HAWASSA UNIVERSITY

COLLEGE OF NATURAL AND COMPUTATIONAL SCIENCES

CHEMISTRY DEPARTMENT

Phytochemical Analysis of The Stem Bark Extract of *Eucalyptus globules* Labill

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

By:

KIDIST BEYENE BAYOU

ADVISOR: TEGENE TESFAYE (PhD)

OCTOBER, 2024

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DECLRATION

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: KIDIST BEYENE BAYOU

Signature: _____

Date: _____

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

Name of advisor: TEGENE TESFAYE (Ph.D)

Signature: _____

Date: _____

ADVISOR'S APPROVAL SHEET

This is to certify that the thesis entitled "**Phytochemical Analysis of The Stem Bark Extract of *Eucalyptus globules* Labill.**" 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 KIDIST BEYENE BAYOU (ID, No Chemk/086/9), 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.

Dr. TEGENE TESFAYE (PhD)	_____	_____
Name of advisor	Signature	Date

EXAMINERS APPROVAL SHEET

Examiners of the final defense by KIDIST BEYENE BAYOU have read and evaluated her thesis entitled "**Phytochemical Analysis of The Stem BarkExtract of *Eucalyptus globules* Labill.**" and examined the candidate. This is therefore to certify that, the thesis has been accepted in partial fulfillment of the requirements for the degree of Master of Science in Chemistry with specialization in Organic Chemistry.

_____	_____	_____
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TABLE OF CONTENTS

Content **Page**

LIST OF TABLES

Table **Page**

LIST OF FIGURES

Figure **Page**

LIST OF SCHEMES

Scheme **Page**

LIST OF ABBREVIATIONS

CC	Column Chromatography
DEPT	Distortion less Enhancement by Polarization Transfer
EO	Essential Oil
GC-MS	Gas chromatography/mass spectrometry
HPLC	High performance liquid chromatography
SE	Semi purified extract
SRE	Sideroxylonal-Rich Extract

ABSTARCT

The traditional medicinal system continues to play an essential role in health care, with about 80% of the world's inhabitants relying mainly on traditional medicines for their primary health care. The genus *Eucalyptus* is known to possess medicinal properties in every part of the plant. *Eucalyptus globules* is one of the medicinal plants that has been claimed to be used traditionally to treat several illnesses such as stomachaches, toothache, snake bite, gonorrhea, evil eyes, diarrhea, skin diseases, and headaches. The aim of this study was to investigate the phytochemicals of the stem bark of *Eucalyptus globules.* The stem bark was chopped into small pieces, and air dried under shade, at room temperature. The complete air-dried stem bark was pulverized into fine powder. The powdered stem bark was extracted successively with three different solvents: hexane, $CHCl_3$:MeOH (v/v, 1:1) and methanol using cold maceration method. The percentage yield shows 0.42%, 2.4%, and 3.22% of hexane, $CHCl_3$:MeOH (v/v, 1:1) and methanolic extracts respectively. Phytochemical screening tests of the crude extracts of the$CHCl_3$:MeOH (v/v, 1:1) and methanolic extracts revealed the presence of the classes of secondary metabolites namely alkaloids, flavonoids, phenols, glycosides, terpenoids, saponins, and tannins. The crude n-hexane extract showed the presence of phenols, cardiac glycosides, terpenoids, steroids, and saponins. The $CHCl_3$:MeOH (v/v, 1:1) extracts were further fractionated with various composition of hexane:ethyl acetate and ethylacetate:methanol solvent systems which resulted in three compounds,**EG-1**, **EG-2**, and **EG-3**. The UV-Vis and IR spectral data of the isolated compounds were generated in addition to their melting points. The structures of the isolated compounds were partially illustrated because of the absence of NMR data. The experimental UV-Vis and IR spectroscopic data were interpreted and compared with literature in order to propose the structure and the identity of the compounds. Hence the isolated compounds **EG-1**, **EG-2** and **EG-3** were proposed to be chrysin (5,7-dihydroxyflavone), purpurin (1,2,4-trihydroxy 9-10 anthraquinone) and 1,2-Dimethoxy-6-methyl-9,10-anthraquinone ,respectively.

Key: Compound isolation, *Eucalyptus globules;* stem bark, *phytochemicals*, secondary metabolites,

1. INTRODUCTION

In this section, general information on the background of the study, statement of the problem, the objectives of the study and significance of the study are presented.

1.1 Background of the study

Medicinal plants have played a role in treating and preventing ailments and diseases, bringing wellness to human beings [1]. The use of medicinal plants by human dates since antiquity and is still used to treat both human and animal ailments in various parts around the globe [2]. In Ethiopia, the use of traditional medicinal plants as alternative curative remedies has a long history, particularly related to those living in the countryside with incomplete coverage by modern medical systems, shortage of pharmaceuticals and unaffordable prices of modern drugs [3–5]. Various medicinal plant species are employed for the prophylaxis, treatment and management of various ailment conditions. One of the most widely used plants in traditional remedies is plants of the *Eucalyptus* genus. *Eucalyptus* is a flowering plant and belongs to the Myrtaceae family with three genera such as *Eucalyptus, Corymbia and Angophora*. There are about 900 species, subspecies and hybrids of *Eucalyptus* globally [6] and become the most planted genus of tree species in the world [7]. Currently, more than hundred *Eucalyptus* species are grown in Africa, seventy of them found in Kenya [8] and about fifty-five cultivated in Ethiopia [9], of which the most widespread species include *Eucalyptus camaldulensis*, *Eucalyptus citriodora*, *Eucalyptus globules*, *Eucalyptus regnans*, *Eucalyptus saligna* and *Eucalyptus tereticornis*. However, *Eucalyptus globules* and *Eucalyptus camaldulensis* are the two dominantly spreading species among nearly fifty-five different species of the genus introduced to Ethiopia [10].

Several species of *Eucalyptus* are used in traditional medicine as antiseptics, and against upper respiratory tract infections, such as common cold, influenza and sinus congestion [11–14]. Particularly, the essential oil obtained from these plants has a therapeutic application in treatment of pulmonary infections by inhalation [15, 16]; for the treatment of gastrointestinal disorders and wound healing, as an herbicide, and against some pests, acaricide, nematicide [17, 18], in perfumery, soap making, as an antioxidant, against some fungi and bacteria that can be pathogenic [19].

Generally, *Eucalyptus* essential oils (EO) are mainly used for its antimicrobial [20, 21], antifungal [22], antiseptic, astringent [23], anti-inflammatory [13], wound healing, and disinfectant [24], and cosmetic and food industries [25].

Eucalyptus globules, also known as Blue Gum, is the main source of eucalyptus oil used globally [26, 27] and the leaves, stems, fruits and flowers are the parts of *Eucalyptus globules* that have been mostly considered as source of essential oils [13, 28, 29]. These essential oils that are extracted from *Eucalyptus globules* leaves are known to be a rich source of traditional medicines with a variety of biological activities. The phytochemical components of the leaves, fruits, flowers, and stems of *Eucalyptus globules* have been mostly studied, but to the best of my knowledge, no report is available on the phytochemical analysis of the *Eucalyptus globules* stem-bark grown in Kebado town, Dara wereda, south Sidama, Sidama region. Therefore, it is worthwhile to carry out phytochemical analysis on the stem-bark of *Eucalyptus globules* cultivated in at that place.

1.2. Statement of the problem

Eucalyptus globules have been utilized for medicinal purposes, for the treatment of various ailments with its leaves, barks, and fruits [30]. The *Eucalyptus* essential oils extracted from leaves, fruits, buds and bark of *Eucalyptus globules* has been found promising as antibacterial, antiseptic, antioxidant, anti-inflammatory, and anticancer agent [31]. Moreover, *Eucalyptus globules* is a rich source of phytochemical compounds as flavonoids, alkaloids, tannins and propanoids, extracted in the leaf, stem and root of the plant [32, 33]. However, phytochemicals contained in the same plant species may vary according to the geographical origin, genetic differences, part of the plant used, method of extraction, age/stage of maturity, and season of harvest [34–41]. Therefore, in this study the investigation of the phytochemical constituents of the stem-bark extract of *Eucalyptus globules* cultivated in Kebado, Dara Wereda, Sidama Region, were done.

1.3. Objectives of the study

1.3.1. General objective

The overall objective of this study is to investigate the chemical constituents of the stem-bark extract of *Eucalyptus globules* Labill.

1.3.2. Specific objectives

- To extract the stem-bark of *Eucalyptus globules* using n-hexane, $CHCl_3$:MeOH (v/v, 1:1) and methanol solvent system
- To do phytochemical screening test of the crude plant extracts in order to identify the classes of secondary metabolites present in the plant stem-bark
- To isolate compounds through column chromatography
- To characterize and elucidate the structures of the compounds isolated from the extracts

1.4. Significance of the study

The study was focused on the identification of secondary metabolites of the stem-bark of the plant that could be responsible for it's widely uses in traditional medicine and give information about type of secondary metabolites in the stem bark of *Eucalyptus globules*. Moreover, the finding of this study would help to serve as baseline for further studies on the isolation of a lead compound for pharmaceutical development.

2. LITERATURE REVIEW

The genus, ethno botany, biological activity and phytochemistry of *Eucalyptus* plant species is reviewed and presented below.

2.1. The genus *Eucalyptus*

The *Eucalyptus* genus is represented by 900 species and subspecies identified throughout the globe [6]. It corresponds to one of the principal genus of the Myrtaceae family with three genera such as *Eucalyptus, Corymbian and Angophora*, native from Australia and cultivated in several countries worldwide [42–47].

Eucalyptus are woody perennial, shrubs to tall trees, with a rapid growth to attain a gigantic size and mostly evergreen [48]. It is now widely and successfully planted in India, South Africa, Zimbabwe, Kenya, Uganda, and Tanzania [49]. According to Liu and Li, [50], the major *Eucalyptus* growing countries currently are China, India, and Brazil, respectively. Currently, more than 100 *Eucalyptus* species are grown in Africa, 70 of them found in Kenya [8] and about 55 cultivated in Ethiopia [9], of which the most widespread species include *Eucalyptus camaldulensis*, *Eucalyptus citriodora*, *Eucalyptus globules*, *Eucalyptus regnans*, *Eucalyptus saligna* and *Eucalyptus tereticornis*. However, *Eucalyptus globules* and *Eucalyptus camaldulensis* are the two dominantly spreading species among nearly 55 different species of the genus introduced to Ethiopia [51].

2.2. Ethnobotany of Eucalyptus

Eucalyptus species have been shown to possess several medicinal properties, such as antiseptic, antioxidant, antimicrobial, acaricidal, insecticidal, and herbicidal activities, and are also used in the production of paper, timber, honey, and essential oil [42]. Particularly, the species *Eucalyptus cinerea*, *Eucalyptus camaldulensis*, and *Eucalyptus globules* are the ones which have received more attention in terms of their essential oil components. Other therapeutic activities, such as anti-inflammatory, astringent and healing properties, have also been reported for *Eucalyptus* species [52–55].

Eucalyptus cinerea is known to possess the highest content of 1,8-cineole (1,8-epoxy-pmenthane) better known as eucalyptol [56–60]. This compound is being used in medicinal, perfumery and flavour preparations [61]. In ancient times, aboriginal people used *Eucalyptus* plant for several purposes, mostly as medicine and as food [62, 63].

Eucalyptus cinerea essential oil has also been found as an effective insecticide againstthe house fly, *Musca domestica* Linnaeus, offering a natural pesticide towards houseflies.

Eucalyptus camaldulensis is widely used in traditional medicine to treat a variety of disease conditions including colds, asthma, coughs, diarrhea and dysentery, hemorrhage, laryngalgia, laryngitis, sore throat, spasm, trachagia and vermifuge [64]. A decoction of the plant is used to treat enteric infections including diarrhea and dysentery, constipations and other stomach problems, asthma, oral thrush, boils, sores, skin and wound infections, asthma, bronchitis, eczema and athletes foot [65, 66]. *Eucalyptus camaldulensis* also used to treat gastrointestinal disorders [12, 67]. In addition, a decoction of *Eucalyptus camaldulensis* leaves is reported to be a remedy for sore throat and other bacterial respiratory and urinary tracts infections. The poultice of the leaves is applied over wounds and ulcers. The leaves essential oils have been used in the treatment of lung diseases and were stated to have antitubercular effect [67, 68].

In Ethiopia, fresh leaves of *Eucalyptus camaldulensis* and *Eucalyptus globules* are used to treat stomach ache, evil spirit/swells and wounds [69, 70]. In Nigeria the resinous exudates from *Eucalyptus camaldulensis* trunk, commonly called "zaity", is taken orally to cure bladder infections [71]. The sticks of this species have been used as a tooth cleaner to prevent tooth decay and periodontitis [72]. *Eucalyptus camaldulensis* gum also used to treat sore throat and diarrhea, while the smoke of burnt leaves was inhaled to treat respiratory problems in Sudan; decoctions from leaves were prepared with sugar for stomach ache in Senegal. Similarly, fresh leaves of *Eucalyptus globulus* is used to treat rabies and common cold or cough [69,70,73], foot smell, skin diseases [74], fever, colds, antitussive, cough, grippe, respiratory ailments, hair care, migraine, thorax ailments [75].

2.3 Biological Activities of some selected species of the genus *Eucalyptus*

Biological activities *Eucalyptus cinerea, Eucalyptus camaldulensis, and Eucalyptus globules* are reviewed under this subtopic.

2.3.1. Biological activities of *Eucalyptus cinerea*

Eucalyptus cinerea essential oil has antibacterial activity against gram-positive and negative pathogenic bacteria [25, 34, 76, 77], and has anti-inflammatory activities [78–81]. *Eucalyptus cinerea* essential oil has also nematicidal [82], and antioxidant [83] activities that make it a benign agricultural agent.

2.3.2. Biological activities of *Eucalyptus camaldulensis*

Several studies have reported that essential oils from the leaves of *Eucalyptus camaldulensis* showed antimicrobial [53, 84–86], anti-oxidant [87–92], antifungal [93, 94, 95], analgesic and anti-inflammatory effects [13], antioxidative or antiradical [96] activities.

2.3.3. Biological activities of *Eucalyptus globules*

The essential oil extracted from *Eucalyptus globules* leaves is known to be a rich source of traditional medicines with a variety of biological activities. It is widely used to treat common cold, pulmonary tuberculosis, diabetes, nasal congestion, bronchial disease, asthma and is also used as disinfectant, antioxidant, and antiseptic agent, especially in the treatment of upper respiratory tract infections [97]. The oil is applied externally to relief rheumatism as a counterirritant and for the certain skin diseases [27, 97]. The essential oil of *Eucalyptus globules* leaves has several leading benefits that play a role in the medical world as an antibacterial [85, 98, 99], anti-fungal [100], anti-oxidant, [101, 102], anti-inflammatory [13, 80] and a cytotoxic antitumor effect [103–105].

2.4. Phytochemistry of some selected species of the genus *Eucalyptus*

The review of the phytochemistry of *Eucalyptus cinerea, Eucalyptus camaldulensis, and Eucalyptus globules* are presented under this subtopic.

2.4.1. Phytochemistry of *Eucalyptus cinerea*

The essential oils extracted from the leaves of *Eucalyptus cinerea* contained a complex mixture of essential oils consisting mainly of oxygenated monoterpenes such as 1,8-cineole, *trans*-pinocarveol, *α*-terpineol, 4-terpineol, *cis*-jasmone, pinocarvone, fenchol, camphor, cumin aldehyde, *α*-terpinyl acetate and mono terpene hydrocarbons such as camphene , *α*-phellandrene, *α*-pinene, *p*-cymene, limonene followed by oxygenated sesquiterpenes borneol, spathulenol, viridiflorol, globulol, epiglobulol, *β*-eudesmol and sesquiterpene hydrocarbons such as germacrene *β*- aromadendrene,viridiflorene, bicyclogermacrene, *β*-caryophyllene [34, 100, 106]. In general, the chemical composition of the essential oils extracted from the leaves of *Eucalyptus cinerea* is characterized by large amount of1,8-cineole **(1)** better known as eucalyptol and camphene **(2)** [34,107-112] and other constituents including *α*-pinene **(3)**, *p*-cymene **(4)**, *α*-terpineol **(5)**, t*rans*-pinaocarveol **(6)**, *α*-phellandrene **(7)**, cuminal **(8)**, globulol **(9)**, limonene **(10)**, aromadendrene **(11)**, spathulenol **(12)**, and terpinene-4-ol **(13)** (Figure 1**)** [107].

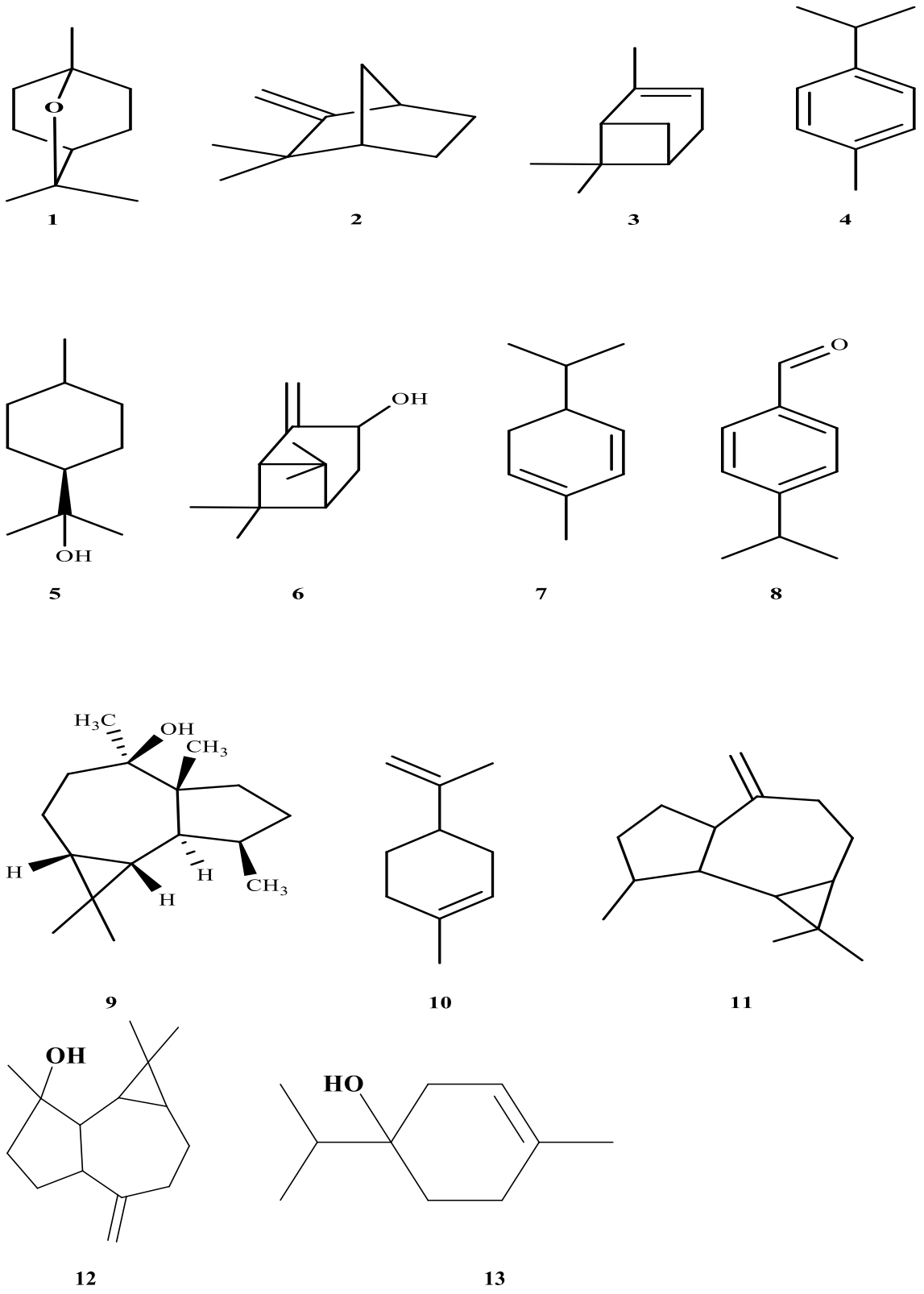


Figure 1. The Chemical structures ofcompounds identified from the essential oil of *Eucalyptus cinerea* leaf

As per existing literature, the essential oil from *Eucalyptus cinerea* has been only focused on its leaves. There are no studies regarding the chemical constituents of essential oils extracted from the stem, seeds, and roots of *Eucalyptus cinerea*. However, the chemical composition of the essential oils of aerial parts (leaves, flowers and fruits) of *Eucalyptus cinerea* contained *α*-pinene, limonene, 1,8-cineole, *α*-terpineol, and *α*-terpinyl acetate [25,111,113] as the main components.

Eucalyptus cinerea is rich in bioactive compounds and the hydroethanolic extract (EtOH-H_2O) of *Eucalyptus cinerea* leaves and its fractions (ethyl acetate fraction) contained polyphenols, flavonoids, quinones, terpenoids, alkaloids, and tannins [107]. In a study conducted by Soliman, F.M. *et al.* [114], two acyl phloroglucinol compounds namely; sideroxylonal B **(14)** and macrocarpal A (**15)** were isolated from the Sideroxylonal-Rich Extract (SRE), dried chloroform: methanol (80:20) extract of the air-dried powdered leaves, of the juvenile leaves of *Eucalyptus cinerea* cultivated in Egypt. In a similar study, compound ent-catechin **(16)** (Figure 2) was isolated from the semi purified extract (SE) of *Eucalyptus cinerea* leaves [115].

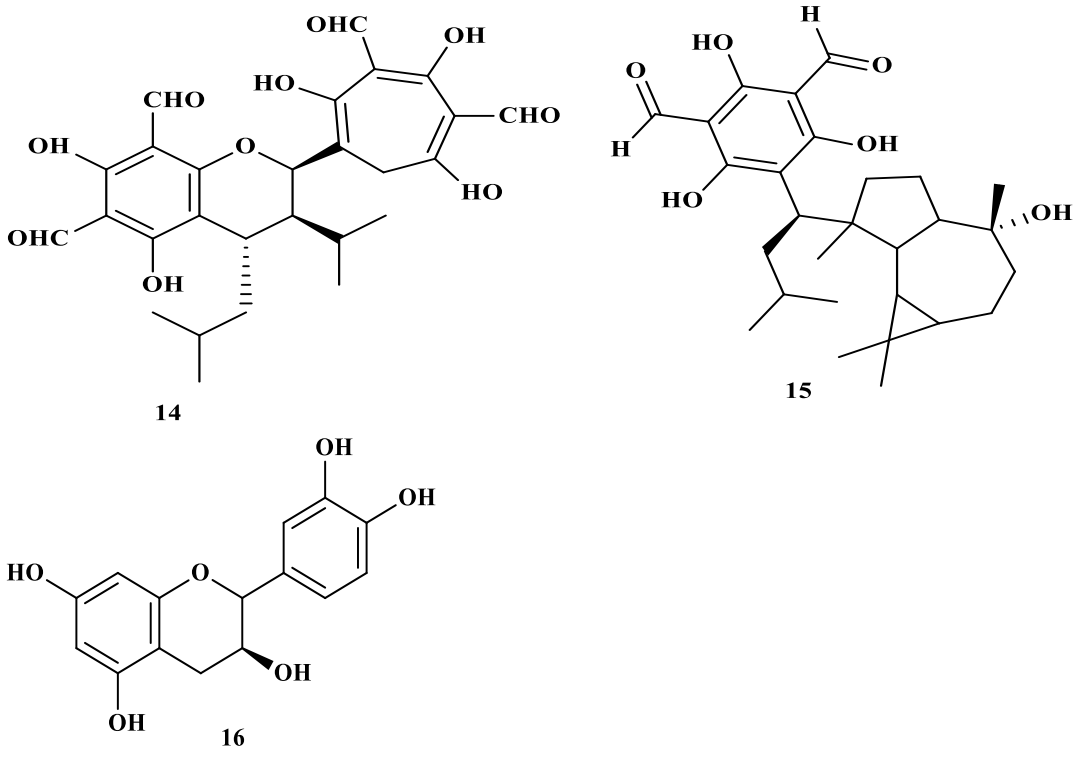


Figure 2: Chemical structures of isolated compounds fromthe leaves of *Eucalyptus cinerea*

2.4.2. Phytochemistry of *Eucalyptus camaldulensis*

In general, studies reported that the main components of the leaf essential oil of most *Eucalyptus camaldulensis* were 1, 8-cineole **(1)**, *α*-pinene **(3)**, *β*-pinene **(17)**, *α*-terpineol **(5)**, globulol **(9)**, borneol **(18)**, aromadendrene **(11)**, eudesmol **(19)** and terpinen-4-ol **(13)** (Figure 3**)** [118-120]. Other studies reported that 1,8-cineole is the major constituent [45,121-125]; spathulenol and *p*-cymene as the major components [126]; while *α*-pinene, *p*-cymene and *α*-phellandrene were considered the principal constituents [127]. In another studies, 1,8-cineole and *α*-terpineol are the main compounds [87]; 1,8-cineole, *α*-pinene and limonene were main compounds [128]; *p*-cymene, 1,8 cineole, limonene and *α*-pinene were main compound [129]; 1,8 cineole, *β*-pinene, *α*-terpinene **(20)** and *p*-cymene were main compounds [130]; ethanone, eucalyptol, *β*-caryophyllene **(21)** and carvacrol **(22)** as main components, while pulegone **(23)**, thujone **(24)**, *γ*-terpinene **(25)**, nerolidol **(26)** as the minor components [84], *α*-pinene, *p*-cymene, *α*-phellandrene, 1,8-cineole, *α*-terpinene, and limonene as main components [131], and *p*-cymene, *γ*-terpinene, *α*-pinene, 1,8-cineole, terpinen-4-ol, *α*-terpineol, carvacrol, and thymol **(27)** as the major components [132]. This variation in the chemical composition of the leaf essential oil of *Eucalyptus camaldulensis* samples is due to edaphic and climatic factors, seasonal and diurnal emission activity cycles [129, 133–135]. However, the chemical composition of the essential oil extracted from the leaves of *Eucalyptus camaldulensis* depends on the plant origin as well as seasonal variation throughout the plant's vegetative cycle [117].

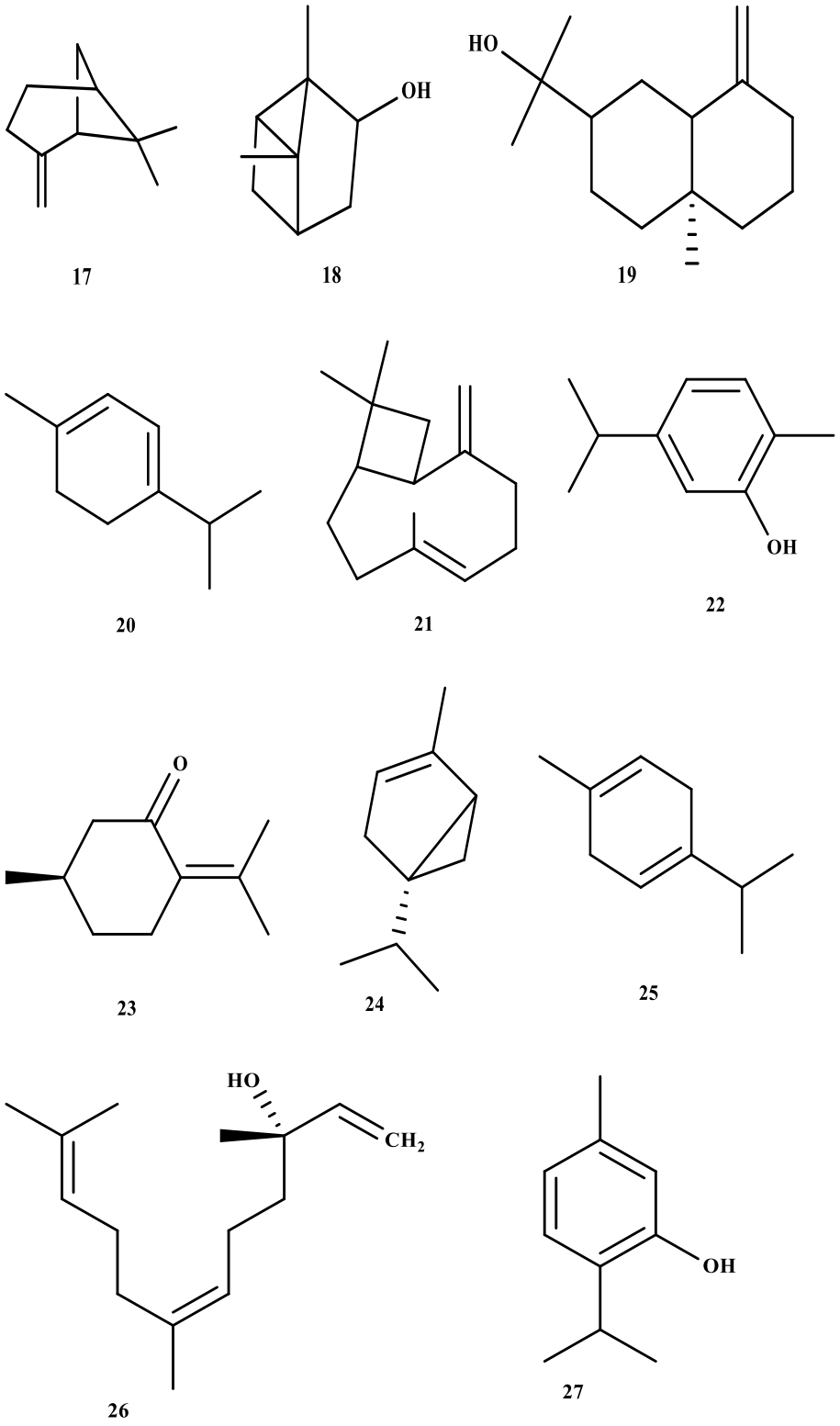


Figure 3. Chemical structures of some of the compounds identified from the essential oil of the leaf of *Eucalyptus camaldulensis*

Previous studies on the essential oil of *Eucalyptus camaldulensis* flowers revealed the presence of 1, 8-cineole **(1)**, *β*-pinene **(3)**, and spathulenol as the most abundant constituents [136]. The major components of the fruits essential oil were aromadendrene, *α*-pinene, drimenol **(28)**, and cubenol **(29)** [137].

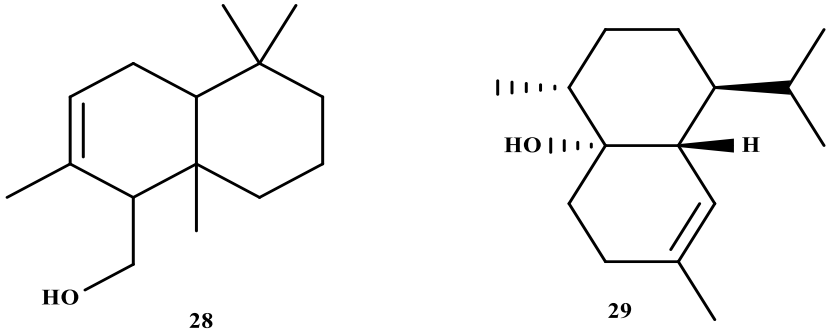


Figure 4. Chemical structures of some of the compounds isolated from the essential oil of the fruit of *Eucalyptus camandulensis*

The phytochemical screening of ethanol, methanol, and petroleum ether leaf extracts from Nigeria contained in moderate to high amount secondary metabolites: alkaloids, saponins, tannins, flavonoids, steroid, carbohydrates, and cardiac glycosides, and not anthraquinones [138].

Similarly, the secondary metabolites screening of *Eucalyptus camaldulensis* leaf extracts confirmed presence of tannin, saponins, and cardiac glycosides [139]; the chloroform and methanol extracts of leaf contained alkaloids, cardiac glycosides, steroids, tannins, flavonoids, saponins and terpenoids [86]; n-hexane, chloroform, and methanol extracts of leaf also showed the presence of tannins and saponins [68]. Crude methanolic leaves extract of *Eucalyptus camaldulensis* contained anthraquinones, flavonoids, saponins, and terpenoids [140]. Similarly, the aqueous, hexane, ethyl acetate and chloroform leave extracts showed the presence of anthraquinones [141].

Phytochemical screening of the crude stem barks methanol extract of *Eucalyptus camaldulensis* indicated presence of saponins, flavonoids, tannins, and also volatile oils [142]. Similarly, the phytochemical composition of the crude water extracts of the stem-bark contained tannins, saponins, phenols, and glycosides; n-hexane, chloroform, and methanol extracts of stem bark showed the presence of tannins and saponins [68]; chloroform and methanol extracts from stem bark contained secondary metabolites such as alkaloids, cardiac glycosides, steroids, tannins, flavonoids [86].

Phytochemical screening of the crude ethanol extracts of the roots of *Eucalyptus camaldulensis* contained tannins and glycosides, while its water extracts of the roots contained tannins, saponins, phenols, and glycosides [143]. A study revealed the isolation, identification, and confirmation of compounds: gallic acid **(30)**, taxifolin **(31)**, methyl gallate **(32)**, quercetin **(33)**, luteolin **(34)**, and hesperidin **(35)** from ethyl acetate extract of *Eucalyptus camaldulensis* leaves [144]. Similarly, scopoletin **(36)** and chlorogenic acid **(37)** were the isolated from the ethyl acetate fraction of the ethanol extract of *Eucalyptus camaldulensis* leaves [145], stigmasterol **(38)** was isolated from the hexane fraction of fruits of *Eucalyptus camaldulensis* [146], while ellagic acid, protocatechuic acid, flavonol, ellagitannin, quercetin, and gallic acid were identified in the stem bark ether extracts of *Eucalyptus camaldulensis* [147] (Figure 5).

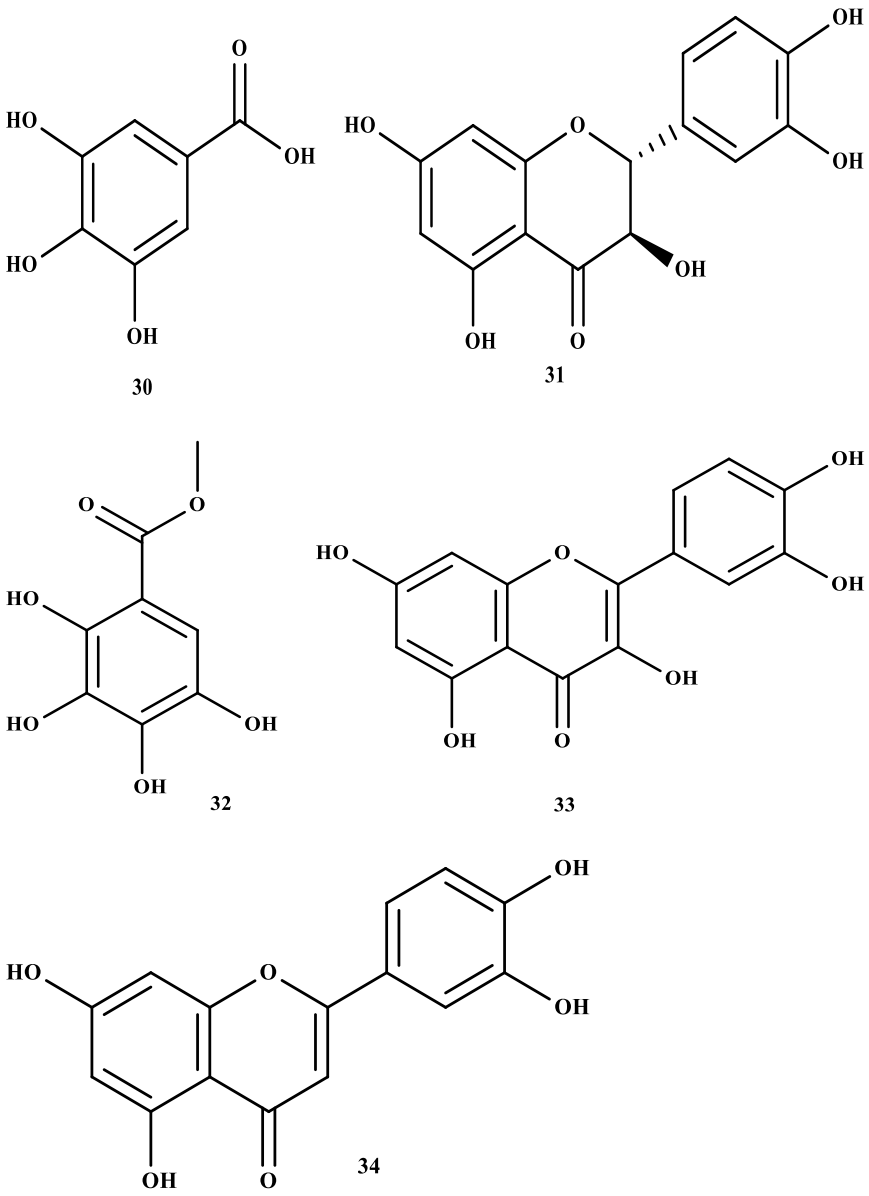


Figure 5. Chemical structures of compounds isolated from the leaf of *Eucalyptus camandulensis*

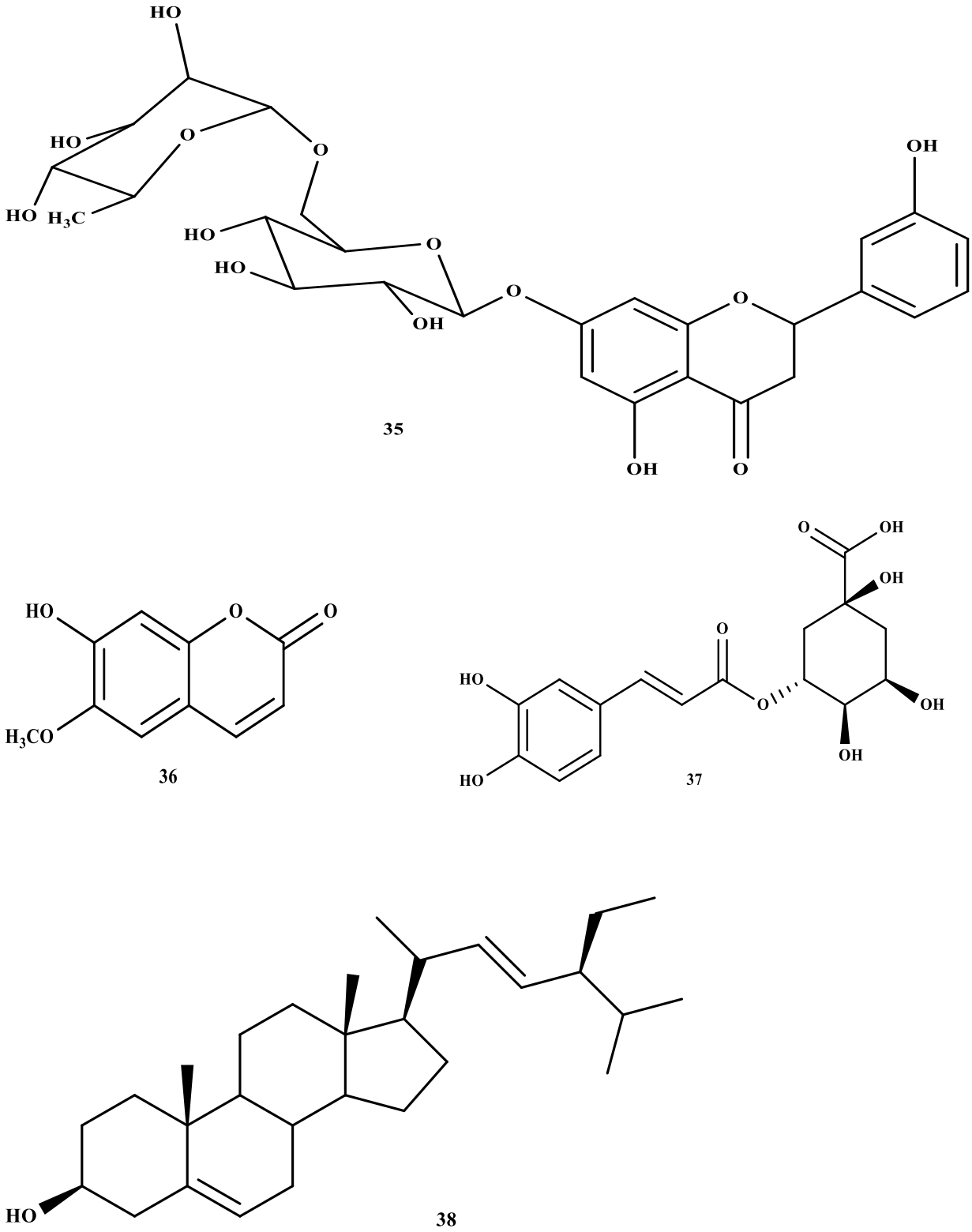


Figure 6. Chemical structures of compounds isolated from the leaf of *Eucalyptus camandulensis*

2.4.3. Phytochemistry of *Eucalyptus globules*

The chemical composition of the *Eucalyptus globules* essential oil is a complex mixture of substances, commonly contains camphor, borneol, 1,8-cineole, spathulenol, *α*-terpineol, *α*-pinene, limonene, tricyclene, camphene, globulol, *o*-cimene, *cis*-*o*-cymene, *α*-terpinyl acetate, *p*-cymene, *β*-myrcene, solanone, *β*-pinen,*α*-pinene, *o*-cymene, limonene, 1,8-cineole with varying concentrations [91, 93, 148-155].

Similar investigation of essential oil composition of *Eucalyptus globules* leaf identified major chemical components such as *α*-pinene, *β*-pinene, *β*-myrcene, *α*-phellandrene, *p*-cymene, *D*-limonene, eucalyptol, *γ*-terpinene, isopinocarveol, pinocarvone, terpinene-4-ol, *trans*-*p*-Mentha-1(7), 8-dien-2-ol **(39)**, *α*-terpineol, aromandendrene, alloaromadendrene, viridiflorene, and epiglobulol **(40)** with different percentages [156]. Furthermore, the essential oil from the leaves of *Eucalyptus globules* reported to contain constituents such as *γ*-cadienene **(41)**, *α*-gurjuene **(42)**, linalool oxide **(43)**, *α*-pinene, *β*-pinene, aromadendrene, *γ*-elemene **(44)**, 1,8-cineole, globulol,pipertone **(45)**, *α*-,*β*- and *γ*-terpinen 4-ol, fenchol, linalool **(46)**, boreneol, caryophyllene **(47)**, citronellal **(48)**, cuminaldhyde **(49)**, citral **(50)**, geranyle acetate **(51)**, cinnamic acid **(52)**, eudesmyl acetate **(53)**, epi-globulol, ledol **(54)**, *S*-guaiazulene **(55)**, quercetin, rutin, caffeic **(56)**, *α*-pinocarvone, virdifloral **(57)**, homoserin, cyanin, chrysanthemin, malvidin, peonin, *β*-selinene **(58)**, delphindin **(59)**, *cis*-pinocarveol, cynaidin, 8-demethylsideroxylin, quercetol, alloaromadendrene, ferulic **(60)**, gallic, keracyanin **(61)** (Figure 7), gentisic and protocateuchic acids [157–159]**.**

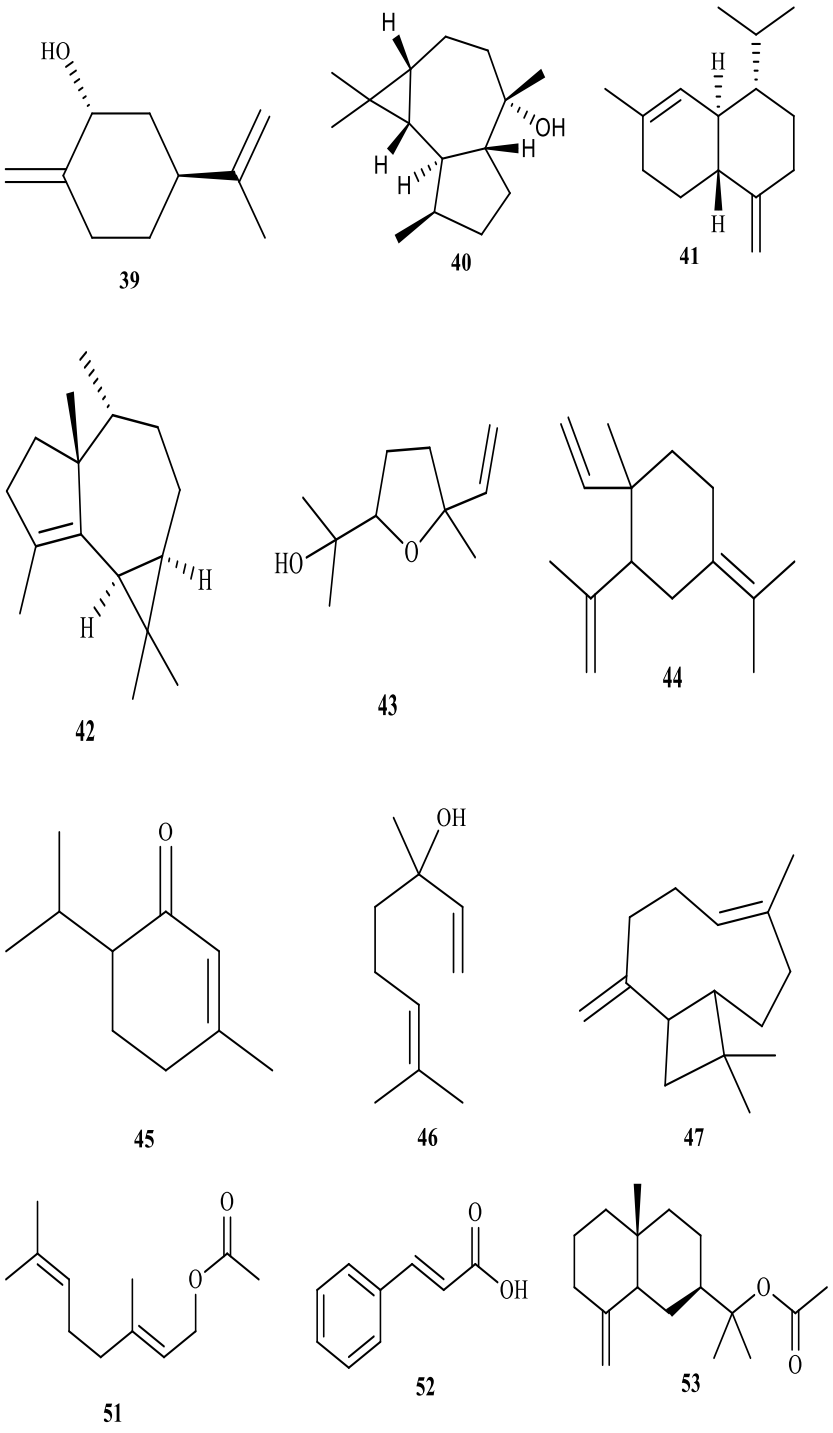


Figure 7. Chemical structures of some of the compounds identified in the essential oil of the leaf of *Eucalyptus globules*

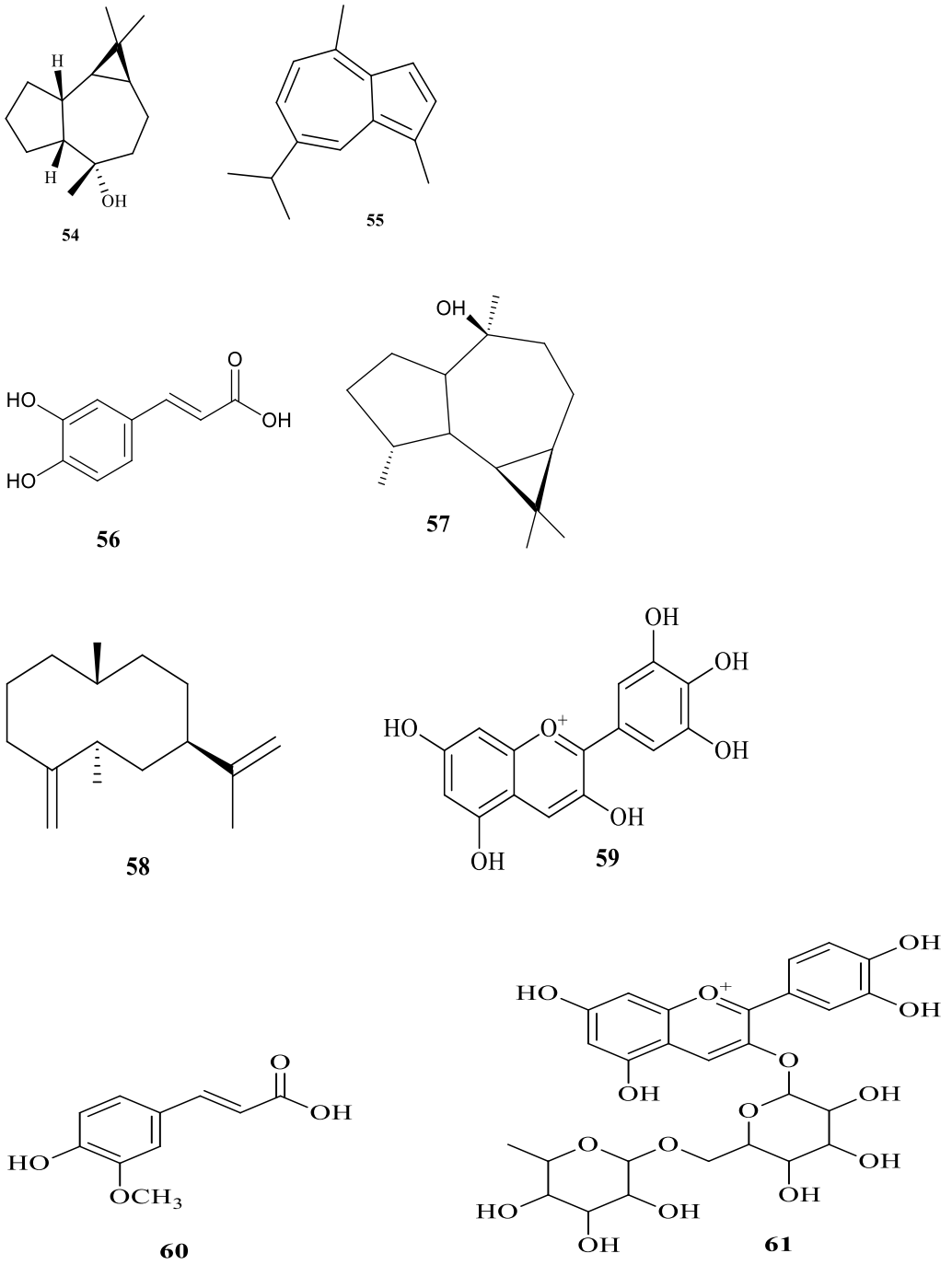


Figure 8. Chemical structures of some of the compounds identified in the essential oil of the leaf of *Eucalyptus globules*

The essential oil of fruit, bud and branch oils is known to contain α-thujene, 1, 8-cineole (**1)** and aromadendrene **(11)** with varying concentrations [144]. Phytochemicals such as cardio glycosides, saponins, alkaloids, terpenoids, tannins and phenolic compounds were observed in methanolic extract of *Eucalyptus globules* leaf [160], while the acetone extract of *Eucalyptus*

globules leaves showed the presence of phyto constituents such as alkaloids, flavonoids, phenols, tannins, quinones, glycosides, steroids, terpenoids, volatile acids and leucoanthocyanides [161]. In a similar study, the major phytochemicals found in methanol and acetone extract of *Eucalyptus globules* leaves were found to be flavonoids, glycosides, proteins and amino acids, saponins, steroids, phenolic compounds/tannins, and terpenoids [162].

Phytochemical analysis of an ethanolic extract of *Eucalyptus globules* leaves by HPLC (High performance liquid chromatography) contained mainly kaempferol, quercetin, and myrecetin, while the chemical constituent of ethanolic extract of *Eucalyptus globules* leaves obtained through GC-MS (Gas chromatography/mass spectrometry) showed the presence of *α*-pinene, piperitone, 1,8-cineol, limonene, *p*-cymene, *α*-terpineol, globulol, spathulenol, methane 1,2,3 triol, *γ*-terpenene, and sabinol [29]. Similarly, the same instrumental analysis of the methanol, chloroform, and hexane extracts of *Eucalyptus globules* leaves led to the identification of 1,8-cineol as major constituents in all the three extracts, while *α*-pinene, *cis*-verbenol, *α*-guaiene, *α*-terpineol acetate, and spathulenol were more common in methanol and chloroform extracts, while aromadendren, epiglobulol and chrysanthenone are more common in methanol and hexane extracts and *o*-ocimene was only found in chloroform and hexane extracts [163]. The above instrumental analysis of a dichloromethane extract of *Eucalyptus globules* leaf revealed the presence of major compounds such as *α*-deudesmol**,** *α*-phellandrene and *β*-pinene, while tridecane and 4-terpineol were found in trace amounts. The other compounds identified were: *α*-pinene, camphene, myrcene, *p*-cymene, limonene, *γ*-terpinene, terpinolene, undecane, endofenchol, camphor, borneol, *α*-terpineol, *α*-copaene, *α*-gurjunene, (*E*) caryophyllene, aromadendrene, *α*-selinene, *δ*-armophene, *α*-eudesmol, and globulol [164].

Compound such asstigmasterol, β-D-glucopyranoside **(62)**, ursolic acid **(63)**,*α*-amyrin, *α*-amyrin acetate, 4', 5, 7-trimethoxykaempferol **(64)**, genistein **(65)**, epicatechin **(66)**, naringenin **(67)**, catechin **(68)**, octyl-*β*-D-glucopyranoside,7, 8-dihydroxycoumarin **(69)** (Figure 9) were isolated from the ethyl acetate fraction of the methanolic extract of the whole plant of *Eucalyptus globules* [165].

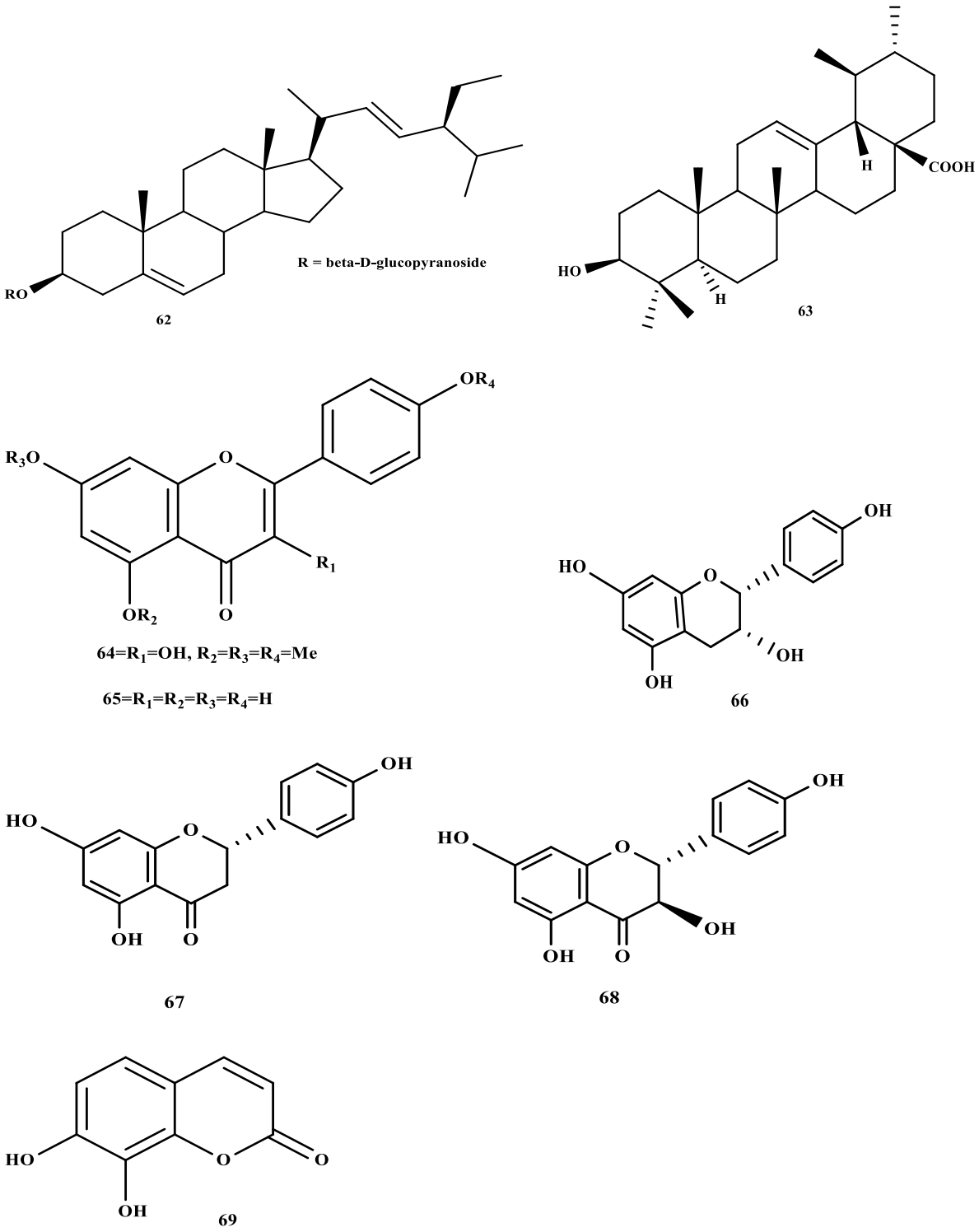


Figure 9. The chemical structures of the compounds isolated from the whole part of *Eucalyptus globulus*

3. MATERIALS AND METHODS

All the materials and methods used in this research are presented below.

3.1. Materials

3.1.1. Chemicals

The chemicals that were used in this experiment were n-hexane, chloroform, and methanol for the extraction of the stem bark of *Eucalyptus globules*plant. Solvents such as n-hexane, ethyl acetate, and methanol were used as a solvent system in TLC and column chromatography. Pre-coated thin layer chromatography (TLC) on aluminum foil was used to monitor the fraction similarities which might result in fraction combinations. Silica gel 120 mesh size (250 µm) was used for column chromatography.

Reagents such as acetic anhydride, sulfuric acid, glacial acetic acid, dilute sodium hydro oxide, dilute hydrochloric acid, solution of potassium bismuth iodide (or Dragendroff's reagent), ferric chloride, nitric acid, ammonium hydroxide, ammonia, benzene, and Molish's reagent were used for the detection of phytochemicals in the crude extracts. All the solvents and chemicals used in this research were analytical grade and were obtained from Ran-chem. CO. Ltd. Company, Addis Ababa, Ethiopia.

3.1.2. Instruments

Mortar and pestle were used to crush the sample; Orbital Shaker was used to continuously shake the sample with the extraction solvent; Rotary evaporator specification was used to concentrate the extract and recover the solvent. Analytical balance (ADAM AFR-110L) was used to measure the powder sample; Melting points were measured on METTLER TOLEDO Model MP30 melting point apparatus. UV chamber (uvltec) was used to view spots on the developed TLC plates; UV spectra of the samples (ethanol) were recorded on T80+ UV-VIS spectrophotometer; IR spectra of the samples (KBr pressing) were recorded on PerkinElmer, Frontier FTIR spectrophotometer.

3.1.3 Plant material collection and authentication

The stem bark of the plant was collected from its natural habitat in July 2024 from Kebado kebele, Dara district, Sidama region, Ethiopia. The taxonomic identification of the plant species was confirmed by botanist Retta Regassa, Hawassa College of Teacher Education.

3.2 Methods

3.2.1Plant material preparation

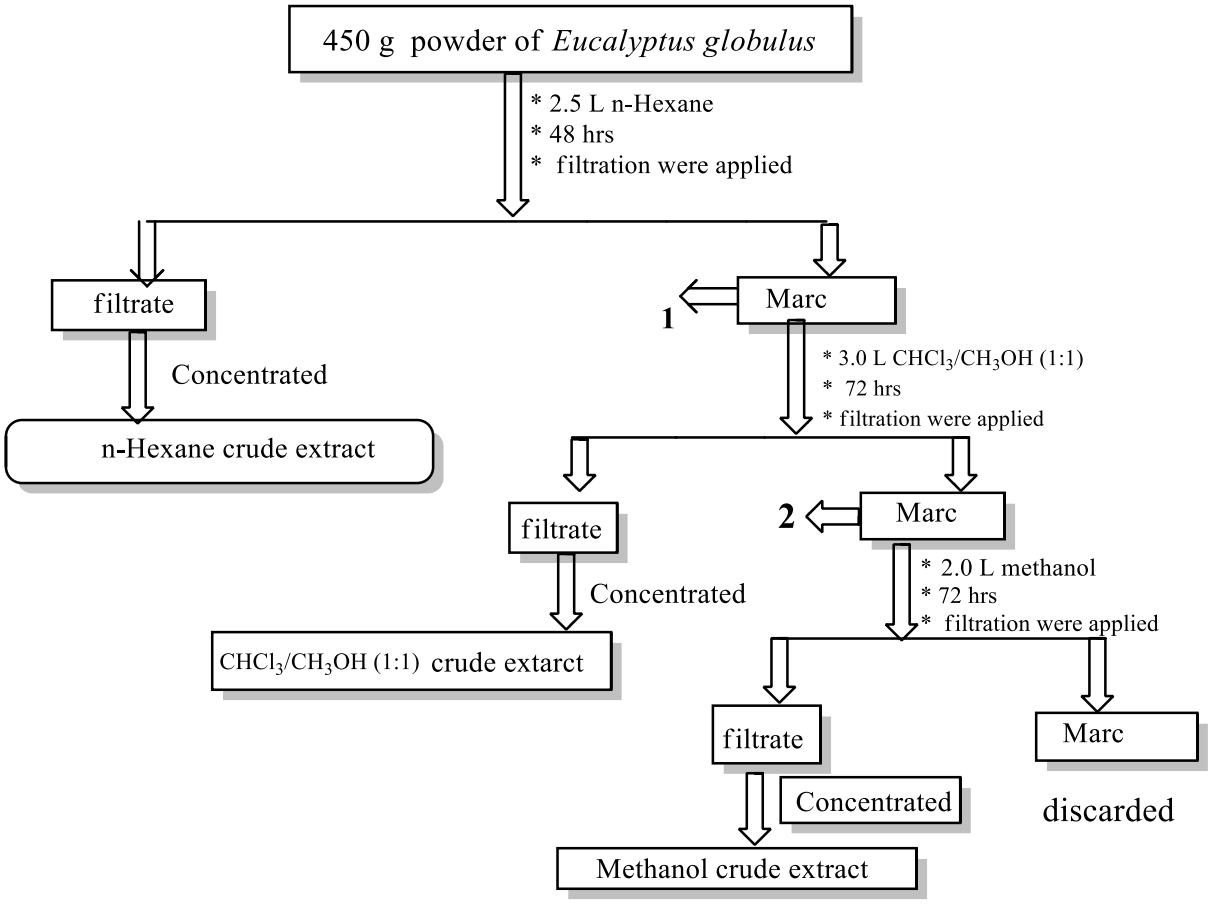
The collected stem bark of *Eucalyptus globules* was washed thoroughly with distilled water to remove dust particles, and placed in shaded area under aerated condition for two days at room temperature. The semi-dried stem bark was chopped into small pieces, and air dried under shade. The complete air-dried stem bark was pulverized into fine powder using a mortar and pestle and was kept in air-tight non-transparent plastic bottle until solvent extraction.

3.2.2 Extraction of crude material

Eucalyptus globule powder (450 g) was weighed and subsequently transferred into an Erlenmeyer flask covered with aluminum foil and was cold macerated in 2.5 liters of n-hexane in an orbital shaker for 48 hours. The mixture was filtered through Whatman No. 1 filter paper and the solvent was removed under reduced pressure using a rotary evaporator at 40 °C and 120 rpm. The concentrated extract was weighed and stored in the refrigerator until further analysis, while the marc was used for further extraction using $CHCl_3$:MeOH (v/v, 1:1). The marc was soaked in 3.0 liters of $CHCl_3$:MeOH (v/v, 1:1) and was put on an orbital shaker at room temperature for a period of 72 hours. The mixture was filtered through Whatman No. 1 filter paper and the solvent was evaporated under reduced pressure using a rotary evaporator. The concentrated extract was weighed and stored in the refrigerator until further analysis, while the marc was used for further extraction using methanol. The marc from the second extraction was soaked in 2.0 liters of methanol and was put on an orbital shaker at room temperature for a period of 72 hours. The mixture was filtered through Whatman No. 1 filter paper and the solvent was evaporated under reduced pressure using a rotary evaporator at 40 °C and 120 rpm. The concentrated extract was weighed and stored in the refrigerator until further analysis, while the marc was discarded. The percentages of extract yield for the three extracts were calculated using following formula:

$$\text{Extraction yield} = \frac{\text{weight of dry extract}}{\text{weight of dried plant sample}} \times 100$$

The steps applied in the extraction of the powder stem barks of *Eucalyptus globules* were summarized in Scheme 1.



Scheme 1. General scheme on the extraction of crude material from *Eucalyptus globulus* stem bark

3.2.3. Solvent system selection for chromatographic separation

The TLC profile of crude extract was checked using various proportions and mixture of n-hexane, chloroform, methanol, ethyl acetate solvent systems so as to obtain a good solvent system for the chromatographic separation.

3.2.4. Phytochemical screening

Investigation of the classes of secondary metabolites was carried out for all the extracts as per the standard methods reported in the literature [166–168].

Test for alkaloids

Extract (0.5 mg) was taken, and 3 mL of methanol was added to it. Then 300 µL of acetic acid was added to it and then a solution of ammonium hydroxide was added drop by drop. The appearance of precipitate indicates the presence of alkaloids.

Test for flavonoids

Extract (1.0 mg) was taken and placed into a test tube. Then few drops of sodium hydroxide solution were added and shaken. Emergence of intense yellow color that turns to colorless after adding dilute acid implies the existence of flavonoids.

Test for phenols

The crude extract of the plant material was treated with 3 to 4 drops of ferric chloride solution, or dissolving 5mg of dry extract in 0.5 mL of 1% ferric chloride solution. The formation of bluish-black color indicates the presence of phenolic compounds.

Test for glycosides

Extract (0.5 mg) was treated with 0.2 mL glacial acetic acid and then drop wisely 3.5% ferric chloride was added to the solution. This will be layered with 1.0 mL of conc. sulfuric acid. A reddish-brown ring that occurs at the interface indicates the presence of cardiac glycosides.

Test for terpenoids

Extract (5.0 mg) was mixed with chloroform (2.0 mL), and concentrated sulphuric acid (3.0 mL) was carefully added to form a layer. A reddish-brown coloration of the inter face shows a positive result for the presence of terpenoids.

Test for tannins

About 2.5 mg of each plant extract was boiled in 5mLof water in a test tube and then filtered through Whatman's no.1 filter paper. Two to three drops of 0.1% ferric chloride added and read for brownish green or a blue-black precipitate indicating a positive result.

Test for saponins

Extract (0.5 mg) was taken in a test tube and then 5.0 mL of distilled water was added to it. The solution was vigorously shaken and the formation of stable foam indicates the presence of saponins.

Test for steroids

The crude plant extracts (1.0 mg) was dissolved in 10.0 mL of chloroform and to it an equal volume of concentrated sulfuric acid was added from sides of the test tube. The upper layer turns into red and the sulfuric acid layer shows yellow with green fluorescence. This indicates the presence of steroids.

3.2.5. Compound isolation

Once the appropriate solvent system is identified, fractionation of crude extract was conducted. The column was packed with 60 g silica gel as the stationary phase which was wetted using n-hexane to achieve least polarity to the mobile phase during the beginning of elution. The mobile phase for elution was fixed based on the TLC results obtained in the course of solvent selection. Dried $CHCl_3$:MeOH (v/v, 1:1) crude extract (8.0 g) was adsorbed on small amount of silica gel. The adsorbed crude extract was added into the column that was already packed with silica gel and eluted with increasing gradient of ethyl acetate in n-hexane and ethyl acetate in methanol.The elution process was started by n-hexane, followed by hexane:ethyl acetate and ethyl acetate: methanol in various ratios. A number of fractions were collected for each type of solvent systems used for fractionation which led to a total of 76 fractions (Table 1).

Table 1. Solvent system used for fractionation

Solvent system	Proportions (v/v, mL)	Volume (mL)	# of fractions collected
n-hexane :ethyl acetate	300 : 0	300	1-6
n-hexane :ethyl acetate	285 : 15	300	7-12
n-hexane :ethyl acetate	270 : 30	300	13-18
n-hexane :ethyl acetate	255 : 45	300	19-24
n-hexane :ethyl acetate	240 : 60	300	25-30
n-hexane :ethyl acetate	225 : 75	300	31-36
n-hexane :ethyl acetate	210 : 90	300	37-42
n-hexane :ethyl acetate	195 : 105	300	43-48
n-hexane :ethyl acetate	80 : 120	200	49-52
n-hexane :ethyl acetate	60 : 140	200	53-56
n-hexane :ethyl acetate	50 : 150	200	57-60
n-hexane :ethyl acetate	40 : 160	200	61-64
n-hexane :ethyl acetate	0: 100	100	65-66
ethyl acetate:methanol	80:20	100	67-68
ethyl acetate:methanol	70:30	100	69-70
ethyl acetate:methanol	50:50	100	71-72
ethyl acetate:methanol	30:70	100	73-74
ethyl acetate:methanol	0:100	100	75-76

Fractions that showed single spot and have the same color and Rf, on the developed TLC plate were combined The fractions were concentrated to dryness under reduced pressure using a rotary evaporator at 40 °C and 120 rpm. The dried fractions were weighed to get the amount of the compound isolated which afterwards was kept in refrigerator until further analysis. Fractions that showed two or more than two spots on the developed TLC plate were left out as they required further column chromatography separation with various combinations of solvents. Because of limited time and finance further fractionation of fractions having more than one spot on the TLC were not performed.

3.2.6. Structure elucidation

UV-visible and IR spectroscopic data of all the isolated compounds were generated and their melting points were also determined. These data of the isolated compounds were used to suggest the structures and identity of the isolated compounds by comparing with literature data.

The UV-vis and IR spectroscopic data were also systematically interpreted in order to identify the functional groups of the isolated compounds.

4. RESULTS AND DISCUSSION

The results obtained from this study were presented and discussed as shown below.

4.1 Extraction yield

The amounts of the crude extracts for each solvent and their extraction yields (or percentage yields (%w/w)) are shown in Table 2.

Table 2. Extraction yields of the crude extract of *Eucalyptus globules*

Extract	Mass (g)	% Yield
Hexane	1.9	0.42
$CHCl_3$:MeOH (v/v, 1:1)	10.8	2.40
Methanol	14.5	3.22

The % yields of the extracts has shown increment in the order: methanol > $CHCl_3$:MeOH (v/v, 1:1) > n-hexane, as shown in Table 2. Thus, methanol was relatively efficient in extracting phytochemicals from the stem bark of *Eucalyptus* when compared to $CHCl_3$:MeOH (v/v, 1:1) and n-hexane solvent systems. This tell us that the stem bark of *Eucalyptus globules* is enriched with polar secondary metabolites which was further supported by previously reported results on the extraction of *Eucalyptus globules* leaves using methanol 9.54%, chloroform 7.64%, and ethyl acetate 7.1% [160]. In another study, acetone extraction of *Eucalyptus globules* leaves produced a yield of 28.1%, whereas ethanol extraction produced a yield of 17.5% [161].

4.2 Classes of secondary metabolites identified

All the crude extracts were tested for the presence of the classes of secondary metabolites, which were evidenced with remarkable color changes or precipitations. The results of the screening test were presented in Table 3.

Table 3. Phytochemical screening results of crude extracts

Secondary metabolites	n-hexane	$CHCl_3$:MeOH (v/v, 1:1)	Methanol
Alkaloids	–	+	+
Flavonoids	–	+	+
Phenols	+	+	+
Glycosides	+	+	+
Terpenoids	+	+	+
Saponins	+	+	+
Steroids	+	+	+
Tannins	–	+	+

Absent = (–), Present = (+)

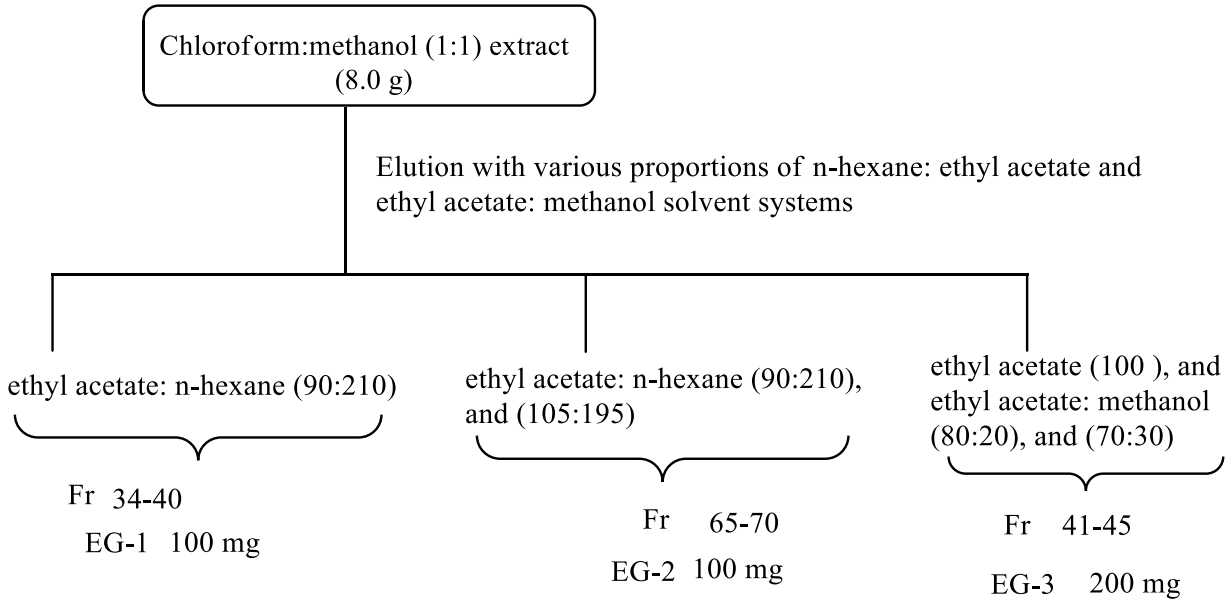
The phytochemical screening test confirmed the presence of the classes of secondary metabolites (Table 3). The phytochemicals present in the$CHCl_3$:MeOH (v/v, 1:1) and methanolic extracts were found to be the same (Table 3). Similar study showed that tannins, saponins, cardiac glycosides, phenolic compounds, and terpenoids were observed in methanolic extract of *Eucalyptus globules* leaves [160, 169]. Phytochemicals such as phenolic compounds, cardiac glycosides, terpenoids, and steroids were also observed in n-hexane extract of *Eucalyptus globules* leaves [169].

Most phytochemicals or secondary metabolites naturally occur in different parts of medicinal plants, such as leaves, fruits, roots, flowers, and seeds [170,171]. For instance, alkaloids have several beneficial properties such as anti-inflammatory, antimicrobial, anthelmintic, and antidiarrhoeal [172, 173]. Similarly, flavonoids showed anti-allergic, anti-cancer, antioxidant, anti-inflammatory, anti-viral antimicrobial, and antidiarrhoeal activities [173–175]. Therefore, alkaloids and flavonoids present in the *Eucalyptus globules* stem bark explain the traditional use of the plant for the treatment of microbial and fungal infections [20–22] and enteric infections such as diarrhea and dysentery. Phenolic compounds show antioxidant, anti-inflammatory, and anti-carcinogenic activities. Simple phenolics showed antimicrobial, anthelmintic, and antiseptic activities [173, 176], which is in agreement with the traditional use of the plant as an antiseptic, astringent [23], and disinfectant [24]. Terpenoids have antimicrobial and insecticidal properties and are used as pesticides and fungicides in agriculture and horticulture [173, 177–179]. Saponins also have antimicrobial, anti-viral, anti-inflammatory, and antioxidant activities, along with their use as a molluscicide, insecticide, and ichthyoid [180]. The presence of terpenoids and saponins in plants justifies their traditional use of the *Eucalyptus* as an herbicide, acaricide, and nematicide [17, 18]. Furthermore, the use of the plant as an astringent [23], anti-inflammation [13], wound healer, and disinfectant [24] is due to the presence of tannins. These phytochemicals are used as astringents against diarrhea [181], a diuretic against stomach and duodenal tumors [182], and anti-inflammatory [183].

4.3 Compounds isolated

Chromatographic separation of the $CHCl_3$:MeOH (v/v, 1:1) crude extract gave three compounds. Fractions 34-40 were combined as they gave a single spot and weighed to give 100 mg of white solid compound labeled as **EG-1**.Compound **EG-1**has shown an Rf value of 0.42 (n-hexane:ethyl acetate 4:1) and has a melting point of 284 °C. Fractions 65-70 were combined, concentrated and weighed to give 100 mg of white solid compound labeled as **EG-2**.

This compound has shown an Rf value of 0.41 (n-hexane:ethyl acetate 3:1) and has a melting point of 265 °C. The third compound labeled **EG-3** is a white solid that was found from fractions 41-45 (200 mg). This compound has shown an Rf value of 0.46 (n-hexane:ethyl acetate 4:1) and has a melting point of 192 °C. A summary of the procedures followed to isolate compounds **EG-1**, **EG-2**, and **EG-3** is shown in Scheme 2.



Scheme 2. Schematic diagram of the key steps on the isolation of compounds from $CHCl_3$:MeOH (v/v, 1:1) extract

4.4 Structure elucidation of the isolated compounds

UV-Vis and IR data were generated for all isolated compounds. The interpretation of the UV-Vis and IR spectral data of the compounds are discussed in the sub-sections below (Section 4.4.1 and 4.4.2).

4.4.1 UV-Vis spectra of the isolated compounds

The UV spectrum of compound **EG-1** (Appendix 1) showed absorbance of peaks λ_{max} at 240 nm and 283 nm indicating the presence of Π – Π* transition of conjugated dienes and n – Π* transition of C=O respectively.

The UV spectrum of compound **EG-2** (Appendix 2) showed absorbance of peaks λ_{max} at 238.7 nm and 287.8 nm indicating the presence of Π – Π* transition of conjugated dienes and n – Π* transition of C=O.

The UV spectrum of compound **EG-3** (Appendix 3) showed absorbance of peaks λ_{max} at 240 nm and 284 nm indicating the presence of Π – Π* transition of conjugated dienes and n – Π* transition of C=O bond in aromatic groups respectively.

4.4.2 IR spectra of the isolated compounds

Compound **EG-1**(Appendix 4) IR spectrum showed a broad band in the region of 3600-3200 cm^{-1} for the hydroxyl (OH) stretching, and 2942 cm^{-1} and 2847 cm^{-1} for aliphatic C-H stretching vibrations. Carbonyl (C=O) stretching vibrations was also seen at 1726 cm^{-1}, aromatic C-C stretch (in–ring) vibrations were also seen at 1458 cm^{-1}. Thus, compound **EG-1** has hydroxyl, carbonyl and aromatic functional groups.

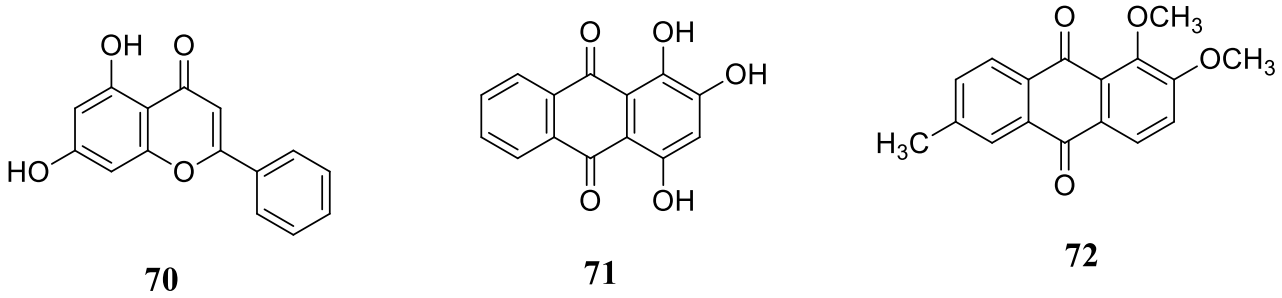
Compound **EG-2** (Appendix 5) IR spectrum showed a broad peak in the region of 3430 and 3294 cm^{-1} for the O-H stretches, and 2940 cm^{-1} and 2874 cm^{-1} for aliphatic C-H stretching vibrations. The absorptions around 1698 and 1644**c**m^{-1}corresponds to the C=O stretching connected to aromatic, and 1380 **c**m^{-1}foraromatic C=C stretching. Thus, compound **EG-2** has hydroxyl, carbonyl and aromatic functional groups.

Compound **EG-3** (Appendix 6) IR spectrum showed a medium broad band in the region of 3600-3200 cm^{-1}suggested an OH stretches and 2940 and 2888 cm^{-1} for aliphatic C-H stretching vibrations. The weak peak at1740, 1708, and 1644 cm^{-1} could be attributed to C=O stretching connected to aromatic ring. An aromatic C=C stretching vibration was also seen at 1458 cm^{-1}. Thus, compound **EG-3** has hydroxyl, carbonyl, and aromatic functional groups. In general, the IR and UV-Vis spectroscopic data of the isolated compounds contains information regarding the type of functional groups expected in the compounds. Therefore, we need other spectroscopic data such as H-NMR, C-NMR, DEPT and MS to fully elucidate the structures of the isolated compounds.

Table 4. Comparison of melting point, UV-Vis, and IR experimental data to literature

Cpd	Mp(°C) (exp.)	Mp (C°) (litr.) [184–186]	UV (nm) (exp)	UV (nm) (litr.) [184– 187]	IR (exp) cm^{-1}	IR (litr.)cm^{-1} [186–189]	Functional group.
EG-1	284	284-286	240 283	212, 268, and 313.5	3480 3392	3080; 3009	O–H
					2942 2847	2945	Aliphatic C-H
					1726	1655	C=O
					1630 1458	1610	aromatic C=C
EG-2	265	265-270	238.7 287.8	254 416	3430 3294	3377 3043	O–H
					2940 2874		Aliphatic C-H
					1698; 1644	1668; 1619	C=O
					1464 1380	1468; 1457 1383; 1331	aromatic C=C
EG-3	192	193-196	240 284	257, 341, 373	3458 3292	3081	O–H
					2954 2888	2945	Aliphatic C-H
					1740; 1708; 1644	1666 1601	C=O
					1460		Aromatic C=C

Based on Table 4, the chemical structure of the isolated compounds EG-1 chrysin (5,7-dihydroxyflavone) **(70)**, EG-2 purpurin (1,2,4-trihydroxy -9,10 anthraquinone) **(71)** and EG-3 1,2-Dimethoxy-6-methyl-9, 10-anthraquinone **(72)** were proposed and were shown in Figure 10.



70 **71** **72**

Figure 10: Proposed chemical structure of the isolated compounds (EG-1, EG2, EG-3)

Chrysin (5,7-dihydroxyflavone) is a dihydroxyflavone found in numerous plants species such as *Oroxylum indicum, Cytisus multiflorus, Mangifera indica, Desmos cochinchinensis, Scutellaria bornmuelleri, Pyrus pashia,Teloxys graveolens, Alpinia oxyphylla* [190], *and many* fruits [191,192], passion flowers such as *Passiflora coerulea* [193] and even mushrooms [194].

Chrysin (EG-1) has been shown to possess pharmacological properties such as antioxidant and anti-inflammatory activities [190]. Moreover, chrysin has shown gastrointestinal protective properties in gastric ulcers, diarrhea, colitis, and inflammatory bowel disease [195]. Respiratory tract protective effects of chrysin have been witnessed in airway inflammation, pulmonary hypertension, pleurisy and lung injury, lung fibrosis, pulmonary edema, pulmonary arterial hypertension, asthma, chronic obstructive pulmonary disease, allergic inflammation, and pneumonia [196]. Skin protective effects of chrysin have been observed in cases of psoriasis, atopic dermatitis, photo-aging and melanogenesis, and leishmaniasis [197, 198]. In this study, chrysin was suggested to be one of the compounds isolated from the stem bark extract of *Eucalyptus globules* that may explain the use of the plant for the treatment of a variety of disease conditions including colds, asthma, cough, diarrhea and dysentery, hemorrhage, laryngalgia, laryngitis, sore throat, spasm, trachagia and vermifuge [64], constipation and other stomach problems, oral thrush, boils, sores, skin and wound infections, bronchitis, eczema and athletes foot [65, 66], and skin diseases [74], fever, antitussive, grippe, respiratory ailments, migraine, thorax ailments [75].

Purpurin (EG-2) belongs to the class of organic compounds known as hydroxyl anthraquinones. It is a naturally occurring hydroxyanthraquinone mostly isolated from the roots of a perennial herb *Rubia cordifolia* (madder plant). This compound has been reported to exist in the root of *Rubia sikkimensis* Kurz [199], *Rubia cordifolia* Linn [199-201], *Rubia tinctorum* L. [199, 201-203] and *Rubia* peregrina L. [199,201].

Purpurin possesses diverse pharmacological properties, including antioxidant, antimicrobial, neuroprotective, antiadipogenic, anticancer, anti-inflammatory, anti-dysentery, and antifungal [204- 206]. In this study, purpurin was suggested to be one of the compounds isolated from the stem bark extract of *Eucalyptus globules,* which may justify the use of the plant as a traditional medicine to treat stomach problems, oral thrush, skin and wound infections, foot smell [65, 66, 74], fever, antitussive, grippe or flu, respiratory ailments, migraine, and thorax ailments [75].

Compound 1,2-dimethoxy-6-methyl-9,10-anthraquinone is a naturally occurring anthraquinone mostly isolated from the roots of *Rennellia elliptica* Korth., whichis one of the species in the genus *Rennellia* [207]. The root extract of *Rennellia elliptica* was reported to be antimalarial [208] and antioxidant [209]. In this study, this compound is suggested to be one of the compounds isolated from the stem bark extract of *Eucalyptus globules,* which may justify its medicinal uses to treat fever, cough, thorax ailments [75], urinary and respiratory infections, inflammation [15, 16], and its use as antioxidant [96].

To the best of my knowledge, the identity of the isolated compounds EG-1 chrysin (5,7-dihydroxyflavone), EG-2 purpurin or (1,2,4-trihydroxy -9,10 anthraquinone), and EG-3 (1,2-Dimethoxy-6-methyl-9, 10-anthraquinone) haven’t been reported in *Eucalyptus globules* extracts of different parts including bark, leaf, root, fruits or seeds, and flower.

5. CONCLUSION AND RECOMMENDATIONS

4.1 Conclusion

In the present study, the extraction of phytochemicals from the stem bark of *Eucalyptus globules* was satisfactorily performed. The extraction process was conducted using cold maceration technique with three different solvents: hexane, $CHCl_3$:MeOH (v/v, 1:1) and methanol sequentially. The percentage yield shows 0.42% of hexane extracts 2.40% of $CHCl_3$:MeOH (v/v, 1:1) extracts, and 3.22% of methanolic extracts. Alkaloids, flavonoids, phenols, glycosides, terpenoids, saponins, and tannins were identified on the crude extracts of the plant. The presence of these classes of secondary metabolites suggested the anti-allergic, anti-cancer, antioxidant, anti-inflammatory, anti-viral, insecticidal, and antimicrobial activity of the stem barks of *Eucalyptus globules* extract. Moreover, three compounds **EG-1**, **EG-2** and **EG-3**were isolated from the $CHCl_3$:MeOH (v/v, 1:1) crude extract. Partial structure elucidation and comparing the experimental spectroscopic data and the melting points with that of literature the isolated compounds **EG-1**, **EG-2** and **EG-3** were proposed to be chrysin (5,7-dihydroxyflavone), purpurin (1,2,4-trihydroxy-9,10-anthraquinone) and 1,2-Dimethoxy-6-methyl-9,10-anthraquinone respectively.

4.2 Recommendations

- Spectroscopic data such as H^1-NMR, C^{13}-NMR, DEPT and MS should be carried out to fully illustrate the structures of the isolated compounds.
- The hexane and methanol crude extract should also be fractionated using various solvent systems to isolate compounds. Furthermore, the fractionation of fractions having more than one spot on the TLC should also be performed to completely analyze the stem bark extract of the plant.
- Pharmacological studies of the plant should be investigated so as to establish the traditional uses of the plant and to identify bioactive molecules that are used for medicinal purposes.

REFERNCES

1. Mickymaray, S. Efficacy and mechanism of traditional medicinal plants and bioactive compounds against clinically important pathogens. *Antibiotics.* **2019**, *8*, 1–57.
2. Thakur, M.; Asrani, R.K.;Thakur, S.; Sharma, P.K.; Pati, R.D.; La, B.; Parkash, O. Observations on traditional usage of ethnomedicinal plants in humans and animals of Kangra and Chamba districts of Himachal Pradesh in north-western Himalaya, India. *J. Ethnopharmacol.* **2016**, *191*, 280–300.
3. Araya, S.; Abera, B.; Giday, M. Study of plants traditionally used in public and animal health management in Seharti Samre District, Southern Tigray, Ethiopia.*J. Ethnobiol. Ethnomed.* **2015**, *11*, 1–25.
4. Belayneh, A.; Asfaw, Z.; Demissew, S.; Bussa, N.F. Medicinal plants potential and use by pastoral and agro-pastoral communities in Erer Valley of Babile Wereda, Eastern Ethiopia. *J. Ethnobiol. Ethnomed.* **2012**, *8*, 2–11.
5. Zerabruk, S.; Yirga, G. Traditional knowledge of medicinal plants in Gindeberet district, Western Ethiopia. *South African Journal of Botany.* **2012**, *78*, 165–169.
6. Boland, D.J.; Brooker, M.I.H,; Chippendale, G.M.;Hall, N.; Hyland, B.P.M.; Johnson, R.D. *Forest trees of Australia*, 5th ed., Melbourne: CSIRO Publishing, Australia, **2006**, p336.
7. Teketay, D. Facts and experiences on Eucalypts in Ethiopia and elsewhere: Ground for making life informed decisions. *Walia.* **2000**, *21*, 25–46.
8. Oballa, P.;Chagala-odera, E.; Wamalwa, L.; Oeba,V.; Mutitu, E.;Mwangi,L. The performance of Eucalyptus Hybrid clones and local landraces in various agro-ecological zones in Kenya. International Service for the acquisition of Agri-Biotechnology Applications (ISAA). **2005**.
9. Tadesse, M.; Inga, H.; Ensermu, K.; Sue, E.;Eva, P.*Flora of Ethiopia and Eritrea: Gentianaceae to Cyclocheilaceae,*Addis Abeba University, Ethiopia, **2005**, *5*, p157.
10. Lemenih, M.; Kassa, H. Re-Greening Ethiopia: History, challenges and lessons. Forests. **2014**, *5*, 1896–1909.
11. Kayani, S.; Ahmad , M.; Zafar, M.; Sultana, S.; Khan, M.P.Z.; Ashraf, M.A.;Hussain, J.; Yaseen, G. Ethnobotanical uses of medicinal plants for respiratory disorders among the inhabitants of Gallies–Abbottabad, Northern Pakistan. *J. Ethnopharmacol.* **2014**, *156*,

47–60.

12. Knezevic, P.; Aleksic, V.; Simin, N.; Svircev, E.; Petrovic, A.;Mimica-Dukic, N. Antimicrobial activity of *Eucalyptus camaldulensis* essential oils and their interactions with conventional antimicrobial agents against multi-drug resistant Acinetobacter baumannii. *J. Ethnopharmacol.* **2016**, *178*, 125–36.
13. Silva, J.; Abebe, W.; Sousa, S.M.; Duarte, V.G.; Machado,M.L.; Matos, F.A. Analgesic and antiinflammatory effects of essential oils of Eucalyptus. *J. Ethnopharmacol.* **2003**. *89*, 277–283.
14. Williams, L.R.; Stockley, J.K.; Yan,W.; Home,V.N. Essential oils with high antimicrobial activity for therapeutic use. *Int. J. Aromather.* **1998**, *8*, 30–40.
15. Low, D.; Rawal, B.D.; Griffin, W.J. Antibacterial action of the essential oils of some Australian Myrtaceae with special references to the activity of chromatographic fractions of oil of *Eucalyptus citriodora*. *Planta Med.* **1974**, *26*, 184–189.
16. Hasegawa, T.; Takano, F.; Takata, T.; Niiyama,M.; Ohta,T.Bioactive monoterpene glycosides conjugated with gallic acid from the leaves of Eucalyptus globules.*Phytochemistry.* **2008**, *69*, 747–753.
17. Barbosa, L.C.A.; Filomeno, C.A.; Teixeria, R.R. Chemical variability and biological activities of Eucalyptus spp. essential oils. *Molecules.* **2016**, *21*, 1671–1703.
18. Zhou, L.; Li, J.; Kong, Q.;Luo, S.; Wang, J.; Feng, S.; Yuan, M.;Chen, T.; Yuan, S.; Ding, C.Chemical composition, antioxidant, antimicrobial, and phytotoxic potential of *Eucalyptus grandis* × *E. urophylla* leaves essential oils. *Molecules.* **2021**, *26*, 1450–1462.
19. Dhakad, A.K.; Pandey, V.V.; Beg, S.; Rawat, J.M.; Singh, A. Biological, medicinal and toxicological significance of Eucalyptus leaf essential oil: a review. *J Sci Food Agri.* **2018**, *98*, 833–48.
20. Bachir, R.G.; Benali, M. Antibacterial activity of the essential oils from the leaves of *Eucalyptus globulus* against Escherichia coli and Staphylococcus aureus. *Asian Pac J Trop Biomed.* **2012**, *2*, 739–742.
21. Mulyaningsih, S.; Sporer, F.; Reichling, J.; Wink, M. Antibacterial activity of essential oils from Eucalyptus and of selected components against multidrug-resistant bacterial pathogens. *Pharmaceutical Biology.* **2011**, *49*, 893–899.
22. Gakuubi, M. M.; Maina, A.W.; Wagacha, J.M. Antifungal activity of essential oil of *Eucalyptus camaldulensis* Dehnh. against selected *Fusarium* spp. *International Journal of Microbiology.* **2017**, *17*, 1–7.

23. Marzoug, H.N.B.; Romdhane, M.; Lebrihi, A.; Mathieu, F.; Couderc, F.; Abderraba, M.; Larbi Khouja, M.; Bouajila, J.*Eucalyptus oleosa* essential oils: Chemical composition and antimicrobial and antioxidant activities of the oils from different plant parts (stems, leaves, flowers and fruits). *Molecules.* **2011**, *16*, 1695–1709.
24. Valeriano, C.; Oliveira, T.L.C.; Carvalho, S.M.; Cardoso, M.G.; Alves, E.; Piccoli, R.H. The sanitizing action of essential oil-based solutions against Salmonella enterica serotype Enteritidis S64 biofilm formation on AISI 304 stainless steel. *Food Control.* **2012**, *25*, 673–677.
25. Silva, S.M.; Abe, S.Y.; Murakami, F.S.; Frensch, G.; Marques, F.A.; Nakashima, T. Essential oils from different plant parts of *Eucalyptus cinerea F. Muell. Ex Benth.* (Myrtaceae) as a source of 1, 8-cineole and their bioactivities. *Pharmaceuticals.* **2011**, *4*, 1535–1550.
26. Nordqvist, J. *Eucalyptus: What are the health benefits? Medical news today*: https://www.medicalnewstoday.com/articles/266580.php. (Accessed on June, 2024).
27. Van Wyk, B.E.; Wink, M. *Medicinal plants of the world*, Pretoria: Briza Publications; **2017**, p480.
28. Abbasi, N.; Khalighi, Z.; Eftekhari, Z.; Bahmani, M. Extraction and phytoanalysis of chemical compounds of *Eucalyptus globulus* leaf native to Dehloran, Ilam Province, Iran by HS-SPME and GC-MS. *Adv. Anim. Vet. Sci.* **2020**, *8*, 647-652.
29. Ullah, A.; Anjum, A.A.; Rabbani, M.; Nawaz, M.; Ashraf, M.; Ijaz, M.; Ali, A.; Rashid, A.; Najeeb, I.; Pervez, A. Phytochemical composition and In-vitro activity of ethanolic extract of *Eucalyptus globulus* leaves against multidrug resistant poultry pathogens. *Cell Mol Biol (Noisy le Grand).* **2021**, *67*, 159-164.
30. Bachir, R.G.; Benali, M. Antibacterial activity of leaf essential oils of *Eucalyptus globulus* and *Eucalyptus camaldulensis*. *Afr. J. Pharm. Pharmacol.* **2008**, *2*, 211-215.
31. Maria, G.V.I.; Claudia, L.; Clara, M. Beneficial and Healthy Properties of Eucalyptus Plants: A Great Potential Use. *The Open Agriculture Journal.* **2016**, *10*, 52-57.
32. Boulekbache-Makhlouf, L.; Meudec, E.; Mazauric, J.P.; Madani, K.; Cheynier, V. Qualitative and semi-quantitative analysis of phenolics in *Eucalyptus globules* leaves by high-performance liquid chromatography coupled with diode array detection and electro spray ionization mass spectrometry. *Phytochemical Analysis.* **2013**, *24*, 162–170.
33. Dixit, A.; Rohilla, A.; Singh, V. *Eucalyptus globules*: A new perspective in therapeutics. *Int J Pharm Chem Sci*. **2012**, *1*, 1678-83.

34. Elaissi, A.; Salah,K.H.; Mabrouk, S.; K. M. Larbi,K.M.; Chemli, R.; Harzallah-Skhiri, F. Antibacterial activity and chemical composition of 20 Eucalyptus species' essential oils. *Food Chemistry.* **2011**, *129*, 1427–1434.
35. Ben Jemâa, J.M.; Haouel, S.; Bouaziz, M.; Khouja, M.L. Seasonal variations in chemical composition and fumigant activity of five Eucalyptus essential oils against three moth pests of stored dates in Tunisia. *J Stored Prod Res.* **2012**, *48*, 61–7.
36. Chennoufi, R.; Morizur, J.; Richard, H.; Sandret, F. Study of Eucalyptus globules essential oils from Morocco (young and adult leaves). *Rivista Italiana EPPOS.* **1980**, *62*, 353–7.
37. Zrira, S.; Elamrani, A.; Benjilali, B. Chemical composition of the essential oil of Pistacia lentiscus L. from Morocco—a seasonal variation. *Flavour Fragr J.* **2003**, *18*, 475–80.
38. Zrira, S.; El Khirani, F.; Benjilali, B. Essential oils of six xerophyl Eucalyptus species: effect of location on the yield and the chemical composition. *Acts. Inst.Agron.Veto(Morocco).* **1994**, *14*, 5–9.
39. Elaissi, A.; Rouis, Z.; Mabrouk, S.; Bel Haj Salah, K.; Aouni, M.; Larbi Khouja, M.; Farhat, F.; Rachid Chemli, R.; Harzallah-Skhiri, F.Correlation between chemical composition and antibacterial activity of essential oils from fifteen Eucalyptus species growing in the Korbous and Jbel Abderrahman Arboreta (North East Tunisia). *Molecules.* **2012**, *17*, 44–57.
40. Barra, A. Factors affecting chemical variability of essential oils: a review of recent developments, *Nat. Prod. Commun.* **2009**, *4*, 1147-1154.
41. Khammassi, M.;Mighri, H.; Ben Mansour, M.; Amri, I.; Jamoussi, B.; Khaldi, A.Metabolite profiling and potential antioxidant activity of sixteen fennel (Foeniculumvulgare Mill.) populations wild-growing in Tunisia. *South Afr. J. Bot.* **2022**, *148*, 407–414.
42. Flores, T.B.; Alvares, C.A.; Souza, V.C.; Stape, J.L. *Eucalyptus in Brazil: climatic zoning and identification guide*; Piracicaba: IPEF, **2018**, p447.
43. Gilles, M.; Zhao, J.; An, M.; Agboola, S. Chemical composition and antimicrobial properties of EOs of three Australian Eucalyptus species. *Food Chem.* **2010**, *119*, 731-737.
44. Pereira, V.; Dias, C.; Vasconcelos, M.C.; Rosa, E.; Saavedra, M.J. Antibacterial activity and synergistic effects between *Eucalyptus globulus* leaf residues (EOs and extracts) and antibiotics against several isolates of respiratory tract infections (Pseudomonas

aeruginosa). *Ind. Crops Prod.* **2014**, *52*, 1-7.

45. Bello, M.O.; Olabanji, I.O.; Ibrahim, A.O.; Yekeen, T.A.; Oboh, L.M. Nutraceuticals in leaves of *Eucalyptus citriodora* and *Eucalyptus camandulensis*. *Food Sci.* **2013**, *62*, 17873-17876.
46. Tyagi, A.K.; Malik, A. Antimicrobial potential and chemical composition of *Eucalyptus globulus* oil in liquid and vapor phase against food spoilage microorganisms. *Food Chem.* **2011**, *126*, 228-235.
47. Araujo, F.O.L.; Rietzler, A.C.; Duarte, L.P.; Silva, G.D.F.; Carazza, F., Filho, S.A.V. Chemical constituents and ecotoxicological effect of the volatile oil from leaves of *Eucalyptus urograndis* (Mirtaceae). *Quím. Nova.* **2010**, *33*, 1510-1513.
48. Chandorkar, N.; Tambe, S.; Aminb, P.; Madankar, C. A systematic and comprehensive review on current understanding of the pharmacological actions, molecular mechanisms, and clinical implications of the genus Eucalyptus. *Phytomedicine Plus.* **2021**, *1*, 1-20.
49. Kilimo, T. Eucalyptus Hybrid Clones in East Africa; Meeting the Demand for Wood through Clonal Forestry Technology. Occassional Paper No.1, **2011**.
50. Liu, H.; Li, J. The study of the ecological problems of eucalyptus plantation and sustainable development in Maoming Xiaoliang. *Journal of Sustainable development.* **2010**, *3*, 197–200.
51. Lemenih, M.; Kassa, H. Re-Greening Ethiopia: History, challenges and lessons. *Forests.* **2014**, *5*, 1896–1909.
52. Salehi, B.; Sharifi-Rad, J.; Quispe, C.; Llaique, H.; Villalobos, M.; Smeriglio, A.; Trombetta, D.; Ezzat, S.M.; Salem, M.A.; Zayed, A.; Castillo, C.M.S.; Yazdi, S.E.; Sen, S.; Acharya, K.; Sharopov, F.; Martins, N. Insights into Eucalyptus genus chemical constituents, biological activities and health-promoting effects. *Trends Food Sci Technol.* **2019**, *91*, 609–24.
53. Cimanga, K.; Kambu, K.; Tona, L.; Apers, S.; De Bruyne, T.; Hermans, N.; Totté, J.; Pieters, L.; Vlietinck, A.J. Correlation between chemical composition and antibacterial activity of essential oils of some aromatic medicinal plants growing in the Democratic Republic of Congo. *J Ethnopharmacol.* **2002**, 79, 213-220.
54. Döll-Boscardin, P.M.; Sartoratto, A.; Maia, B.;, Paula, J.P.; Nakashima, T.; Farago, P.V.; Kanunfre, C.C. In Vitro Cytotoxic Potential of Essential Oils of *Eucalyptus benthamii* and Its Related Terpenes on Tumor Cell Lines. *Evid Based Complement Alternat Med.* **2012**, *12*, 1-8.

55. Döll-Boscardin, P.M.; Borsato, D.M.; Mirante, D.C.; Nakashima, T.; Budel, J.M.; Kovaliczn, R.A.; Maia, B.; Paula, J.P.; Farago, P.V. Essential oil from leaves of *Eucalyptus benthamii*: Secretory structures, volatile composition and biological activities. *Planta Medica.* **2015**, *81*, 1–9.
56. Sayonara, M.S.; Simone, Y.A.; Fábio, S.M.; Gustavo, F.; Francisco, A.M.; Tomoe, N. Essential Oils from Different Plant Parts of *Eucalyptus cinerea F. Muell. ex Benth.* (Myrtaceae) as a Source of 1,8-Cineole and Their Bioactivities. *Pharmaceuticals.* **2011**, *4*, 1535–1550.
57. Zrira, S.; Bessiere, J.M.; Menut, C.; Elamrani, A.; Benjilali, B. Chemical composition of the essential oil of nine Eucalyptus species growing on Morrocco. *Flavour Fragr. J.* **2004**, *19*, 172–175.
58. Babu, G.D.K.; Singh, B. Simulation of Eucalyptus cinerea oil distillation: A study on optimization of 1,8-cineole production. *Biochem. Eng. J.* **2009**, *44*, 226–231.
59. Zrira, S.; Bessiere, J.M.; Menut, C.; Elamrani, A.; Benjilali, B. Chemical composition of the essential oil of nine Eucalyptus species growing in Morocco. *Flavour Fragr. J.* **2004**, *19*, 172–175.
60. Dhakad, A.K.; Pandey, V.V.; Beg, S.; Rawat, J.M.; Singh, A. Biological, medicinal and toxicological significance of Eucalyptus leaf essential oil: a review. *Journal of the Science of Food and Agriculture.* **2018**, *98*, 833–848.
61. Small, B.E.J. The Australian Eucalyptus oils industry: an overview. *Australian Forestry Journal.* **1981**, *44*, 170–177.
62. Chevallier, A. *Encyclopaedia of medicinal plants.* St Leonards, NSW: Dorling Kindersley Pty Ltd, **2001**, p410.
63. Kluthe, B.M.G.; Chen, D.K. Eucalyptus sp. at the intersection of environment and culture in Kenya. *Ethnobiology Letters.* **2017**, *8*, 15–22.
64. Duke, J.A.; Wain, K.K. Medicinal plants of the world. Computer index with more than 85,000 Entries, **1981**, *3*, p1654.
65. Mavundza, E.J.; Street, R.; Baijnath, H. A review of the ethnomedicinal, pharmacology, cytotoxicity and phytochemistry of the genus Euphorbia in southern Africa. *South African Journal of Botany.* **2022**, *144*, 403–418.
66. Duke, J.A.; Ayensu, E.S. *Medicinal plants of China, Algonae, Mich,* Reference Publications, 2nd edition, Medicinal plants of the World, no. 4., **1985**.
67. Adeniyi, B.A.; Odufowoke, R.O.; Olaleye, S.B. Antibacterial and gastro protective

properties of *Eucalyptus torelliana F. Muell* crude extracts. *Int. J. Pharmacol.* **2006**, *2*, 362–365.

68. Adeniyi, C.B.A.; Lawal, T.O.; Mahady, G.B. In vitro susceptibility of Helicobacter pylori to extracts of *Eucalyptus camaldulensis* and *Eucalyptus torelliana*. *Pharmaceutical Biology.* **2009**, *47*, 99–102.
69. Mersha, A.E.; Ensermu, K.; Gemedo, D. Ethnobotanical study of medicinal plants in Guji Agro-pastoralists, Blue Hora District of Borana Zone, Oromia Region, Ethiopia. *J. Med. Plants Stud.* **2016**, *4*, 170–184.
70. Zenebe, G.; Zerihun, M.; Solomon, Z. An Ethnobotanical Study of Medicinal Plants in Asgede Tsimbila District, Northwestern Tigray, Northern Ethiopia. *Ethnobotany Research & Applications.* **2012**, *10*, 305–320.
71. Abubakar, E.M.M. Antibacterial potential of crude leaf extracts of Eucalyptus camaldulensis against some pathogenic bacteria. *Afr. J. Plant Sci.* **2010**, *4*, 202–209.
72. Bukar, A.; Danfillo, I. S.; Adeleke, O. A.; Ogunbodede, E.O. Traditional oral health practices among Kanuri women of Borno State, Nigeria. *Odontostomatol. Trop.* **2004**, *27*, 25–31.
73. Genene, B.; Ramachandra, R.P. Ethnobotanical Study of Medicinal Plants Used to Treat Human Ailments by Guji Oromo Tribes in Abaya District, Borana, Oromia, Ethiopia. *Univers. J. Plant Sci.* **2015**, *3*, 1–8.
74. Nigussie, A. Ethnobotanical Studies on Traditional Medicinal Plants Used in the Treatment of Humans' Skin Diseases in Ethiopia: A Review. *Clin Dermatol J.* **2024**, *9*, 1–24.
75. Mustapha, A.; Abdelhamid, El. M.; Fouad, M.; Hassan, B.; Baha, S.; Khalil, C. An ethnobotanical survey of medicinal plants used in the Tata Province, Morocco. *Int. J. Med. Plants. Res.* **2012**, *1*, 99–123.
76. Franco, J.; Nakashima, T.; Franco, L.; Boller, C. Chemical composition and antimicrobial in vitro activity of the essential oil *Eucalyptus cinerea F. Mull. ex Benth.*, Myrtaceae, extracted in different time intervals. *Braz J Pharmacog.* **2005**, *15*, 191–4.
77. Sebei, K.; Sakouhi, F.;Herchi,W.; Khouja, M.L.; Boukhchina, S. Chemical composition and antibacterial activities of seven Eucalyptus species essential oils leaves. *Biol Res.* **2015**, *48*, 1–5.
78. Levison, K.K.; Takayama, K.; Isowa, K.; Okabe, K.; Nagai, T. Formulation optimization of indomethacin gels containing a combination of three kinds of cyclic monoterpenes as

percutaneous penetration enhancers. *J Pharm Sci.* **1994**, *83*, 1367–72.

79. Levison, K.K.; Takayama, K.; Isowa, K.; Okabe, K.; Nagai, T. Formulation optimization of indomethacin gels containing a combination of three kinds of cyclic monoterpenes as percutaneous penetration enhancers. *J. Pharm. Sci.* **1994**, *83*, 1367–1372.
80. Juergens, U.R.; Dethlefsen, U.; Steinkamp, G.; Gillissen, A.; Repges, R.; Vetter, H. Anti-inflammatory activity of 1,8-cineole (eucalyptol) in bronchial asthma: a double blind placebo-controlled trial. *Respiratory Medicine.* **2003**, *97*, 250–256.
81. Santos, F.A.; Rao, V.S.N. Anti-inflammatory and antinociceptive effects of 1, 8-cineole, a terpenoid oxide present in many plant essential oils. *Phytotherapy Research.* **2000**, *14*, 240–244.
82. Sangwan, N.K.; Verma, B.S.; Verma, K.K.; Dhindsa, K.S. Nematicidal activity of some essential plant oils. *Pesticide Science.* **1990**, *28*, 331–335.
83. Grassmann, J.; Hippeli, S.; Dornisch, K.; Rohnert, U.; Beuscher, N.; Elstner, E.F. Antioxidant properties of essential oils. Possible explanation for their anti-inflammatory effects. *Arzneimittel Forschung Drug Research.* **2000**, *50*, 135–139.
84. Akin, M.; Aktumsek, A.; Nostro, A. Antibacterial activity and composition of the essential oils of *Eucalyptus camaldulensis Dehn.*and *Myrtus communis L.* growing in Northern Cyprus. *Afr. J. Biotechnol.* **2010**, *9*, 531–535.
85. Bachir Raho, G.; Benali, M. Antibacterial activity of leaf essential oils of *Eucalyptus globulus* and *Eucalyptus camaldulensis. Int. J. Pharm. Pharmacol.* **2011**, *2*, 001–005.
86. Usman, H.N.; Majiya, H.; Kolawole, H.A; Legbo,M.I.; Musa, A.D. Evaluation of phytochemical analysis and antibacterial potential of different solvent extracts of leaves and stem bark of *Eucalyptus camaldulensis* against selected pathogenic bacteria. *Biokemistri.* **2021**, *33*, 301–310.
87. Noumi, V.D.; Nguimbou, R.M.; Tsague, M.V.; Deli, M.; Rup-Jacques, S.; Amadou, D.; Baudelaire,E.N.; Sokeng, S.; Njintang, N.Y. Phytochemical Profile and *In Vitro* Antioxidant Properties of Essential Oils from Powder Fractions of *Eucalyptus camaldulensis* Leaves. *Am. J. Plant Sci.* **2021**, *12*, 329–346.
88. Salem, M.Z.; Ashmawy, N.A.; Elansary, H.O.;El-Settawy, A.A. Chemotyping of Various Eucalyptus Species Grown in Egypt and Antioxidant and Anti-bacterial Activities of its Respective Essential Oils. *Nat. Prod. Res.* **2015**, *7*, 681–685.
89. Wei, A.;Shibamoto, T. Antioxidant Activities and Volatile Constituents of Various Essential Oils. *J. Agri. Food Chem.* **2007**, *55*, 1737–1742.

90. Miguel, M.G. Antioxidant Activity of Medicinal and Aromatic Plants. A Review. *Flavour Frag. J.* **2010**, *25*, 291–312.
91. Bicas, J.L.; Neri-Numa, I.A.; Ruiz, A.L.T.G.; De Carvalho, J.E.; Pastore, G.M. Evaluation of the Antioxidant and Antiproliferative Potential of Bioflavors. *Food Chem. Toxicol.* **2011**, *49*, 1610–1615.
92. Moon, H.K.; Kang, P.; Lee, H.S.; Min, S.S.;Seol, G.H. Effects of 1, 8-Cineole on Chronic Exposure to Nicotine in Rats. *J. Pharm. Pharmacol.* **2014**, *66*, 688–693.
93. Ramezani, H.; Singh, H.P.; Batish, D.R.;Kohli, R.K. Antifungal activity of the volatile oil of *Eucalyptus citriodora*. *Fitoterapia.* **2002**, *73*:261–262.
94. Su, Y.C.; Ho, C.L.; Wang, E.I.; Chang, S.T. Antifungal activities and chemical compositions of essential oils from leaves of four eucalyptus. *Taiwan J for Sci.* **2006**, *21*, 49–61.
95. Rakotonirainy, M.S.; Lavédrine, B. Screening for antifungal activity of essential oils and related compounds to control the biocontamination in libraries and archives storage areas. *Int BioDeterior Biodegrad.* **2005**, *55*:141–147.
96. Siramon, P.; Ohtani, Y. Antioxidative and antiradical activities of *Eucalyptus camaldulensis* leaf oils from Thailand. *J Wood Sci.* **2007**, *53*, 498–504.
97. Song, A.; Wang, Y.; Liu, Y. Study on the chemical constituents of the essential oil of the leaves of Eucalyptus globulus Labill from China. *Asian J. Tradit. Med.* **2009**, *4*, 134–140.
98. Damjanović-Vratnica, B.; Dakov, T.; Šuković, D.; Damjanović, J. Antimicrobial effect of essential oil isolated from *Eucalyptus globulus Labill.* from Montenegro Czech. *J. Food Sci.* **2011**, *29*, 277–284
99. Ghalem, B.R.; Mohamed, B.Antibacterial activity of leaf essential oils of *Eucalyptus globulus* and *Eucalyptus camaldulensis*. *Afr. J. Pharmacy Pharmacol.* **2008**, *2*, 211–215.
100. Barbosa, L.C.A.; Filomeno, C.A.;Teixeira, R.R. Chemical variability and biological activities of Eucalyptus spp. essential oils. Molecules. **2016**, *21*, 1–13.
101. Akolade, J.O.; Olajide, O.O.; Afolayan, M.O.; Akande, S.A.; Idowu, D.I.; Orishadipe, A.T. Chemical composition, antioxidant and cytotoxic effects of *Eucalyptus globulus* grown in north-central Nigeria. *J. Nat. Prod. PlantRec.* **2012**, *2*, 11–8.
102. González-Burgos, E.; Liaudanskas, M.; Viškelis, J.; Žvikas, V.; Janulis, V.;GómezSerranillos, M.P. Antioxidant activity, neuroprotective properties and bioactive constituents analysis of varying polarity extracts from *Eucalyptus globulus* leaves. *J. Food Drug Anal.* **2018**, *26*, 1293–1302.

103. Adnan, M. Bioactive potential of essential oil extracted from the leaves of *Eucalyptus globulus* (Myrtaceae). *J. Pharmacogn. Phytochem.* **2019**, *8*, 213–216.
104. Teixeira, A.; Dacunha, D.C.; Barros, L.; Caires, H.R.; Xavier, C.P.R.; Ferreira, I.C.F.R.;Vasconcelos, M.H. *Eucalyptus globulus* Labill. decoction extract inhibits the growth of NCI-H460 cells by increasing the p53 levels and altering the cell cycle profile. *Food and Function.* **2019**, *10*, 3188–3197.
105. Ghareeb, M.A.; Sobeh, M.; El-Maadawy, W.H.; Mohammed, H.S.; Khalil, H.; Botros, S.;Wink, M. Chemical profiling of polyphenolics in *Eucalyptus globulus* and evaluation of its hepato–renal protective potential against cyclophosphamide induced toxicity in mice. *Antioxidants.* **2019**, *8*, 415–430.
106. Elaissi, A.; Marzouki, H.; Medini, H.; Khouja, M. L.; Farhat, F.; Lynene, F.;Skhiri - Harzallah, F.; Chemli, R.Variation in volatile leaf oils of 13 Eucalyptus species harvested from Souinet Arboreta (Tunisia).*Chem. Biodiversity*. **2010**, *7*, 909–921.
107. Yosra, K.; Karama, Z.B.; Fatma, R.; Mohamed, T.; Slim, T. Efficacy of *Eucalyptus cinerea* as a Source of Bioactive Compounds for Curative Biocontrol of Crown Gall Caused by Agrobacterium tumefaciens Strain B6. *BioMed Research International.* **2017**, *1*, 1–10.
108. Li, H.; Madden, J.L.; Potts, B.M. Variation in volatile leaf oils of the Tasmanian Eucalyptus species II subgenus Symphyomyrtus. *Biochem Syst Ecol.* **1996**, *24*, 547–569.
109. Zrira, S.; Bessiere, J.M.; Menut, C.; Elamrani, A.; Benjilali, B. Chemical composition of the essential oil of nine Eucalyptus species growing in Morocco. *Flavour Fragr J.* **2004**, *19*, 172–175.
110. Dunlop, P.J.; Bignell, C.M.; Hibbert, D.B.; Brooker, M.I.H. Use of gas chromatograms of the essential leaf oils of the genus Eucalyptus for taxonomic purposes: *E. subser. Euglobulares* (Blakely). *Flavour Fragr J.* **2003**, *18*, 162–169.
111. Babu, G.D.K.; Singh, B. Simulation of *Eucalyptus cinerea* oil distillation: A study on optimization of 1, 8 cineole production. *Biochem. Eng. J.* **2009**, *44*, 226–231.
112. Elaissi, A.; Rouis, Z.; Ben Salem, A.N.; Mabrouk, S.; Ben Salem, Y.; Haj Salah, B.K.; Aouni, M.; Farhat, F.; Chemli, R.; Harzallah-Skhiri, F.; Larbi Khouja, M. Chemical composition of 8 eucalyptus species' essential oils and the evaluation of their antibacterial, antifungal and antiviral activities. *BMC Complement. Altern. Med.* **2012**, *12*, 1–15.

113. Burt, S. Essential oils: Their antibacterial properties and potential applications in foods—A review. *Int. J. Food Microbiol.* **2004**, *94*, 223–253.
114. Soliman, F.M.; Fathy, M.M.; Salama, M.M.; Al-Abd, A.M.; Saber, F.R.; El-Halawany, A.M. Cytotoxic activity of acyl phloroglucinols isolated from the leaves of *Eucalyptus cinerea F. Muell. ex Benth.* cultivated in Egypt. *Sci. Rep.* **2014**, *4*, 1–6.
115. Silva, S.M.; Abe, S.Y.; Bueno, F.G.; Lopes, N.P.; deMello, J.; Nakashima, T. Direct proof by C^{13}-NMR of semi-purified extract and isolation of ent -Catechin from leaves of *Eucalyptus cinerea*. *Phcog Mag.* **2014**, *10*, 191–4.
116. Abd El-Mageed, A.A.; Osman, A.K.; Tawfik, A.Q.; Mohammed, H.A. Chemical composition of the essential oils of four *Eucalyptus* species (Myrtaceae) from Egypt. *Research Journal of Phytochemistry.***2011**, *5*, 115–122.
117. Flamini, G.; Cioni, P.L.; Morelli, I.; Maccioni, S.; Baldini, R.Phytochemical typologies in some populations of M. communis L. on Caprione Promontory (East Liguria, Italy). *Food Chem.* **2004**, *85*, 599–604.
118. Barbosa, A.C.L.; Filomeno, A.C.; Teixeira, R.R. Chemical Variability and Biological Activities of Eucalyptus spp. Essential Oils. *Molecules.* **2016**, *21*, 1671–1704.
119. Jemaa, J.M.B.; Haouel, S.; Bouaziz, M.; Khouja, M.L. Seasonal variations in chemical composition and fumigant activity of five Eucalyptus EOs against three moth pests of stored dates in Tunisia. *J. Stored Prod. Res.* **2012**, *48*, 61–67.
120. Al-Snafi, E.A. The pharmacological and therapeutic importance of Eucalyptus species grown in Iraq. *IOSR Journal of Pharmacy.* **2017**, *7*, 72–91.
121. Salem, M.Z.M.; Zidan, Y.E.; Mansour, M.M.A.; El Hadidi, N.M.N.; Abo Elgat, W.A.A. Antifungal activities of two essential oils used in the treatment of three commercial woods deteriorated by five common mold fungi. *Int. Biodeterior. Biodegrad.* **2016**, *106*, 88–96.
122. Oyedeji, A.O.; Ekundayo, O.; Olawore, O.N.; Adeniyi, B.A.; Koenig, W.A. Antimicrobial activity of the Eos of five Eucalyptus species growing in Nigeria. *Fitoterapia.* **1999**, *70*, 526–528.
123. Batista-Pereira, L.G.; Fernandes, J.B.; Silva, M.F.G.F.; Vieira, P.C.; Bueno, O.C.; Correêa, A.G. Electrophysiological responses of Attasexdens rubropilosa workers to EOs of Eucalyptus and its chemical composition. Z. *Naturforsch.* **2006**, *61*, 749–755.

124. Batista-Pereira, L.G.; Fernandes, J.B.; Correa, A.G.; da Silva, M.F.G.F.; Vieira, P.C. Electrophysiological responses of Eucalyptus brown looper Thyrinteina arnobia to EOs of seven Eucalyptus species. *J. Braz. Chem. Soc.* **2006**, *17*, 555–561.
125. Ghasemi, V.; Moharramipour, S.; Tahmasbi, G. Biological activity of some plant EOs against Varroa destructor (*Acari: Varroidae*), an ectoparasitic mite of *Apis mellifera* (Hymenoptera: Apidae). *Exp. Appl. Acarol.* **2011**, *55*, 147–154.
126. Verdeguer, M.; Blazquez, M.A.; Boira, H. Phytotoxic effects of Lantana camara, Eucalyptus camaldulensis and Eriocephalus africanus EOs in weeds of Mediterranean summer crops. *Biochem. Syst. Ecol.* **2009**, *37*, 362–369.
127. Cheng, S.S.; Huang, C.G.; Chen, Y.J.; Yu, J.J.; Chen, W.J.; Chang, S.T. Chemical compositions and larvicidal activities of leaf EOs from two Eucalyptus species. *Bioresour. Technol.* **2009**, *100*, 452–456.
128. Francisco, J.D.C.; Järvenpää, E.P.; Huopalahti, R.; Sivik, B. Comparison of *Eucalyptus camaldulensis* Dehn. Oils from Mozambique Obtained by Hydrodistillation and Supercritical Carbon Dioxide Extraction.*J Agric Food Chem.* **2001**, *49*, 2339–2342.
129. Nah-Traore, Bouaré, S.; Sidibe, L.; Somboro, A.A.; Fofana, B.; Tangara, O.; Diallo, D.; Chalchat, J.C.;Figuiredo, G. Antimicrobial Activity of Essential Oils of *Eucalyptus camaldulensis* from Mali. *Asian J. Plant Sci. Res.* **2014**,*4*, 69–73.
130. Pagula, F.P.; Kurkcuoglu, M.;Baser, C.K.H. Essential Oil Composition of *Eucalyptus camaldulensis Dehn.*from Mozambique. *J. Essent. Oil Res.* **2000**, *12*, 333–335.
131. Cheng, S.S.; Huang, C.G.; Chen, Y.J.; Yu, J.J.; Chen, W.J.; Chang, S.T. Chemical compositions and larvicidal activities of leaf essential oils from two eucalyptus species. *Bioresour Technol.* **2008**, *99*, 3617–22.
132. Siramon, P.; Ohtani, Y. Antioxidative and antiradical activities of *Eucalyptus camaldulensis* leaf oils from Thailand. *J. Wood Sci.* **2007**, *53*, 498–504.
133. Tchoumbougnang, F.; Jazet, D.P.M.; Sameza, M.L.; Nkouaya, M.E.G.; Tiako, F.G.B.; Amvam Zollo, P.H.;Menut, C. Larvicide Activity on Anopheles gambiae Giles and Chemical Composition of Essential Oils Extracted from Four Plants Grown in Cameroon. *Biotechnology Agronomy Society and Environment*. **2009**, *13*, 77-84.
134. Jouault, S. *The Quality of the Essential Oils and Its Influence on Their Efficiency and Toxicity*. Thesis, Pharmacological Faculty, University of Lorraine, Nancy, **2012**, p137.
135. He, C.; Murray, F.; Lyons, T. Monoterpene and isoprene emissions from 15 Eucalyptus species in Australia. *Atmos Environ.* **2000**, *34*, 645–55.

136. Giamakis, A.; Kretsi, O.; Chinou, I.; Spyropoulos, C.G. *E. camaldulensis*: volatiles from immature flowers and high production of 1, 8-cineole and *β*-Pinene by in vitro cultures. *Phytochemistry.* **2001**, *58*, 351-355.
137. El-Ghorab, A.H.; El-Massry, K.F.; Fadel, H.M. The Egyptian *Eucalyptus camaldulensis* var. brevirostris: chemical compositions of the fruit volatile oil and antioxidant activity. *Flavour Frag. J.* **2002**, *17*, 306–312.
138. Chuku, A.; Ogbonna, A.I.; Obande, G.A.; Namang, M.; Ahmad, I.R.Antimicrobial effects of leaves of *Eucalyptus camaldulensis* on some microbial pathogens. *Eur. J. Med. Plants.* **2016**, *14*, 1–8.
139. Ayepola, O.O.; Adeniyi, B.A. The antibacterial activity of leaf extracts of *Eucalyptus camaldulensis* (Myrtaceae). *J. Appl. Sci. Res.* **2008**, *4*, 1410–1413.
140. Sabo, V.A.; Petar Knezevic, P. Antimicrobial activity of *Eucalyptus camaldulensis Dehn.*plant extracts and essential oils: A review. *Ind. Crop. Prod.* **2019**, *132*, 413–429.
141. Abdulmumin, Y.; Abdulmumin, T.M.; Muhammad, I.A.; Murtala, M.; Dalhatu, M.M.; Amina, L.A.; Bichi, S.A.; Sarki,S.I. Bioassay Guided Fractionation, Phytochemicals and Toxicity Evaluation of *Eucalyptus camaldulensis*Leave Extracts. *SARJNP*. **2021**, *4*, 75–82.
142. Islam, F.; Khatun, H.; Khatun, M.; Ali, S.M.; Khanam, J.A. Growth inhibition and apoptosis of Ehrlich ascites carcinoma cells by the methanol extract of *Eucalyptus camaldulensis*. *Pharm. Biol.* **2014**, *52*, 281–290.
143. Shagal, M.H.; Kubmarawa, D.; Tadzabia, K.; Dennis, K.I. Evaluation of phytochemical and antimicrobial potentials of roots, stem-bark and leaves extracts of *Eucalyptus camaldulensis*. *Afr. J. Pure Appl. Chem.* **2012**, *6*, 74–77.
144. Ghareeb, M.A.; Habib, M.R.; Mossalem, H.S.; Abdel-Aziz, M.S. Phytochemical analysis of *Eucalyptus camaldulensis* leaves extracts and testing its antimicrobial and schistosomicidal activities. *Bull. Natl. Res. Cent.* **2018**, *42*, 1–9.
145. Al-Tameemi1, Z.S.; Hamad, M.N. Isolation of Two Phenolic Compounds from the Ethyl Acetate Fraction of Iraqi Eucalyptus *Camaldulensis Dehnh*. IJDDT, **2022**, *12*, 274–280.
146. Al-Tameemi1, Z.S.; Hamad, M.N. Isolation of Three Secondary Metabolites from the *Eucalyptus camaldulensis* Dehnh. plant for the First Time. *IJDDT*. **2022**, *12*, 55–62.

147. Abdelkhalek, A.; Kordy, M.A.; Salem, Z.M.A.; Behiry, I.S. Antiviral, antifungal, and insecticidal activities of Eucalyptus bark extract: HPLC analysis of polyphenolic compounds. *Microbial Pathogenesis.***2020**, *147*, 104383.
148. Joshi, A.; Sharma, A.; Bachheti, R.K.; Pandey, D.P. A comparative study of the chemical composition of the essential oil from *Eucalyptus globulus* growing in Dehradun (India) and around the world. *Orient. J. Chem.* **2016**, *32*, 331–340.
149. Derwich, E.; Benziane, Z.; Chabir, R.; Bouseta, A. Antifungal activity and gas chromatography coupled with mass spectrometry (GC-MS) leaf oil analysis of essential oils extracted from *Eucalyptus globulus* (Myrtaceae) of north centre region of Morocco. *Afr. J. Pharm. Pharmacol.* **2013**, *7*, 1157–1162.
150. Luís, Â.; Duarte, A.; Gominho, J.; Domingues, F.; Duarte, A.P. Chemical composition, antioxidant, antibacterial and anti-quorum sensing activities of *Eucalyptus globulus* and *Eucalyptus radiata* essential oils. *Ind. Crops Prod.* **2016**, *79*, 274–282.
151. Bachheti, R.K. Chemical composition and antibacterial activity of the essential oil from the leaves of *Eucalyptus globulus* collected from Haramaya University, Ethiopia. *Der Pharma Chem.* **2015**, *7*, 209–214.
152. Kassahun, A.; Feleke, G. Chemical composition and physico-chemical analysis of *Eucalyptus globulus* leave and oil. *Sci. J. Chem* .**2019**, *7*, 36–38.
153. Ait-ouazzou, A.; Conchello, P. Chemical composition and antimicrobial activity of essential oils of Thymus algeriensis, *Eucalyptus globulusand Rosmarinus officinalis* from Morocco. *J. Sci. Food Agric.* **2011**, *91*, 2643–2651.
154. Maciel, M.V.; Morais, S.M.; Bevilaqua, C.M.L.; Silva, R.A.; Barros, R.S.; Sousa, R.N.; Sousa, L.C.; Brito,E.S.; Souza-Neto,M.A. Chemical composition of Eucalyptus spp. essential oils and their insecticidal effects on Lutzomyia longipalpis. *Veterinary Parasitology.* **2010**, *167*, 1–7.
155. Abbasi, N.; Khalighi, Z.; Eftekhari, Z.; Bahmani, M.Extraction and phytoanalysis of chemical compounds of *Eucalyptus globulus* leaf native to Dehloran, Ilam Province, Iran by HS-SPME and GC-MS. *Research Article.* **2020**, *8*, 647–652.
156. Shiferaw, Y.; Kassahun, A.; Tedla, A.; Feleke, G.; Ayalew, A. Investigation of Essential Oil Composition Variation with Age of *Eucalyptus Globulus* Growing in Ethiopia. *Nat Prod Chem Res.* **2019**, *7*, 1–5.

157. Kumar, P.; Mishra, S.; Malik, A.; Satya, S. Compositional analysis and insecticidal activity of *Eucalyptus globulus* (family: Myrtaceae) essential oil against housefly (Musca domestica). *Acta Trop.* **2012**, *122*, 212–218.
158. Stackpole, D.J.; Vaillancourt, R.E.; Alves A.; Rodrigues, J.; Potts, B.M. Genetic Variation in the Chemical Components of *Eucalyptus globulus* Wood. *G3 (Bethesda).* **2011**, *1*, 151–159.
159. Gouveia, S.; Fernández, C.C.; Sanromán, M.A.; Moldes, D. Enzymatic polymerisation and effect of fractionation of dissolved lignin from *Eucalyptus globulus* Kraft liquor. *Bioresour Technol.* **2012**, *121*, 131–138.
160. Gurav, N.V.; Gade, R.M.;Choudhari, R.J. Phytochemical and Thin Layer Chromatographic Analysis of Chloroform and Methanol Extracts of *Azadirachta indica* and *Eucalyptus globulus* Leaves. *Int. J. Plant Soil Sci.* **2023**, *35*, 502–513.
161. Abirami, S.; NishanthinI, K.; Poonkothai, M.Antimicrobial activity and phytochemical screening of the leaf extracts of *Eucalyptus globulus*.*Int J Curr Pharm Res*. **2017**, *9*, 85–89.
162. Suman, P.; Keshamma, E. Qualitative and Quantitative Phytochemical Analysis and Evaluation of Biological Activity of Leaf Extract of *Eucalyptus globulus*. *Int .J. Curr. Microbiol. App. Sci.* **2019**, *8*, 3442–3449.
163. Nile, S.H.; Keum, Y.S. Chemical composition, antoxidant, anti-inflammatory and antitumor activities of *E. globulus Labill*. *Indian J. Exp. Biol.* **2018**, *56*, 734–742.
164. Mworia, J. K.; Kibiti, C. M.; Ngugi, M. P.;Ngeranwa, J. N. Antipyretic Potential of Dichloromethane Leaf Extract of *Eucalyptus globulus* (Labill) and *Senna didymobotrya* (Fresenius) in Rats Models. *Heliyon.* **2019**, *5*, 1–8.
165. Ibrahim, M.; Ambreen, S.; Hussain, A.; Hussain, N.; M. Imran, M.; Ali, B.; Sumrra, S.H.; Yousuf, M.;Rehmani, F.S. Phytochemical Investigation on *Eucalyptus globulus* Labill. *Asian J. Chem.* **2014**, *26*, 1011–1014.
166. Prashant, T.; Bimlesh, K.; Mandeep, K.; Gurpreet, K.; Haleen, K. Phytochemical screening and extraction. *Inter. Pharma. Sci.* **2011**, *1*, 98–104.
167. Ganesh, S.; Vennila, J.J. Phytochemical Analysis of *Acanthus ilicifolius* and *Avicennia officinal*By GC-MS. *Res. J. Phytochem.* **2011**, *5*, 60–65.
168. Okamoto, Y.; Suzuki, A.; Ueda, K.; Ito, C.; Itoigawa. M.; Furukawa, H.; N, K. Anti-Estrogenic Activity of Prenylated Isoflavones from *Millettia pachycarpa*: Implications for Pharmacophores and Unique Mechanisms. *J. Health. Scie.* **2006**, *52*,

186–191.

169. Ishnava, B.K; Chauhan, J.B.; Barad, M.B. Anticariogenic and phytochemical evaluation of Eucalyptus globules. Saudi J Bio Sci, **2012**, *20*, 69-74.
170. Jamshidi-Kia, F.; Lorigooini, Z.; Amini-Khoei, H. Medicinal Plants: Past History and Future Perspective. *Journal of Herbmed Pharmacology*, **2018**, *7*, 57-90.
171. Parameswari, P.; Devika, R.; Vijayaraghavan, P. *In Vitro* Anti-Inflammatory and Antimicrobial Potential of Leaf Extract from *Artemisia nilagirica (Clarke) Pamp*. *Saudi Journal of Biological Sciences*, **2019**, *26*, 460- 463.
172. Debnath, B.; Singh, W.S.; Das, M.; Goswami, S.; Singh, M. K.; Maiti, D.; Manna, K. Role of plant alkaloids on human health: A review of biological activities. *Materials today chemistry*, **2018**, *9*, 56-72.
173. Tiwari, P.; Kumar, B.; Kaur, M.; Kaur, G.; Kaur, H. Phytochemical screening and Extraction: A Review. *Internationale Pharmaceutica Sciencia.* **2011**, *1*, 98-106.
174. Guardia, T.; Rotelli, A. E.;Juarez, A. O.; Pelzer, L. E. Anti-inflammatory Properties of Plant Flavonoids. Effects of Rutin, Quercetin and Hesperidin on Adjuvant Arthritis in Rat. *Farmaco*, **2001**, *56*, 683-7.
175. Hertog, M. G.; Kromhout, D.; Aravanis, C.; Blackburn, H.; Buzina, R.; Fidanza, F. Flavonoid Intake and Long-Term Risk of Coronary Heart Disease and Cancer in the Seven Countries Study. *Archives of Internal Medicine*, **1995**, *155*, 381-6.
176. Park, E. S.; Moon, W. S.; Song, M. J.; Kim, M. N.; Chung, K. H.; Yoon, J. S. Antimicrobial Activity of phenol and Benzoic Acid Derivatives. *International biodeterioration and biodegradation*, **2001**, *47*, 209-14.
177. Kataev, V. E.; Strobykina, I. Yu.; Andreeva, O. V.; Garifullin, B. F.; Sharipova, R. R.; Mironov, V. F.; Chestnova, R.V. Synthesis and Antituberculosis Activity of Derivatives of Stevia Rebaudiana Glycoside Steviolbioside and Diterpenoid Isosteviol Containing Hydrazone, Hydrazide, and Pyridinoyl Moieties. *Russian Journal of Bioorganic Chemistry*, **2011**, *37*, 483-91.
178. Böhme, K.; Velázquez, J. B.; Calo-Mata., P. Antibacterial, Antiviral and Antifungal Activity of Essential Oils: Mechanisms and Applications. In Antimicrobial Compounds. Berlin: *Springer*, **2014**, 51-81.
179. Gutiérrez-del-Río, I.; Fernández, J.; Lombó, F. Plant nutraceuticals as antimicrobial agents in food preservation: terpenoids, polyphenols and thiols. *International journal of antimicrobial agents*, **2018**, *52*, 309-315.

180. Desai, S. D.; Desai, D. G.; Kaur, H. Saponins and their biological activities. *Pharma Times*, **2009**, *41*, 13-16.
181. Fujiki, H.; Imai, K.; Nakachi, K.; Shimizu, M.; Moriwaki, H.; Suganuma, M. Challenging the Effectiveness of Green Tea in Primary and Tertiary Cancer Prevention. *Journal of Cancer Research and Clinical Oncology*, **2012**, *138*, 1259-70.
182. Trouillas, P.; Calliste, C. A.; Allais, D. P.; Simon, A.; Marfak, A.; Delage, C.; Duroux, J. L. Antioxidant, Anti-inflammatory and Antiproliferative Properties of Sixteen Water Plant Extracts Used in the Limousin Countryside as Herbal Teas. *Food Chemistry,* **2003**, *80*, 399-407.
183. Brito Arias, M. Synthesis and Characterization of Glycosides. Springer, **2007**, ISBN 9780387262512.
184. Beg, F.; Bhatia, A.M.; Baig, I.U. Biological Activity of Modified Chrysin. *International Journal of Multidisciplinary and Current Research.* **2014**, *2*, 126-130.
185. Caro, Y., Anamale, L., Fouillaud, M. et al. Natural hydroxyanthraquinoid pigments as potent food grade colorants: an overview. *Nat. Prod. Bioprospect.* **2012**, *2*, 174–193.
186. Osman, C.P.; Ismail, N.H.; Ahmad, R.; Ahmat, N.; Awang, K.; Jaafar, F.M. Anthraquinones with Antiplasmodial Activity from the Roots of Rennellia elliptica Korth. (Rubiaceae). *Molecules.* **2010**, *15*, 7218-7226.
187. Ranjitha, S.; Rajarajan, G.; Gnanendra, T.S.; Anbarasan, P.M.; Aroulmoji, V. Structural and optical properties of Purpurin for dye-sensitized solar cells. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy.* **2015**, *149*, 997–1008.
188. Watroly, M.N.; Sekar, M.; Fuloria, S.; Gan, S.H.; Jeyabalan, S.; Wu, Y.S.; Subramaniyan, V.; Sathasivam, K.V.; Ravi, S.; Rani, I.M.; Lum, P.T.; Vaijanathappa, J.; Meenakshi, U.D.; Mani, S.; Fuloria, N.K. Chemistry, Biosynthesis, Physicochemical and Biological Properties of Rubiadin: A Promising Natural Anthraquinone for New Drug Discovery and Development. *Drug Design, Development and Therapy*. **2021**, *15*, 4527–4549.
189. Vahur, S.; Teearu ,A.; Peets, P.; Joosu, L.;Leito, I. ATR-FT-IR spectral collection of conservation materials in the extended region of 4000-80 cm^{-1}. *Anal Bioanal Chem.* **2016**, *408*, 3373-9.
190. Talebi, M.; Talebi, M.; Farkhondeh, T.; Kopustinskiene, D.M.; Simal-Gandara, J.; Bernatoniene, J.; Samarghandian, S. An updated review on the versatile role of chrysin in neurological diseases: Chemistry, pharmacology, and drug delivery

approaches.*Biomedicine & Pharmacotherapy*. **2021**, *141*, 1-13.

191. Song, W.J.; Li, Y.H.; Wang, J.G.; Li, Z.Y.; Zhang, J.Q. Characterization of nucleobases and nucleosides in the fruit of Alpinia oxyphylla collected from different cultivation regions. *Drug Test Anal.* **2014**, *6*, 239-245.
192. Qing, Z.J.; Yong, W.; Hui, L.Y.;Yong, L.W.;MLong, L.H.; Ao, D.J.; Pei Li Xia, P.L. Two new natural products from the fruits of Alpinia oxyphylla with inhibitory effects on nitric oxide production in lipopolysaccharide activated RAW264.7 macrophage cells. *Arch Pharm Res.* **2012**, 35, 2143-2146.
193. Medina, J.H.; Paladini, A.C.; Wolfman, C.;Levi de Stein, M.; Calvo, D.; Diaz, L.E.; Peña, C. Chrysin (5,7-di-OHflavone), a naturally occurring ligand for benzodiazepine receptors, with anticonvulsant properties. *Biochem Pharmacol.* **1990**, *40*, 2227-2231.
194. Anandhi, R.; Annadurai, T.; Anitha, T.S.;Muralidharan, A.R.; Najmunnisha, K.; Nachiappan, V.; Thomas, P.A.; Geraldine, P. Antihypercholesterolemic and antioxidative effects of an extract of the oyster mushroom, Pleurotus ostreatus, and its major constituent, chrysin, in Triton WR-1339-induced hypercholesterolemic rats. *J Physiol Biochem.* **2013**, *69*, 313-323.
195. Meckes,M.; Paz, D.; Acosta, J.; Mata, R. The effects of chrysin and pinostrobin, two flavonoids isolated from Teloxys graveolens leaves, on isolated guinea-pig ileum.*Phytomedicine*. **1998**, *5*, 459–463.
196. Abd El-Hady, F.K.; Shaker, K.; Imhoff, J.F.; Zinecker, H. Bioactive metabolites from propolis inhibit superoxide anion radical, acetylcholinesterase and phosphodiesterase (PDE4). *Int. J. Pharm. Sci. Rev. Res.* **2013**, *21*, 338–344.
197. Kurek-Gorecka, A.; Gorecki, M.; Rzepecka-Stojko, A.; Balwierz, R.; Stojko, J. Bee products in dermatology and skin care. *Molecules*. **2020**, *25*, 556-572.
198. Zhu, L.; Lu, Y.; Yu, W.G.; Zhao, X.; Lu, Y.H. Anti-photoageing and antimelanogenesis activities of chrysin. *Pharm. Biol.* **2016**, *54*, 2692–2700.
199. Mouri, C.; Laursen, R.A. Identification of anthraquinone markers for distinguishing Rubia species in madder-dyed textiles by HPLC. *Microchimica Acta.* **2012**, *179*, 105-113.
200. Zeng, W.; Shen, C.; Mo, S.; Ni, C.; Lin, Y.; Fang, Y.; Yang, H.; Luo, G.; Xiao, L.; Zhan, R.; Yan, P. The Effective Treatment of Purpurin on Inflammation and Adjuvant-Induced Arthritis. *Molecules.* **2023**, *28*, 366-381.
201. Wouters, J. High Performance Liquid Chromatography of Anthraquinones: Analysis of

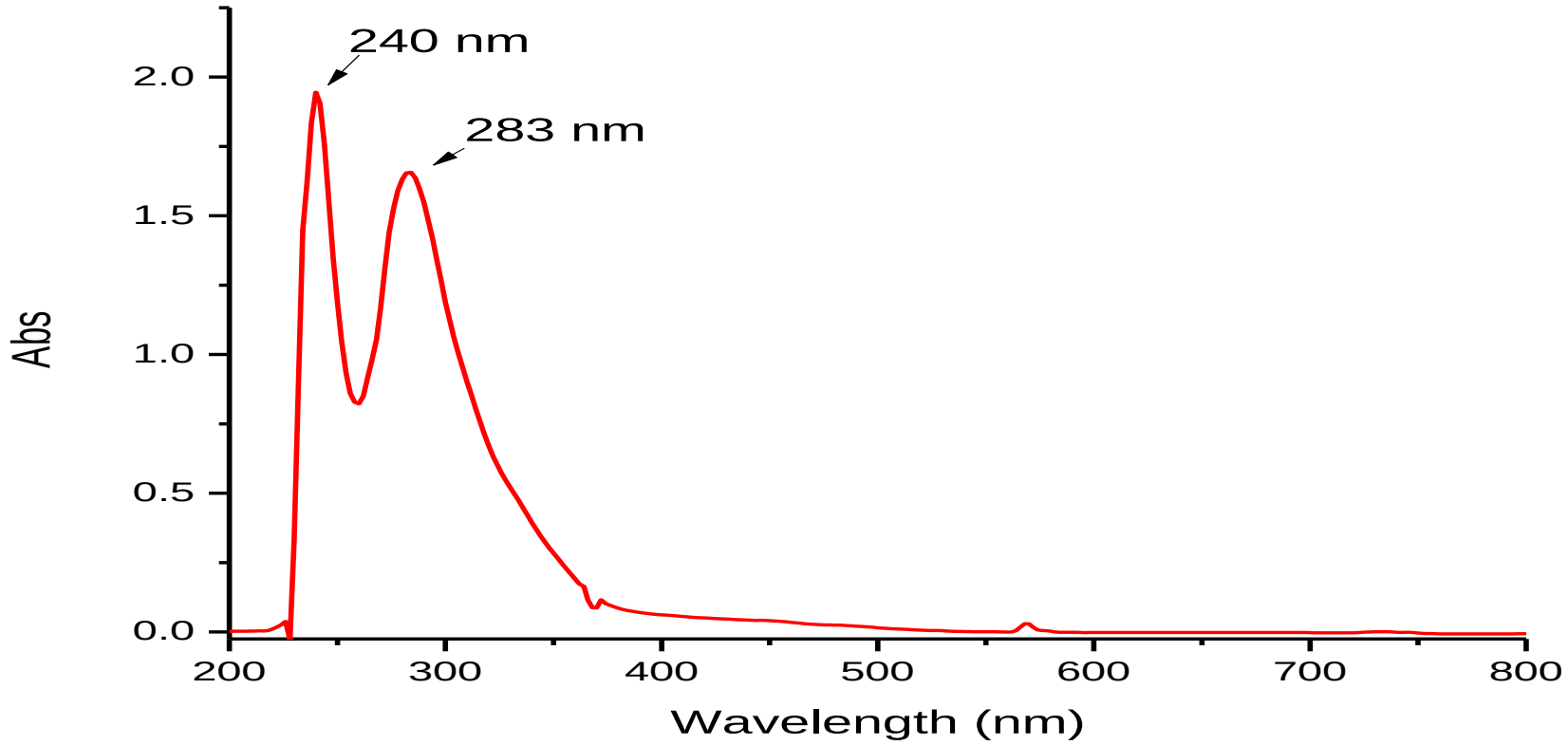
Plant and Insect Extracts and Dyed Textiles. *Studies Conserv.* **1985**, *30*, 119-128.

202. Marković;Z.; Manojlović, N.; Svetlana, J.; Živić, M.HPLC, UV–vis and NMR spectroscopic and DFT characterization of purpurin isolated from Rubia tinctorum L.*Hem. ind.* **2013**, *67*, 77–88.
203. Han, Y.S.; der Heijden, R.V.; Verpoorte, R. 2001. Biosynthesis of anthraquinones in cell cultures of Rubiaceae. *Plant Cell Tiss. Org.* **2001**, *67*, 201-220.
204. Singh, J.; Hussain, Y.; Luqman, S.; Meena, A. Purpurin: A natural anthraquinone with multifaceted pharmacological activities. *Phytotherapy Research.* **2020**, *35*, 2418–2428.
205. Zengin, G.; Degirmenci, N.S.; Alpsoy, L.; Aktumsek, A. Evaluation of antioxidant, enzyme inhibition, and cytotoxic activity of three anthraquinones (alizarin, purpurin, and quinizarin). *Hum. Exp. Toxicol.* **2016**, *35*, 544–553.
206. Nam, W.; Nam, S.H.; Kim, S.P.; Levin, C.; Friedman, M. Anti-adipogenic and anti-obesity activities of purpurin in 3T3-L1 preadipocyte cells and in mice fed a high-fat diet. *BMC Complement. Altern. Med.* **2019**, *19*, 364.
207. Suratman, S. The Indonesian Species of Rennellia Korth. (Rubiaceae). *Journal of Biological Diversity*. **2008**, *9*, 259–263.
208. Osman, C.P.; Ismail, N.H.; Ahmad, R.; Ahmat, N.; Awang, K.; Jaafar, F.M. Anthraquinones with antiplasmodial activity from the roots of Rennellia elliptica Korth. (Rubiaceae). Molecules. **2010**, *15*, 7218–7226.
209. Ahmad, R.; Mahbob, E. N. M.; Noor, Z. M.; Ismail, N. H.; Lajis, N. H.; Shaari, K. Evaluation of antioxidant potential of medicinal plants from Malaysian Rubiaceae (subfamily Rubioideae). *African Journal of Biotechnology*. **2010**, *9*, 7948- 7954.

APPENDICES

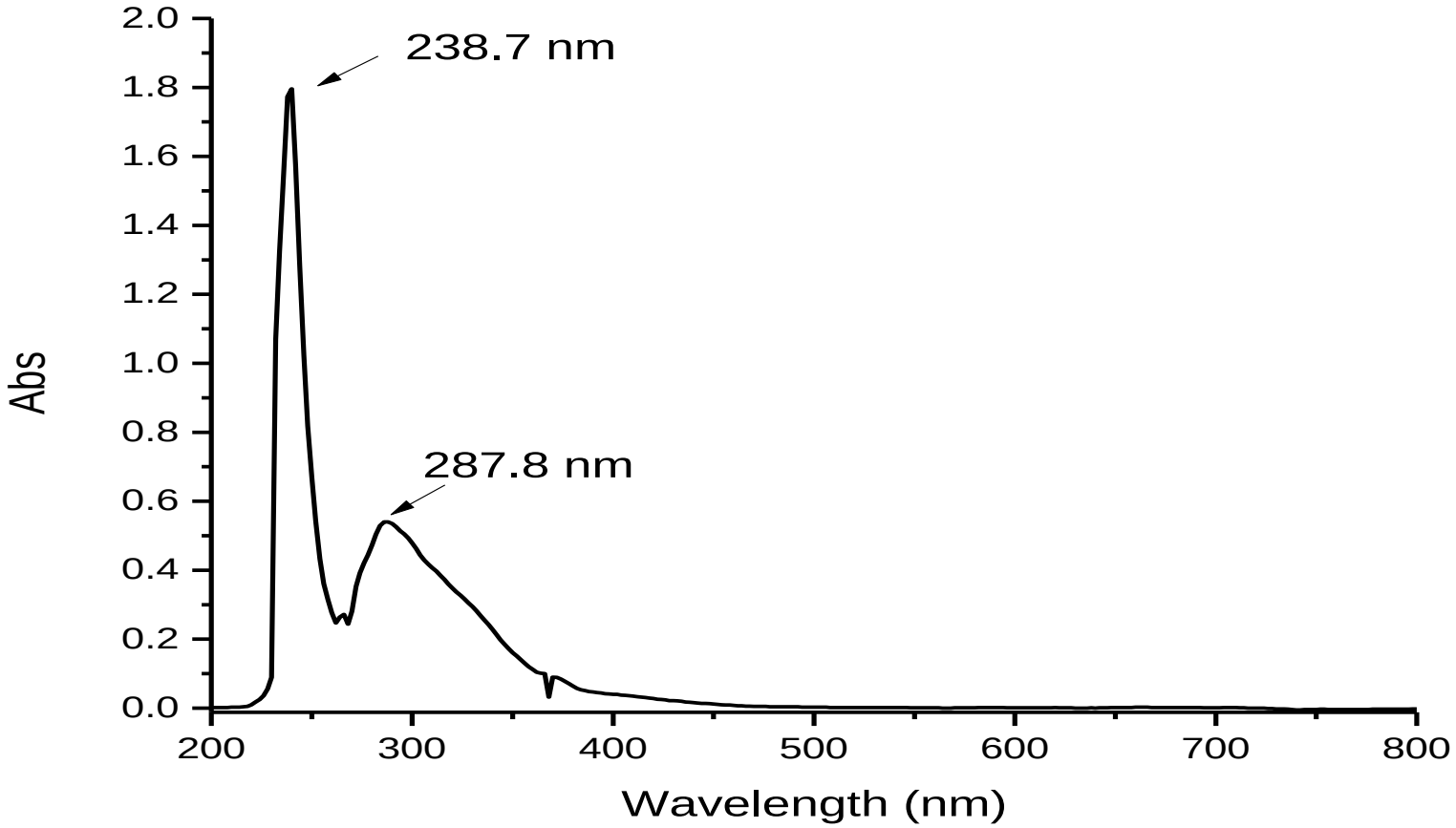
Appendix 1: UV-Vis Spectra of Isolated Compounds

UV-Vis spectra of compound 1 (EG-1)



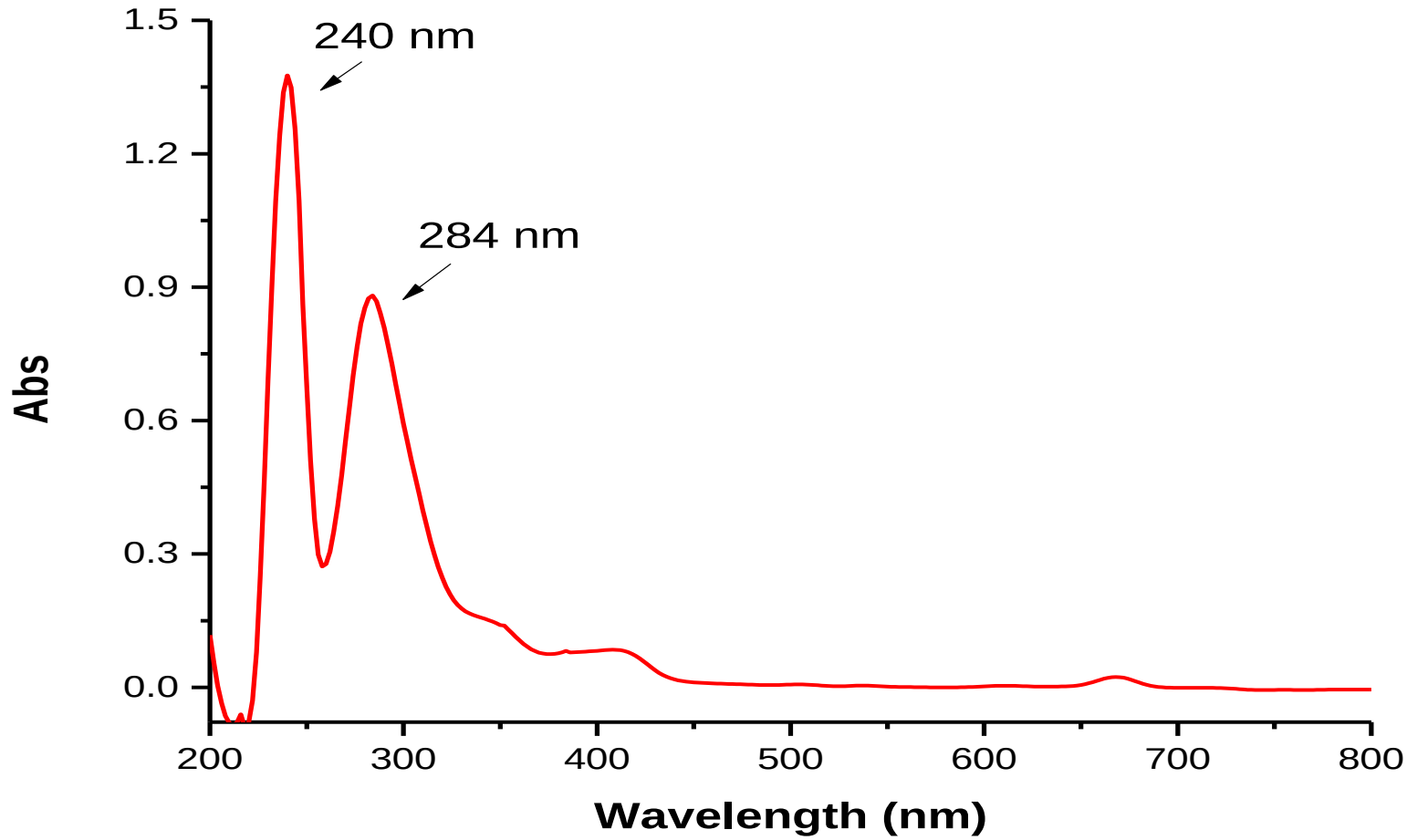
Appendix 2: UV-Vis Spectra of Isolated Compounds

UV-Vis spectra of compound 2 (EG-2)



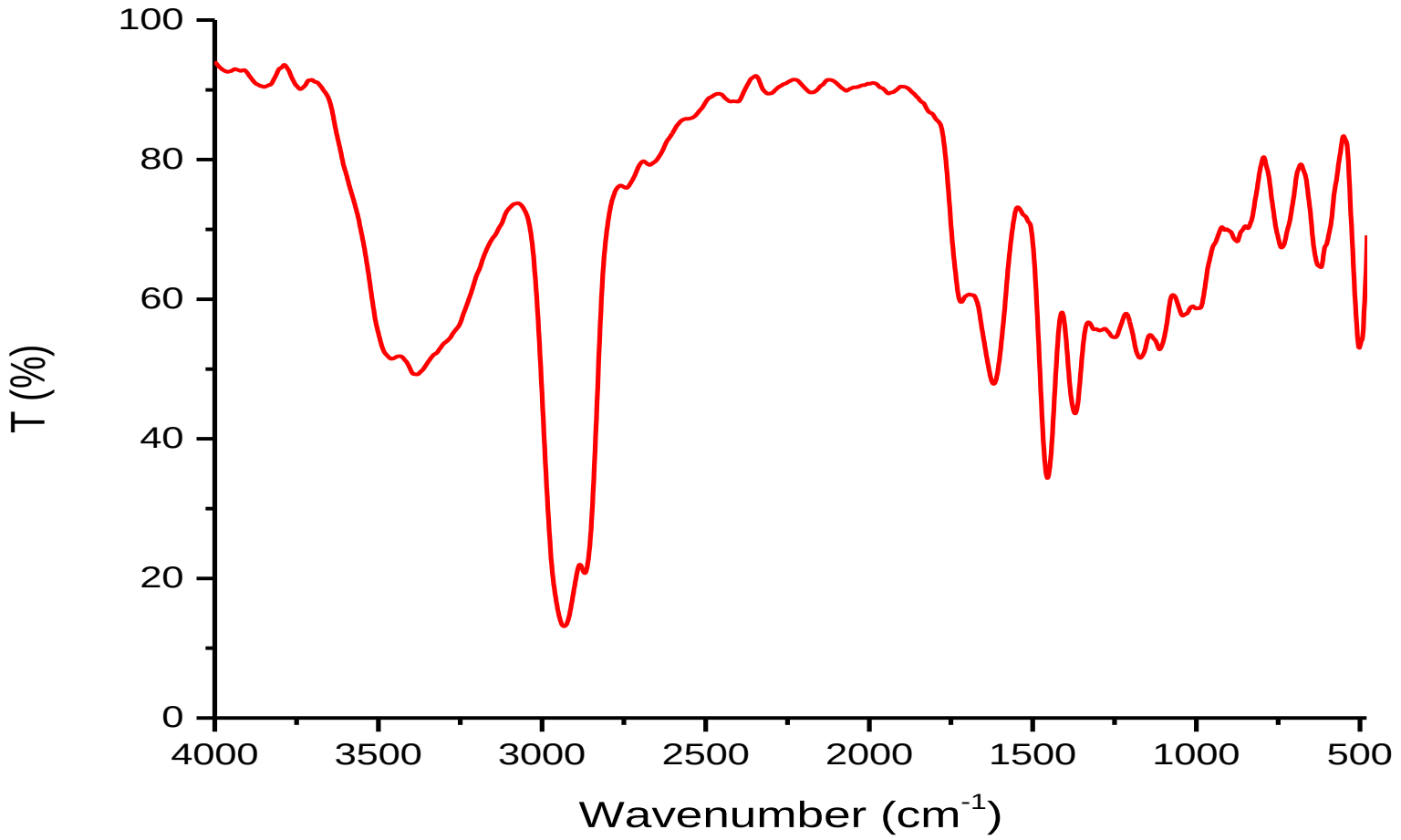
Appendix 3: UV-Vis Spectra of Isolated Compounds

UV-Vis spectra of compound 3 (EG-3)



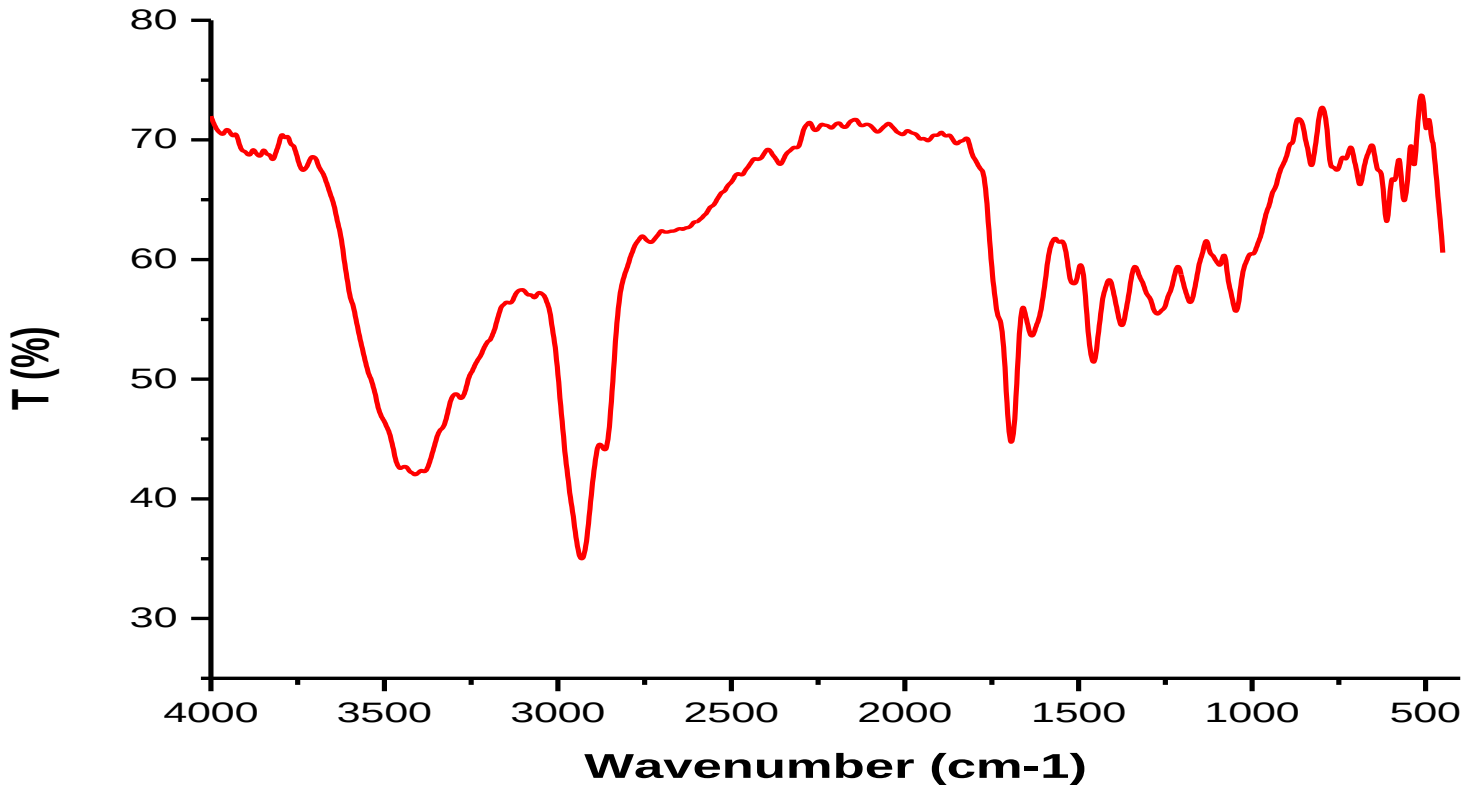
Appendix 4: IR Spectra of Isolated Compounds

IR spectra of compound 1 (EG-1)

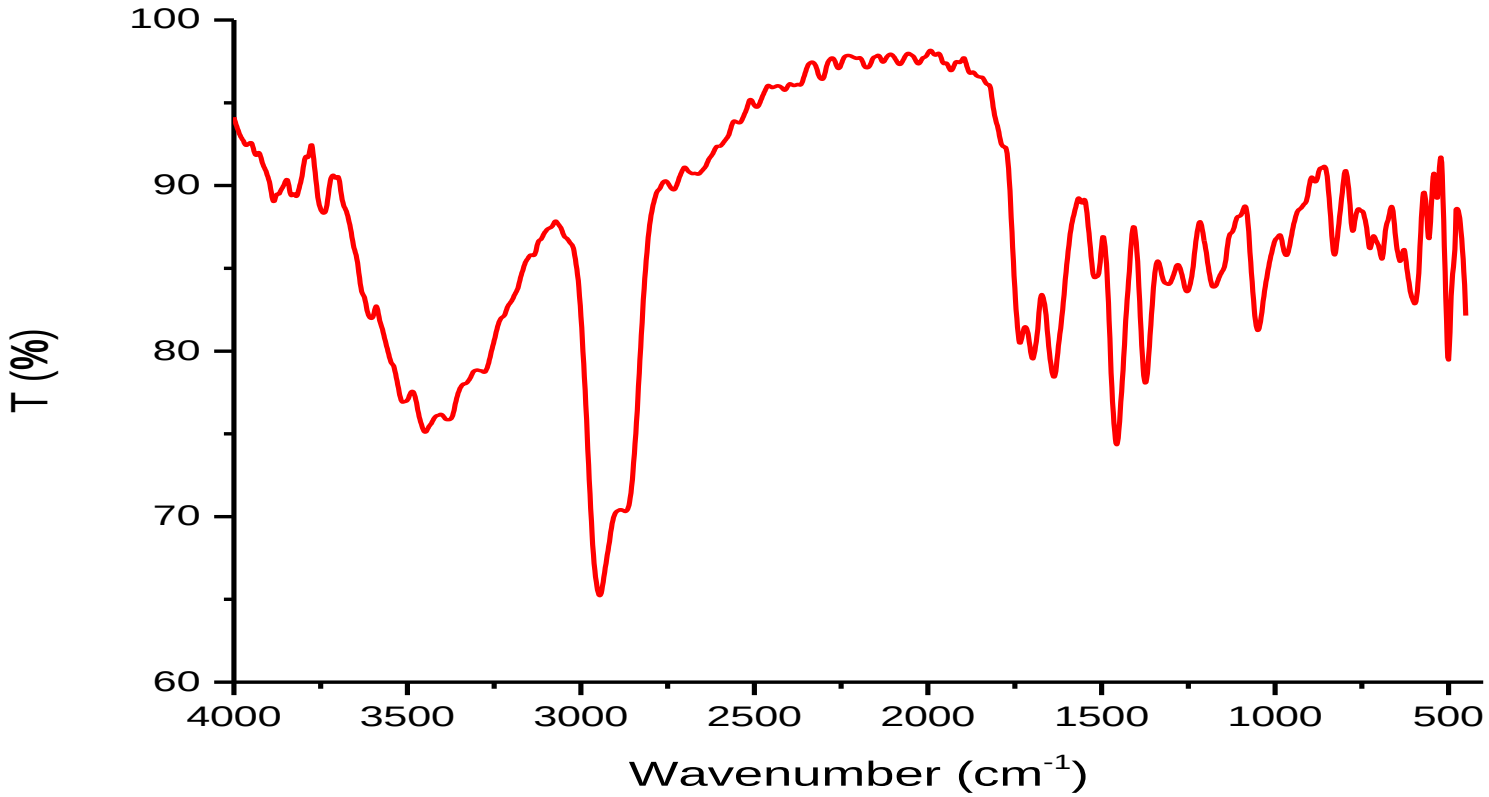


Appendix 5: IR Spectra of Isolated Compounds

IR spectra of compound 2 (EG-2)

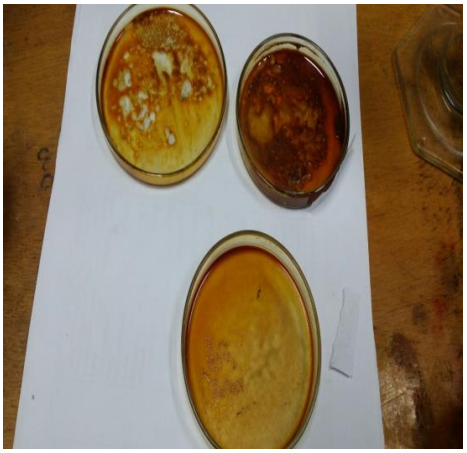


Appendix 6: The IR spectra of compound 3 (EG-3)



Appendix 7: Laboratory equipment and sample photos taken during the experiment sessions

a) Sample (powder)preparation and extraction photos

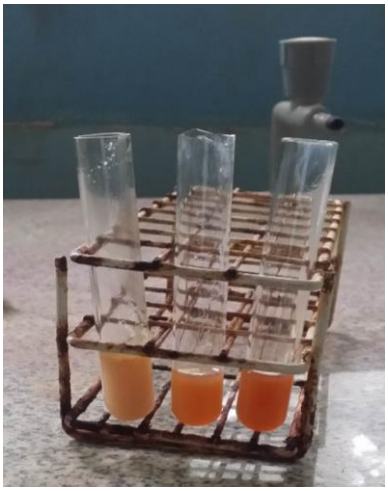


b) Filtration and concentrating the crude extract using rotary evaporator

c)Researcher and solvents used for extraction and fractionation

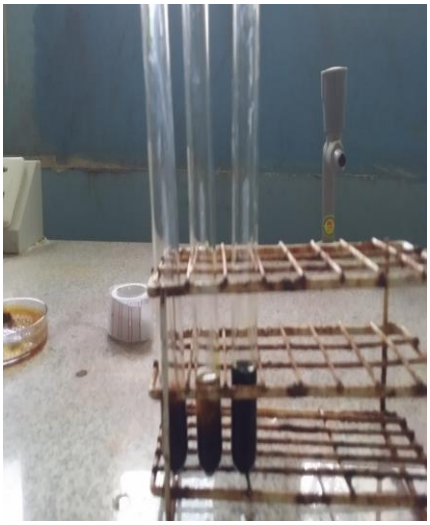
d)(colour less ,+ result for flavonoide)

e)redish browen coloration (+ test terpenoid)



f)Appearance of Precipitate(+ test result for alkaloid)

g)Phytochemical screening test result yellow with green flurescent (+ test for stroid), h)green or blue black precipitate (+ test for tennine)

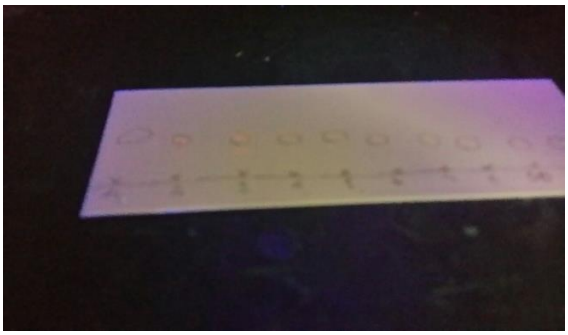


i)formation of stable foam (+ test for saponins)

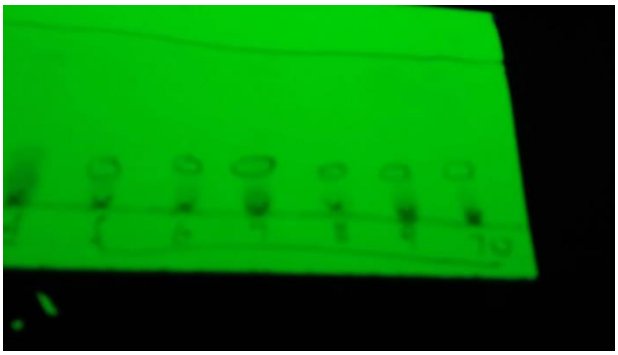
j)a reddish browen ring (glycoside)

k)formation of bluish black (phenol)

l)TLC profile of fraction(34-40) collection from the column



m)TLC profile of fraction (41-50) collected from column



n) TLC profile of fraction (65-70).