HAWASSA UNIVERSITY

COLLEGE OF NATURAL AND COMPUTATIONAL SCIENCES,

DEPARTMENT OF CHEMISTRY

PHYTOCHEMICAL INVESTIGATION OF THE STEM BARK OF *STEREOSPERMUM KUNTHIANUM*

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

BY:

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OCTOBER, 2024

HAWASSA, ETHIOPIA

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DECLARATION

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

Name: Abatihun Shitie

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Date:_____

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

Name of advisor: Dr. Tegene Tesfaye

Signature: _____

Date:_____

HAWASSA, ETHIOPIA

ADVISOR'S APPROVAL SHEET-1

This is to certify that the thesis entitled "Phytochemical investigation of the stem bark of *Stereospermum kunthianum*" submitted in partial fulfillment of the requirements for the degree Master of Science in Chemistry graduate program of the Department of Chemistry, Hawassa university, and is a record of original research carried out by Abatihun Shitie (ID, No Chem/k/046/09), 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.

Tegene Tesfaye (PhD) _____ _____

Name of advisor Signature Date

EXAMINER 'S APPROVAL SHEET-1

We, the undersigned, members of the Board of Examiners of the final open defense by Abatihun Shitie, have read and evaluated his thesis entitled" Phytochemical investigation of the stem bark of *Stereospermum kunthianum*," and examined the candidate. This is, therefore, to certify that the thesis has been accepted in partial fulfillment of the requirements for the degree.

Tegene Tesfaye (PhD)	_____	_____
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Final approval and acceptance of the thesis is contingent upon the submission of the final copy of the dissertation to the scool of Graduate studies (SGS) through the Department/School Graduate Committee (DGC/SGC) of the of candidate's department.

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LIST OF ABBREVIATIONS

CC	Column Chromatography
DCM	Dichloromethane
DMSO	Dimethyl Sulphoxide
HPLC	High performance liquid chromatography
IR	Infra Red
Rf	Retention factor
TLC	Thin Layer Chromatography
Uv	Ultraviolet
WHO	World Health Organization

TABLE OF CONTENTS

Contents **Page**

List of Tables

Table **Page**

List of Figures

Figure **Page**

ABSTRACT

*The traditional medicinal system continues to play an essential role in health care, with about 80% of the world's inhabitants relying mainly on traditional medicines for their primary health care. The genus Stereospermum is known to possess medicinal properties in every part of the plant. Stereospermum kunthianum is one of the medicinal plants that has been claimed to be used traditionally to treat several illnesses such stomachaches, toothache, snake bitze, gonorrhea, evil eyes, diarrhea, skin diseases, and headaches. This study was conducted to investigate "Phytochemical constituents of stem bark of Stereospermum kunthianum". The stem bark (500 g) of Stereospermum kunthianum was extracted with n-hexane, chloroform/methanol (v/v, 1:1) and methanol solvent systems sequentially using maceration technique and resulted in 1.8 g, 9.8 g and 15 g crude extract respectively. Phytochemical screening was carried out by standard procedures and the result showed the presence of the classes of secondary metabolites such as alkaloids, tannins, saponins, steroids, flavonoids, phenols, glycosides, and terpenoids.The chloroform/methanol (v/v, 1:1) crude extract was subjected to column chromatography that resulted in three compounds coded as **SK1**,**SK2** and **SK3**. The structures of the isolated compounds was partially characterized by generating UV and IR data,systematically interpreting the data,melting point and comparing the data with literature.The FTIR analysis revealed that **SK1**, **SK2** and **SK3** are in good agreement with solanidanes, α-amyrin and Betulin respectively.*

Key;word *Stereospermum kunthianum,phytochemical,FTIR,solanidanes,α-amyrin and Betulin.*

1. INTRODUCTION

1.1. BACK GROUND OF THE STUDY

A medicinal plant is any plant which, in one or more of its organs, contains substances that can be used for therapeutic purposes, or which are precursors for chemo-pharmaceutical semi-synthesis."When a plant is designated as medicinal it is implied that the said plant is used as a drug or therapeutic agent or an active ingredient of a medicinal preparation. Medicinal plants have been identified and used throughout human history. Plants have the ability to synthesize a wide variety of chemical compounds that are used to perform important biological functions, and to defend against attack from predators such as insects, fungi and herbivorous mammals, since ancient civilization, herbs and plants products have been used for treatment of wide range of diseases. Especially, people living in rural areas have been using indigenous plants as medicines [1]. Therefore, the World Health Organization (WHO) is now actively encouraging the use of herbal medicines, which they have been traditionally used for centuries. The HPLC is one of the most convenient and comprehensive and separation technique for separating individual components in plant extracts which has a great importance in relation to authentication, quantification and fingerprinting in the herbal industry [2].

Stereospermum, a genus (Family; Bignoniaceae) of at least 24 species distributed from Africa and Madagascar to South East Asia [3]. Some species in the genus *Stereopermum*, such as *Stereopermum acuminatissimum*, *Stereopermum cylindricum*, *Stereopermum kunthianum*, *Stereopermum personatum*, *Stereopermum suaveolens*, *Stereopermum tetragonum* and *Stereopermum zenkeri* were concentrated quinines as the main ingredient were illustrated [4].

Stereospermum kunthianum Cham (Family; Bignoniaceae) is a plant that has its different parts used in traditional medicine for the treatment of different ailments and conditions such as wounds, ulcers, gastritis, bronchitis and other pain and inflammatory related health conditions. It is a deciduous shrub or small tree widely spread across Africa with some species distribution in Asia. The species is well spread all over the Sahel region [5].

Stereospermum kunthianum Cham (Family; Bignoniaceae) is a small woody tree of about 5 or 15 m high and diameter 25 cm. It is found in the Sudano-Guinea savannah regions of Africa and Asia, where the plant parts are used to treat various ailments [6]. It has thin, grey-black bark,

smooth or flaking in patches, the trunk is rarely straight, with twisted branches with abundant, fragrant, precocious, pink or purplish flowers, making the tree a spectacular sight. The alternate leaves are imparipinnate compound and some 25 cm long; leaflets are nearly opposite with one terminal leaflet, and with short, soft hairs, oblong to oblong-elliptic in shape, green and hairless above, yellowish-green with prominent venation below, apex somewhat attenuate, and the base tapering. The leaf margin may be entire or sometimes toothed in coppice shoots, while petiolules are virtually absent. Petioles may be up to 7 cm long, and are caniculate. Immature leaves are occasionally toothed and hairy [7].

1.2. STATEMENT OF THE PROBLEM

In developing countries, such as Ethiopia, traditional medicine has played a significant role in treating health problems in both livestock and humans but the knowledge and the practice of utilization is transferred orally from one generation to the next through professional healers, knowledgeable elders and/or ordinary people. This inhibits the standardization and international recognition of traditional medicine in Ethiopia. All *Stereospermum* species that are found in Ethiopia are well studied on root and root bark parts. Phytochemical investigation on the stem-bark parts of the plant is not studied so far, in Ethiopia. Therefore, the aim of the present study will be to identify the chemical constituents of the stem-bark of *Stereospermum kunthianum* and perhaps substantiate the traditional use of the plant on the basis of the medicinal importance of the chemical constituents, using previous studies from literature.

1.3. OBJECTIVES OF THE STUDY

1.3.1. General objective

The main objective of this study is investigation of the phytochemical constituents from crude extracts of stem bark of *Stereospermum kunthianum*.

1.3.2. Specific objectives

- To collect the stem bark of the plant for authentication and phytochemical analysis.
- To extract the plant part using solvents such as n-hexane, chloroform: methanol (1:1, v/v) ratio and methanol using maceration technique.
- To carry out phytochemical screening tests on the crude extracts.
- To characterize the compounds isolated from the extracts using spectroscopic (IR and UV-Vis) data.

1.4. Significance of the study

The finding of this study would help to

- Serve as baseline for further studies on different plant parts of *Stereospermum kunthianum.*
- Give some information about the chemical constituents of the stem bark of *Stereospermum kunthianum.*
- Give some information about isolated compound from stem bark of *Stereospermum kunthianum.*

2. LITERATURE REVIEW

In this section a review of the genus *Stereospermum* and its ethnobotanical uses, phytochemistry and biological activity of selected *Stereospermum* species is presented.

2.1. The Genus *Stereospermum*

Stereospermum, a genus (Family; Bignoniaceae) of at least 24 species distributed from Africa and Madagascar to Southeast Asia [3]. The genus *Stereospermum* is further organized into species including *Stereospermum acuminatissimum, Stereospermum binhchauensis, Stereospermum annamens*e, *Stereospermum arcuatum, Stereospermum Boivini, Stereospermum chelonoides, Stereospermum cylindricum, Stereospermum euphorioides, Stereospermum suaveolens*, [8]. The genus *Stereospermum* is found in dry areas of deciduous forest, woodland, bush, rocky outcrops, termite mounds and margins of evergreen forests. The species is well spread all over the Sahel region and is often found near streams. *Stereospermum kunthianum* species distributed Democratic Republic of Congo, Djibouti, Eritrea, Ethiopia, Kenya, Mozambique, Senegal, Somalia, South Africa, Sudan, Tanzania, Uganda, Zambia and Zimbabwe [8-12].

2.2. Ethnobotany

The leaves of *Stereospermum* is used to treat otalgia, odantalgia, rheumatalgia, malarial fever, wounds, chronic dyspepsia, and anti-pyretic. Its root is used to treat bitter, astringent, acrid, anodyne, appetizer, constipating, diuretic, lithotropic, expectorant, cardio tonic, aphrodisiac, anti-inflammatory, anti-bacterial, febrifuge and tonic, anti-emetic, anti-pyretic, asthma and cough. Its flower is used to treat burning sensation, vitiated condition of pittavata, cardiopathy, hiccough and general debility. Its fruit is used to treat vitiated condition of pitta and vata and its seed is used to treat external application in hemicranias [9]. *Stereospermum kunthianum* pods are chewed with salt to treat coughs and are used in treatment of ulcers, leprosy, skin eruptions and venereal diseases, while the stem bark decoction or infusion is used to cure bronchitis, pneumonia, cough, rheumatic arthritis and dysentery [6]. The roots and leaves have been found useful in treating venereal diseases, respiratory ailments, and gastritis [6].

2.3 Phytochemistry and Biological Activities of *Stereospermum*

The review of the phytochemistry and biological activities of some selected species of *Stereospermum* is presented below.

2.3.1. *Stereospermum colais*

Stereospermum colais is a large straight stemmed deciduous tree 18-30 m in height and2.8 m in girth found throughout in moist regions of India up to an altitude of about 1200 m, chiefly in deciduous forests. Phytochemical screening of the class of secondary metabolites of crude extract of *Stereospermum colais* leaves, revealed the presence of flavonoid, alkaloid, quinones, cardiac glycosides, terpenoids, and tannins [15, 16]. *Stereospermum colais* (Bignoniaceae) leaves extract reported best antioxidant activity [17], stem bark contains pharmacologically active constituents which possess analgesic activity justifying its popular use in treatment of painful conditions [18].*Stereospermum colais* may be interesting for incorporation in pharmaceutical preparations for human health, since it can suppress hyperglycaemia, and or as food additives due to its antiradical efficiency [19]. Even though, b biological activities of *Stereospermum colais* were reported, to the best of our knowledge there was no on its phytochemical compostion.

2.3.2. *Stereospermum chelonoides*

Stereospermum chelonoides is a middle-sized tree having young shoots covered with viscid pubescence, leaflets 3-5 pair, elliptic, shortly acuminate, often serrulate, blade 3-6 inch long, rough on the upper [20, 21]. This plant of stem bark contains tannins, flavonoids, alkaloids and quinines [22, 23]. M.Saifur R. *et al* [24] isolated β-sitosterol (Fig. 1) from stem bark of *Stereospermum chelonoides* dichloromethane / methanol crude extract. M.Saifur R.*et al* [24], studied on the ethyl acetate and dichloromethane on stem bark crude extracts and reported its anti-microbial activity against a wide range of bacteria and fungi by disc diffusion method.

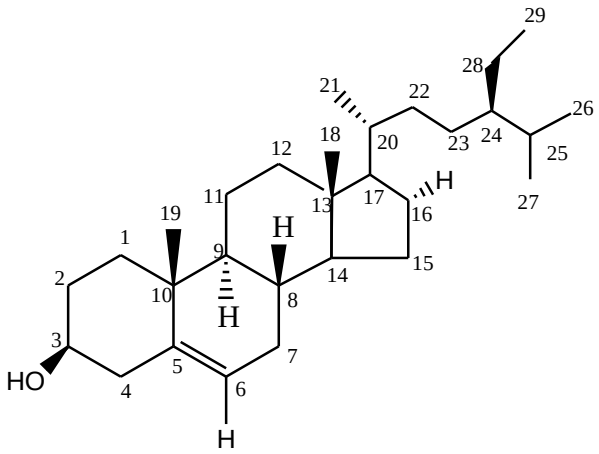
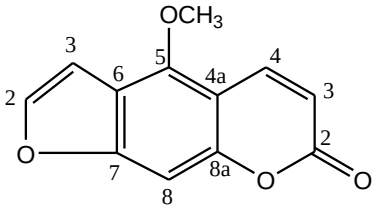


Figure 1. Chemical structure of **1** isolated from the stem bark of *Stereospermum chelonoides.*

2.3.3. *Stereospermum binhchauensis*

Stereospermum binhchauensis V.S. Dinh, T. *et al* [25, 26] reported indicate good cytotoxic activity and isolation of ten compounds such as bergapten (**2**), lupeol (**3**), 5,7-dihyroxychromone (**4**),oleanolic acid (**5**), luteo-lin (**6**), naringenin 7-O-b-D-glucopyranoside (**7**),eriodictyol 7-O-b-D-glucopyranoside (**8**), luteolin 7-O-b-D-glucopyra-noside (**9**), 6-O-p-trans-coumaroylcatalpol (**10**) (Figure 2) from leaves of *Stereospermum binhchauensis*.



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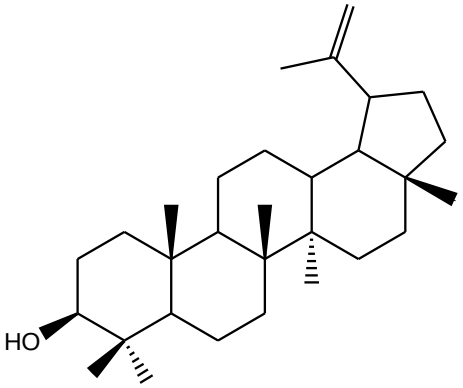

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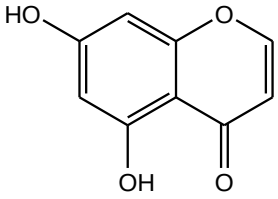

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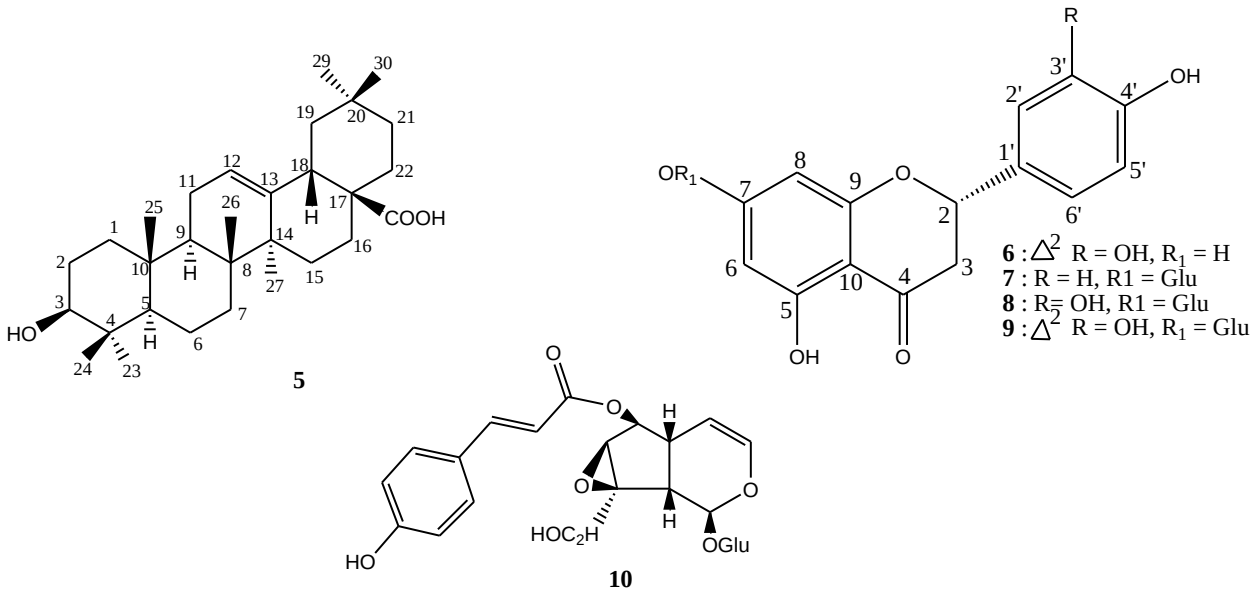


Figure 2. Chemical structures of compounds isolated from the leaves of *Stereospermum binhchauensis.*

2.3.4. *Stereospermum suaveolens*

Stereospermum suaveolens DC,is an important medicinal plant in India. The plant has been studied for many pharmacological actions like analgesic, wound healing, antidyspeptic, astringent and liver stimulant**,** only few were concerned with isolation of active compounds. Abdul S. *et al* [27], isolated three compounds from roots of *Stereospermum suaveolens* **11**, **12** and **13** (figure 3)

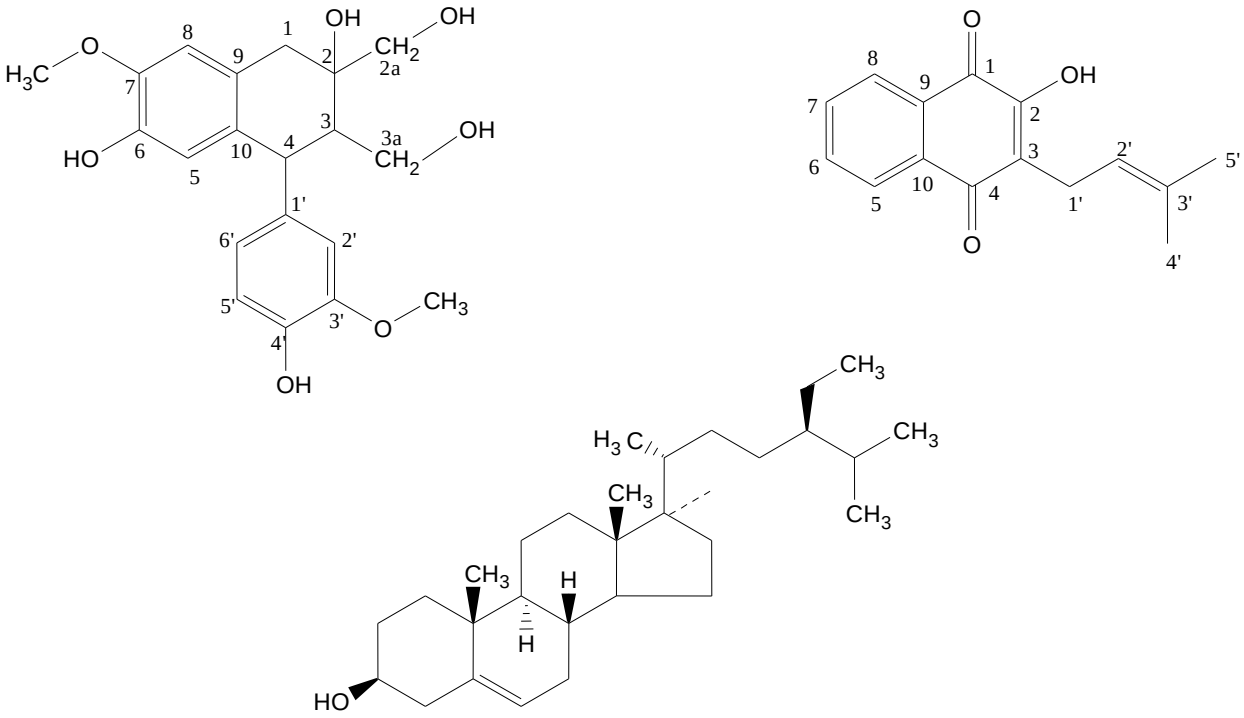


Figure 3. Chemical structures of compounds isolated from the roots of *Stereospermum suaveolens*

2.3.5. Stereospermum kunthianum

Stereospermum kunthianum is an important medicinal plant. Reports indicates the presence of phenolic compounds and flavonoids, tannins, saponins, flavoniods, terpenoids, polyphenols, higher fatty acids, coumarins, sterols, naphthaquinones, anthraquinone and glycosides in the roots bark of the plant [13, 14, 28, 29, and 30]. The methanol extract of the root bark of *Stereospermum kunthianum* yielded in compound (**14**), stigmasterol (**15**) and β-sitosterol), lupeol (**16**) (figure 4) [31-33].

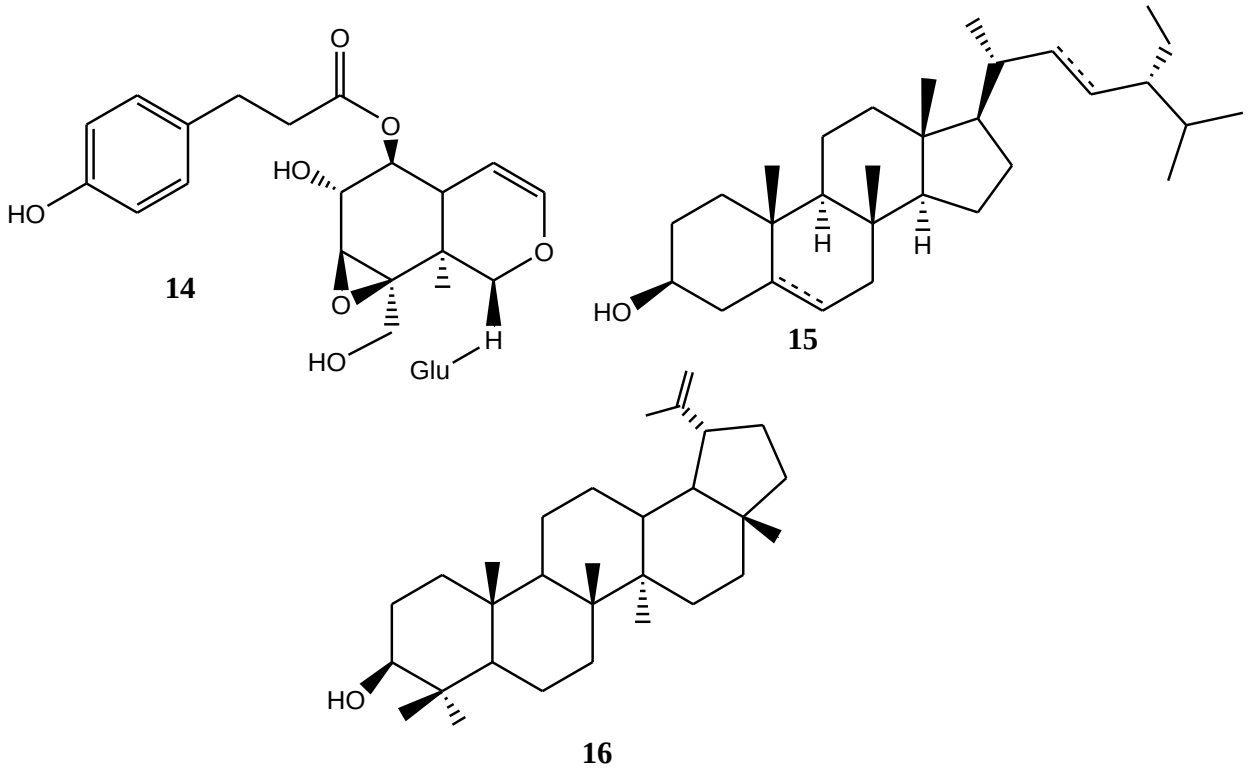


Figure 4. Chemical structures of compounds isolated from the root bark of *Stereospermum kunthianum.*

Scientific investigation of ethnomedicinal claims of *Stereospermum kunthianum* has been carried out pharmacologically. Some of the pharmacologic parameters investigated using crude extracts or isolated compounds were antibacterial, anti-inflammatory, analgesic, antiplasmodial, antdiarrhoeal, antconvulsant and antioxidant activities [34]. Therefore, the aim of the present study was to identify the chemical constituents of the stem-bark of *Stereospermum kunthianum* and substantiate the traditional use of the plant on the basis of the medicinal importance of the isolated compounds from previous studies.

3. MATERIALS AND METHODS

In this section the chemicals, the instruments used and the methods employed on the study are presented.

3.1. Chemicals and Instruments

3.1.1. Chemicals

n- hexane, chloroform and methanol, were used for gradient extraction and phytochemical analyses, reagent like Dragendroff's, dilute sodium hydroxide, ferric chloride, concentrated hydrochloric acid, concentrated sulphuric acid,glacial acetic acid,acetic anhydride and chloroform were used. All chemicals are analytical grade. The chemicals were purchased from Ran chem. Co. Ltd. Agents, Addis Ababa, Ethiopia.

3.1.2. Instruments

Rotary evaporator (Heidolph, UK) was used for concentration of crude extracts. Grant (GLS 400) thermostatic bath shaker was used for extraction. Oven (model N50L GENLAB WIDNES ENGLAND) was used for drying beakers and conical flasks. Analytical Balance ADAM (AFP-110L) was used for measuring plant material. An infrared (IR) spectrum was obtained from Perkin Elmer BX infrared spectrometer (400- 4000cm^{-1}) and model or specification of the Uv instrument to determine the absorbance of a compound.

3.1.3 Collection and authentication of plant material

The stem bark of *Stereospermum kunthianum* was collected in May 23,2024 from WelmaraWereda,West Shewa Zone, Oromia region.Welmara is found in between Mulo and Sebeta. It is 30-km West Addis Ababa, Capital city of Ethiopia. The sample was authenticated by botanist Mr. Reta Regassa, Department of Biology at Hawassa teacher training college.

3.2. Methods

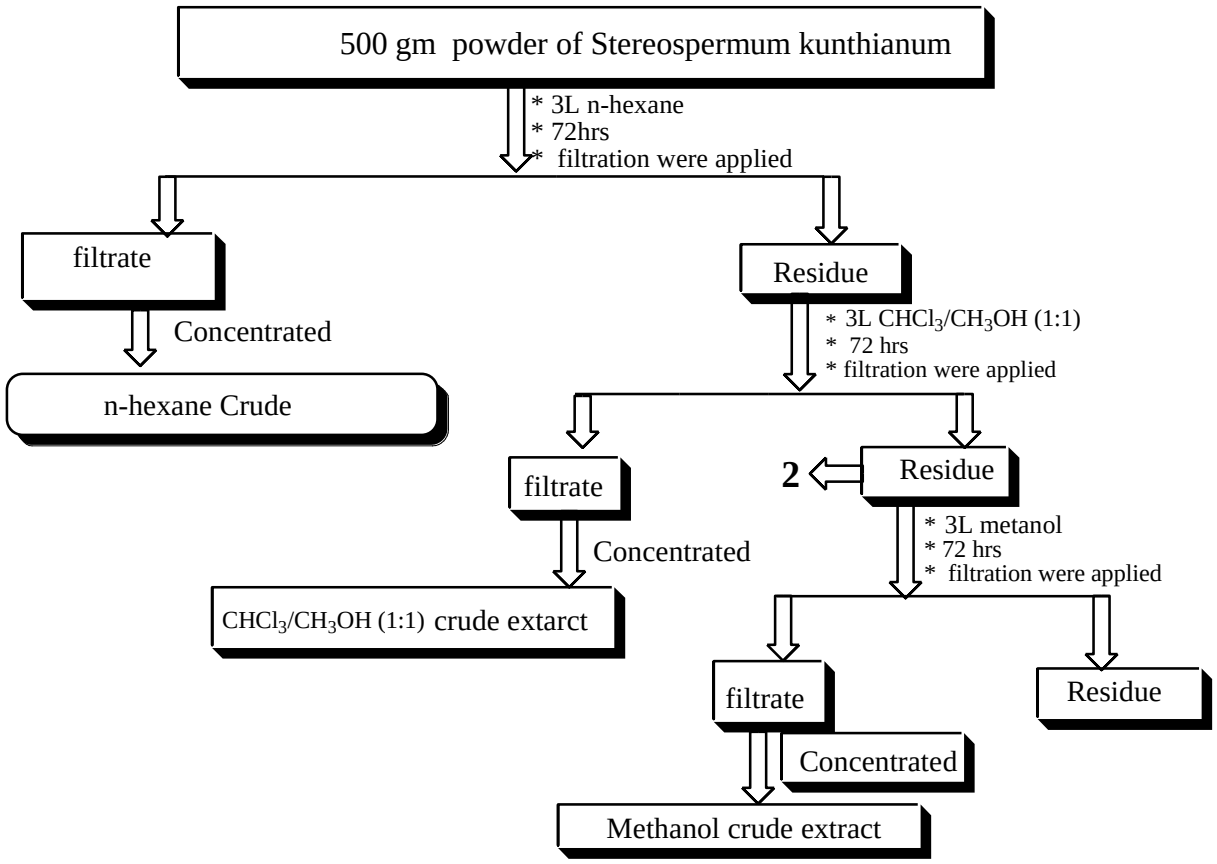
3.2.1 Plant material preparation

The collected plant material was chopped into small pieces and air-dried under shade for 50 days and milled to suitable size by using mortar and pestle.

3.2.2. Extraction of crude material

The stem bark (500 g) of *Stereospermum kunthianum* was sequentially extracted with n-hexane, chloroform/methanol (1:1, v/v) and methanol using maceration technique for 72 hrs with continuous shaking, on an orbital shaker. The extracted matter was filtered using Whatman No.1 filter paper, and the residual solvent in each gradient extract was removed using Rotary evaporator under reduced pressure. The crude extracts of each solvent was stored in hood until it became dry scheme 1.

Scheme 1. General outline for the extraction of stem bark *Stereospermum kunthianum.*



3.2.3. Phytochemical screening

Investigation of the class of secondary metabolites was carried out for all the extracts as per the standard methods reported in the literature [35-38]. The n-hexane/ethyl acetate solvent mixture showed relatively good separation of components of the crude extracts of chloroform/methanol on TLC plates.

3.2.3.1. Detection of Alkaloids: 0.1g crude extracts was dissolved individually in dilute hydrochloric acid and filtered by using filter paper.

Dragendroff's Test: Filtrates was treated with Dragendroff's reagent (solution of Potassium Bismuth Iodide). Formation of red precipitate indicates the presence of alkaloids.

3.2.3.2. Detection of Flavanoids

Alkaline Reagent Test: : 0.1g crude extracts were treated with 3-4 drops of sodium hydroxide solution. Formation of intense yellow color, which becomes colorless on addition of 1-2 drops dilute acid, indicates the presence of flavonoids.

3.2.3.3. Detection of phenols

Ferric Chloride Test: : 0.1g crude extracts were treated with 3-4 drops of ferric chlorides solution. Formation of green or bluish black color indicates the presence of phenols.

3.2.3.4. Detection of Glycosides

Keller-Killani test: : 0.1g crude extracts were treated with, glacial acetic acid, traces of ferric chloride and concentrated Sulphuric acid was added to extract formation of reddish brown color at the junction of two layers and the upper layer turn bluish green indicates the presence of glycosides.

3.2.3.5. Detection of Terpenoids

Salkowski test: : 0.1g crude extracts were treated with chloroform and filtered by using filter paper. The filtrates were treated 1-3 drops of concentrated H_2SO_4, shaken and allowed to stand.Appearance of a reddish brown coloration indicates the presence of terpenoids.

3.2.3.6. Detection of Tannins

0.1g crude extracts was boiled for 20 min in a boiling tube by using distilled water and then filtered by using filter paper, 1-4 drops of 0.1% ferric chloride ($FeCl_3$) solution was added and mixed well and allowed to stand some time. Appearance of brownish green or blue black color indicates confirmation of tannins.

3.2.3.7. Detection of Saponins

Froth test: : 0.1g crude extracts was dissolved in 20 ml distilled water and this was shaken in a graduated cylinder for 15 min. Formation of 1 cm layer of foam indicates the presence of saponins.

Foam test: 0.5 gm of extracts was shaken with 2ml of water. If foam produced persists for ten minutes it indicates the presence of saponins.

3,2.3.8. Detection of Steroids

Liebermann Burchard reaction: 200 mg plant extract was treated in 10 ml chloroform then filtered by using filter paper. In 2 ml filtrate, 2 ml acetic anhydride and 2 ml concentrated H_2SO_4 was added. Appearance of a blue-green ring indicates the presence of steroids.

3.2.4 Solvent selection for column chromatography

The TLC profiles of the crude extracts were analyzed in various solvent systems by increasing gradient of n-hexane in ethyl acetate with various proportions (90:10, 80:20, 70:30, 60:40, and 50:50% by volume). Accordingly, this solvent mixture was found to be an appropriate solvent to use in chromatographic separation with increasing gradient. The yield of n-hexane extract was too small, the TLC profile analysis was carried out for chloroform/ methanol and methanol extracts in different solvent systems to identify the appropriate solvent system for column chromatography. The TLC profile of chloroform/ Methanol and methanol crude extract was conducted in n-hexane/ethyl acetate, ethyl acetate /chloroform and ethyl acetate/ methanol and many more combination by varying combination ratio. n-hexane/ ethyl acetate showed relatively good separation of components of the crude extract of chloroform/ methanol, whereas crude extract of methanol is not promising on TLC profile showed more disturbance due to polarity of solvent.

3.2.5. Isolation of Compound

Crude extract of chloroform/methanol (1:1, v/v) ratio was selected for column chromatography (CC) based on good TLC profile. Chloroform/methanol (1:1) crude extract (8 g) was adsorbed on silica gel (20 g) and subjected to column chromatographic separation.

The column was packed in wet packing method by using silica gel (110 g) in n-hexane. The column was then eluted with increasing gradient of ethyl acetate in n-hexane. A total of 81 fractions (Table 1) were collected and the components of each fraction were checked by TLC. The spots on the TLC plates were visualized using UV light (at 254nm and 365nm). The collected fractions were concentrated using a rotary evaporator. Fractions with same Rf values on TLC were combined fractions that have some impurities were washed in hexane to remove impurities.

Table 1. Column chromatography fractionations of the crude extract of chloroform/methanol using n-hexane/ethyl acetate solvent system.

Fraction No	Solvent System	Solvent ratio v/v (ml)	Volume of Fraction (ml)
1	n-hexane/ethyl acetate	100:0	30
2	n-hexane/ethyl acetate	95:5	30
3	n-hexane/ethyl acetate	90:10	30
4	n-hexane/ethyl acetate	85:15	30
5	n-hexane/ethyl acetate	80:20	30
6	n-hexane/ethyl acetate	75:25	30
7	n-hexane/ethyl acetate	70:30	30
8	n-hexane/ethyl acetate	65:35	30
9	n-hexane/ethyl acetate	60:40	30
10	n-hexane/ethyl acetate	40:60	30
11	n-hexane/ethyl acetate	35:65	30
12	n-hexane/ethyl acetate	30:70	30
13	n-hexane/ethyl acetate	25:75	30
14	n-hexane/ethyl acetate	20:80	30
15	n-hexane/ethyl acetate	15:85	30
16	n-hexane/ethyl acetate	10:90	30
17	Methanol/ethyl acetate	10:90	30
18	Methanol/ethyl acetate	20:80	30
19	Methanol/ethyl acetate	25:75	30
20	Methanol/ethyl acetate	30:70	30
21	Methanol	50:50	30

3.2.6. Generating spectroscopic data and compound characterization

The spectroscopic data of all the isolated compounds was generated using UV visible and IR spectroscopies. NMR data was not generated due to malfunctioning of the instrument at Addis Ababa University. The structure elucidation of the compounds was performed by comparing the IR data of the isolated compounds to that of literature. The melting points of the isolated compounds are also compared to that of the melting points of compounds from literature. The

compounds having similar experimental IR data and melting points with that of literature were proposed.

4. RESULTS AND DISCUSSION

In this section, yield of crude extract, identified of classes of secondary metabolites and partial characterization of isolated compounds, are presented and discussed.

4.1. YIELD OF EXTRACT

The percentage yield of successive extracts of stem bark of *Stereospermum kunthianum* was presented below (Table 2).

Table 2. The mass of extracted matter in each gradient extract.

No.	**Solvent system used for extraction**	**Mass of Crude extract**	**Yield (%)**
1	n-hexane	1.8	0.36
2	chloroform/methanol (1:1, v/v)	9.8	1.96
3	Methanol	15	3

$$\textbf{Percent yield} = \frac{\textbf{mass of extract}}{\textbf{Mass of plant used for extraction}} \textbf{ X 100}$$

As shown in Table 2, the gradient extraction of the stem bark and roots of *Stereospermum kunthianum* yielded different quantities of extract. The percentage of crude extract of methanol was found to be higher than the other crude extracts. This indicates that the stem bark of *Stereospermum kunthianum* plant material contained mostly polar components, but not used to column chromatographic separation because the crude extract not promising separation on the TLC plates.

4.2. Phytochemical screening

Preliminary phytochemical screening tests were performed as per standard procedure reported in (Section 3.2.2) on the crude extract of n-hexane, chloroform/methanol (1:1) and methanol. The phytochemical screening test was conducted to determine the presence or absence of secondary metabolites, including alkaloids, saponins, flavonoids, phenols, steroids, glycosides, terpenoids, and tannins. The result of the phytochemical screening test was presented below (Table 3).

Table 3. Phytochemical analysis on stem bark of *Stereospermum kunthianum.*

NO	Bioactive constituents	n-hexane extract	Chloroform/methanol Extract	Methanol extract
1	Alkaloids	-	+	+
2	Saponins	-	+	+
3	Flavonoids	-	+	+
4	Phenols	-	+	+
5	Steroids	+	+	+
6	Glycosides	+	+	+
7	Terpenoids	+	+	+
8	Tannins	-	+	-

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

According to the results in the above table (Table 3) n-hexane crude extracts indicates the presence of steroids, glycosides and terpenoids, whereas alkaloids, saponins, flavonoids, phenols and tannins are absent. The chloroform/methanol (1:1, v/v) extract showed the presence of all tested phytochemicals. On the other hand the methanol extract revealed the presence of alkaloids, saponins, flavonoids, phenols, steroids, glycosides and terpenoids and absence of tannins. These compounds are known to be biologically active, and therefore, contribute to the traditional medicinal importance of the plant. The biological activities of the classes of secondary metabolites identified in the plant are presented below.

Phenolic compounds **were reported to have** several biological activities such as increasing bile secretions, reduce blood cholesterol and lipid levels , antimicrobial, antiulcer, anti-inflammatory, antioxidant, cytotoxic, antitumor, and antidepressant activities [36].

Flavonoids have broad biological and pharmacological activities and multiple biological properties including antimicrobial, cytotoxicity, anti-inflammatory as well as antitumor activities, enzyme inhibition, oestrogenic activity, anti-allergic activity, antioxidant activity and vascular activity [36].

Tannins are natural healing, for instance tannin-containing plant extracts are used as astringents, against diarrhea, as diuretics, against stomach and duodenal tumors and as anti-inflammatory, antiseptic, antioxidant and haemostatic pharmaceuticals [36].

Alkaloids are significant role in pharmacological activities including antihypertensive effects anti-arrhythmic effect, anti-malarial activity and anticancer actions [36].

Terpenoids possess various medicinal properties, including anti-carcinogenic, anti-malarial, anti-ulcer, hepatoprotective, antimicrobial, and diuretic activities. Notable examples include the sesquiterpenoid anti-malarial drugs artemisinin and its derivative artesunate, as well as the diterpenoid-based anticancer drugs. [36].

Traditional use of *Stereospermum kunthianum* plant material are chewed with salt to treat coughs and are used in treatment of ulcers, leprosy, skin eruptions and venereal diseases, while the stem bark decoction or infusion is used to cure bronchitis, pneumonia, cough, rheumatic arthritis and dysentery [6]. The roots and leaves have been found useful in treating venereal diseases, respiratory ailments, and gastritis [6]. Stem bark of plant material used to treat Analgesic and anti-inflammatory activities [10]. Hence forth these medicinal important of the plant might be due to the presence of the aforementioned classes of secondary metabolites.

4.3. Isolation of compounds

The crude extract of chloroform/methanol (1:1, v/v) extract (8 g) was subjected to column chromatographic separation using n-hexane and ethyl acetate combination as was discussed in the methods part. This resulted in isolation of three compounds labeled **SK1, SK2** and **SK3**. Fractions 1-10 eluted by n-hexane and ethyl acetate (99:1) were combined based on the similarities of their TLC profiles showed six different kinds of spot but difficult to perform further purification due to the small amount of the yield.

A light yellowish crystalline compound labeled as **SK1** melting point 287.5°C was obtained from combined fractions 22-29, that were eluted with 5% ethyl acetate in n-hexane. The crystal was separated from the solution by removing the solvent and by washing it by n-hexane, repeatedly, to remove impurities. 24.5 mg of light yellowish crystalline compound was obtained. Finally the isolated compound analyzed by TLC to insure the purity of a compound in different solvent system. Its Rf value was determined to be 0.45 in n-hexane ethyl acetate combination (85:15).

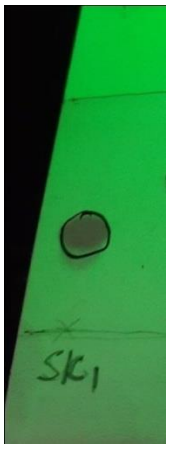


Figure 5. TLC profile of compound **SK1**

Compound labeled as **SK2** was obtained from fractions 36-45 that was eluted with 15% ethyl acetate in n-hexane. 20 mg of yellowish crystalline solid, melting point, 185.5°C. Its Rf value was determined to be 0.65 in n-hexane ethyl acetate combination (80:15%).

Figure 6. TLC profile of compound **SK2**

Compound labeled as **SK3** was isolated from fractions 59-68 that was eluted with 25% ethyl acetate in n-hexane. 25 mg of deep brown solid melting point, 255°C was obtained. Its Rf value was determined to be 0.68 in n-hexane ethyl acetate combination (80:20%).

Figure 7. TLC profile of compound **SK3**

A combined fraction from 30-35, 46-58, 69-74 and 75-87 showed deep yellow, yellow, deep brown and brown color monitored five, seven, nine and six spot respectively, on TLC. All combined fractions difficult to perform further fraction to get a pure compound due to yield and shortage of solvent.

4.4. Structure elucidation of the isolated compounds

The Uv and IR data of the isolated compounds were interpreted systematically comparison of their IR bands and the melting points values with those reported in the literature . The absence of NMR data, however, makes the structure elucidation to be partial and not 100% certain. Compounds that showed similar IR frequencies and melting points to that of literature values were proposed to be the isolated compounds.

4.4.1. Compound SK1

The structural elucidation was done using the data obtained from UV-Vis and IR. Compound **SK1** showed UV absorption peak at 242 nm and 296 nm (Appendix. 1).

Table 4.UV-Vis spectrum for **SK1**

Absorption peak (nm)	Functional group	Electronic transition	Absorption range	Region
242 296	C=O and C=C	n-π*	190-380	(Near) ultraviolet

Compound **SK1** IR spectrum was determined (Appendix. 2) the broad band at 3420.9 cm^{-1} indicates the presence of hydroxyl functional group. The weak band at 3071.6 cm^{-1} represents amines, whereas the bands at 2944.4 cm^{-1} and 2808.5 cm^{-1} indicated C-H stretching of methyl groups and C-H stretching in CH_2 respectively. Sharp peak observed at 1625.0 cm^{-1} indicate the presence of olefinic C=C bond. Sharp peak observed at 1557.0 cm^{-1} indicate N-H bending. sharp peak observed at 1285.2 cm^{-1} represent stretching C-O on carbonyl carbon. The observed data suggested that compound **SK1** could be an alcohol and C=C double bond and the melting point determined 287.5°C.Based on spectroscopic data compound **SK1** near to identical chemical structure with solanidanes (pure solanidanes melting point 286 to 288 °C) [37-40].

Table 5. Comparison of the IR data and melting point of SK1 and data obtained from literature.

Compound	Frequency (cm^{-1})		Functional group
	Experimental	Literature [37-40]	
SK1	3420.9	3432.13	Hydroxyl (OH)
	3081.9	3118.60	Amines (NH_2)
	2944.4, 2808.5	2925.04, 2861.85	CH_3 stretching, C-H stretching in CH_2
	1625.0	1686.91, 1644.2	Olefinic C=C double bond
	1557.0	1564.5, 1542.1, 1508.5	N-H bending
	1285.2	1232.61	C-O stretching
Melting point (°C)	287.5 ^{O}C	286 -288 °C	

.

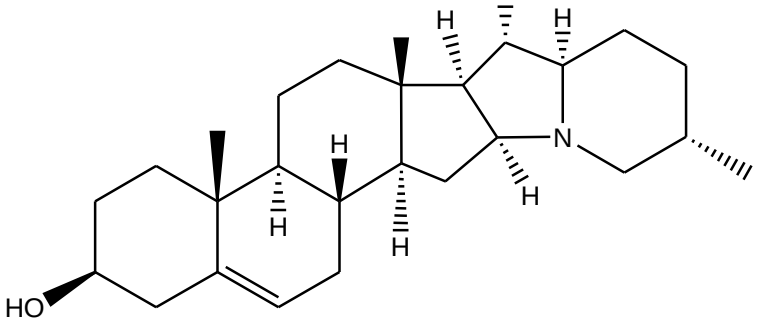


Figure 8. Proposed structure of compound **SK1** (solanidanes)

4.4.2. Compound SK2

The structural elucidation was done using the data obtained from UV-Vis and IR. Compound **SK2** showed UV absorption peak at 242 nm (Appendix. 3).

Table 6 UV-Vis spectrum for SK2

Absorption peak (nm)	Functional group	Electronic transition	Absorption range	Region
242	C=O	n-π*	190-380	(Near) ultraviolet

Compound **SK2** IR spectrum was determined (Appendix. 4) the observed broad band at 3437.7 cm^{-1} indicates the presence of hydroxyl functional group, the bands at 3071.4 cm^{-1} showed the presence C-H stretching vibrations for methylene and the range between 2935 cm^{-1} 2833cm^{-1} represents CH_3 stretching of methyl and C-H stretching in CH_2. Sharp peak observed at 1606.9 cm^{-1} indicate the presence of C=C bond. The sharp peak observed at 1462.1 cm^{-1} represent C-H bending and sharp peak observed at 1300.5 cm^{-1} represent stretching C-O on carbonyl carbon. The observed data suggested that compound **SK2** could be an alcohol and C=C double bond bearing compound and the melting point determined 185.5 $^{\circ}C$, based on spectroscopic data compound **SK2** near to identical chemical structure with (α-amyrin) [41].

Table 7 Comparison of the IR data and melting point of SK2 and data obtained from literature.

Compound	Frequency (cm^{-1})		Functional group
	Experimental	Literature [41]	
SK2	3437.7	3442	Hydroxyl (OH)
	2935.4, 2833	2931, 2860	CH_3 stretching, C-H stretching in CH_2
	2556.3	2693, 2695	
	1726.1	1735	Carbonyl (C=O) groups
	1462.1	1483	C-H bending of unsaturated alkene
	1300.5	1278, 1235	C-O stretching of the carbonyl carbon of carboxylic acid
Melting point (°C)	185.5 ^{O}C	186 ^{O}C	

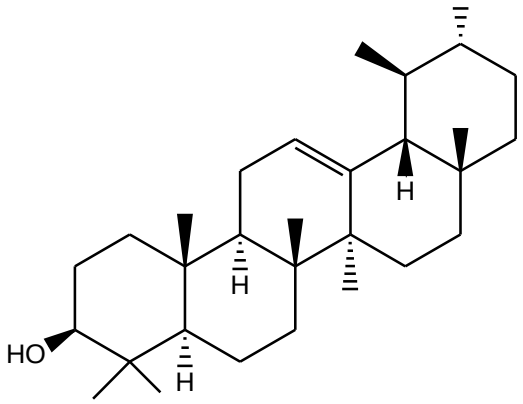


Figure 9. Proposed structure of compound **SK2** (α-amyrin)

α-Amyrin and β-amyrin are widely known as a natural mixture of triterpenes. Their pharmacological potentials have been reported in previous studies, including antitumor, anti-inflammatory, anxiolytic, and hepatoprotective effects. α-Amyrin and β-amyrin can be found in numerous plant species [42-48].

4.4.3. Compound SK3

The structural elucidation was done using the data obtained from UV-Vis and IR. Compound **SK3** showed UV absorption peak at 243 nm (Appendix. 5).

Table 8 UV-Vis spectrum for SK3

Absorption peak (nm)	Functional group	Electronic transition	Absorption range	Region
243	C=O	n-π*	190-380	(Near) ultraviolet

Compound **SK3** IR spectrum was determined (Appendix. 6) the observed broad band at 3434.4 cm^{-1} indicates the presence of hydroxyl functional group. The strong band observed at 2927.9 cm^{-1} and 2847.1 cm^{-1} represents C-H stretching of aliphatic, the bands at 1755.9 cm^{-1} indicated the presence of C=O. Sharp peak observed at 1630.3 cm^{-1} indicate the presence of olefinic C=C bond, The sharp peak observed at 1429.2 cm^{-1} represent C-H bending and sharp peak observed at 1277.7 cm^{-1} represent stretching C-O on carbonyl carbon. The observed data suggested that compound **SK3** could be an alcohol and C=C double bond bearing compound and the melting point determined 255 ^{O}C. Based on spectroscopic data compound **SK3** was proposed to have a chemical structure identical to Betulin [49-50].

Table 9 Comparison of the IR data and melting point of SK3 and data obtained from literature.

Compound	Frequency (cm^{-1})		Functional group
	Experimental	Literature [49-50]	
SK3	3434.8	3434	Hydroxyl (OH)
	2927.9, 2847.1	2924, 2847	Aliphatic C-H stretching
	1755.9	1728	Carbonyl (C=O) stretching vibration
	1603.3	1640	Olefinic C=C double bond
	1429.2	1464	C-H bending
	1277.7	1271	C-O stretching of the carbonyl carbon of carboxylic acid
Melting point (°C)	255°C	256-257 °C	

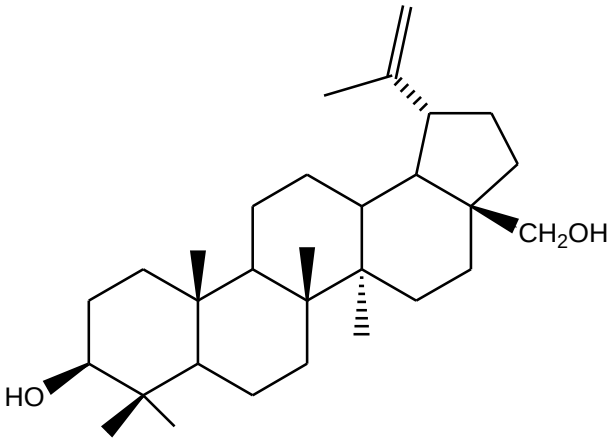


Figure 10. Proposed structure of compound **SK3** (Betulin**)**

Betulin is a pentacyclic triterpene alcohol with a lupane skeleton. It can be isolated from various plants in small amounts, but up to 30% dry weight from the birch bark by extraction with high boiling hydrocarbon solvents or with water azeotropes of alcohols [51]. Betulin has attracted more attention because it has some pharmacological activities, such as antiviral, anti-inflammatory, antitumor activities [52], and protective effects against cadmium (Cd)-induced cytotoxicity [53]. The latest research by Tang et al-showed that betulin can inhibit sterol regulatory element-binding proteins (SREBPs) pathway, decreases the biosynthesis of cholesterol and fatty acid, improves insulin sensitivity and reduces atherosclerotic plaques [54] .

5. CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

This work is the attempt of phytochemical investigation on the stem bark of *Stereospermum kunthianum.* The stem bark of *Stereospermum kunthianum* collected from Welmara Wereda,West Shewa Zone, Oromia region. Phytochemical screening of the stem bark *Stereospermum kunthianum* n-hexane, chloroform/methanol and methanol extract revealed that the presence of alkaloids, saponins, flavonoids, phenols, steroids, glycosides, terpenoids and tannins. Chromatographic separation chloroform/methanol (1:1) ratio yielded compound **SK1** (solanidanes), compound **SK2 (α**-amyrin) and compound **SK3 (** Betulin) that are partial characterized. The spectroscopic data of all the isolated compounds was generated using UV visible and IR. NMR data was not generated due to malfunctioning of the instrument at Addis Ababa University. The structural elucidations of the compounds were performed by comparing the IR and melting points data of the isolated compounds to those reported in the literature.

5.2. Recommendations

Compound **SK1** (solanidanes), compound **SK2 (α**-amyrin) and compound **SK3 (** Betulin) were isolated from the stem bark of *Stereospermum kunthianum* in the present study not characterized fully due to malfunction of NMR, and thus strongly recommended analysing their structures using NMR. These compounds are known to posses' antimicrobial activities of different strains based on literature references. However, due to shortage of time, we couldn't conduct the bioassay of the isolated compounds as well as the crude extract. Thus, we recommend further screening of the extract and compounds against selected strains of microorganism so as to validate the traditional use of the plant. As this work is the only study that attempted to phytochemically analyze the polar extract stem bark of plant material, further study is recommended on other parts of the plant such as aerial parts, leaf, fruits and shoot part of plant material. Finally, the crude extract on TLC plate showed still many unidentified compounds that we have missed during the chromatographic separation. However, due to shortage of time we couldn't continue to isolate and we recommend a continuation of the work using high-tech separation techniques such as HPLC and analysis of their structures using NMR and MS.

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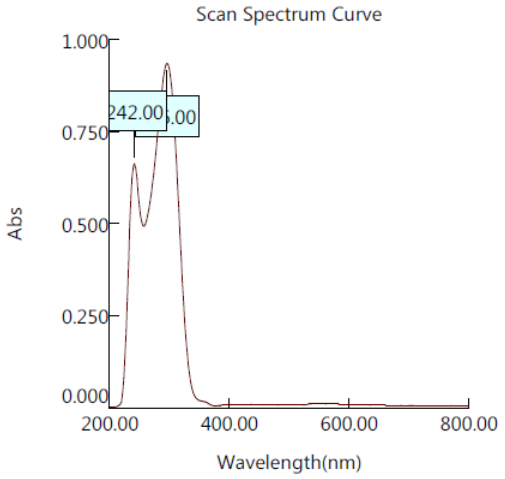
Appendix

Appendix 1: UV-Vis spectrum for **SK1** compound



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- **Scan Spectrum Performance**
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  Measure Mode : Abs
  Interval : 2.00 nm
  Speed : Fast
  Data File : SK1-Untitled3.spd
  Create Date/Time : Monday, August 26, 2024 11:26:33 AM

  Data Type : Manipulated
  Method File:
- **Analyse Note**
  Analyser : Administrator

Sample Name :

Comment :

No.	P/V	Wavelength(nm)	Abs	Comment
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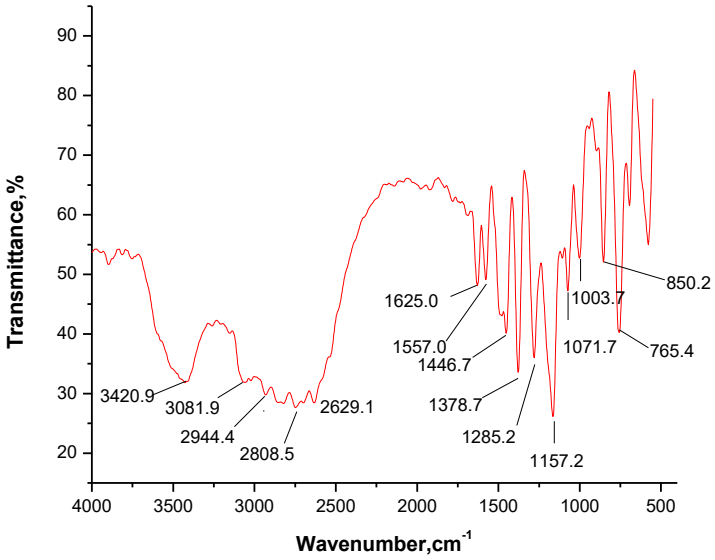
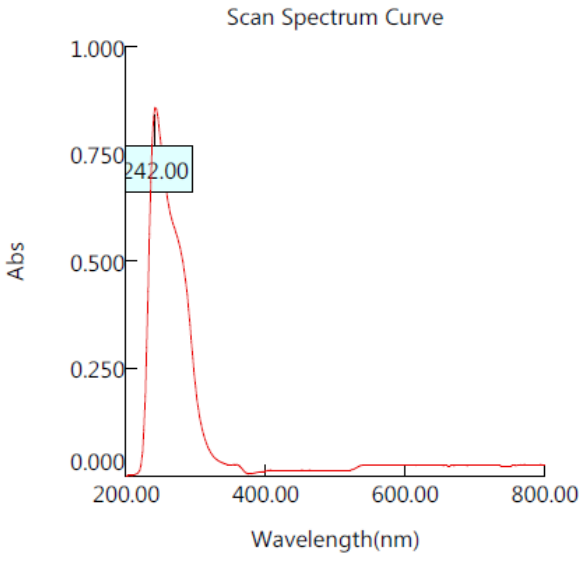
2	Peak	242.00	0.663	

Appendix 2: IR (KBr) spectrum for **SK1** compound



Appendix 3: UV-Vis spectrum for **SK2** compound



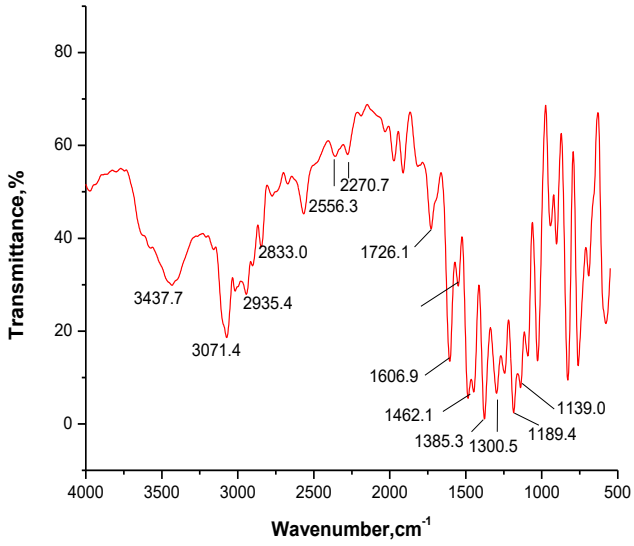
- **Instrument Performance**
  Model : UV-VIS Spectrophotometer
  Number : 16-1884-18-0075
  Spectral Bandwidth : 0.50 nm
- **Scan Spectrum Performance**
  Scan Range : 200.00 to 800.00 nm
  Measure Mode : Abs
  Interval : 2.00 nm
  Speed : Fast
  Data File : SK2-Untitled8.spd
  Create Date/Time : Monday, August 26, 2024 11:44:40 A

  Data Type : Manipulated
  Method File:
- **Analyse Note**
  Analyser : Administrator

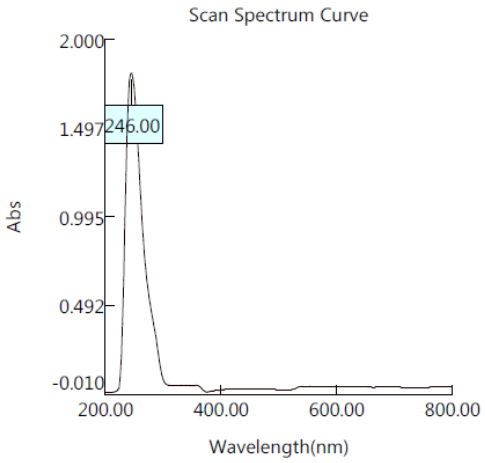
Sample Name :
Comment :

No.	P/V	Wavelength(nm)	Abs	Comment
1	Peak	242.00	0.858	

Appendix 4: IR (KBr) spectrum for **SK2** compound



Appendix 5: UV-Vis spectrum for **SK3** compound



- **Instrument Performance**
  Model : UV-VIS Spectrophotometer
  Number : 16-1884-18-0075
  Spectral Bandwidth : 0.50 nm
- **Scan Spectrum Performance**
  Scan Range : 200.00 to 800.00 nm
  Measure Mode : Abs
  Interval : 2.00 nm
  Speed : Fast
  Data File : SK3-Untitled11.spd
  Create Date/Time : Monday, August 26, 2024 11:53:29 AM

  Data Type : Manipulated
  Method File:
- **Analyse Note**
  Analyser : Administrator

Sample Name :
Comment :

No.	P/V	Wavelength(nm)	Abs	Comment
1	Peak	246.00	1.809	

Appendix 6: IR (KBr) spectrum for **SK3** compound