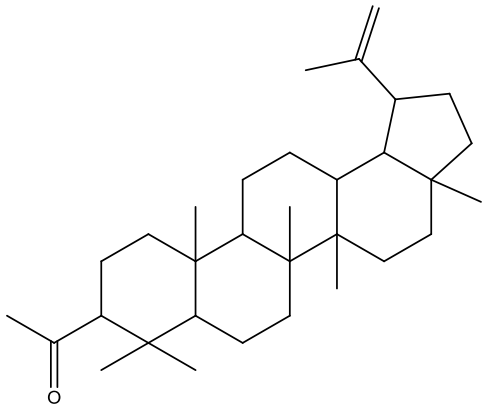


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Figure 16. Proposed structure of compound **EC-1** (Lupeol acetate)

4.4.2. Compound EC-2

Compound **EC-2** was isolated as white crystal from dichloromethane: methanol (1:1 ratio) extract. Structural elucidation of this compound was done based on the spectroscopic data obtained from UV-Vis and IR.

Compound **EC-2**(Appendix 3), was isolated as white crystal solid (280mg) with a melting point of 256-257 °C. TLC showed R_f value 0.6 in n-hexane/ethyl acetate (80:20) solvent system. The structural elucidation was done using the data obtained from UV-Vis and IR. Compound **EC-2** (Appendix 3) the UV absorption maxima at 254nm and 326 nm.

The IR spectrum of **EC-2** compound (appendix 4), showed broad absorption bands at 3444 cm^{-1} indicated the presence of O-H group. The absorptions bands at 2923 and 2848cm^{-1} indicated the presence of C-H stretching for asymmetrical CH_3 and symmetrical CH_2 respectively. The band at 1729cm^{-1} indicated the unsaturation that is C=O absorption band and band at 1021cm^{-1} indicated the presence of C-O stretches. The absorption band at 1473cm^{-1} indicated the presence of C-H bending of CH_2 and absorption band at a 1320cm^{-1} is due to C-H bending [88, 89].

Table 6. Typical Infrared (IR) absorption frequencies of the compound EC-2

Observed frequency (cm-1)	Possible Frequency Range (cm-1) Literature reported data	Assignment
3444	3434	hydroxyl (OH) stretching
2923, 2848	2924, 2847	aliphatic C-H stretching
1729	1728	carbonyl (C=O) stretching vibrations
1679	1640	olefinic C=C double bond
1460	1464	indicates C-H bending
1247	1271	C-O stretching of the carbonyl carbon of carboxylic acid
254-256 °C	256-257 °C	Melting point

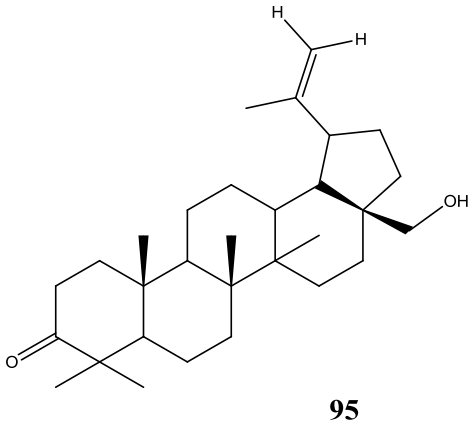

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Figure 17. Proposed structure of compound **EC-2** (Betulone).

4.4.3. Compound EC-3

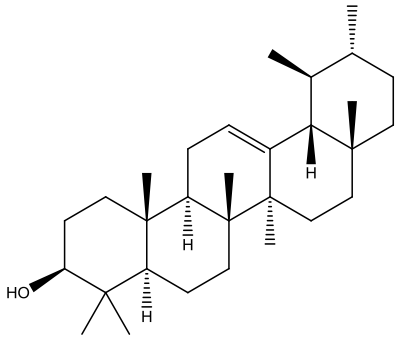
Compound **EC-3** was isolated as yellowsh crystal from dichloromethane: methanol (1:1 ratio) extract. Structural elucidation of this compound was done based on the spectroscopic data obtained from UV-Vis and IR.

Compound **EC-3** (Appendix 5), was isolated as yellowish crystal (320mg) with a melting point of 223-225 °C and R_f value 0.18 in n-hexane/ethyl acetate (80:20) solvent system. The structural elucidation was done using partial data obtained from UV-Vis and IR. Compound **EC-3** (Appendix 5) was isolated as yellowish crystal exhibiting the UV absorption maxima at 260nm and 354 nm.

The IR spectrum (Appendix 6) indicated vibrations at 3478cm^{-1} indicates hydroxyl group (O-H) functional group. Two bands at 2925 and 2861 cm^{-1} showed the presence of both asymmetric and symmetric C-H stretching vibrations for methylene and methyl in the range between 2925-2861cm^{-1}. Bands 1740cm^{-1} indicate C=O stretch. Additionally, the compound shows strong absorption band at 1234cm^{-1} indicating the presence of C-O stretching bond 1475cm^{-1} compare with literature [90].

Table 7. Typical Infrared (IR) absorption frequencies of the compound EC-3

Observed frequency (cm-1)	Possible Frequency Range (cm-1) Literature reported data	Assignment
3470	3442	hydroxyl (OH)
2923, 2861	2931, 2860	CH3 stretching, C-H str. In CH2
2690, 2230	2693, 2693	
1740	1735	carbonyl (C=O) groups
1475	1483	C-H bending of unsaturated alkene.
1268, 1225	1278, 1235	s C-O stretching of the carbonyl carbon of carboxylic acid
186°C	186°C	Melting point



1

Figure 18. Proposed structure of compound **EC-3** (α-amyrin)

4.5 Antibacterial activity test results

The antibacterial activity of the crude extracts from the root of *E. cymosa* and isolated compounds (EC-1, EC-2, and EC-3) was tested at the concentration of (150mg/ml and 100mg/ml) against four pathogenic bacterial strains: two gram-positive *(S.aureus and S. pyogen*) and two gram-negative (*E.coli and P. aeruginosa*) (Appendix 7)

The result showed that dichloromethane, dichloromethane: methanol, and methanol crude extract showed a zone of inhibition ranging from 6 to 7mm for gram-positive bacteria and 7 to 9mm for gram-negative bacteria. Methanol extract showed higher antibacterial activity against gram-positive *S.aureus* (8.5mm) and *S. pyogenes* (9 mm) than gram-negative, *E.coli* (7mm) and *P. auruginosa* (7mm) at the concentration of 150mg/ml. when dichloromethane, dichloromethane: methanol, and methanol extract are compared with each other methanol extract against all selected pathogenic strains, methanol extract showed a relatively higher inhibition zone at both concentrations (150mg/ml and 100mg/ml) (table 4)

The inhibition zone of the isolated compounds was found to be in the range of 8 to 11mm for gram-positive and from 8 to 9.5mm for gram-negative bacteria. The isolated compounds showed significant activities especially EC-3 at 150mg/ml against *S. aureus, S.pyogen, E. coli, and P. aeruginosa* with inhibition zones 11, 10.5, 10, and 9.5mm respectively (table 4), compared with positive control (Tetracycline) (13.5, 13, 12, and 11.5mm respectively). From the results, it was observed that the isolated compounds (EC-1, EC-2, and EC-3) showed a relatively higher

bacterial zone of inhibition as compared with the root extracts from *E. cymosa* (dichloromethane, dichloromethane: methanol, and methanol) in case of tested antibacterial activities. Finally, the observed antibacterial activities from the root of crude extracts and isolated compounds may justify the traditional use of the plant for the treatment of different bacterial infections.

Table 4. Antibacterial activity of plant extracts and compounds against *S. auras*, *E. coli*, *P. aeruginosa*, and *S. pyogen*.

Compounds & conc. (150 mg/mL)		Bacteria species and zone of inhibitions in millimeter (mm)			
		Gram-positive		Gram-negative	
		S. pyogen ATCC19615	*S. aureus* ATCC25923	*E. coli* ATCC25922	*P. aeruginosa* ATCC27853
EC- DCM	*150mg/ml*	7	7	6.5	6
	100mg/ml	6.4	6.4	6	6
EC-DCM/Met	150mg/ml	8.5	8	7	7
	100mg/ml	8	7.1	6.2	6
EC-Methanol	150mg/ml	9	8.5	7	7
	100mg/ml	7	7	6	6.8
EC-1	150mg/ml	9.5	8.5	8	8
	100mg/ml	9	8	7	6
EC-2	150mg/ml	10	9	9	9
	100mg/ml	9	8.5	8.	8
EC-3	150mg/ml	11	10.5	10	9.5
	100mg/ml	10	10	9	9
Tetracycline (+ve control)		13.5	13	12	11.5
DMSO (-ve control)		0	0	0	0

CHAPTER FIVE

5. Conclusion and Recommendation

5.1 Conclusion

In this study, phytochemical screening tests and compound isolation were carried out on the root of *E. cymosa*. Phytochemical screening test of the n-hexane, dichloromethane, dichloromethane: methanol (1:1 by v/v), and methanol extracts of the root of *E. cymosa*. The result revealed the presence of a variety of chemical constituents, alkaloids, terpenoids, saponins, steroids, flavonoids, tannins, phenols, and glycosides. Silica gel column Chromatographic separation of the dichloromethane: methanol (1:1 by v/v) extract has led to the isolation of the mixture of three compounds labeled EC-1, EC-2, and EC-3. Their isolated compounds were determined based on partial characterization spectra (Uv-Vis and FTIR). These isolated compounds were isolated from root of *E. cymosa*.

In vitro test results showed that the crude extracts and isolated compounds were evaluated using the disc diffusion method for their antibacterial activities against four bacterial strains specifically (*S. pyogen* ATCC19615, *S. aureus* ATCC25923, *E. coli* ATCC25922, and *P. aeruginosa* ATCC27853). Evaluation of antibacterial activities revealed that the isolated compound (EC-3) had the highest activity against all the strains especially against (*S. aureus* 11mm zone inhibition). Therefore, the antibacterial activity tests have revealed that the antibacterial activity of the isolated compounds from root *E. cymosa* showed it will be a promising source of bioactive compounds that could be used as lead compounds in selectively search new antibacterial drugs.

5.2 Recommendation

Based on the present study the following recommendations are forwarded:

- Besides the present antibacteria studies on the four microbes the work can be extended on many other microbes.
- It is recommended that further studies should be conducted on anti-oxidant and anticancer activities of this plant.

- It is recommended that further studies spectroscopic techniques such as NMR, HMBC, and HSQC are required to fully characterize the isolated compound.
- More compounds can be isolated from the plant using advanced chromatographic techniques of HPLC.
- It is recommended that further similar studies should be conducted on other parts of the plant such as root bark and fruit.

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Appendix

Appendix 1: The UV spectrum of compound EC-1

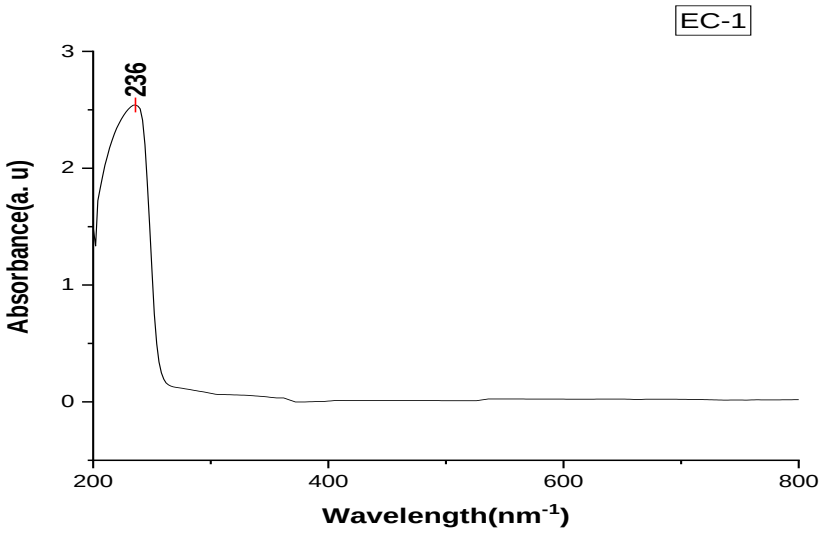

Figure 19. The UV spectrum of compound EC-1

Appendix 2: The IR spectrum of compound EC-1

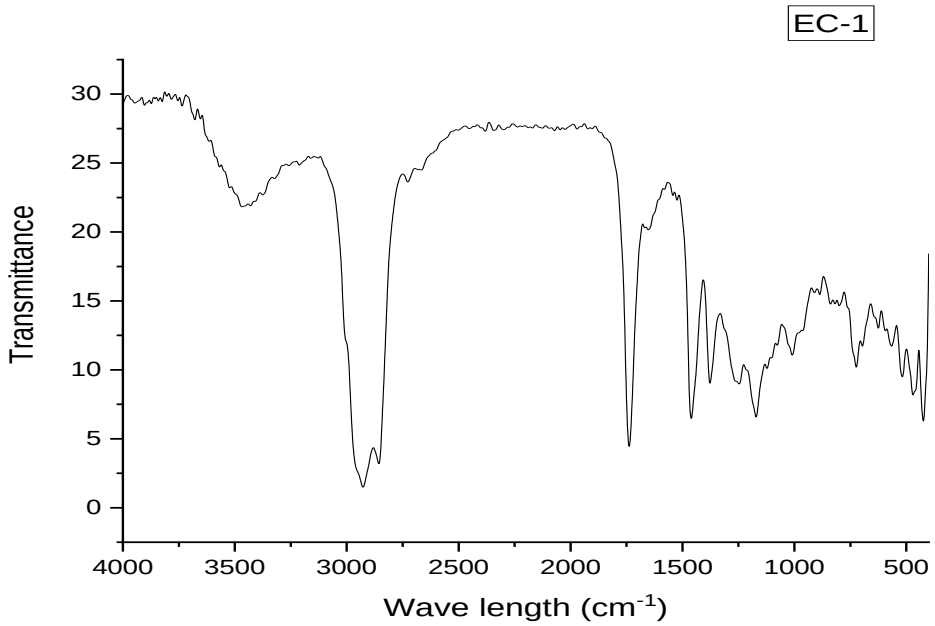

Figure 20. The IR spectrum of compound EC-1

Appendix 3: The UV spectrum of compound EC-2

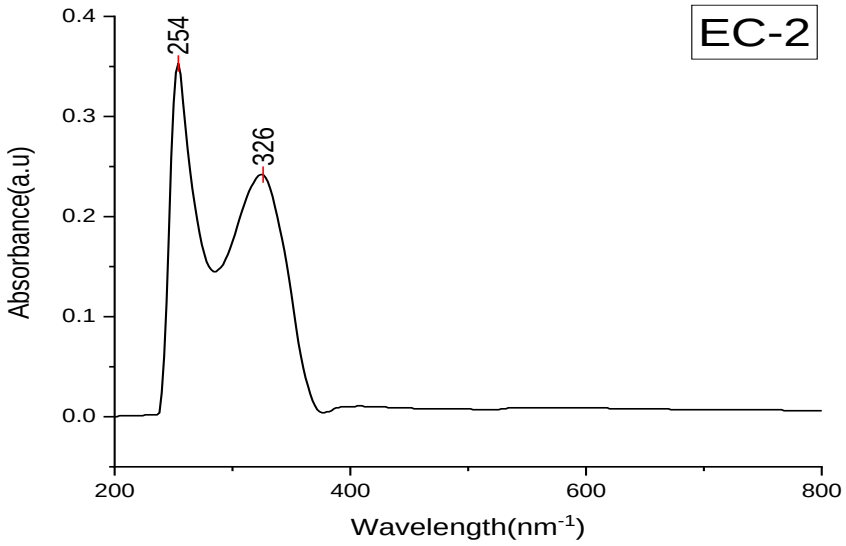

Figure 21. The UV spectrum of compound EC-2

Appendix 4: The IR spectrum of compound EC-2

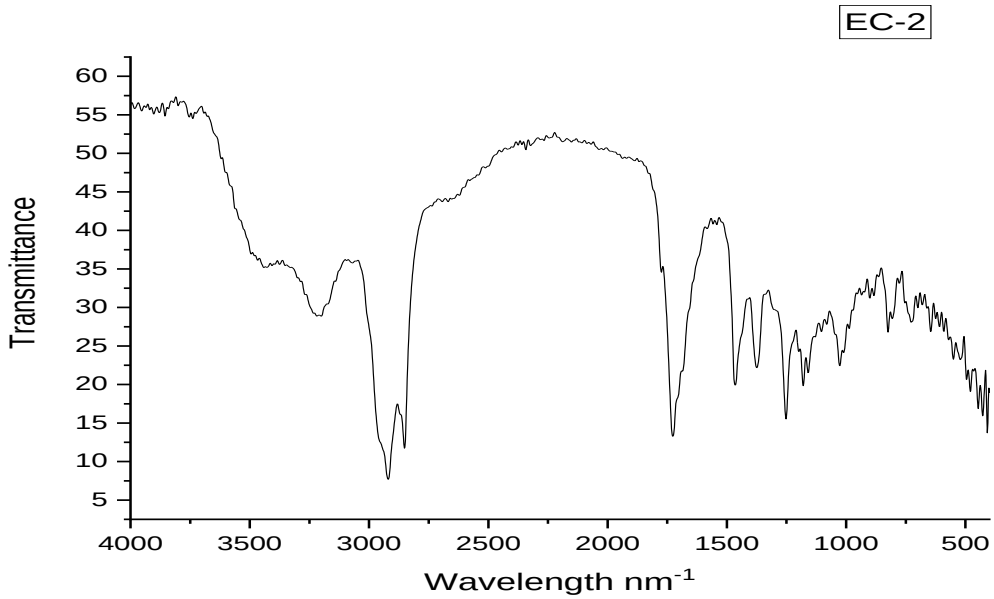

Figure 22. The IR spectrum of compound EC-2

Appendix 5: The UV spectrum of compound EC-3

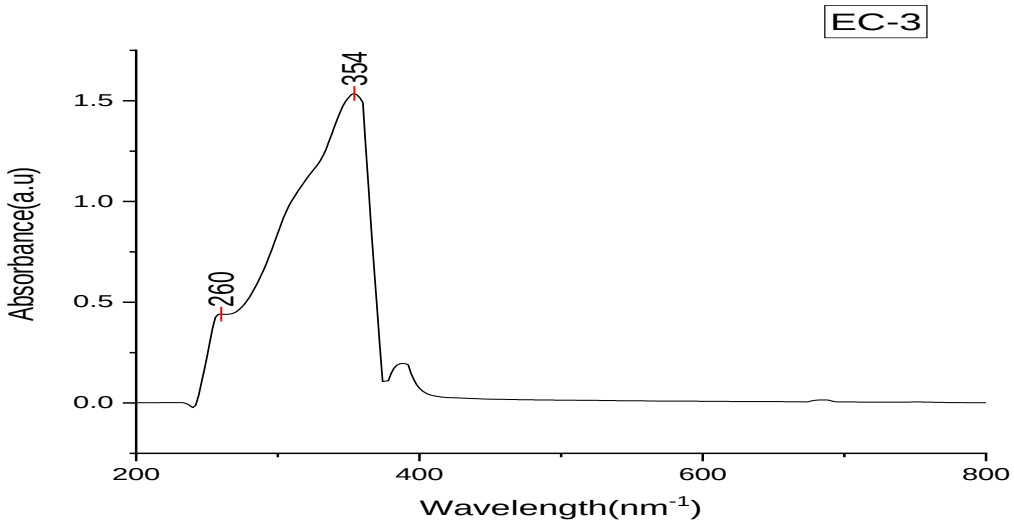

Figure 23. The UV spectrum of compound EC-3

Appendix 6: The IR spectrum of compound EC-3

Figure 24. The IR spectrum of compound EC-3

Appendix 7: The zones of inhibition for extracts, EC-DCM, EC-DCM: M, EC-M and isolated compounds.

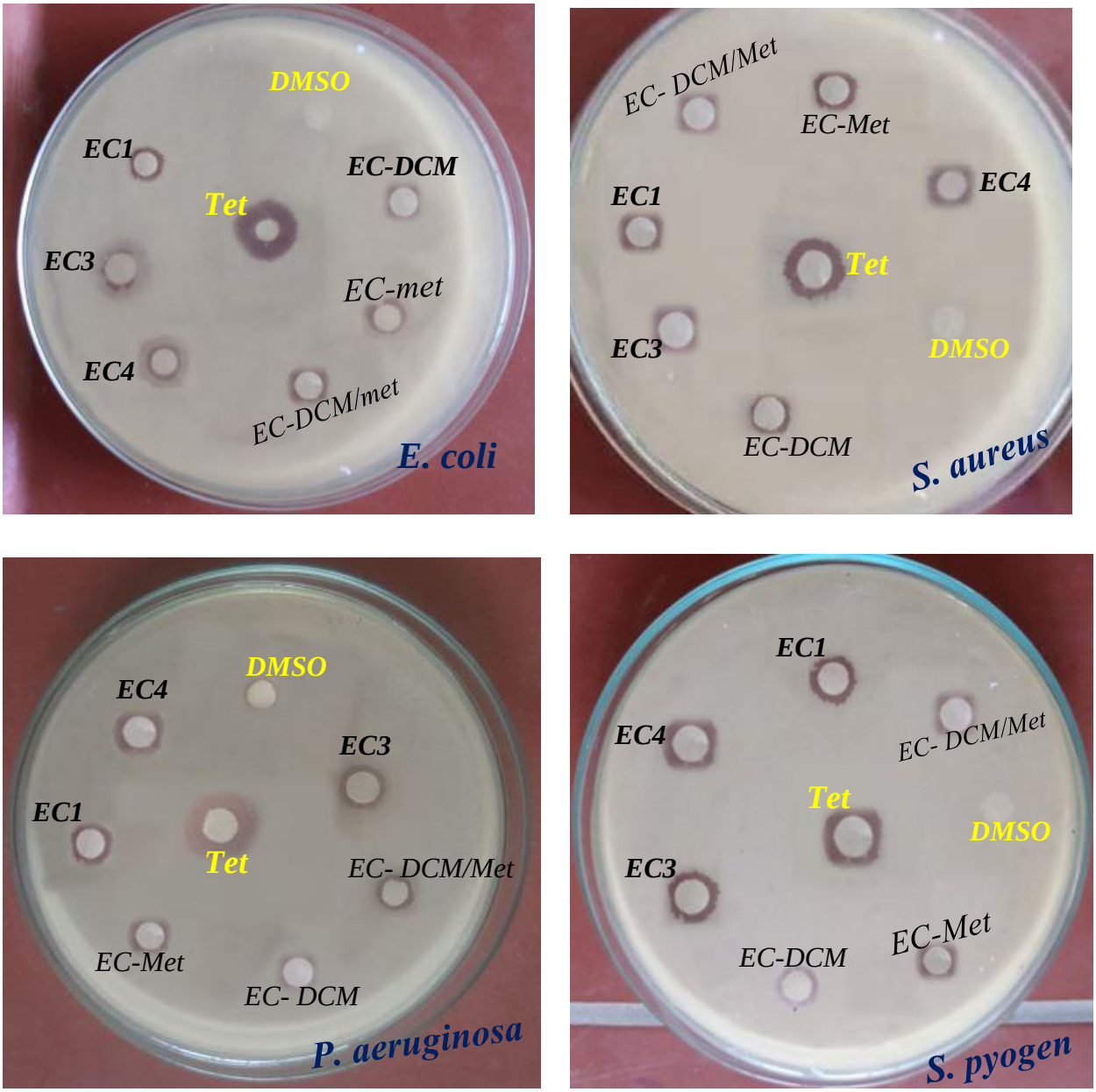

Figure 25. The zones of inhibition for extracts, EC-DCM, EC-DCM: M, EC-M and isolated compounds.

Appendix 8, TLC profile isolated compound EC-1(A), EC-2(B), and EC-3(C).


(A)

(B)

(B)

(C)

Figure 26. TLC profile EC-1(A), EC-2(B), and EC-3(C).