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DEPARTMENT OF CHEMISTRY

PHYTOCHEMICAL INVESTIGATION AND ANTIBACTERIAL ACTIVITY TESTS ON ROOT EXTRACT OF *EUPHORBIA CANDELABRUM*

BY

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JUNE, 2024

HAWASSA, ETHIOPIA

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KIFLE JILO

A THESIS SUBMITTED TO THE DEPARTMENT OF CHEMISTRY, COLLEGE OF NATURAL AND COMPUTATIONAL SCIENCE, SCHOOL OF GRADUATE STUDIES IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN ORGANIC CHEMISTRY

JUNE, 2024

HAWASSA, ETHIOPIA

DECLARATION

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

Name: Kifle Jilo

Signature: _____

This thesis has been submitted for examination with my approval as a University.

Advisor

Name of advisor: Professor Legesse Adane

Signature: _____

APPROVAL SHEET-1

This is to certify that the thesis entitled "**Phytochemical investigation and antibacterial activity on the root extract of *Euphorbia candelabrum***" submitted in partial fulfilment 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, is a record of original research carried out **by Kifle Jilo Jego** (ID, No GPChmR/0007/15), 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.

Professor Legesse Adane	_____	_____
Name of advisor	Signature	Date

APPROVAL SHEET-2

We who are members of the broad range of Examiners of the final defense by Kifle Jilo Jego have read and evaluated this thesis entitled "**Phytochemical investigation and antibacterial activity of the root extract of *Euphorbia candelabrum***". This is therefore to certify that; the thesis has been accepted as partial fulfillment of the requirements for the degree of masters of Science in chemistry with specialization in organic chemistry.

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Name of Internal Examiner	Signature	Date
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ABBREVIATIONS AND ACRONYMS

DMESO	Dimethyl sulfoxide
FT-IR	Fourier Transform Infrared Spectroscopy
IC_{50}	Half maximal inhibition concentration
MIC	Minimum inhibition concentration
TLC	Thin Layer Chromatography
UV-Vis	Ultraviolet Visible
DE	Dexamethasone equivalence
GAE	Gallic acid equivalence
QE	Quercetin equivalence
AE	Atropine equivalence

ABSTRACT

Euphorbia candelabrum is a medicinal plant that is known to be used for the treatment of various diseases such as coughs, tuberculosis, malaria, and wound healing and for treating various skin disorders in different communities. This study aimed to investigate the chemical constituents and characterize the compounds isolated from the root extract of Euphorbia candelabrum using UV-Vis and FT-IR spectroscopy as well as to test its antibacterial activity. Due to its medicinal importance, the plant material was collected from, the Sika kebele, Bule woreda, Gedeo zone, Southern Ethiopian region. The plant material was air dried and sequentially extracted with n-hexane, dichloromethane, dichloromethane/methanol (1:1 v/v) and methanol. Phytochemical screening of the dichloromethane, dichloromethane/methanol (1:1 v/v) and methanol crude extracts revealed the presence of alkaloids, flavonoids, phenols, glycosides, terpenoids, tannins, saponins and steroids, whereas the n-hexane crude extracts contained alkaloids, glycosides, terpenoids, steroids and saponins. The total contents of flavonoids, phenolics, alkaloids, and steroids in the dichloromethane/methanol (1:1 v/v) crude extract were 10.47 mg QE/g, 13.4 mg GAE/g, 1.426 mg AE/g, and 2.91 mg DE/g , respectively. The methanol extract contained 11 mg QE/g, 19 mg GAE/g, and 2.1 mg AE/g of extract for the total of flavonoid, phenolic, and alkaloid contents, respectively. Column chromatographic separation of dichloromethane/methanol (1:1 v/v) resulted in the isolation of three compounds (KJ-1, KJ-2 and KJ-3) and structure of the isolated compounds were not fully characterized due to lack of NMR instrument. The crude extracts and isolated compounds were tested against four bacterial strains in vitro (E. coli, P. aeruginosa, S. aureus and S. pyogen) using the disc diffusion method. The antibacterial activity tests revealed that the isolated compounds had greater activity than did the crude extracts. Notably, compound KJ-3 demonstrated the highest antibacterial activity against all tested bacterial strains than crude extracts and isolated compounds but slightly lower than that of the reference drug tetracycline. The present study indicated that the root of Euphorbia candelabrum could be a good source of compounds for the discovery of antibacterial agents because in vitro tests were carried out on several bacterial strains.

Key words: *Euphorbia candelabrum*, antibacterial activity, phytochemical screening, root extract, Quantitative analysis

1. INTRODUCTION

1.1 Back ground of the study

Medicinal plants, as gifts from nature, have been utilized by humans for centuries to treat both human and animal illnesses, through traditional medicine or traditional healers [1]. Its various parts, such as the root bark, roots and aerial parts, have a long history of use in various people of different cultural and economic backgrounds worldwide. The precise origins of the initial use of medicinal plants remain unknown. It appears that the quest for remedies emerged concurrently across cultures, driven by humanity's desire to heal, whether for magical or religious purposes or due to the discovery of preparations that provided temporary relief [2]. These plants are used to treat specific ailments as well as to provide general tonics, meals, and other methods to enhance the body's immunity and vigor [3]. Some of the most common uses are pain management, digestive health, wound healing, respiratory health, stress relief and relaxation, boosting immunity, cardiovascular health, hormonal balance, antibacterial and antiviral properties, and pain relief in cancer care. These are just a few examples of the broad spectrum of uses of medicinal plants [2, 3].

One hundred seventy (170) nations have documented the utilization of traditional medicine, with acupuncture emerging as the prevalent form of practice in 113 countries. Even in developed countries, there is a growing recognition and integration of traditional medicine into healthcare frameworks [4]. Approximately 80% in developing countries rely on medicinal plants to address their health issues [5]. Many of these plants are easily accessible in rural areas, which make them comparatively more affordable than modern medicine [6].

The medicinal uses of these plants can be attributed to the presence of phytochemical constituents. Phytochemicals are naturally occurring molecules found in different morphological parts of plants, such as seeds, leaves, roots, bark, and aerial parts. These plant parts contain organic compounds known as secondary metabolites, including tannins, glycosides, alkaloids, terpenoids, phenolics, steroids, coumarins, flavonoids, anthraquinones, and others [7]. These secondary metabolites are not essential for the growth, development, or reproduction of an organism. They are produced either as a result of the organism adapting to its environment or as a defense mechanism against predators, thus aiding in the survival of the organism. These secondary metabolites also demonstrate health-promoting and disease-curing properties for humans [5].

Due to their unique chemical composition and diverse modes of actions, medicinal plants have remained essential for drug development and research [8]. With time, these medicinal plants have undergone significant advancements in their ability to target a wide range of biological processes, with some becoming crucial drugs in the healthcare system [7]. The quest for medicinal plants as a source of potential drug candidates is ongoing. There are significant numbers of modern drugs currently on the market that are obtained from medicinal plants. Moreover, many more drug candidates in different phases of drug discovery programs of pharma companies have been obtained from medicinal plants [9]. Some examples of successful drugs derived from medicinal plants are listed below (Table 1, Figure 1).

Table 1 Examples of modern drugs obtained from natural products (medicinal plants)

S/N	Drug name	Derived plant name	Therapeutic uses	Reference
1	Artemisinin (**1**)	*Artemisia annua*	treatment for malaria	[10]
2	Reserpine (**2**)	*Rauvolfia serpentina*	treat high blood pressure and certain psychiatric disorders	[11]
3	Paclitaxel (**3**) and docetaxel (**4**)	*Taxus brevifolia*	used in cancer chemotherapy	[12]
4	Galantamine (**5**)	*Narcissus*	used to treat Alzheimer's disease	[13]
5	Pilocarpine (**6**)	*Pilocarpus microphyllus*	used to treat glaucoma and dry mouth	[14]
6	Withanolides (**7**)	*Withania somnifera*	Used for treat inflammation and oxidative stress	[15]

However, there are still many drugs that are derived from medicinal plants, and ongoing research into plant-based medicine continues to uncover new potential treatments. Additionally, some traditional herbal remedies have been adapted to modern pharmaceuticals. Overall, medicinal plants continue to play an important role in the development of new drugs [13-15].

Ethiopia is home to a rich diversity of medicinal plants that have been used for centuries by traditional healers to treat various ailments [16]. These plants have the potential to be further studied and developed for their medicinal properties. Some of the potential medicinal plants in Ethiopia include:

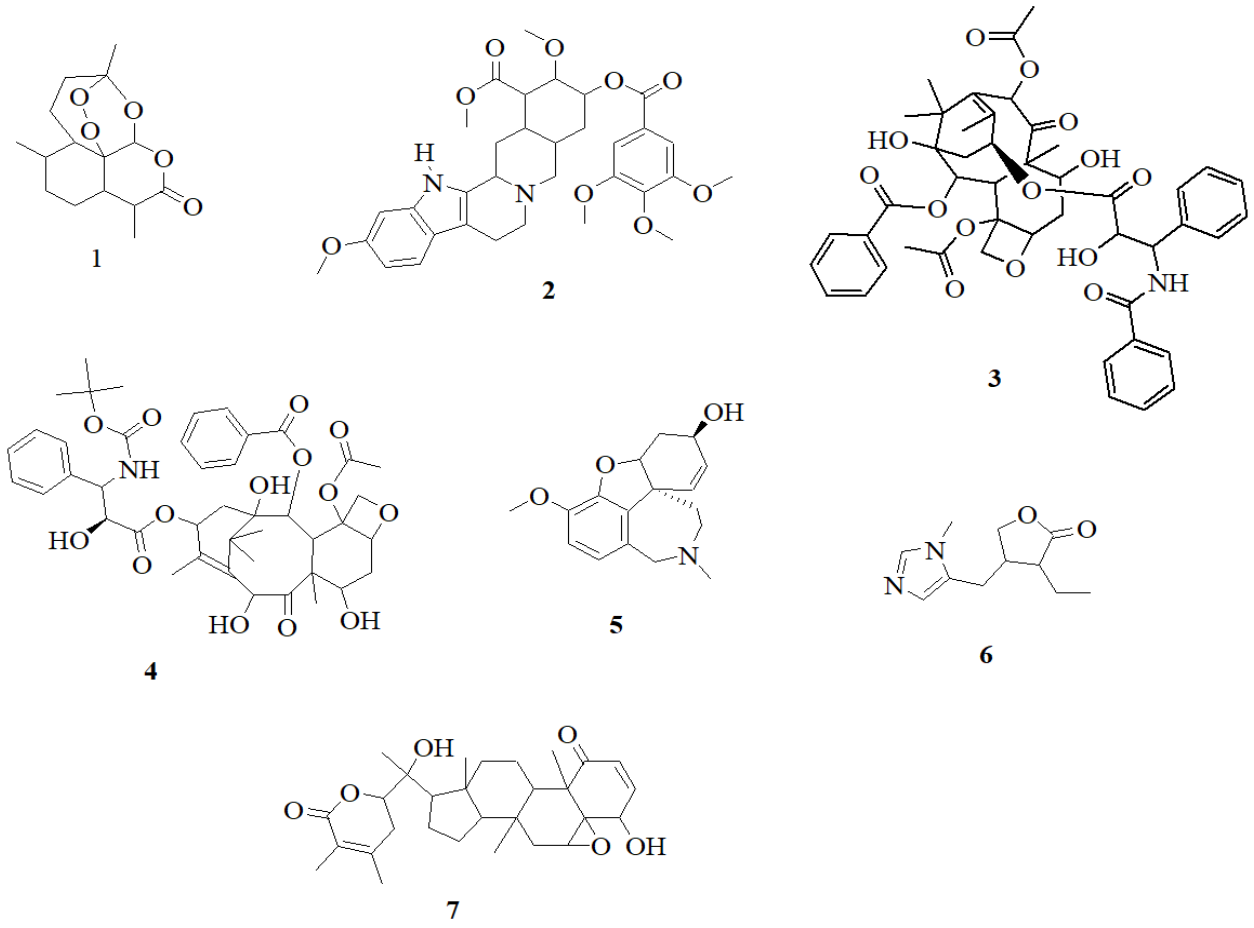


Figure 1 : Example of modern drugs derived from medicinal plants

- *Artemisia annua* (Sweet wormwood): This plant is known for its anti-malarial properties and is used in traditional Ethiopian medicine to treat malaria [17].
- *Rhamnus prinoides* (Abyssinian buckthorn): This plant has been used traditionally to treat various ailments, including skin diseases, stomachaches, and as a laxative [18].
- *Carica papaya* (Papaya): The leaves and fruit of the papaya plant are used in Ethiopian traditional medicine to treat digestive disorders and as a natural remedy for skin conditions [19].
- *Ocimum lamiifolium* (African basil): This plant is used in traditional Ethiopian medicine to treat respiratory infections, coughs, and colds [20].
- *Hagenia abyssinica* (African redwood): The bark of this tree is used in traditional medicine to treat stomach ulcers, diarrhea, and as a general tonic [21].
- *Euphorbia candelabrum* (Abyssinian Euphorbia): This plant has been used in Ethiopian traditional medicine to treat wounds, skin infections, and as a pain reliever. It also has potential anti-inflammatory and analgesic properties that could be further explored for medicinal use [22]. To date however, no phytochemical studies have been carried out on the roots of *Euphorbia candelabrum*.

1.2 Statement of the problem

Nature has provided a vast array of natural remedies for treating various ailments, with plants being a predominant source of new bioactive chemicals. In Ethiopia, traditional medicine has long been used to treat both human and livestock health issues. However, the knowledge and practice of utilizing these plants is passed down orally, hindering standardization and recognition of traditional medicine on an international level.

The main point here is that all *Euphorbia* species found in Ethiopia are not well studied from a photochemical constituent perspective. From *Euphorbia candelabrum* plant, only latex part was studied and other parts were not studied. In addition, the chemical constituents are not well known which makes it difficult to use the plant appropriately and regulate the dose and concentration. Thus, scientific experiments are needed to identify the constituents of the plant in the search for new chemically bioactive drugs. Therefore, the aim of the present study was to identify the chemical constituents and antibacterial activity of the root part of *Euphorbia candelabrum*.

1.3 Objectives

1.3.1 General objective

The overall objective of this study was to investigate the phytochemical constituents and antibacterial activity tests of root extracts of *Euphorbia candelabrum.*

1.3.2 Specific objectives

- To extract root of *Euphorbia candelabrum* using gradient solvent system (n-hexane, dichloromethane, dichloromethane/methanol (1:1 v/v) and methanol)
- To analyze phytochemical screening tests of each extracts
- To determine quantitative analysis of secondary metabolites in extracts
- To isolate compounds using column chromatography technique
- To evaluate anti-bacterial activity of the crude extracts and isolated compounds against different bacterial strains (*Escherichia coli* (gram-negative), *Pseudomonas aeruginosa* (gram-negative), *Staphylococcus aureus* (gram-positive), and *Streptococcus pyogenes* (gram-positive)) by using disc diffusion method
- To characterize the isolated compounds using spectroscopic techniques such as UV-Vis and FT-IR.

1.4 Significance of the study

The study of *Euphorbia candelabrum* provides a strong foundation for further research on various aspects of the plant. By identifying the chemical compounds present in the root extract of *Euphorbia candelabrum* and assessing their antibacterial properties, this study has the potential to significantly contribute to natural product research. This could lead to the development of new antibiotics or antibacterial agents, which is particularly crucial in light of increasing antibiotic resistance and the urgent need for effective treatments for bacterial infections.

Moreover, this study can offer valuable insights for the conservation and sustainable use of medicinal plants, while also making meaningful contributions to the broader field of natural product research. This study can also serve as a reference for other researchers interested in the phytochemical investigation of this plant.

Overall, the findings of this study have the potential to significantly impact the development of new antibacterial treatments and enhance our understanding of the chemical composition of the roots of *Euphorbia candelabrum*.

2. LITERATURE REVIEW

2.1 Botanical description of *Euphorbia*

The genus *Euphorbia* is named after the Greek physician Euphorbus, who served King Juba II of Numidia and Mauritania. Euphorbus reportedly utilized a plant known as *Euphorbia resinifera*, characterized by its milky latex and potent medicinal properties, in his medical treatments. The common name "spurge" originates from the French word "espurgier," meaning "to purge," which reflects the historical use of *Euphorbia* latex as a purgative agent [23]. *Euphorbia* is a genus of succulent plants in the *Euphorbiaceae* family and is known for its diversity, with over 2,000 species. About 1,200 of these species are succulents with unusual shapes and fleshy leaves [24]. The species in this genus, and to a larger extent of the spurge family, are characterized by the production of milky irritant white latex [25]. There are approximately 170 species of *Euphorbia* found in Ethiopia. So the majority of them are likely native to the region. These include *Euphorbia abyssinica, Euphorbia candelabrum, Euphorbia stellispina, Euphorbia erythraea, Euphorbia schimperi,* and *Euphorbia tirucali* [26].

2.2 Ethno medicinal uses of some species in genus *Euphorbia*

The leaves, stem, and root of *Euphorbia ingens* are applied in traditional medicine for the treatment of cancer and other pathologies, including swellings, fistula, lesions, wounds, abscesses, burns and mental disorders [27]. It has cultural significance and also used in traditional medicine in some African communities. In South Africa, the latex sap of *Euphorbia ingens* is used topically to treat skin conditions such as warts, corns, and calluses. The Venda and Sotho use it as a cancer remedy. It is also used as a purgative and emetic in traditional medicine practices [28]. In Namibia, the latex sap of the candelabra tree is used to treat insect bites and stings. The stem and root are used to make a decoction that is taken orally to treat stomach ailments [23]. In Botswana, various parts of *Euphorbia ingens* are used in traditional medicine to treat skin conditions, insect bites, and stomach ailments. In Zimbabwe, the latex sap of the plant is used topically to treat skin conditions, and the stem and root are used to make a decoction for stomach ailments [29].

Euphorbia neriifolia is a plant species that has been used in traditional medicine for the treatment of various diseases. Traditional healers have utilized the sap from injured *Euphorbia neriifolia* stems as a potent laxative and remedy for earaches. It has been used as a strong purgative for various conditions such as liver and spleen enlargement, dropsy, syphilis, general swelling, and leprosy. It has also been found to be beneficial in treating asthma. Practitioners of

Ayurveda and Siddha in India have reported that a mixture of the plant's juice and simple syrup, taken in doses of 10-20 drops three times a day, has been effective in completely curing asthma attacks [30]. In Nigeria, the bark has been used as a strong purgative. The root is considered antiseptic and mixed with black pepper; it is employed in the treatment of snake bites both internally and externally. The leaves are diuretic and heated, squeezed, and the sap taken, sometimes with salt, to treat asthma; wheezing in babies; colds; and stomach upset. The leaves are also used to treat fevers, coughs and colds, and diabetes [31, 32].

The ethno medicinal uses of different parts of *Euphorbia trigona* can vary by region and country. In some African countries, the latex of the plant has been used to treat skin conditions and wounds, as well as digestive issues and respiratory ailments. In other regions, the leaves or stems of the plant may be used in similar ways for traditional medicine purposes. For instance, in Nigeria, the latex of *Euphorbia trigona* has been used to treat skin conditions and wounds, while in Ethiopia, the plant has been used to treat respiratory ailments and digestive problems. In South Africa, the plant has been used for anti-inflammatory properties to alleviate pain and swelling [33-35].

There is no traditional medicinal use of *Euphorbia abyssinica* outside of Ethiopia, and it is considered a poisonous plant by the Food and Drug Administration of the United States [36]. However, in Ethiopian traditional medicine, the plant has been used to treat various diseases, including malaria. The medicinal effects are mainly found in the plant's latex and roots. In local practices, the fresh root is collected, chopped, dried, powdered, and consumed with egg and milk for the treatment of malaria [37].

Euphorbia tirucali owing to a diverse range of chemical components, the traditional medical literature from various regions, particularly tropical and subtropical areas where it is native, is imbued with accounts of its therapeutic potential. For example, in East Africa, the latex is utilized for addressing a spectrum of conditions such as sexual impotence, warts, epilepsy, toothache, hemorrhoids, snake bites, ectoparasite removal, and cough. In India, this plant is integral to traditional households and is used as a remedy for ailments including spleen enlargement, asthma, dropsy, leprosy, biliousness, leucorrhoea, dyspepsia, jaundice, colic, tumors, and bladder stones. The latex, with vesicant and rubefacient properties, is used as an emetic in large doses, a purgative in small doses, and applied for toothaches, earaches, rheumatism, warts, and cough, neuralgia, and scorpion bites [38, 39]. In regions such as Indonesia, the root infusion is used for alleviating bone discomfort, and poultices of the roots or leaves are utilized for nose ulcers, hemorrhoids, and thorn extraction. Wood decoctions are

applied against leprosy and instances of paralysis in the hands and feet following childbirth. Meanwhile, in Java, the plant's latex is employed for treating skin ailments and bone fractures [23, 40].

Euphorbia candelabrum has a longstanding tradition of use for its pain-relieving properties and its potential analgesic effects relevant to conditions such as arthritis and joint discomfort. Its versatile applications include topical use for aiding wound healing, preventing infections, and treating various skin disorders. Furthermore, it has been utilized to address respiratory issues, digestive discomfort, and fungal infections. The plant is also believed to have anti-inflammatory characteristics, potential immune system-boosting properties, and the ability to reduce fever and alleviate pain [41].

The latex is employed for medicinal purposes in Central Africa and by the Loita Maasai people to manage various health issues. In traditional medicine, the plant's sap has been employed to address a wide spectrum of conditions, including coughs, tuberculosis, malaria, and HIV infections. Finally, in traditional Ethiopian medicine, the combination of the plant's sap with clarified honey was used as a purgative for treating syphilis, and when mixed with other medicinal plants, it was applied topically to alleviate symptoms associated with leprosy [42].

2.3 Phytochemical studies of some species in genus *Euphorbia*

Medicinal plants are rich in Phytochemicals constituents. These constituents are non-nutritive chemicals that produce definite physiological effects on human body and protect them from various diseases [43]. Many studies have been conducted on the chemical analysis of *Euphorbia* species. The results showed that there are many chemical molecules. The most encountered are the diterpenoids, the triterpenoids, sterols, alkaloids, flavonoids, phenols and tannins [44].

The phytochemical screening tests of *Euphorbia ingens* revealed that the chloroform, ethyl acetate, and methanol extracts of the plant contain terpenoids, alkaloids, flavonoids, tannins, saponins, sterols, and polyphenols.

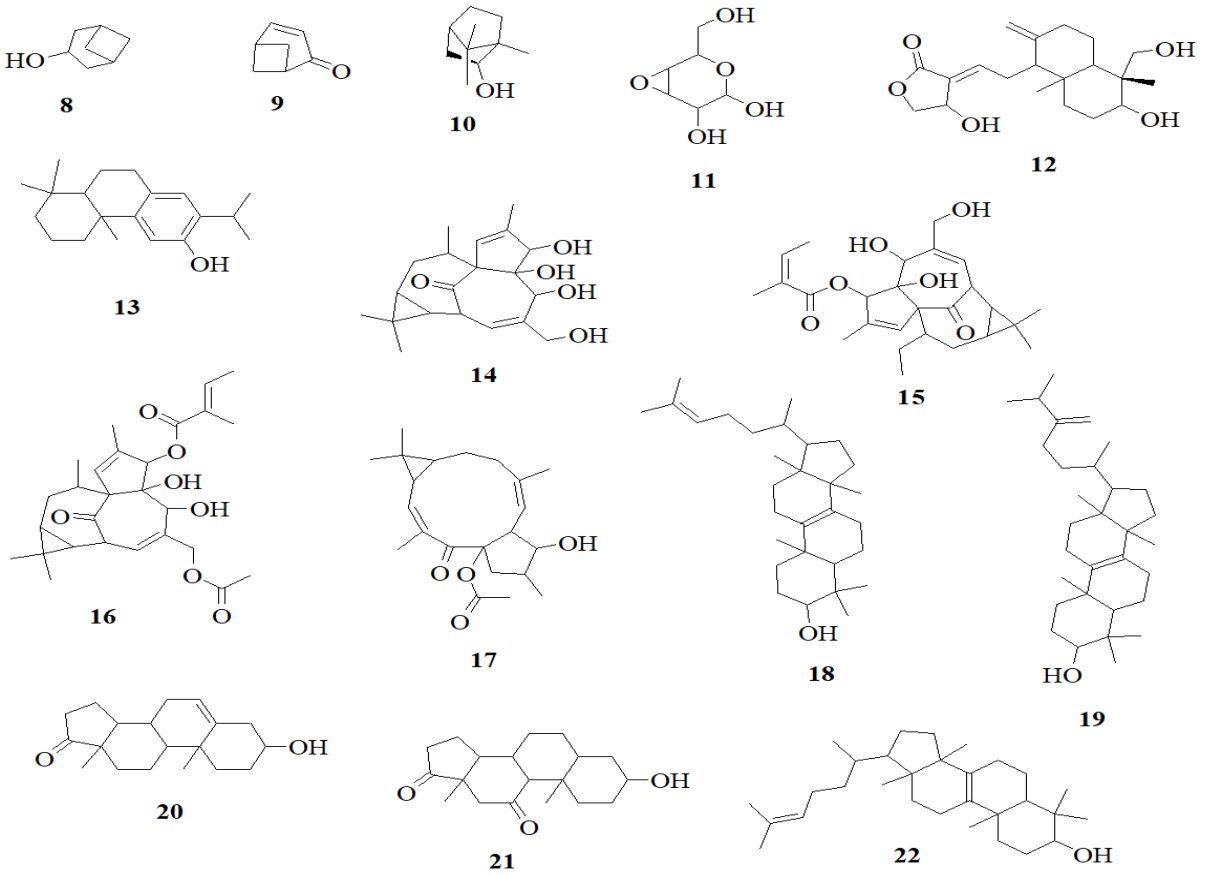


Figure 2 : The chemical structures of compounds isolated from latex, stems, leaves, roots and root bark of *Euphorbia ingens*

Some of the terpenoids and diterpenoids reported from *Euphorbia ingens* roots, extracted using chloroform and methanol, include Bicyclo[3.1.1]heptan-3-ol (**8**), Bicyclo[3.1.1]hept 3-en-2-one (**9**), 2-bornanol (**10**), 3,4-Altrosan (**11**), Andrographolide (**12**), and Ferruginol (**13**) (Figure 2). In *Euphorbia ingens* stems, compounds such as ingenol (**14**), ingenol mebutate (**15**), 20-O-acetyl-ingenol-3-angelate (**16**), and jolkinol D (**17**) was identified (Figure 2). *Euphorbia ingens* latex contained Euphol (**18**) and Euphorbol (**19**) (Figure 2). In ethyl acetate extract of the root barks and leaves of *Euphorbia ingens*, Isolation of sterols such as Prasterone (**20**), 11-oxoandrosterone (**21**), and Lanosterol (**22**) also reported from this plant [45-51].

The results of phytochemical screenings tests of *Euphorbia neriifolia* petroleum ether, chloroform, ethyl acetate, and ethanol extracts of different parts of this plant mainly revealed the presence of proteins, glycosides, tannins, alkaloids, phenols, flavonoids, saponins, cardenoloids, and terpenoids [30].

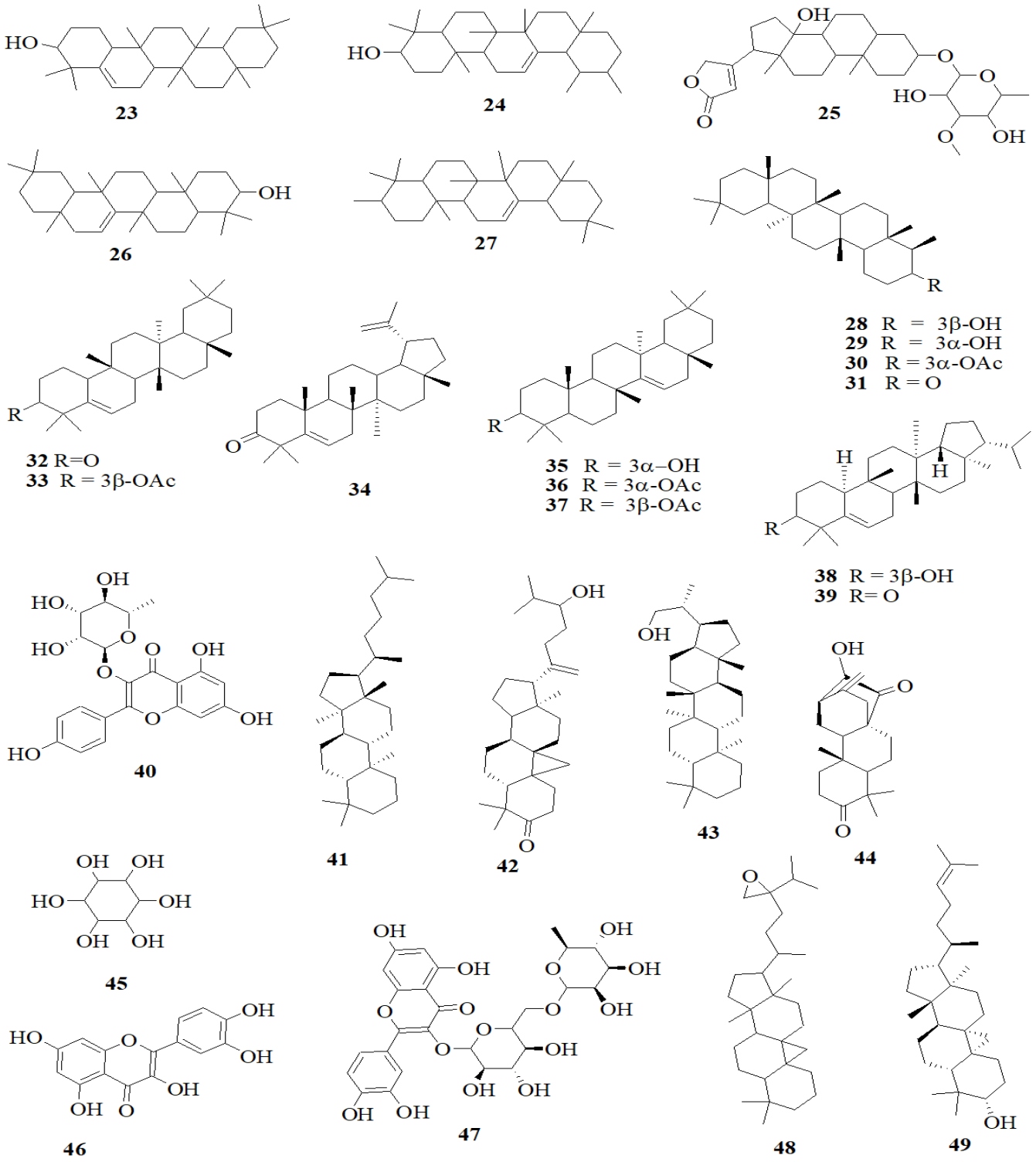
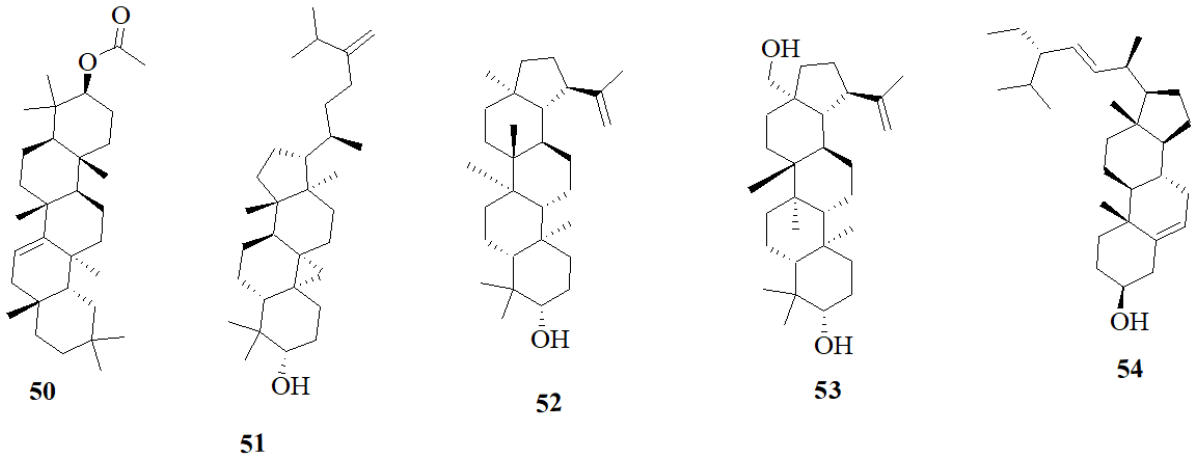


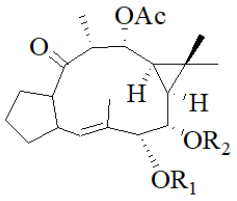
Figure 3 : The chemical structures of compounds isolated from leaves, stem, latex and root of *Euphorbia neriifolia*

Several triterpenoids such as **18** (Figure 2) from latex of *Euphorbia neriifolia,* Glut-5-en-3β-ol (**23**), α-amyrin (**24**), neriifolin (**25**), taraxerol (**26**), β-amyrin (**27**), 3β-friedelanol (**28**), 3α-friedelanol (**29**), 3β-acetoxy friedelane (**30**), friedelin (**31**), glutinone (**32**), glutinol acetate (**33**), lupenone (**34**), epitaraxerol (**35**), epitaraxeryl acetate (**36**), taraxeryl acetate (**37**), 3β-simiarenol

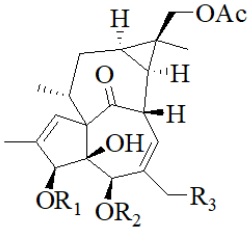
(**38**), simiarenone (**39**), and glycoside afzelin (**40**) (Figure 3) have been reported from the stem and leaves of *Euphorbia neriifolia* [30-31]. Euphane (**41**), Neriifolione (**42**), and neriifoliol (**43**) (Figure 3) were reported from the latex and Antiquorin (**44**) and inositol (**45**) (Figure 3) were reported from the roots of *Euphorbia neriifolia*. Flavonoids such as, Quercetin (**46**), and rutin (**47**) and sterols like 9, 19-cyclolanost-20(21)-en-24-ol-3-one (**48**) and cycloartenol (**49**) (Figure 3) were isolated from leaves of *Euphorbia neriifolia* [30-32, 52]**.**

The preliminary phytochemical screening test of *Euphorbia trigona* revealed the presence of saponins, alkaloids, flavonoids, glycosides, tannin, sterols, and terpenoids [55].





Compound	R_1	R_2
55	Tig	H
56	Tig	Me
57	Bz	Me
58	Ac	Bz
59	Ac	Tig



Compound	R_1	R_2	R_3
60	Ang	H	OAc
61	Ang	Ac	OAc
62	Ang	H	H
63	H	Ang	H

where

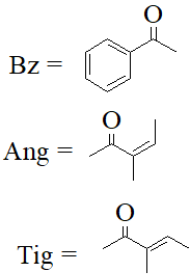


Figure 4 The chemical structures of compounds isolated from aerial parts of *Euphorbia trigona*

Several Triterpenoids such as α-amyrin (**24**), taraxerol (**26**), friedelan-3β-ol (**28**), β-amyrin (**27**), friedelin (**31**), and cycloartenol (**49**) (Figure 3), taraxerol acetate (**50**), 24-methylenecycloartano1 (**51**), lupeol (**52**), betulin (**53**), and stigmasterol (**54**), (Figure 4) diterpene esters of ingol and ingenane types, namely 3,12-diacetate 7-tigliate (**55**), 152 8-O-methylingol 3,12-diacetate 7-tigliate (**56**), 8-O-methyl-ingol 3,12-diacetate 7-benzoate (**57**), ingol 3,7,12-

triacetate 8-benzoate (**58**), and ingol 3,7,12-triacetate 8-tigliate (**59**), ingol 3,7,12-triacetate 8-tigliate (**60**), 17-acetoxyingenol 3-angelate 20-acetate (**61**), 17-Acetoxyingenol 3-angelate 5,20-diacetate (**62**) and 17-acetoxy-20-deoxyingenol 3-angelate (**63**) (Figure 4) were isolated from methanol and ethyl acetate extract of aerial parts of *Euphorbia trigona* [33-35, 53, 54].

Phytochemical screening tests were conducted to determine the presence of secondary metabolites in different parts of *Euphorbia abyssinica*, including the stems, seeds, and latex. The analyses were performed using standard methods on methanol and water extracts. The results of the preliminary phytochemical screening showed that the plant contains alkaloids, cardenolides, saponins, terprnoid, sterols, tannins, flavonoids, coumarins, and carbohydrates [55]. The methanol extract of aerial non-flowering parts including latex of *Euphorbia abyssinica* was analyzed and various compounds including diterpenoid, ingenol esters namely compounds **55**, **56**, **57**, **58** and **59** (Figure 4); triterpenoid like oleanolic acid (**64**), and 3-acetyloxy-(3α)-urs-12-en-28-oic methyl ester (**65**); sterols such as β-sitosterol (**66**), β-Sitosterol -3-O-glucoside (**67**) and lup-20(29)-en-3α,23-diol (**68**) (Figure 5); polyphenols such as 3,3′,4-*O*-Trimethylellagic acid (**69**) , 3,3′-Dimethylellagic acid-4′-*O*-β-d-glucopyranoside (**70**), Methyl gallate (**71**), Gallic acid (**72**), and 1,6-di-*O*-galloyl-d-glucose (**73**) (Figure 5); and flavonoids Kaempferol-3-*O*-α-L-rhamnoside (**74**), Quercitrin (**75**), and Luteolin-7-*O*-glucoside (**76**) Figure 5); unsaturated fatty acid, 8(R)-hydroxy-dec-3(E)-en-oic acid (**77**) (Figure 5) were isolated [36-37, 55-58].

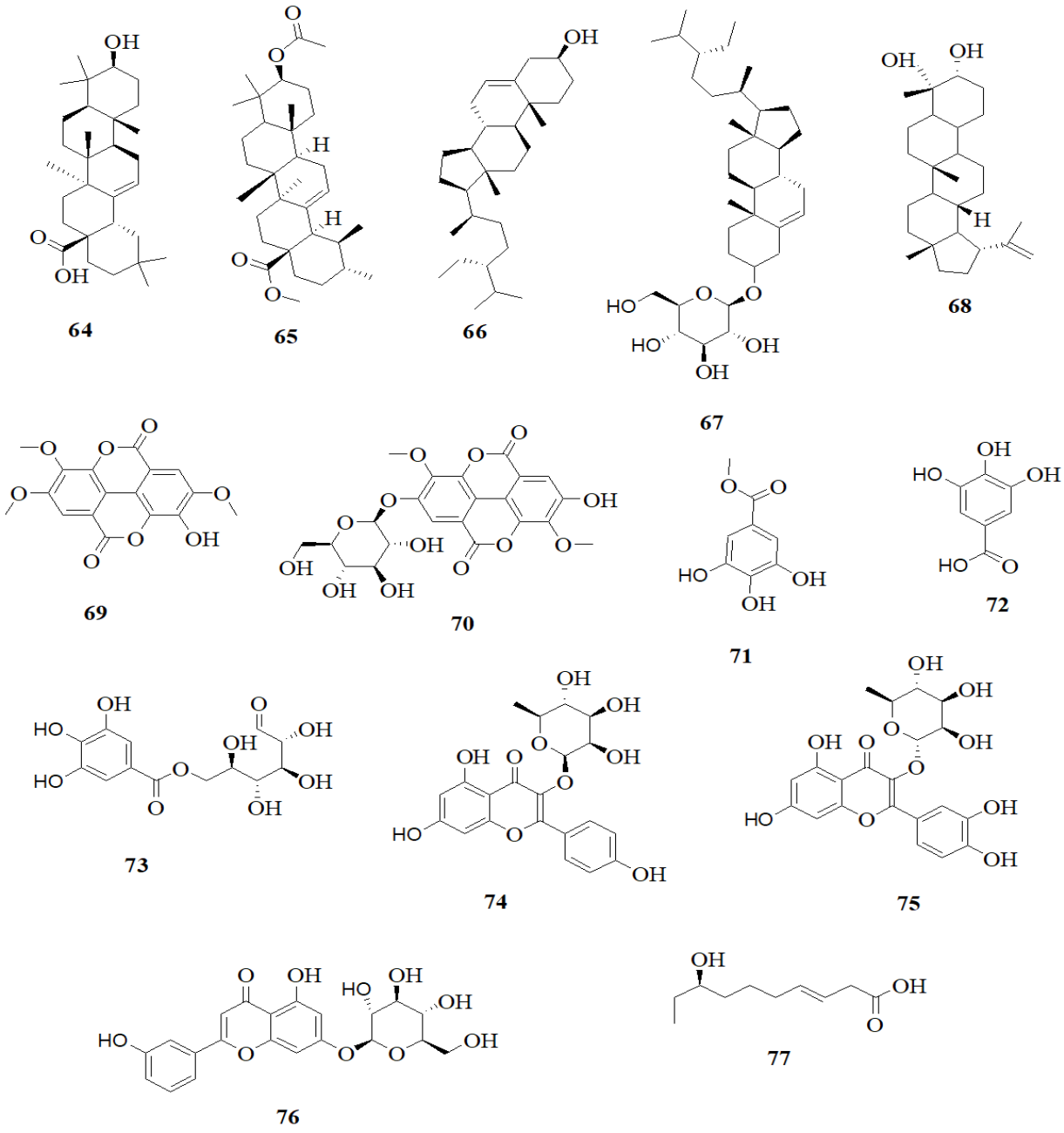


Figure 5 : The chemical structures of compounds isolated from aerial parts of *Euphorbia abyssinica*

Euphorbia tirucalli has been reported to possess flavonoids, diterpenes, phenolic, tannins, steroids, and alkaloids as major phytochemical constituents [59]. The whole plant has been containing 7.4% citric acid (**78**) with some malonic acid (**79**) and succinic acid (**80**) (Figure 6). Latex contains diterpene esters of the phorbol (**81**) (Figure 6) and compound **14** (Figure 2), reported to be highly active carcinogenic and tumor-promoting agents; Sterols Taraxasterol (**82**) and tirucallol (**83**) (Figure 6) are found in fresh latex [60].

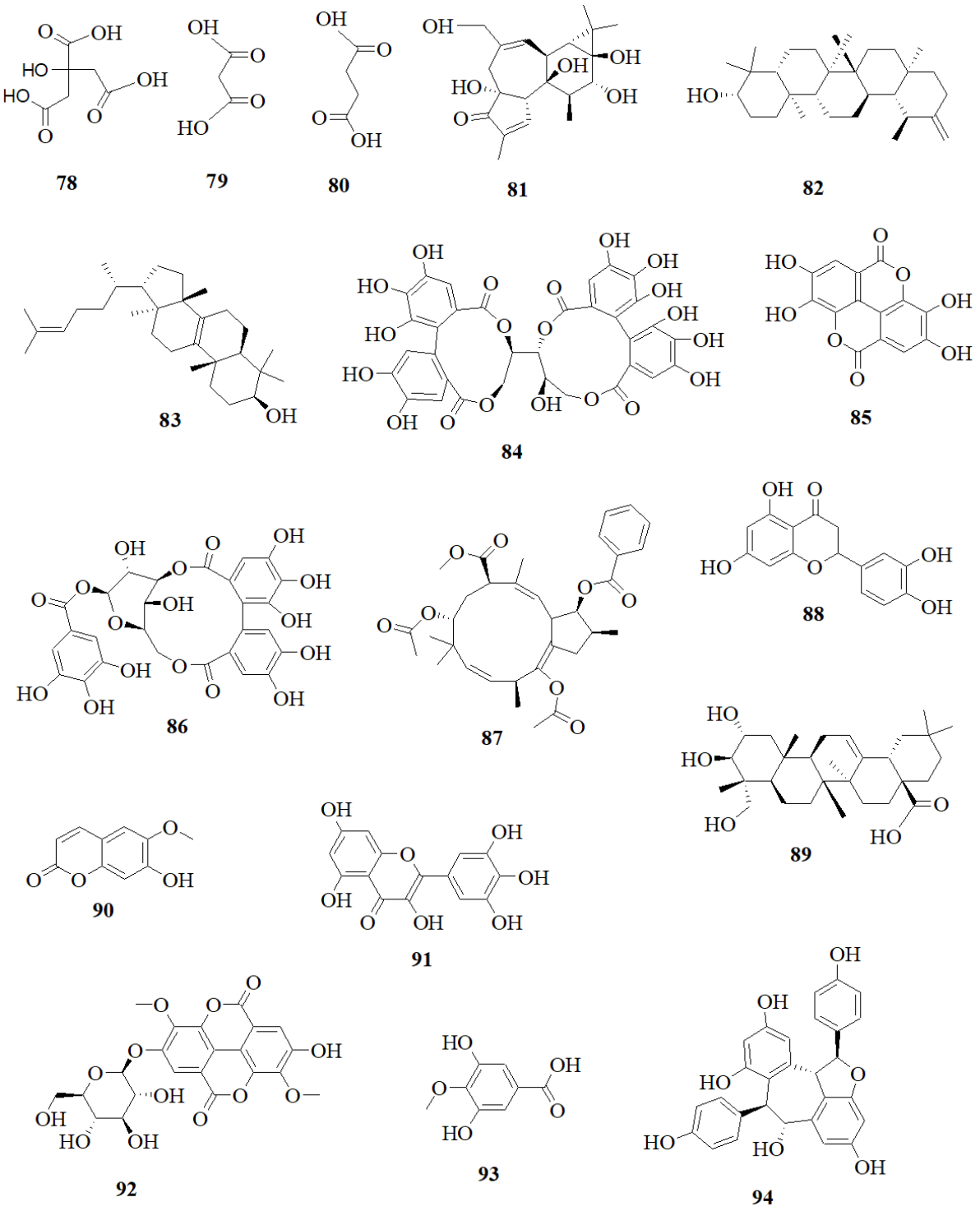


Figure 6 : The chemical structures of compounds isolated from root, stem and latex of *Euphorbia tirucali*

The methanol and chloroform stem extract is reported to possess sterol steroid β-sitosterol (**66**); phenolic compounds casuariin (**84**), ellagic acid (**85**), corilagin (**86**), euphorbin E(**87**) ; flavonoid Eriodictyol **(88**); triterpenoid Arjunolic acid (**89**) ,and coumarin glycoside Scopoletin (**90**), (Figure 6). The ethanolic root extract was analyzed and found to contain various compound like flavonoids such as myricetin (**91**), 3,3'-dimethoxy-4-*O*-α-rhamnopyranoside-ellagic acid (**92**), 4-*O*-methyl-gallic acid (**93**) and ampelopsin A (**94**) (Figure 6) [60-64].

2.4 Biological activities of genus *Euphorbia*

2.4.1 Antiproliferative and cytotoxic activity

The ethyl acetate extract of *Euphorbia ingens* roots has been found to exhibit antiproliferative effects on prostate cancer cells (DU-145) without impacting non-cancerous cells. The IC_{50} value, which indicates the concentration at which 50% of cell growth is inhibited, was less than 10 µg/ml that making it a promising candidate for further study and potential purification as a chemotherapeutic agent. This suggests that *Euphorbia ingens* ethyl acetate extract could be a valuable source for developing a treatment for prostate cancer [65].

2.4.2 Antimicrobial activity

The extracts from the aerial part of *Euphorbia ingens* exhibited promising activity against the Gram-positive bacteria *Bacillus subtilis* and *Staphylococcus aureus* in both the disc diffusion and microplate assay. The dichloromethane extract of the aerial part of *Euporbia ingens* showed good activity (5 mm and 12.85 mg/ml) against *Staphylococcus aureus*, but only slight activity (2 mm and 25.7 mg/ml) against *Bacillus subtilis*. The ethyl acetate extract showed significant activity against *Bacillus subtilis* (7 mm and 6.59 mg/ml) and *Staphylococcus aureus* (10 mm and 6.59 mg/ml) with slight activity (1 mm and 13.2 mg/ml) against *Escherichia coli* [45, 66].

The acetone extracts from the stem of *Euphorbia tirucalli* exhibited inhibitory effects against all the microorganisms tested. *Escherichia coli* exhibited notable sensitivity to the acetone extract, with a minimum inhibitory concentration (MIC) of 500 µg. *Candida albicans, Aspergillus niger*, and *Aspergillus fumigatus* demonstrated MICs of 750 µg for the acetone extract. The chloroform extracts displayed reactivity against *Bacillus subtilis, Escherichia coli, Proteus vulgaris, Staphylococcus aureus, Aspergillus niger,* and *Candida albicans*. The MICs for the chloroform extract were 250 µg for *Proteus vulgaris*, 500 µg for *Escherichia coli* and *Staphylococcus aureus*, 750 µg for *Bacillus subtilis* and *Candida albicans*, and 1000 µg for *Aspergillus niger*. The methanol extracts also exhibited activity against *Bacillus subtilis, Escherichia coli, Enterococcus faecalis, Staphylococcus aureus,* and *Candida albicans*. The MICs for the methanol extract were 500 µg for *Escherichia coli* and *Staphylococcus aureus*, 750 µg for *Bacillus subtilis* and *Enterococcus faecalis*, and 1000 µg for *Candida albicans*. Interestingly, the petroleum ether and hexane extracts did not demonstrate activity against any of the microorganisms under study. This suggests that the active compounds in *Euphorbia tirucalli* are polar in nature [67-68].

2.4.3 Anti-viral activities

Some isolated compounds were tested for antiviral potential against human coronavirus (HCoV) activity using Actinomycin D as the positive control. The results of the assay showed that small differences in the structural features of the tested compounds greatly influenced their antiviral activity. Among the friedelane derivatives, the orientation of the C-3 proton had a dramatic effect on the antiviral activity of compounds **28** and **29** (Figure 3) which is epimers. Comparing the activity data of compound **28** (cell survival %, 132.4) with that of compound **30** (cell survival %, 80.9) and positive control Actinomycin D (cell survival %, 69.5) (Figure 3) indicated that the acetyl group negatively impacted the antiviral activity. Additionally, compound **35** (cell survival %, 111) (Figure 3), a taraxerane derivative, was found to be the most active derivative in this group, and the data suggested that the acetyl group was not essential for the antiviral activity of taraxerane derivatives, unlike in the case of friedelane derivatives [30, 69-70].

The antiviral potential of petroleum ether and dichloromethane-methanol extracts derived from *Euphorbia tirucalli* latex, focusing on tobamoviruses such as tobacco and tomato mosaic viruses. The study utilized extract concentrations at 50, 100, and 150 ppm. At 150 ppm, the petroleum ether extract exhibited an 80% defensive action against the tomato mosaic virus, while the dichloromethane-methanol extract demonstrated an 81% defensive action against the tobacco mosaic virus. Additionally, the lytic effect of herpes simplex virus type-2 was assessed using the endpoint titration technique. The study established the maximum lytic effect of the water and methanol extracts (50% at 100 µg/ml and 70% at 100 µg/ml respectively) of *Euphorbia tirucali.* These values indicate that both the water and methanol extracts of *Euphorbia tirucali* have significant lytic activity against HSV-2 *in vitro*. The methanol extract was found to be more potent than the water extract, with a maximum lytic effect [71].

2.4.4 Anticancer activity

The in-*vitro* cytotoxicity of compound **18** (Figure 2) was evaluated using the murine F1B16 melanoma cell line. The results showed that the 50% inhibition concentration was 173.78 mg/ml. The anticancer activity of methanolic extracts of *Euphorbia neriifolia* against the B16F10 melanoma cancer cell line was also tested. The extract exhibited significant cytotoxicity in the concentration range of 10–1,000 µg/mL using SRB and MTT assays. The 50% inhibition concentration (IC_{50}) of the methanolic extract was 198.26 µg/mL by SRB assay and 212.78 µg/mL by MTT assay [70]. These values indicate that both compound **18** and the methanolic extract have significant cytotoxic activity against B16F10 melanoma cells [72].

The researchers tested the cytotoxic effects of the compounds on keratinocytes in *vitro*, using ingenol mebutate as a positive control. They found that two ingenane derivatives (**60** and **61**) (Figure 4) had significantly stronger cytotoxic effects (IC_{50} values 0.39 μM and 0.32 μM, respectively) on keratinocytes compared to ingenol mebutate (IC_{50} value 0.84 μM). These compounds could be further studied as potential alternatives to Picato (which contains ingenol mebutate), especially since Picato has been withdrawn from the market in the European Union. These compounds also show promise as potential therapeutic agents for actinic keratosis. Their structural similarity to ingenol mebutate and higher keratinocyte inhibitory activity make them promising candidates. This increased activity is likely due to the presence of acetoxy groups, which are known to be essential for activating PKC. Therefore, have a biological effect [34, 53].

2.4.5 Antidiabetic activity

The impact of the ethanol extract of *Euphorbia neriifolia* leaves on a rat model with diabetes induced by alloxan was investigated. Various parameters including oral glucose tolerance test, fasting blood glucose level, and serum lipid levels were measured. The results indicated a decrease in fasting blood glucose levels after 60 minutes of administering the extract during the oral glucose tolerance test. After 15 days, animals treated with the extract showed the greatest reduction in fasting blood glucose levels at a dose of 400 mg/kg. Additionally, the extract led to a reduction in serum lipid levels, comparable to those of the normal group of rats. These results suggest that the ethanol extract of *Euphorbia neriifolia* leaves has potential as a natural antidiabetic and hypolipidemic agent [73].

2.4.6 Hepatoprotective activity

The researchers tested the hepatoprotective activity of the ethyl acetate extract of the entire *Euphorbia neriifolia* plant, along with other indigenous plants. They determined the LD_{50} value in albino mice to be 1624 ± 36 mg/kg. The extract was administered orally to rats (300 mg/100 gm b.w.) and mice (30 mg/100 gm b.w.) one hour before administering CCl_4. After 48 hours, the researchers observed mortality and sacrificed the animals for liver morphology and histology. The results showed that the ethyl acetate extract of *Euphorbia neriifolia* did not protect the liver from CCl_4 intoxication, as there was no significant reduction in prolonged hexobarbital sleeping time and mortality compared to the negative control. Additionally, the extract did not prevent the increase in liver weight and histological changes induced by CCl_4. These results suggest that the ethyl acetate extract of the entire *Euphorbia neriifolia* plant does not have hepatoprotective activity against CCl_4-induced liver damage in rats and mice [74].

2.4.7 Wound healing activity

The wound healing activity of the aqueous extract of *Euphorbia neriifolia* latex was tested on guinea pigs with 8 mm diameter wounds on their dorsal surface. The sterile aqueous extract of latex was applied twice daily for 4 and 7 days. The results showed that the *Euphorbia neriifolia* latex increased collagen and DNA content; improving tensile strength, epithelization, and angiogenesis, indicating potential wound healing properties [75].

2.4.8 Anti-inflammatory activity

The anti-inflammatory effect of compound **18** (Figure 2) was assessed by evaluating carrageenan-induced mechanical hyperalgesia at doses of 30 and 100 mg/kg. The evaluation involved examining keratinocyte-derived chemokines (IL-1b and IL-6) and tumor necrosis factor-alpha, which are known to inhibit myeloperoxidase activity. The results of the study supported the potential therapeutic application of euphol in the management of inflammatory conditions [76]. Additionally, anti-inflammatory activity of petroleum ether, dichloromethane-methanol, and aqueous extracts of *Euphorbia tirucalli* latex using carrageenan-induced paw edema, with ibuprofen as the standard drug was evaluated. The study revealed that the petroleum ether, dichloromethane-methanol, and aqueous extracts notably ($P < 0.01$) inhibited inflammatory edema in rats at dosages of 30, 100, and 300 mg/kg, respectively. A p-value of less than 0.01 ($P < 0.01$) suggests that the difference in anti-inflammatory activity between the extracts and the control group is statistically significant. This means that the observed effects are unlikely to have occurred due to random chance alone, and there is a high level of confidence that the extracts indeed possess anti-inflammatory properties [77].

2.4.9 Antioxidant activity

The antioxidant potential of the aerial parts of *Euphorbia tirucalli* was evaluated using the DPPH and nitric oxide scavenging assays, with ascorbic acid serving as the standard for both assessments. The IC_{50} value for the DPPH and nitric oxide scavenging activities were determined as 15.893 and 29.759 µg/mL, respectively. Additionally, the IC_{50} value for the DPPH assay of the hydro alcoholic and ethyl acetate extracts of the aerial parts of *Euphorbia tricalli* were determined as 69.599 and 20.455 µg/mL, respectively. Similarly, the nitric oxide scavenging assay yielded IC_{50} values of 17.017 and 17.562 µg/mL for the hydro alcoholic and ethyl acetate extracts, respectively. The IC_{50} values for the DPPH and nitric oxide scavenging activities indicate the effectiveness of the tested extracts in scavenging these free radicals. The lower IC_{50} values for the DPPH and nitric oxide scavenging activities of the ethyl acetate extract compared

to the hydro alcoholic extract suggest that the ethyl acetate extract of *Euphorbia tricalli* may have stronger antioxidant properties [68, 76].

The antioxidant activity of the methanol aerial part extract of *Euphorbia trigona* was evaluated using various in *vitro* assays, including DPPH radical scavenging, ABTS radical scavenging, and ferric reducing antioxidant power (FRAP) assays. The extract demonstrated strong DPPH radical scavenging activity with an IC_{50} value of 28.6 μg/mL, as well as potent ABTS radical scavenging activity with an IC_{50} value of 15.2 μg/mL. These findings suggest that the methanol aerial part extract of *Euphorbia trigona* has potential as a natural antioxidant agent [78].

2.4.10 Anti-malarial activity

The 80% methanolic crude extract from the roots of *Euphorbia abyssinica* demonstrated significant dose-dependent chemosuppressive anti-malarial effects on the body weights of Plasmodium berghei infected mice. Mice treated with this extract exhibited notable chemosuppressive anti-malarial activities ($P < 0.001$) compared to mice treated with distilled water. Specifically, mice treated with 400 and 600 mg/kg of the extract displayed significant chemosuppressive anti-malarial activities ($P < 0.01$ and $P < 0.001$, respectively) in comparison to those treated with 200 mg/kg of the extract [55].

3. MATERIALS AND METHODS

3.1 Materials

3.1.1 Chemicals

Different solvents such as n-hexane (UV spectroscopy and HPLC, 99.8 %), dichloromethane (UV spectroscopy and HPLC, 99.8 %), and methanol (UV spectroscopy and HPLC, 99.9 %) were utilized for gradient extraction, while ethyl acetate (UV spectroscopy and HPLC, 99.9 %) and n-hexane (UV spectroscopy and HPLC, 99.8 %) were used for column elution. Pre-coated TLC (silica gel, UV 254) was employed for chromatographic analyses to determine phytochemicals in a given extract. Various reagents including Dragendroff's reagent, Folin-ciocalteu reagent, Liebermann burchard's reagent, sodium hydroxide, ferric chloride, concentrated hydrochloric acid, concentrated sulfuric acid, glacial acetic acid, ammonia, dexamethasone, atropine, Gallic acid, aluminum chloride, sodium carbonate, citric acid, chloroform, benzene and Bromocresol green were also be utilized for phytochemical screening and quantitative analyses.

3.1.2 Instruments

The rotary evaporator (Heidolph, UK) was used for concentrating crude extracts, and a Grant (GLS 400) thermostatic bath shaker for macerating plant materials. An oven (model N50L, GENLAB, WIDNES, ENGLAND) was used to dry glassware and remove moisture which can interface with sample and an Analytical Balance ADAM (AFP-110L) was used for measurements. The isolated pure compounds were characterized using UV-Vis (T80 UV/VIS spectrophotometer, PG instruments Ltd), and FT-IR spectrometer (KBr pellet) from Perkin Elmer BX (400-4000 cm^{-1}). Additionally, iodine and UV chambers (Uvitec) were used for checking out purity of isolated compounds.

3.1.3 Plant material collection

The root of *Euphorbia candelabrum* was collected in February, 2024 from Sika Kebele, Bule Woreda, Gedeo zone, Southern Ethiopia Regional state, Ethiopia. The area is about 387 km south of Addis Ababa, capital of Ethiopia and 121 km from Hawassa city. The plant material was authenticated in Addis Ababa, Department of Biology herbal botanist.

3.2 Methods

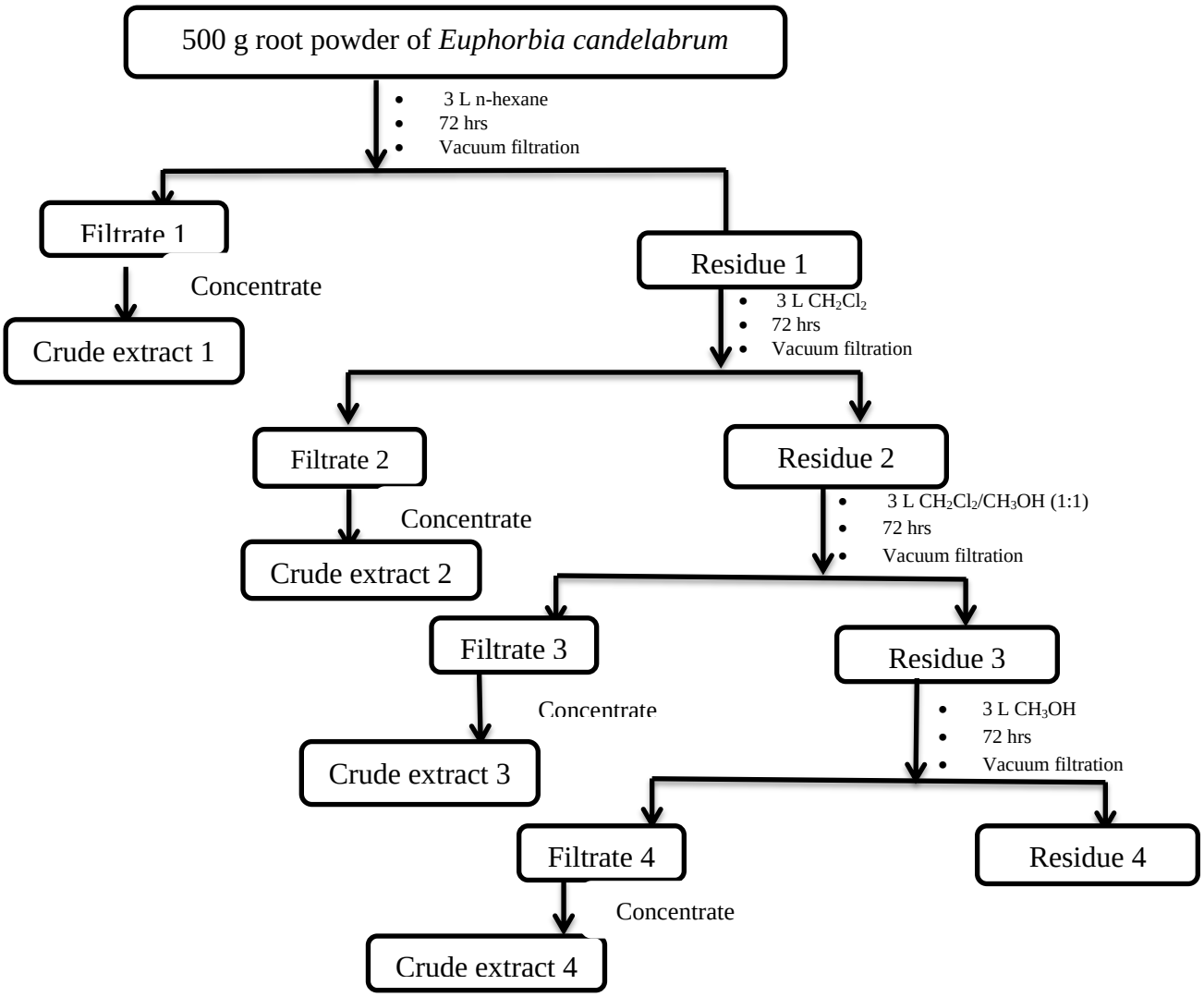
3.2.1 Preparation of plant specimen

The collected plant material (root part) was washed with tap water and chopped into small pieces and air-dried at room temperature for one month. The dried plant material was milled to a suitable size with a grinding machine at the Department of Plant Science, College of Agriculture and Natural Science, Hawassa University to facilitate the extraction process. The sample was stored on refrigerator until used for extraction or use.

3.2.2 Extraction

The powdered root of *Euphorbia candelabrum* (500 g) was soaked in 3 L of n-hexane at room temperature in Erlenmeyer flask.

Scheme 1: General outline for the extraction of root of *Euphorbia candelabrum*



After shaking the contents well, the flask containing the solution was placed on an orbital shaker and agitated at the speed of 120 revolutions per minute for 72 hours. Then the solution was

filtered using Whatman No.1 filter paper and the filtered solution was concentrated using a Rotary evaporator at temperature of 39 ^{0}C and at reduced pressure. The crude extract of solvent was stored in a hood until it becomes dry and ready to determine the weight and safe for further analysis. The marc left was sequentially extracted with dichloromethane, dichloromethane/methanol (1:1 v/v) and methanol following similar procedures used above (Scheme 1)

3.2.3 Phytochemical screening tests

Phytochemical screening was carried out for all the extracts as per the standard methods reported in the literature [79-82].

1. **Test for alkaloids:** 1 ml 1% HCl was added to 0.1 g of the extract dissolved in methanol in a test tube. The mixture was heated for 20 minutes, cooled, and filtered. Then, 1 ml of the filtrate was tested with 0.5 ml of Dragendorff's reagent. The formation of a reddish-brown precipitate indicates the presence of alkaloids (Appendix 1).
2. **Test for flavonoids**

   Alkaline Reagent Test: 0.1 g extracts were dissolved with 4 drops of sodium hydroxide solution. The formation of an intense yellow color, which becomes colorless with the addition of 2 drops of dilute acid, indicates the presence of flavonoids (Appendix 1).
3. **Test for phenols**

   Ferric Chloride Test: 0.1 g extracts were dissolved with 4 drops of ferric chloride solution. The formation of green or bluish-black color indicates the presence of phenols (Appendix 1).
4. **Test for glycosides**

   Keller-Killani test: 1 ml of glacial acetic acid, traces of ferric chloride, and concentrated sulfuric acid were added in to 0.1 g of the extracts. The formation of a reddish-brown color at the junction of two layers and the upper layer turning bluish-green indicated the presence of glycosides (Appendix 1).
5. **Test for Terpenoids**

   Salkowski test: 0.2 g extracts were dissolved with chloroform and filtered using Whatman No. 1 filter paper. The filtrates were then tested with 3 drops of concentrated sulfuric acid (H_2SO_4), shaken, and allowed to stand. The appearance of a reddish-brown color indicates the presence of terpenoids (Appendix 1).
6. **Test for tannins:** 0.1 g extracts were boiled for 20 minutes in a boiling tube with 20 ml of distilled water. The resulting solution was then filtered using Whatman No. 1 filter paper. Next, 3 drops of 0.1% ferric chloride ($FeCl_3$) solution were added and thoroughly mixed,

then allowed to stand for some time. The appearance of a brownish-green or blue-black color confirms the presence of tannins.

7. **Test for saponins**

   Froth test: 0.1 g extracts were diluted with 10 ml distilled water and shaken in a test tube for 15 minutes. The formation of a 1 cm layer of foam indicates the presence of saponins.

   Foam test: 0.1 g of extracts was shaken with 2ml of distilled water. If foam is produced and persists for ten minutes, indicates the presence of saponins (Appendix 1).

8. **Test for steroids**: 2 ml of acetic anhydride was added to a test tube containing 0.2 grams of extract dissolved in methanol. Next, 2 ml of sulfuric acid (H_2SO_4) was added to the mixture, and the color change from violet or blue-green was observed to confirm the presence of steroids (Appendix 1).

9. **Test for anthraqunines**

   Borntrager's test: 0.1 g extracts were dissolved in 10 ml of water and the mixture was shaken and filtered. The residue was washed with 3 ml of benzene. Subsequently, 5 ml of 10% ammonia solution was added to the filtrate. The mixture was shaken, and the presence of a pink, red, or violet color in the ammonical (lower) phase indicates the presence of free anthraqunines (Appendix 1).

3.2.4 Quantitative analysis of secondary metabolites

Quantitative analysis is an important tool for determining the quantity of phytochemical constituents present in plant extracts. The total flavonoid content, total alkaloid content, total phenolic content and total steroid content of the extracts obtained from the roots of *Euphorbia candelabrum* were estimated by employing standard procedures reported in literatures [83-87]. Other secondary metabolites were not quantified in this study due to the lack of necessary reagents and instrumentation. For instance, the determination of total tannins requires measuring the absorbance at 120 nm in Vacuum Ultraviolet (VUV) Spectrophotometer.

I. Determination of total flavonoids content

Total flavonoid content was determined by aluminum chloride method using quercetin as standard. 10 mg of quercetin was taken and dissolved in 10 ml of methanol. Then, the solution was pipetted out 0.4, 0.8, 0.12, 0.16, and 0.20 mL of the quercetin stock solution into a series of 100 mL volumetric flasks and made up the mark with methanol. This gives a series of standard solutions with concentrations of 4, 8, 12, 16, and 20 µg/ml of quercetin. In each flask, 0.3 ml 5% sodium nitrite, 0.3 mL of 10% aluminum chloride solution and 2 ml of 1 M sodium hydroxide

solution was added stepwise as described below and the standard solutions were stored in the dark at a cool temperature for five minutes.

The methanol extract of 10 mg was dissolved in 10 mL of methanol in 25 ml volumetric flask. And then, 0.3 mL of 5% sodium nitrite solution was added, mixed well and allowed to stand for 5 minutes. Then, 0.3 ml of 10% aluminum chloride solution was added and mixed well. After allowed to stand for 6 minutes, 2 ml of 1 M sodium hydroxide solution was added and diluted with 10 ml methanol. The solutions were kept for 15 minutes at room temperature. The blank solution was prepared in the same manner as described earlier without quercetin. The absorbance of solutions was measured at 510 nm three times and the mean value was obtained. The same procedure was repeated with dichloromethane/methanol (1:1 v/v) extract using dichloromethane/methanol (1:1 v/v).

II. Determination of total phenolic content

Folin-Ciocalteu spectrophotometric method was used to determine the total phenolic content. 10 mg of extract was dissolved in 10 ml of methanol in a 25 ml volumetric flask. 1 ml of Folin-Ciocalteu reagent was added and shaken well. After 5 minutes, 10 ml of 5% sodium carbonate was added to the mixture. The volume was made up to 25 ml with distilled water.

A set of reference standard solutions was prepared using gallic acid as the standard. 50 mg of gallic acid was dissolved in 50 ml of methanol and made up to 50 ml in a standard flask. The concentration of gallic acid in the standard stock was 1 mg/ml. 5 ml of the standard solution was diluted to 100 ml with methanol in a standard flask. Therefore, the concentration of gallic acid in the working standard solution was 50 µg/ml. 5, 10, 15, 20, and 25 µg/ml standards were prepared from the working standard by serial dilution. A blank solution was also prepared in the same manner as described above.

The prepared test and standard solutions were incubated for 90 minutes at room temperature. The absorbance of the test and standard solutions was recorded against the blank at 725 nm three times and the mean value was obtained. The same procedure was repeated for dichloromethane/methanol (1:1 v/v) crude extract using dichloromethane/methanol (1:1 v/v) solvent.

III. Determination of total alkaloid content

10 mg of the methanol crude extract was dissolved in 10 ml of 2N HCl and then filtered. 1 ml of solution was transferred in to separatory funnel and washed with 30 ml chloroform. Then, 5 ml

of Bromocresol green and 5 ml of phosphate buffer were added to the solution. The mixture was shook and yellow colored complex was extract with 1, 2, 3 and 4 ml chloroform by vigorous shaking in separatory funnel. The same procedure was repeated for dichloromethane/methanol (1:1 v/v) crude extract.

10 mg of atropine was dissolved in 100 ml distilled water. Then, accurately measured aliquots (10, 15, 20, 25, and 30 ml) were transferred into separating funnels and 5 ml phosphate buffer of pH 4.7 and 5 ml Bromocresol green solutions was added to each funnels. And then, the solution was extracted with 1, 2, 3 and 4 ml chloroform (in parts 10 ml). The extracts were collected in 10 ml beaker and then diluted with chloroform. The absorbance of the test and standard solutions was determined against the blank at 470 nm using a UV-visible spectrophotometer three times and the mean value was obtained.

IV. Total steroids content

25 mg of dichloromethane extract was diluted to in 25 ml volumetric flask using 25 ml chloroform. Then, 1 ml solution was taken and evaporates to dryness. 0.5 ml glacial acetic acid and 5 ml Liebermann burchard's reagent was added and kept for 20 minutes. The same procedure was repeated for dichloromethane/methanol (1:1 v/v) crude extract.

To prepare a series of standard solutions of dexamethasone, 50 mg of dexamethasone was dissolved in 50 ml chloroform. From the stock solution, 20 ml was taken and made up the volume to 50 ml with chloroform. This would result in a solution with a concentration of 400 µg/ml. From the 400 µg/ml solution, aliquots of 6, 7, 8, 9, and 10 ml was taken and each aliquot evaporated to dryness. To each of the dried residues, 0.5 ml of glacial acetic acid and 5 ml of Liebermann-Burchard's reagent was added. The volume of each solution adjusted to 10 ml with chloroform and allowed to stand for 20 minutes before measuring the absorbance. The absorbance of the test and standard solutions was determined against the blank at 618 nm using a UV-visible spectrophotometer three times and the mean value was obtained.

3.2.5 Isolation of compounds

An appropriate solvent was selected for elution after carrying out TLC profile of extracts in various combinations of solvents. The solvent system that showed good resolution of the components of the crude extract on TLC was selected for isolation of compounds in column chromatography. Therefore, the dichloromethane/methanol (1:1 v/v) crude extract weighing 10 g was absorbed onto 20 g of silica gel (mesh size 60-120). Then, the sample was subjected to column chromatography that was preloaded with 100 g silica gel, which was used as the

stationary phase. The elution process was started with n-hexane and followed by mixture of n-hexane and ethyl acetate with increasing polarity. The TLC profile of each fraction was checked and those fractions with the same TLC profiles were combined and concentrated using rotary evaporator. The isolated pure compounds (KJ-1, KJ-2 and KJ-3) were subjected to spectral analysis and anti-bacterial test. The spectral (UV-Vis and FT-IR) data were used to predict structures of the isolated compounds.

3.2.6 Antibacterial activity test

The test organisms that were used to check the antibacterial activity of the crude extracts and isolated compounds were *Escherichia coli* (gram-negative), *Pseudomonas aeruginosa* (gram-negative), *Staphylococcus aureus* (gram-positive), and *Streptococcus pyogenes* (gram-positive). The antibacterial activity tests were determined using agar disc diffusion method.

The bacterial stock cultures were maintained on nutrient agar slats, which were stored at 40 °C. The test solutions were prepared by dissolving 150 mg of the test samples to achieve a concentration of 150 mg/ml in DMSO. The same concentration of tetracycline was used as the positive control, whereas DMSO was used as the negative control. A freshly grown liquid culture of the test pathogen solution of similar turbidity with 0.5 McFarland was seeded on Muller-Hinton agar plates. The plates were shaken gently to allow even mixing of the bacterial cells and agar. Sterile paper discs were separately soaked in the above stock solutions (samples and standards) and then applied to the seeded plates at equidistance. Control experiments were carried out under similar conditions using tetracycline for antibacterial activity as a standard drug. The plates were then inverted and incubated at 37 °C for 24 hours. After the incubation period, the plates were observed for a clearance zone around the disks. Clear inhibition zones formed around the discs, indicating the presence of antibacterial activity, as measured in millimeters [37]. Each experiment was carried out in triplicate. The mean inhibition zone of each test sample was taken to evaluate the antibacterial activity. The inhibition zones produced by the plant extracts were compared with the inhibition zones produced by commercial standard antibiotics.

4. RESULTS AND DISCUSSION

4.1 Determination of extraction yield

The extraction yield is a measure of the solvent efficiency to extract specific components from the original materials. Depending on the method indicated (section 3.2.3) the crude extracts was carried and allowed to dry completely. The percentage yield of successive extracts of root of *Euphorbia candelabrum* was determined using the following formula (Equation 1).

$$\text{Percentage yield} = \frac{\text{Weight of the crude extract (g)}}{\text{Grounded plant material used for extraction (g)}} \times 100\% \quad \text{..... (Equation 1)}$$

Table 2 : The mass of crude extract in each gradient extract

S/No	Solvent system used for extraction	Mass of crude extract	Yield %
1	n-hexane	0.9 g	0.18
2	Dichloromethane	6.8 g	1.36
3	Dichloromethane/Methanol (1:1)	15.4 g	3.13
4	Methanol	11.4 g	2.39

As presented above (Table 2), the gradient extractions of the roots of *Euphorbia candelabrum* using four different solvents produced different yields. Among four crude extracts, the percentage of crude extracts of dichloromethane/methanol (1:1 v/v) was found to be greater than that of the other crude extracts. This indicated that most of the components of the extracts could be medium polar. Therefore, dichloromethane/methanol (1:1 v/v) extract was selected for column chromatographic isolation of compounds due to its high yield and clear spot on TLC.

4.2 Phytochemical screening

Phytochemical screening tests were carried out on n-hexane, dichloromethane, dichloromethane/methanol (1:1 v/v) and methanol root extracts of *Euphorbia candelabrum* using a standard procedure to identify the class of secondary metabolites. The results of the tests are presented below (Table 3)

Table 3 : Phytochemical analysis of the root extracts of *Euphorbia candelabrum*

Secondary metabolites	Solvent used for extraction			
	n-hexane	Dichloromethane	Dichloromethane/methanol 1:1 v/v	Methanol
Alkaloids	+	+	+	+
Flavonoids	-	+	+	+
Phenols	-	+	+	+
Glycosides	-	+	+	+
Terpenoids	+	+	+	+
Tannin		+	+	+
Saponnins	+	+	+	+
Steroids	+	+	+	+
Anthraqunines	-	-	-	-

Key: (+) indicate present of constituent and (-) indicate absent of constituent

The results revealed that the *Euphorbia candelabrum* root extract of dichloromethane, dichloromethane/methanol (1:1 v/v) and methanol contains secondary metabolites such as alkaloids, flavonoids, phenols, glycosides, terpenoids, tannins, saponins, steroids and absence of anthraqunines whereas n-hexane crude extracts contain alkaloids, glycosides, terpenoids, steroids, saponins and absence of flavonoids, phenols, tannin and Anthraqunines (Table 3).

According to the above phytochemical observations, *Euphorbia candelabrum* is plant rich in many secondary metabolites which could be attributed to be its traditional medicinal uses and such studies may lead to drug discovery and development. These compounds are biologically active secondary metabolites that exhibit antimicrobial activity through various mechanisms. Phenolic compounds are well-known for their biological activities, such as increasing bile secretion, reducing blood cholesterol and lipid levels, and displaying antimicrobial properties. Glycosides are diverse group of natural chemicals with a wide range of medicinal uses. They are characterized by their unique glycone-aglycone structure and have been shown to be effective in treating a variety of health conditions, including bacterial infections, cancer, inflammation, cardiovascular disease, and neurodegenerative disorders [81, 82].

Flavonoids also possess diverse biological and pharmacological activities, including antimicrobial, cytotoxic, anti-inflammatory, and antitumor, enzyme inhibition, estrogenic, anti-allergic, antioxidant, and vascular activities. Tannins are natural healers with uses such as astringents, treatment for diarrhea, diuretics, stomach and duodenal tumor remedies, and as anti-inflammatory, antiseptic, antioxidant, and hemostatic pharmaceuticals. Alkaloids play a significant role in pharmacological activities such as antihypertensive effects, antiarrhythmic

effects, antimalarial activity, and anticancer actions. Terpenoids have medicinal properties like ant carcinogenic, antimalarial, anti-ulcer, hepaticidal, antimicrobial, or diuretic activity [82, 88].

Steroids are a diverse group of organic compounds with a wide range of biological activities. They are used to treat a variety of medical conditions, including inflammatory diseases, certain types of cancer, autoimmune diseases, and hormonal deficiencies. Saponins have medicinal properties like hypolipidemic and cholesterol-lowering, anticancer, antiviral, free radical scavenging effects, adjuvant effects and anti-inflammatory [89]. The presence of such important classes of compounds in different extracts obtained from the root extract of *Euphorbia candelabrum* can be used as evidence for the current use of the plant by traditional healers.

Preliminary phytochemical analysis was conducted on *Euphorbia candelabrum* latex using gradient solvents (hexane and acetone) for extractions. The results revealed that the n-hexane extract contained the same secondary metabolites as the root extract of *Euphorbia candelabrum*, namely alkaloids, glycosides, terpenoids, steroids, and saponins. However, flavonoids, phenols, and tannins were absent in the n-hexane extract. The acetone extract contained the same secondary metabolites as the root extracted with dichloromethane, dichloromethane/methanol (1:1 v/v), and methanol, including alkaloids, flavonoids, phenols, glycosides, terpenoids, tannins, saponins, and steroids [52].

4.3 Quantitative phytochemical analysis of secondary metabolites

I. Total flavonoid and phenolic content

The total flavonoid content was determined by the aluminum chloride method using UV–Vis spectroscopy. The results are expressed as mg of quercetin equivalents (QE) per gram of extract. The dichloromethane/methanol (1:1 v/v) and methanol crude extract concentrations were first determined using equation 2 below. The standard curve equations derived from the curve of quercetin, $y = 0.015x + 0.006$ with an R^2 value of 0.983 and $y = 0.018x + 0.024$ with an R^2 value of 0.988 (Appendix 2), along with the absorbance values at 510 nm for each sample recorded in Table 4, were utilized to calculate the concentration of each extract. This approach enabled us to determine the concentrations of the dichloromethane/methanol (1:1 v/v) and methanol extracts based on the absorbance values obtained and the standard curve equations derived from the quercetin curve. Two standard curve equations were utilized in this study due to the different solvents required for each extract sample and standard preparation. For instance, methanol extraction requires methanol for both sample and standard preparation. The concentrations

obtained were 10.47 µg/ml for the dichloromethane/methanol (1:1 v/v) extract and 11 µg/ml for the methanol extract.

$$A = \varepsilon cl \quad \text{…… (Equation 2)}$$

Where: A = absorbance, ε = absorptivity, c = concentration and l = cell length

The total flavonoid content was determined by using equation 3 below, and the total flavonoid contents in the dichloromethane/methanol (1:1 v/v) and methanol root extracts were 10.47 mg QE/g and 11 mg QE/g of extract, respectively. In this study, the methanol crude extract exhibited a greater flavonoid content than did the dichloromethane/methanol (1:1 v/v) extract.

Table 4 : Absorbance of standard and sample solutions of gallic acid and quercetin

Gallic acid standard			Quercetin standard		
Conc. µg/ml	Absorbance at 725 nm	Absorbance at 725 nm	Conc. µg/ml	Absorbance at 510 nm	Absorbance at 510 nm
5	0.113 ± 0.004	0.092 ± 0.003	4	0.110 ± 0.003	0.080 ± 0.003
10	0.331 ± 0.003	0.251 ± 0.003	8	0.163 ± 0.002	0.119 ± 0.003
15	0.543 ± 0.003	0.423 ± 0.003	12	0.231 ± 0.002	0.176 ± 0.002
20	0.766 ± 0.004	0.632 ± 0.002	16	0.324 ± 0.002	0.247 ± 0.001
25	0.964 ± 0.004	0.853 ± 0.005	20	0.398 ± 0.003	0.318 ± 0.002
Dichloromethane/methanol (1:1 v/v)crude extract		0.408 ± 0.007	Dichloromethane/methanol (1:1 v/v) crude extract		0.183 ± 0.002
Methanol crude extract	0.71 ± 0.008		Methanol crude extract	0.222 ± 0.002	

The higher flavonoid content in the methanol crude extract compared to the dichloromethane/methanol (1:1 v/v) extract can be attributed primarily to the higher polarity of methanol. Methanol's polarity enables it to effectively extract polar compounds such as flavonoids, which contain hydroxyl (-OH) groups that can form hydrogen bonds with the solvent. In contrast, dichloromethane is a non-polar solvent better suited for extracting non-polar compounds. In summary, the polarity of the solvent and its ability to form hydrogen bonds with flavonoids are crucial factors in determining the extraction efficiency. Methanol's higher polarity and stronger solvent-solute interactions make it a more effective solvent for extracting flavonoids compared to dichloromethane/methanol (1:1 v/v).

$$\text{Total content} = C \times V/M \quad \text{……. (Equation 3)}$$

where: C = the concentration of the sample, V = volume of solvent used to dissolve the sample and M = initial mass of sample used

The total phenolic contents in dichloromethane/methanol (1:1 v/v) and methanol root extracts of *Euphorbia candelabrum* were determined by the Folin-Ciocalteu method using gallic acid as a standard in milligrams of gallic acid equivalent per gram (mg GAE/g) of the extract. The concentrations of dichloromethane/methanol (1:1 v/v) and methanol were determined by using equation 2. According to the calibration curve of gallic acid ($y = 0.038x-0.12$, $R^2 = 0.983$, and $y = 0.042x-0.09$, $R^2 = 0.988$) (Appendix 3) and the results of the samples recorded (Table 4) at 510 nm, the dichloromethane/methanol (1:1 v/v) was 13.4 µg/ml, and the methanol crude extract concentration was 19.047 µg/ml.

The total content of phenolic was determined by using equation 3, and the results showed that the methanol extract had 19 milligrams of gallic acid equivalent per gram (mg GA/g) and that the dichloromethane/methanol (1:1 v/v) extract had 13.4 milligrams of gallic acid equivalent per gram (mg GA/g) of extract. Compared with the dichloromethane/methanol (1:1 v/v) crude extract, the methanol extract exhibited the highest total phenolic content. This suggests that the greater the polarity of the extraction solvent is, the greater the content of phenolics in the extracts.

II. Total alkaloid and steroid content

The total alkaloid content of the dichloromethane/methanol (1:1 v/v) and methanol root extracts of *Euphorbia candelabrum* were determined using UV–Vis spectroscopy. The results are expressed as milligrams of atropine equivalent per gram (mg AE/g) of extract. The concentration of the samples were calculated using equation 2 from the regression equation of the calibration curve ($y = 0.068x - 0.04$, $R^2 = 0.996$) (Appendix 4), and the absorbance of samples at 470 nm were recorded (Table 5). The concentrations obtained were 1.426 µg/ml and 2.073 µg/ml for the dichloromethane/methanol (1:1 v/v) and methanol crude extracts, respectively. The regression equation of the calibration curve was obtained from the absorbance results (Table 5) of the atropine standard data recorded.

The total alkaloid content of the samples were determined using equation 3, and the total contents of the dichloromethane/methanol (1:1 v/v) and methanol extracts were 1.426 mg AE/g and 2.073 mg AE/g of extract, respectively. Therefore, the methanol crude extract had a greater content of alkaloids than did the dichloromethane/methanol (1:1 v/v) extract. This may be due to

methanol is a polar solvent, while dichloromethane is a nonpolar solvent. Polar alkaloids are more soluble in polar solvents, so they should be more concentrated in the methanol extract.

Table 5 : Absorbance of standard and sample solutions of steroids and alkaloids

dexamethasone standard at 618 nm		Atropine standard at 470 nm	
Conc. µg/ml	Absorbance at 618 nm	Conc. µg/ml	Absorbance at 470 nm
2.4	0.133 ± 0.004	1	0.024 ± 0.003
2.8	0.167 ± 0.003	1.5	0.054 ± 0.003
3.2	0.207 ± 0.004	2	0.089 ± 0.003
3.6	0.244 ± 0.002	2.5	0.121 ± 0.002
4	0.277 ± 0.003	3	0.161 ± 0.003
Dichloromethane/methanol (1:1v/v) crude extract	0.182 ± 0.003	Dichloromethane/methanol (1:1 v/v) crude extract	0.057 ± 0.003
Dichloromethane crude extract	0.313 ± 0.003	methanol crude extract	0.101 ± 0.008

The total steroid content of dichloromethane/methanol (1:1 v/v) and dichloromethane crude extracts of *Euphorbia candelabrum* were determined using a UV-Vis spectrometer at 618 nm. Dexamethasone was used as the standard, and the absorbance was recorded for each standard and samples (Table 5). The concentration of the samples were obtained from the regression equation of the calibration curve ($y = 0.089x - 0.0774$, $R^2 = 0.996$) (Appendix 5) and the absorbance of each sample by using equation 2. The values obtained for dichloromethane/methanol (1:1 v/v) and dichloromethane crude extracts were 2.914 µg/ml and 4.352 µg/ml, respectively.

The total steroid content was calculated using the equation 3. The results obtained for the dichloromethane/methanol (1:1 v/v) and dichloromethane extracts were 2.91 mg DE/g and 4.35 mg DE/g of extract, respectively. When comparing the two extracts, the dichloromethane crude extract had a higher steroid content than the dichloromethane/methanol (1:1 v/v) extract. Due to their lipophilicity, steroids dissolve readily in nonpolar solvents like dichloromethane. This solvent's efficient extraction of lipids and steroids results in a crude extract with higher steroid concentrations. The dichloromethane/methanol (1:1 v/v) extract, on the other hand, has both nonpolar and polar properties, allowing it to extract a wider range of compounds. However, this may come at the expense of steroid extraction efficiency. Since methanol is a polar solvent, it can extract polar compounds, diluting the overall steroid content in the extract.

Furthermore, the total content of flavonoids, phenolics, alkaloids, and steroids from *Euphorbia candelabrum* was determined for the first time in this study. These investigations represent the first reported quantitative analysis of secondary metabolites from *Euphorbia candelabrum*. The

findings revealed that the methanol crude extract contained the highest quantities of phenolics, flavonoids, and alkaloids, while the dichloromethane crude extract contained the highest quantity of steroids.

This finding is also comparable to that reported for another *Euphorbia* species, *Euphorbia hirta*. The total flavonoid content and phenol content of the methanol root extract of *Euphorbia hirta* were reported as catechol equivalents using a standard curve ($Y = 0.0059X + 0.029$, $R^2 = 0.9932$) and gallic acid equivalents using a standard curve ($Y = 0.0073X + 0.1003$, $R^2 = 0.987$), respectively. The roots had flavonoid contents of 4.350 mg CEQ/g and phenolic contents of 18.315 mg GAE/g [82]. When compared with the methanol root extract of *Euphorbia candelabrum*, *Euphorbia candelabrum* had slightly greater flavonoid (4.4 mg QE/g) and phenolic (19 mg GAE/g) content than *Euphorbia hirta*. But, alkaloid and steroid contents have not been determined.

4.4 Isolation of the compounds using column chromatography

After packing the column with a sample, the elution process began with solvents starting from an n-hexane/ethyl acetate composition and gradually increasing in polarity, as detailed in the previous section (3.2.6). This sequential elution process yielded a total of 176 fractions, each with a volume of 30 ml, which were collected and then reduced to five fractions. These fractions were concentrated using a rotary evaporator and those with no spots or with complex patterns of several spots were not combined and discarded. The details regarding the number of fractions, solvent system used, ratio, and number of spots collected are presented in Table 6.

Fractions 147-174 eluted with n-hexane/ethyl acetate (55:45) were combined based on the similarities of their TLC profiles. This led to the isolation of 610 mg of orange color solid compound labelled KJ-1 with an R_f value of 0.53 in a 55:45 n-hexane/ethyl acetate solvent system.

A total of 500 mg of an intense yellow crystalline solid compound labelled KJ-2 was obtained by combining fractions 102-115 that were eluted with an n-hexane/ethyl acetate (65:35) solvent system. Its R_f value was determined to be 0.58 in a 65:35 n-hexane/ethyl acetate solvent system.

A total of 220 mg of a deep yellow crystalline solid compound labelled KJ-3 was obtained by combining fractions 78-81, which were eluted with an n-hexane/ethyl acetate (70:30) solvent system, and its R_f was determined to be 0.56 for the 70:30 n-hexane/ethyl acetate mixture.

The compound KJ-4 was obtained from a combined fraction of 41-43 that was isolated with n-hexane/ethyl acetate (80:20) as the solvent. This led to the isolation of 100 mg of white crystal, and its R_f was determined to be 0.55 for the 80:20 n-hexane/ethyl acetate mixture.

White amorphous solid 100 mg labelled KJ-5 was isolated from a combined fraction of 34-38 that was eluted with n-hexane/ethyl acetate (85:15) as the solvent. Its R_f value was determined to be 0.52 in an 85:15 n-hexane/ethyl acetate solvent system.

Table 6 : The fractions of the dichloromethane/methanol extract (1:1 v/v) collected

Fractions	Solvent system	Ratio	No. of spots
1-9	n-hexane/ethyl acetate	100:0	No spot
10-19	n-hexane/ethyl acetate	95:5	No spot
20-28	n-hexane/ethyl acetate	90:10	No spot
29-33	n-hexane/ethyl acetate	85:15	Two spots
34-38	n-hexane/ethyl acetate	85:15	One spot
39-40	n-hexane/ethyl acetate	85:15	Two spots
41-43	n-hexane/ethyl acetate	80:20	One spot
44-46	n-hexane/ethyl acetate	80:20	Two spots
47-60	n-hexane/ethyl acetate	80:20	Three spots
61-75	n-hexane/ethyl acetate	75:25	Three spots
76-77	n-hexane/ethyl acetate	70:30	Two spots
78-81	n-hexane/ethyl acetate	70:30	One spot
82-90	n-hexane/ethyl acetate	70:30	Two spots
91-95	n-hexane/ethyl acetate	65:35	Three spots
96-101	n-hexane/ethyl acetate	65:35	Two spots
102-115	n-hexane/ethyl acetate	65:35	One spot
116-122	n-hexane/ethyl acetate	60:40	Two spots
13-140	n-hexane/ethyl acetate	60:40	Three spots
141-146	n-hexane/ethyl acetate	55:45	Two spots
147-174	n-hexane/ethyl acetate	55:45	One spot
175-176	n-hexane/ethyl acetate	55:45	No spot

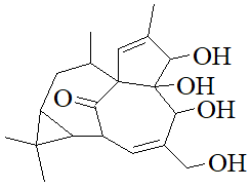
4.5 Partial characterization of the isolated compounds

As presented in the previous section (section 4.3), five compounds (KJ-1, KJ-2, KJ-3, KJ-4 and KJ-5) were isolated from the crude dichloromethane/methanol (1:1 v/v) extract of the root of *Euphorbia candelabrum*. Three compounds (KJ-1, KJ-2 and KJ-3) were characterized using spectroscopy (UV-Visible and FT-IR). The rest (KJ-4 and KJ-5) were not characterized due to small quantities for spectral analysis. The structural elucidation of the compounds is briefly presented in the following sub sections.

4.5.1 Partial characterization of KJ-1

Compound KJ-1 has a melting point of 152-158 ^{0}C. The UV–visible spectrum (Appendix 6) showed absorption bands at λ_{max} 279 nm and 314 nm, which suggested the presence of a conjugated system, such as an alpha, beta-unsaturated ketone or aldehyde. The two distinct absorption bands confirm that the compound contains a conjugated system, such as a chromophore with extended Π-electron delocalization.

The FT-IR (KBr) spectrum (Appendix 7) showed intense and broad absorption bands at 3404 cm^{-1} and intense sharp absorption bands at 1714 cm^{-1} corresponding to the stretching vibrations of the hydroxyl (OH) and carbonyl (C=O) groups, respectively. The appearance of bands at 2921 cm^{-1} and 2854 cm^{-1} could be attributed to the C-H stretching of methylene and methyl groups, respectively. The absorption bands at 1598 cm^{-1} and 1514 cm^{-1} could be attributed to C=C stretching. The presence of peaks at 1464 cm^{-1} and 1378 cm^{-1} suggested C-H bending of methylene and methyl groups, respectively. According to spectral data the compound KJ-1may be Ingenol (compound **14)**, which has melting point 153 ^{0}C. It was reported that, this compound was found in many *Euphorbia* species such as *Euphorbia ingens*, *Euphorbia matabelensis* and *Euphorbia tirucali* [45, 60]. Further analysis using an NMR spectrophotometer is necessary.



14

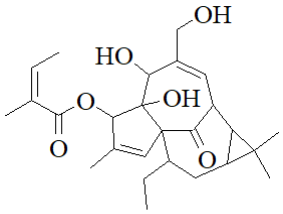
Figure 7 : The chemical structure of Ingenol

4.5.2 Partial characterization of KJ-2

Compound KJ-2 has a melting point of 194-198 ^{0}C. UV-vis absorption spectra (Appendix 8) showed bands or λ_{max} at 259 nm, 282 nm and 321 nm, which confirmed the presence of a conjugated system. This indicates that the compound has absorption peaks at these wavelengths when exposed to light. These three wavelengths indicate that three distinct absorption bands may be conjugated, carbonyl groups and extended π-systems of the compound.

The FT-IR spectrum (Appendix 9) showed a strong broad and intense absorption band at approximately 3436 cm^{-1}, indicating the presence of a hydroxyl (OH) group in the compound. The strong absorption bands at 2933 cm^{-1} and 2850 cm^{-1} revealed the presence of C-H stretching vibrations of methylene and methyl groups, respectively and the strong intense absorption band at 1730 cm^{-1} showed the existence of carbonyl group (C=O) in the compound. The absorption

bands around 1632 cm^{-1} and 1513 cm^{-1} suggested the presence of double (C=C) bonds. The peaks at 1464 cm^{-1} and 1375 cm^{-1} indicate the occurrence of C-H bending of methylene and methyl groups, respectively. The bands at approximately 1228 cm^{-1} revealed the presence of C-O stretching in the compound. The spectral data suggest that compound KJ-2 may be ingenol mebutate (compound **15)**, a known compound with a melting point of 198-202 °C. Compound **15** has been previously reported in several *Euphorbia* species, including *Euphorbia ingens*. To confirm compound KJ-2, further analysis using an NMR spectrophotometer is necessary [45].



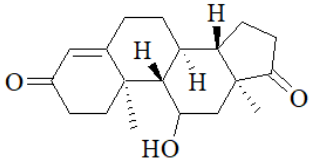
15

Figure 8 : The chemical structure of Ingenol mebutate

4.5.3 Partial characterization of KJ-3

Compound KJ-3 has a melting point of 180-184 ^{0}C. The UV-visible spectra (Appendix 10) showed an absorption band at λ_{max} 286 nm which suggested the presence of a conjugated system in the compound, such as an aromatic ring or an aldehyde carbonyl group (C=O).

The IR spectrum (Appendix 11) showed an absorption band at 3445 cm^{-1} indicating the presence of O-H stretching. The bands at 2921 cm^{-1} and 2861 cm^{-1} indicated the presence of C-H stretching of methylene and methyl group respectively. The absorption bands at 1732 cm^{-1} and 1667 cm^{-1} indicate the absorption of the carbonyl and α,β-unsaturated carbonyl (C=O), respectively in the compound.. The bands at 1455 cm^{-1} and 1377 cm^{-1} suggest C-H bending of methylene and methyl group, respectively. The spectral data suggest that compound KJ-3 may be 11-hydroxtandrostenedione (**95**), a compound that has a melting point of 189-191 °C. Notably, this compound has also been isolated from the plant species *Euphorbia heterophylla* [91]. To confirm compound KJ-2, further analysis using an NMR spectrophotometer is necessary



95

Figure 9 : Chemical structure of 11-hydroxtandrostenedione

4.6 Antibacterial activity tests

The antibacterial activity of the crude extracts (dichloromethane, dichloromethane/methanol (1:1 v/v) and methanol) from the roots of *Euphorbia candelabrum* and the isolated compounds (KJ-1, KJ-2 and KJ-3) were tested at a concentration of 150 mg/ml against four pathogenic bacterial strains: two gram-positive bacteria (*Staphylococcus aureus* and *Streptococcus pyogen*) and two gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*) (Appendix 8). These activities were performed at the microbiology laboratory of the Department of Biology, Adama Science and Technology University. The antibacterial activity was determined by measuring the inhibition zone in diameter and all zones of inhibition were measured using a ruler. Each experiment was carried out in triplicate and the results are expressed as the mean ± standard deviation (Table 7).

Table 7 : Antibacterial inhibition zones of crude extracts and isolated compounds of *Euphorbia candelabrum*

Samples and conc. (150 mg/mL)	Bacteria species and zone of inhibitions in millimeter (mm)			
	E. coli ATCC25922	*S. aureus* ATCC25923	*P. aeruginosa* ATCC27853	*S. pyogenes* ATCC19615
1	6.5 ± 0.08	7 ± 0.10	6 ± 0.09	7 ± 0.11
2	7 ± 0.13	8 ± 0.08	7 ± 0.10	8 ± 0.06
3	7 ± 0.05	7 ± 0.10	6.5 ± 0.05	7.5 ± 0.06
4	9 ± 0.06	10 ± 0.09	8.5 ± 0.05	10.5 ± 0.03
5	8 ± 0.11	9 ± 0.08	8 ± 0.08	9.5 ± 0.06
6	10 ± 0.10	11 ± 0.05	9 ± 0.10	11.5 ± 0.07
Tetracycline (+vecontrol)	12 ± 0.04	12.5 ± 0.06	11.5 ± 0.06	13.5 ± 0.08
DMSO (-ve control)	0	0	0	0

Key: +ve = positive, -ve = negative, 1 = dichloromethane crude extract, 2 = methanol crude extract, 3 = dichloromethane/methanol (1:1 v/v) crude extract, 4 = compound KJ-1, 5 = compound KJ-2, 6= compound KJ-3, DMSO = dimethyl sulfoxide, Sd = Standard deviation, *E. coli = Escherichia coli*, *P.aeruginosa = Pseudomonas aeruginosa*, *S.aureus = Staphylococcus aureus*, *S.pyogenes = Streptococcus pyogenes*

The results revealed that the crude extracts (dichloromethane, methanol and dichloromethane/methanol (1:1 v/v)) had inhibition zones ranging from 6 mm to 7 mm for the gram-negative bacteria and from 7 mm to 8 mm for the gram-positive bacteria. Dichloromethane and methanol extracts showed greater antibacterial activity against gram-positive bacteria than against gram-negative bacteria at a concentration of 150 mg/ml. The dichloromethane/methanol 1:1 v/v crude extract showed greater antibacterial activity against the gram-positive bacteria S. *pyogenes* (7.5 mm) than against gram-negative bacteria such as *E. coli* (7 mm) and *P. aeruginosa* (6.5 mm). The gram-positive bacteria *S. aureus* (7 mm) showed the same antibacterial activity as the gram-negative bacteria *E. coli* (7 mm) but had greater antibacterial activity than *P.*

aeruginosa (6.5 mm). When the crude extracts were compared with each other against all selected pathogenic strains, the methanol extract showed a relatively greater inhibition zone due to the high content of flavonoids and phenolics (Table 7).

Three compounds isolated from dichloromethane/methanol (1:1 v/v) labeled as KJ-1, KJ-2 and KJ-3 showed good activity against all the tested human pathogenic bacteria. The inhibition zones for gram-negative bacteria ranged from 8 mm to 10 mm, and those for gram-positive bacteria ranged from 9 mm to 11.5 mm. All the compounds displayed greater inhibitory activity against gram-positive bacteria compared to gram negative-bacteria (Table 5).

The compound KJ-3 showed an inhibition zone of 10 mm against *E.coli* while crude extracts (dichooromethane, methanol and dihloromethane/methanol 1:1 v/v) and isolated compounds (KJ-1 and KJ-2) had an inhibition zone of 6.5 mm, 7 mm, 7 mm, 9 mm and 8 mm, respectively, against the same bacteria. For *P.aeruginosa*, the inhibition zone of KJ-3 was 9 mm, while those of dichloromethane, methanol, dichloromethane/methanol (1:1 v/v), KJ-1 and KJ-2 were 6 mm, 7 mm, 6.5 mm, 8.5 mm, and 8 mm, respectively. For *S.aureus*, the zone of inhibition of KJ-3 was 11 mm, while those of the other tested extracts and isolated compounds (dichloromethane, methanol, dichloromethane/methanol (1:1 v/v), KJ-1 and KJ-2) were 7 mm, 8 mm, 7 mm, 10 mm and 9 mm, respectively. For S.*pyogenes* bacteria, inhibition zone of KJ-3 was 11.5 mm while dichloromethane, methanol, dichloromethane/methanol 1:1 v/v, KJ-1 and KJ-2 was 7 mm, 8 mm, 7.5 mm, 10.5 mm, 9.5 mm and 11.5 mm respectively. Therefore, KJ-3 showed greater antibacterial activity against *E.coli*, *P.aeruginosa*, *S.aureus* and *S.pyogenes* than did the other isolated compounds and crude extracts.

The antibacterial activity of dichloromethane, methanol, and dichloromethane/methanol (1:1 v/v) extracts from *Euphorbia candelabrum* was determined for the first time. Additionally, the antibacterial activity of an isolated compound from the dichloromethane/methanol (1:1) extract was also evaluated. These investigations represent the first reported studies on the antibacterial activity of these extracts and isolated compound from *Euphorbia candelabrum*.

The antibacterial activity of *Euphorbia candelabrum* root extract is comparable to that reported for another *Euphorbia* species, *Euphorbia hirta*. Using the disc diffusion method, the methanol root extract of *Euphorbia hirta* exhibited larger inhibition zones against gram-positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*) than Gram-negative bacteria (*Escherichia coli* and *Klebsiella pneumoniae*). The inhibition zones were 21 mm, 20 mm, 15 mm, and 12 mm, respectively. The standard antibiotics chloramphenicol showed inhibition zones ranging from 21

to 26 mm. In contrast, the inhibition zone of methanol (negative control) was negligible for all tested microorganisms. In both *Euphorbia candelabrum* and *Euphorbia hirta* methanol root extract, gram-positive bacteria had greater inhibition zones than gram-negative bacteria and *Staphylococcus aureus* had greater inhibition zones than all tested bacteria. Therefore, *Euphorbia candelabrum* methanol root extract had comparable antibacterial activity to the methanol root extract of *Euphorbia hirta* [90].

5. CONCLUSION AND RECCOMENDATION

5.1 Conclusion

This study is the first to report phytochemical studies and antibacterial activities on the root extracts as well as three isolated compounds from *Euphorbia candelabrum*. The phytochemical screening test of methanol, dichloromethane and dichloromethane/methanol (1:1 v/v) crude extracts of *Euphorbia candelabrum* confirmed the presence of alkaloids, flavonoids, phenols, glycosides, terpenoids, tannins, saponins and steroids, while n-hexane crude extracts confirmed the presence of alkaloids, glycosides, terpenoids, steroids and saponins. The total flavonoid, phenolic, alkaloid and steroid contents in the dichloromethane/methanol (1:1 v/v) and methanol extracts were determined. Methanol extract has high content of phenolic, flavonoid and alkaloid than dichloromethane/methanol (1:1 v/v) extract.

The chromatographic separation of dichloromethane/methanol (1:1 v/v) root extract of *Euphorbia candelabrum* resulted in isolation of three compounds and their structures were not determined due to the lack of NMR instrument. The antibacterial activities tests of the isolated compounds and crude extracts against two gram-positive bacteria (*S. aureus* ATCC25923 and S. *pyogenes* ATCC19615) and two gram-negative bacteria (*E. coli* ATCC25922 and *P. aeruginosa* ATCC27853) were evaluated via the disc diffusion method. Among the crude extracts, the methanol extract moderately inhibited the growth of both gram-positive and gram-negative bacteria. Compared with the crude extracts and isolated compounds, the KJ-3 compound inhibited the growth of both gram-positive and gram-negative bacteria more strongly. The observed antibacterial activities support the traditional use of this plant for various ailments. Therefore, *Euphorbia candelabrum* root can be a potential source of natural antibacterial agents.

5.2 Recommendation

Based on the present study, the following recommendations are forwarded

- In this study, a phytochemical investigation of the root extract of *Euphorbia candelabrum* was carried out. Thus, we recommended conducting phytochemical investigations on the root bark extract of *Euphorbia candelabrum* by using different solvent systems and separation techniques.
- In this study, the structure of the isolated compounds was not characterized due to the lack of an NMR instrument. Therefore, we recommend characterizing the compounds using NMR, x-ray crystallography, and other instruments.
- *In vitro* antibacterial activity tests using several bacterial strains are recommended to discover lead compounds from the root of *Euphorbia candelabrum* in search of new antibacterial agents.
- Crude TLC analysis revealed unidentified compounds missed during chromatographic separation. We recommend continued investigation using advanced separation techniques like RP-HPLC to identify these compounds.
- Quantitative analysis of total content of tannin, saponins, terpenoids and glycoside were not determined in this study due to lack of instrument and reagents. We recommended conducting quantitative analysis of the total content of tannin, saponins, terpenoids and glycoside.
- Antioxidant activity tests are recommended by using different methods that can be used to measure antioxidant activity to discover antioxidants.
- Additional studies are needed with this plant to evaluate for *in vivo* assay.

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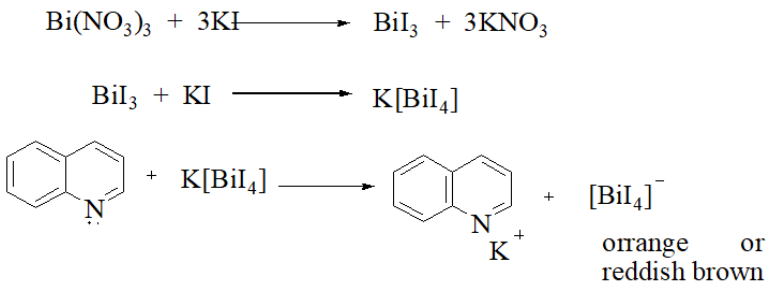
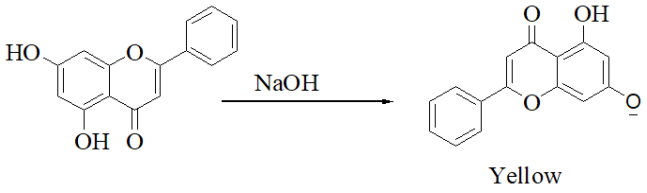
APPENDICES

Appendix 1 : Chemical reactions of phytochemical screening test

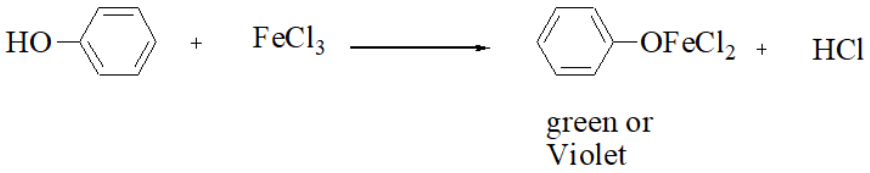
I. Test for alkaloids



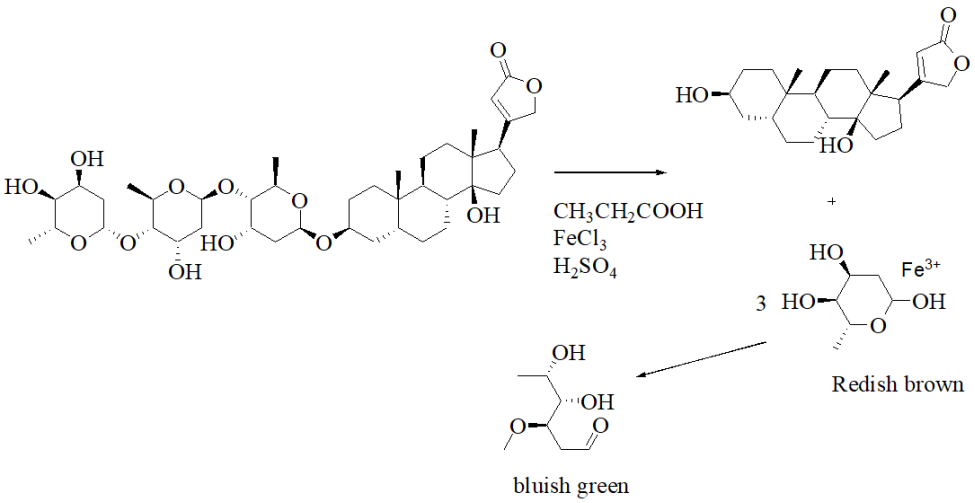
II. Test for flavonoids



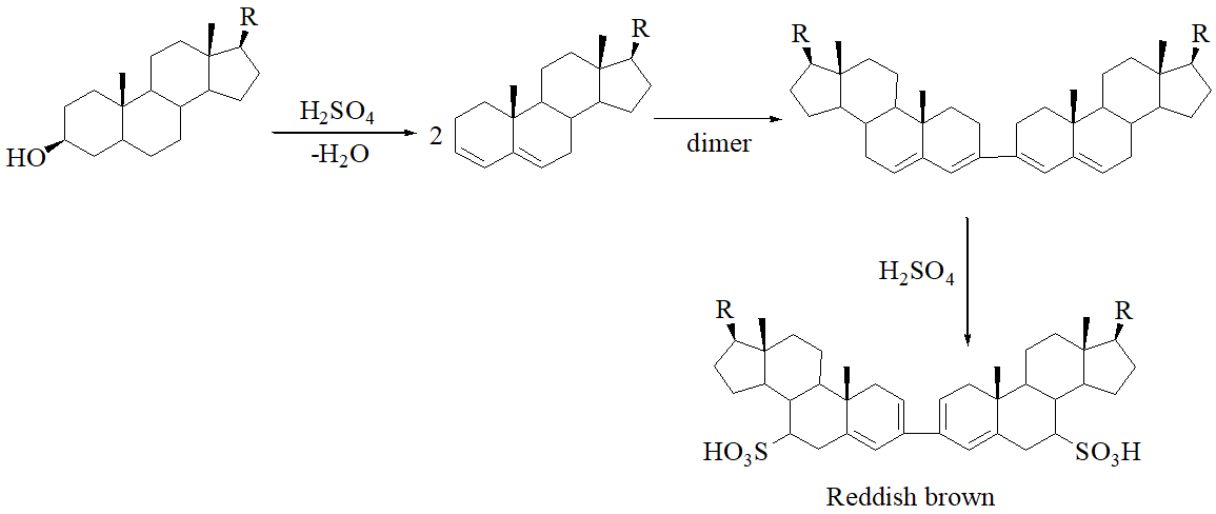
III. Test for phenols



IV. Test for glycosides

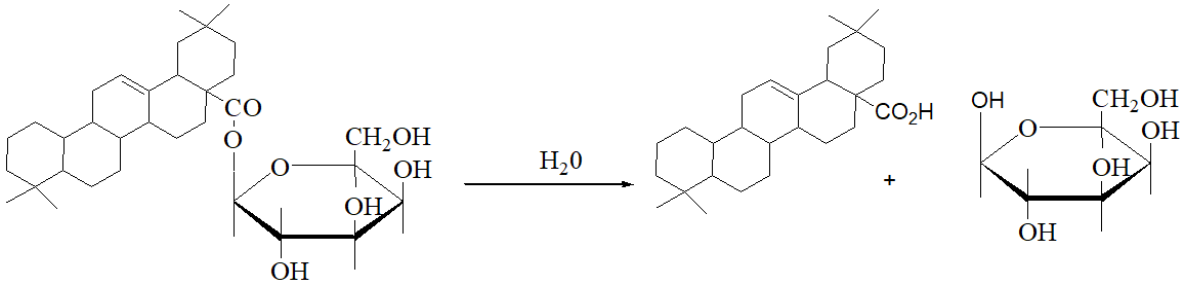


V. Test for terpenoids

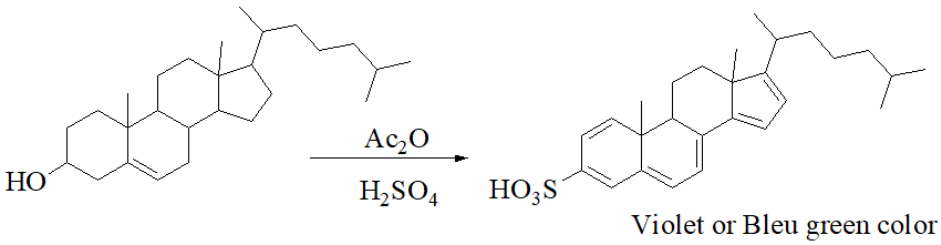


Reddish brown

VI. Test for saponin

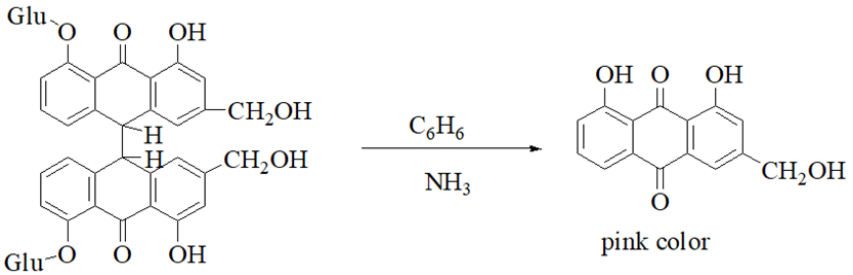


VII. Test for steroids



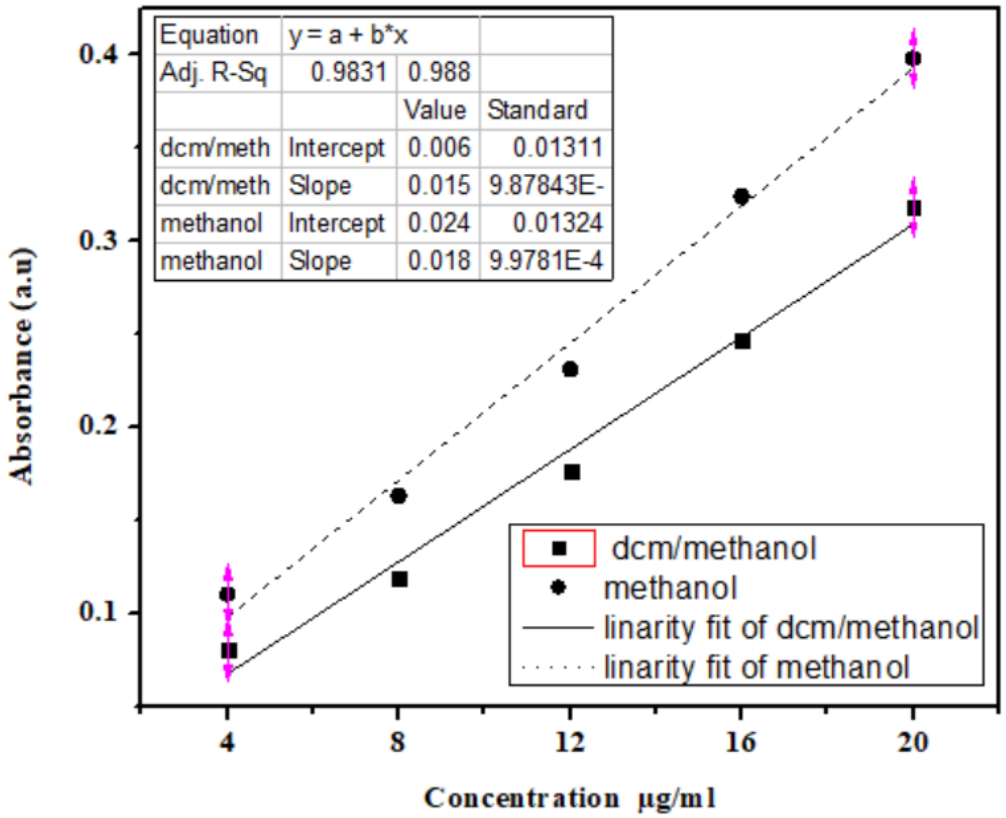
Violet or Bleu green color

VIII. Test for anthraquinones

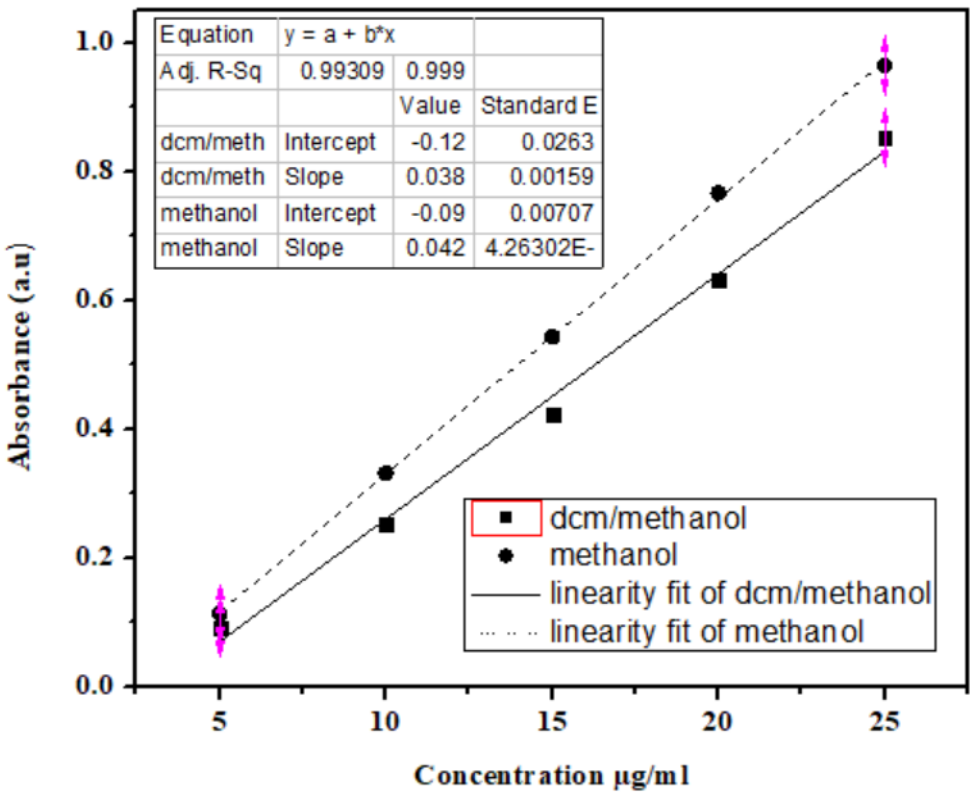


pink color

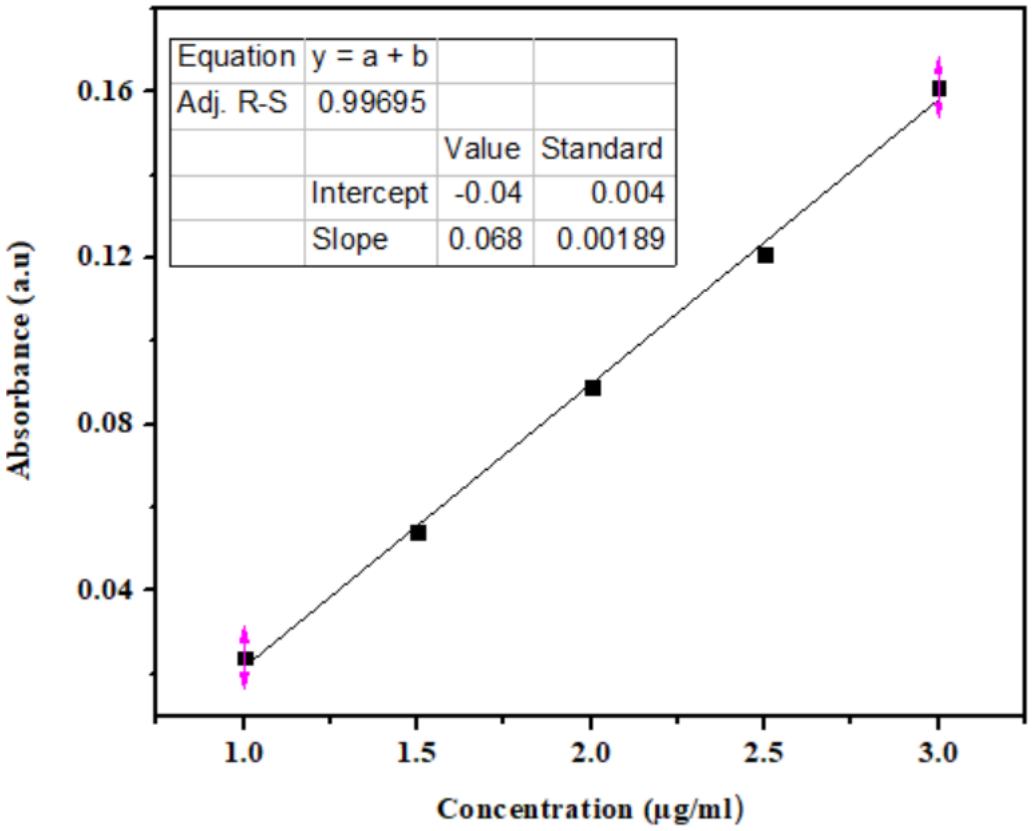
Appendix 2 : The standard curve of quercetin



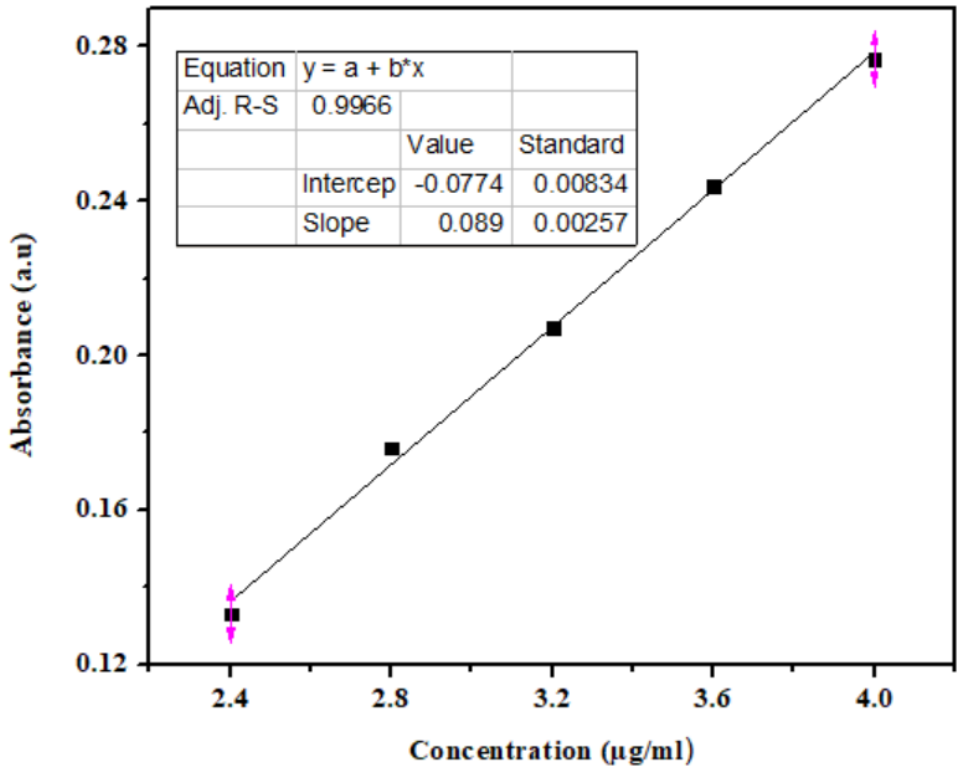
Appendix 3 : The standard curve of gallic acid



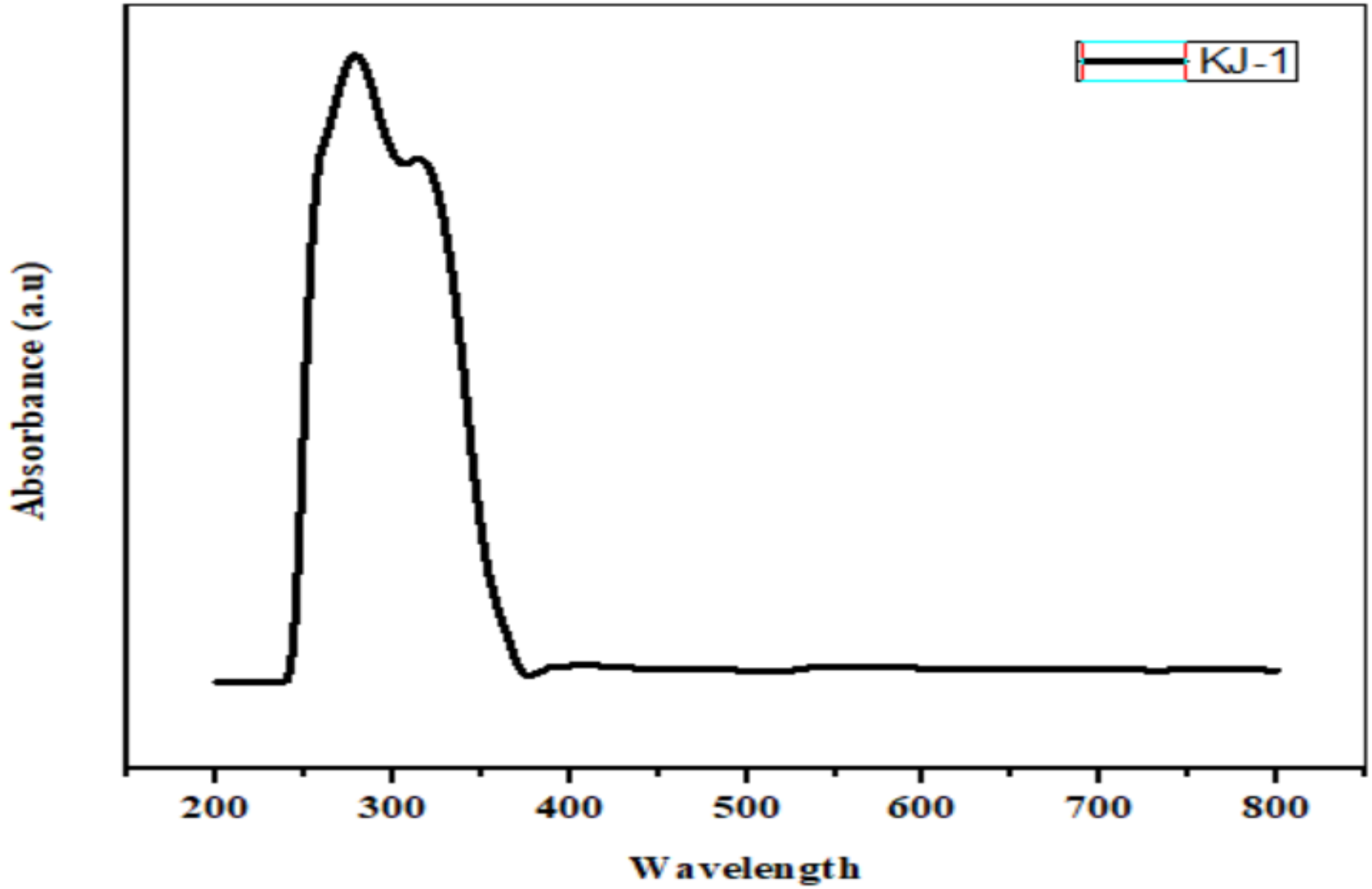
Appendix 4 : The standard curve of Atropine



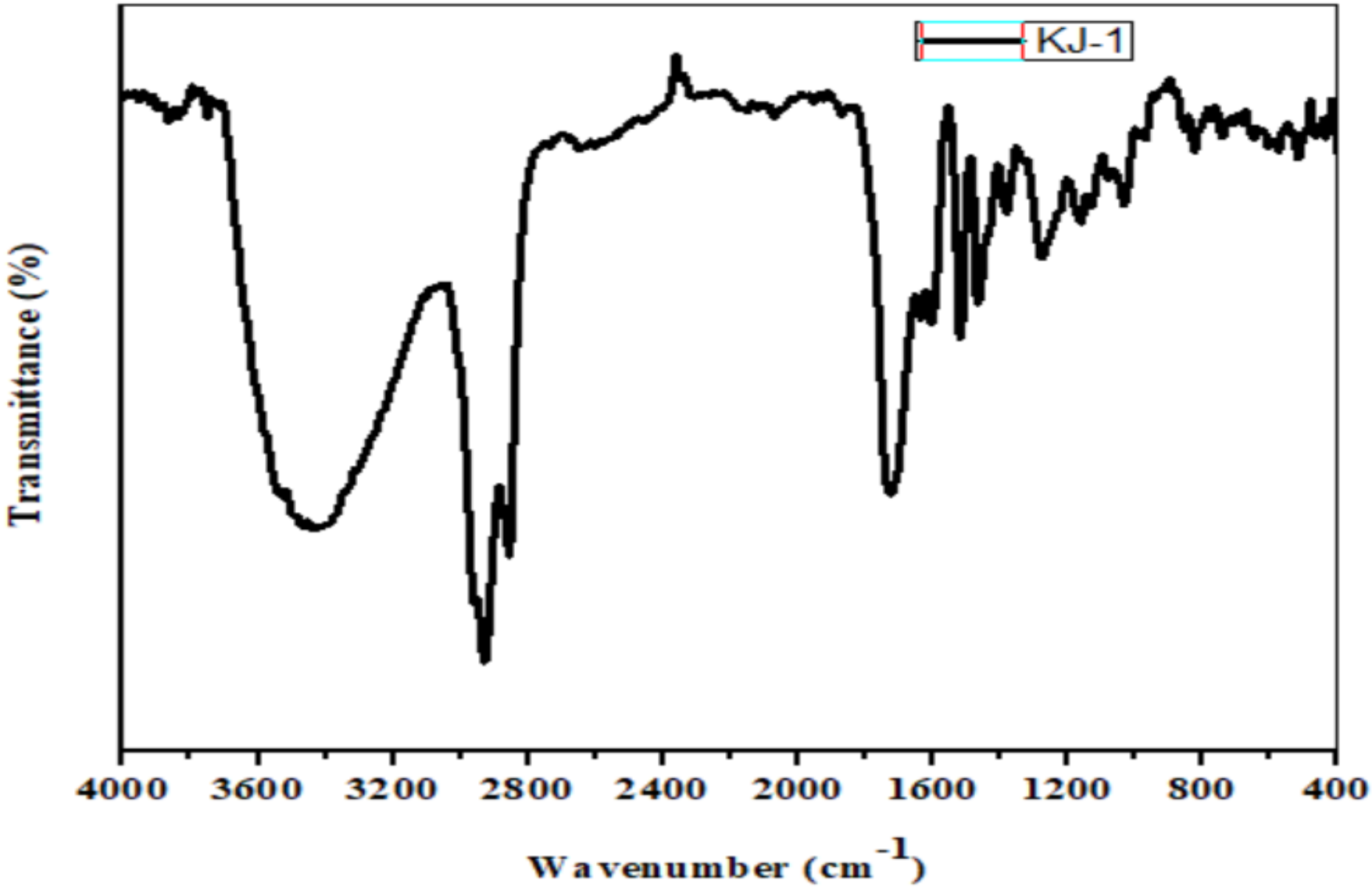
Appendix 5 : The standard curve of dexamethasone



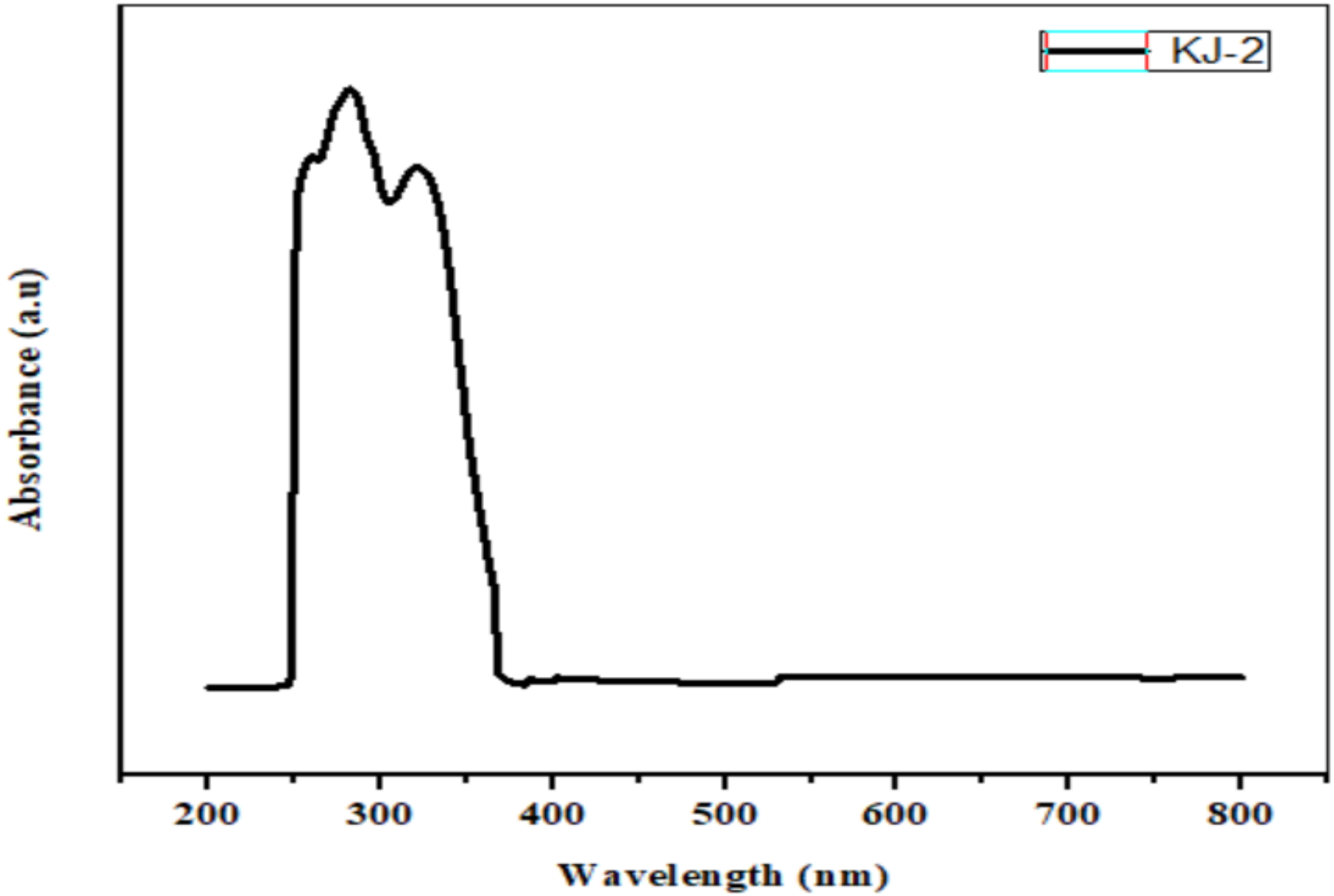
Appendix 6 : The UV-Vis absorption spectrum of compound KJ-1



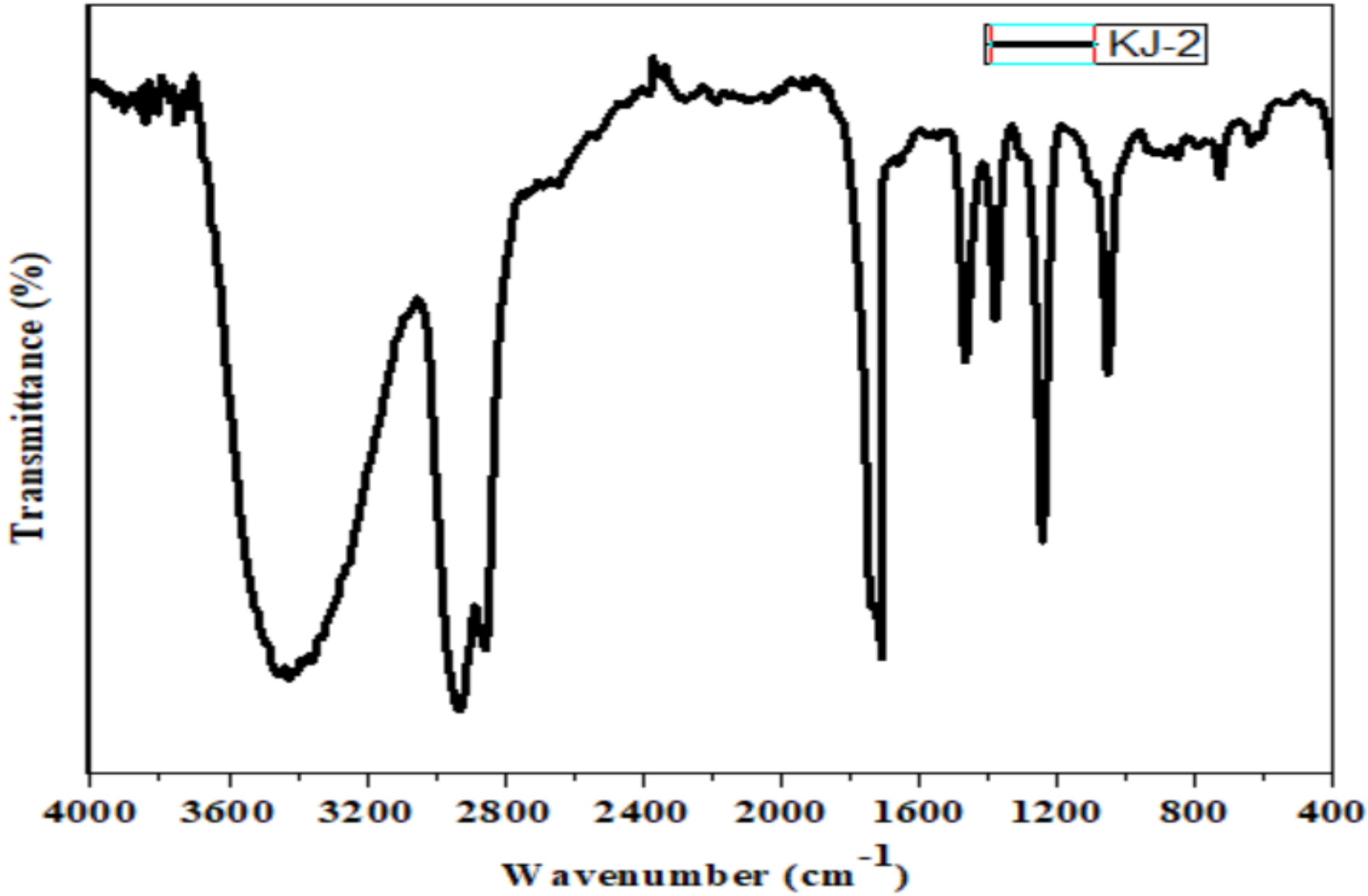
Appendix 7 : The FT-IR absorption spectrum of compound KJ-1



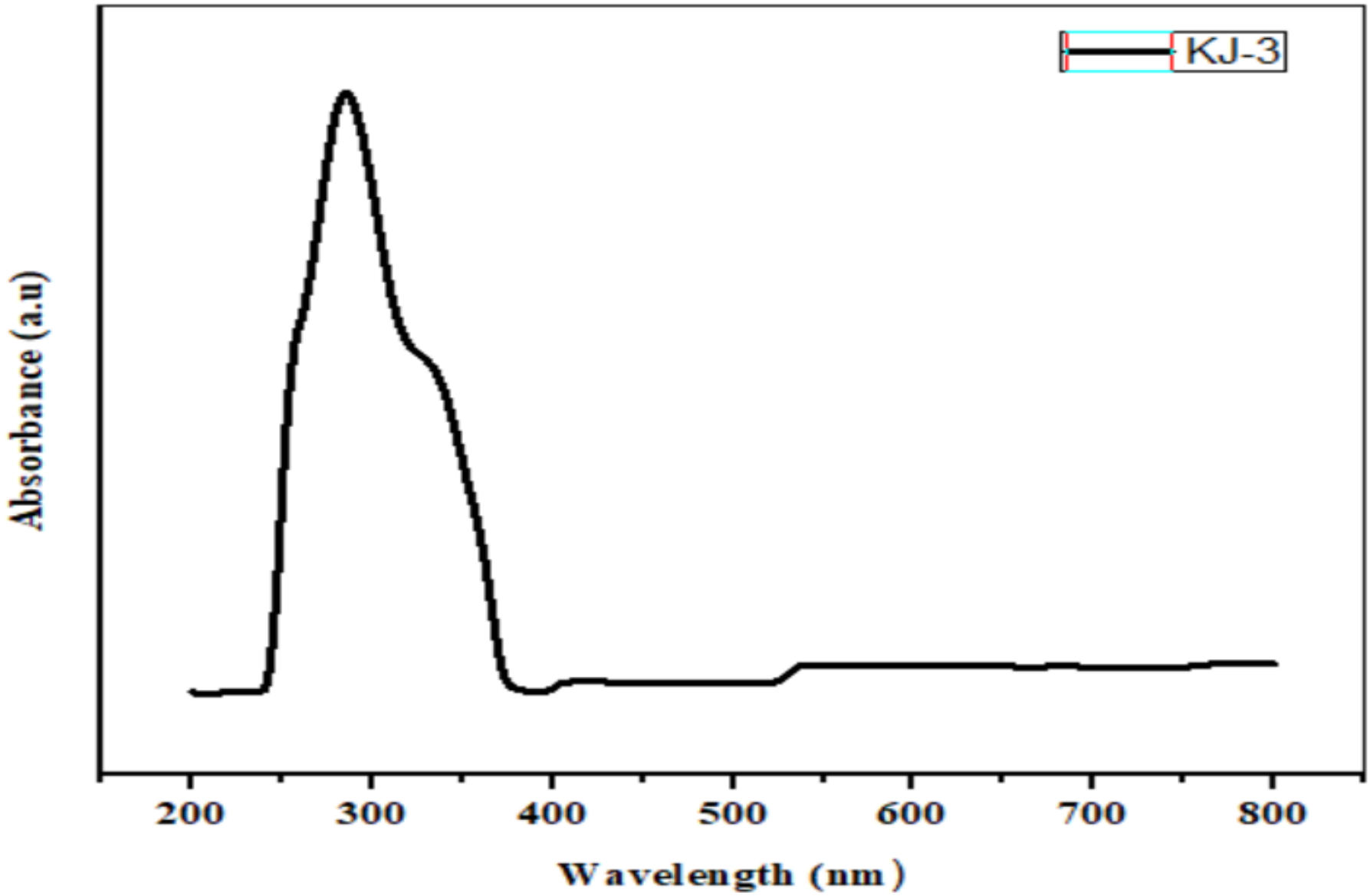
Appendix 8 : The UV-Vis absorption spectrum of compound KJ-2



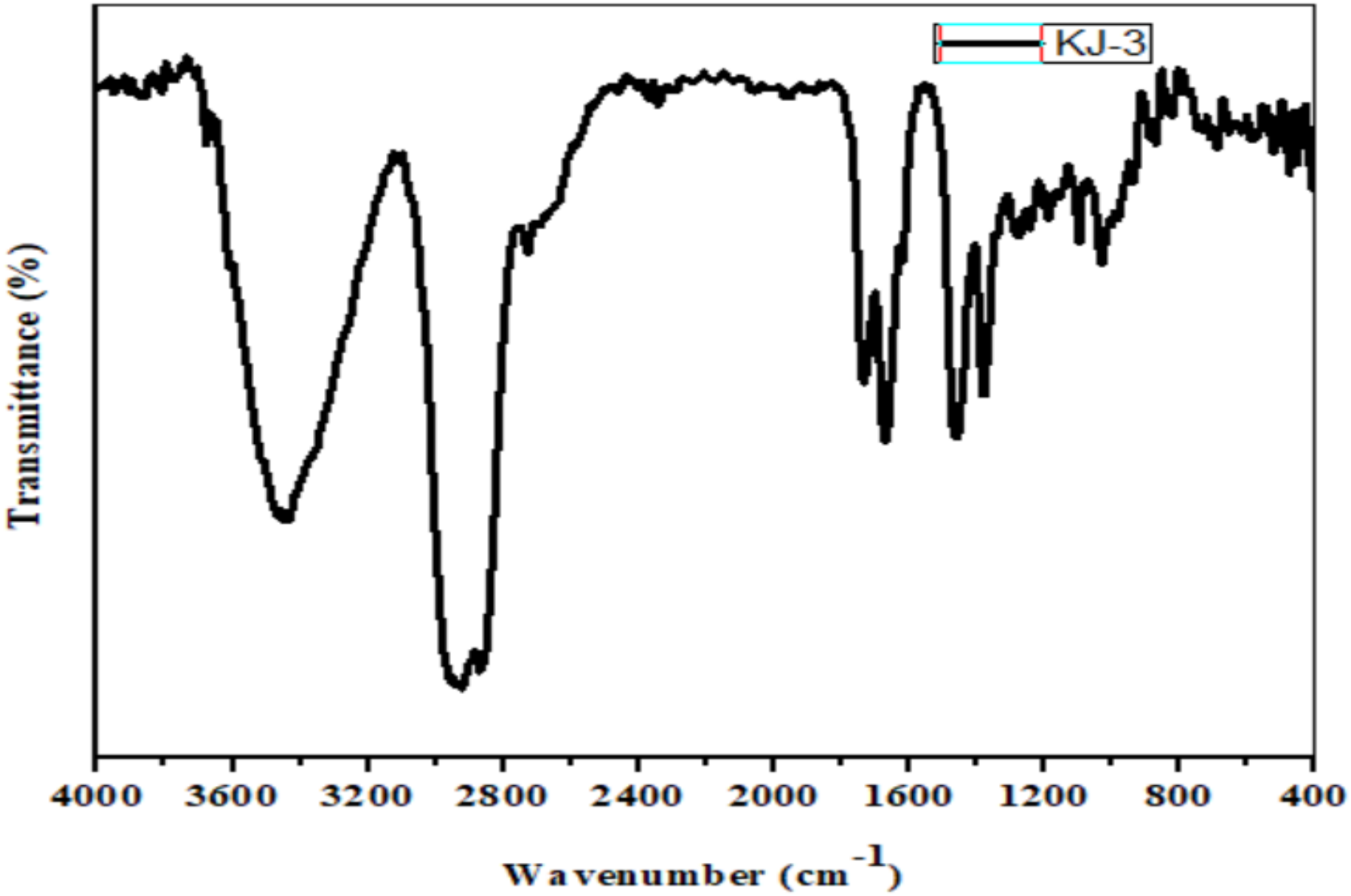
Appendix 9 : The FT-IR absorption spectrum of compound KJ-2



Appendix 10 : The UV-Vis absorption spectrum of compound KJ-3



Appendix 11 : The FT-IR absorption spectrum of compound KJ-3



Appendix 12 : Photo of antibacterial activity test