ADDIS ABABA UNIVERSITY
COLLEGE OF NATURAL AND COMPUTATIONAL SCIENCE
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MSc Thesis

Preliminary Phytochemical Investigation of the Root of *Verbascum sinaiticum* (Scrophulariaceae)

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This thesis research prepared by Genet Kassaye entitled "Preliminary Phytochemical Investigation of the Root of *Verbascum sinaiticum* (Scrophulariaceae)".

The undersigned certify that they have read and hereby recommend for acceptance by the

Department of Chemistry as fulfilling the requirements for the degree of Master of Science in Chemistry with the rules and regulations of Addis Ababa university.

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3. _____ (Examiner)	_____	_____

Declaration

I declare that this thesis, entitled Phytochemical Investigation of *Verbascum sinaiticum*, is my original thesis under the supervision of Dr. Kibrom Gebrehiwot, Department of Chemistry, Addis Ababa University, and that all sources of materials used for this thesis have been duly acknowledged. I solemnly declare that this thesis is not submitted to any other institution, anywhere for the award of any academic degree, diploma, or certificate.

Name: Genet Kassaye

Signature: _____

Dedication

This Thesis work is dedicated to my family specially to my children Daniel Jobrie and Biniam Jobrie for their encouragement and support to come to this level.

Acknowledgement

I would like to express my sincere gratitude to my thesis advisor Dr. Kibrom Gebrehiwot, for his invaluable guidance, support, and encouragement throughout this research. His expertise and mentorship have been instrumental in shaping this work. I am also deeply grateful to the Department of Chemistry Addis Ababa University, for providing me with the necessary resources and facilities to conduct this research. I also express my acknowledgements to the FDRE Ministry of Education for their sponsorship. Finally, I would like to acknowledge the support of my family and friends.

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Acronyms

^{1}H NMR	Proton Nuclear Magnetic Resonance
^{13} C NMR	Carbon Nuclear Magnetic Resonance
DEPT	Distortion less Enhancement by Polarization Transfer
Rf	Retention Factor
Uv-vis	Ultraviolet-visible
HPLC	High Performance Liquid Chromatography
TLC	Thin Layer Chromatography
COSY	Correlation Spectroscopy
CC	Column Chromatography
HSQC	Heteronuclear Single Quantum Correlation
HMBC	Heteronuclear Multiple Bond Correlation
δ	Delta (chemical shift)
s	singlet
d	doublet
m	multiplet
t	triplet
ppm	Parts Per Million
WHO	World Health Organization
V. sinaiticum	*Verbascum sinaiticum*

Abstract

The focus of this study was preliminary phytochemical investigation of the methanol extracts of the root of *V. sinaiticum* (Scrophulaceae). The plant is traditionally used in Ethiopia for various medicinal purposes including wound healing anti-microbial activities. The extraction and characterization of bioactive compounds were conducted using a Cyclohexane: ethyl acetate (2:1) solvent system. The extracts were then analyzed using various spectroscopic methods such as TLC, ^{1} H-NMR, ^{13} C NMR and DEPT-135, to elucidate the structural characteristics of the isolated compounds. The isolated compound from the root extract was identified as 9, 12, 15-octadecatrienoic acid or α-linolenic acid (ALA), an essential omega-3 fatty acid known for its potential health benefits. ALA has been linked to various health advantages, including cardiovascular health, diabetes management, renal health, and anti-inflammatory properties.

Keywords: *Verbascum sinaiticum*, phytochemical composition, α-linolenic acid, omega-3 fatty acid, traditional medicine, health benefits, and acetone-d6.

CHAPTER ONE
INTRODUCTION

1.1 Background of the study

Plants are valuable and fundamental to almost all life on earth. Humans consume plants as a source of food and shelter. Plants uptake carbon dioxide to store carbon and produce oxygen in air. Plants can also use as medicine, food utensils, shelter, clothing, fuel, firewood for cooking, timber for construction, and as fodder for cattle. They are also; maintain soil fertility and for protecting erosion and for controlling environmental balance of the temperature of the earth.

Moreover, plants have played important role in maintaining human health and improving the quality of human life for thousands of years. Generally, they are source of Traditional medicine, which is set of knowledge, ability or skill and cultural practices that based on different ethnic experiences.

According to WHO TRADITIONAL MEDICINE STRATEGY definition (2014- 2023): - The practice of traditional medicine (TM) is not new. It is the sum of all the information, expertise, and methods:- whether explicable or not derived from the theories, convictions, and first hand experiences of various cultures and applied to the preservation of health as well as the avoidance, detection, enhancement, or management of physical and mental disorders [1].

As noted by Meresa and colleagues (2017), since the beginning of human civilization, medicinal plants have been used by mankind for curing a variety of disease and pain. This is why medicinal plants have played a key role in the worldwide maintenance of health. There is traditional medical knowledge and practices around the world that are relevant to human health. The process of extracting natural goods is the oldest human activity. Plant extracts and isolated products have been used for centuries in agriculture, medicine, cosmetics, and diet supplements [2].

According to a study conducted by the research team (Bruce N et al., 1993), various research have indicated that a wide variety of plants contain antioxidants. Foods, which are rich in antioxidants can fight free radicals and make them harmless by breaking down their molecular structure. Thus, by postponing or preventing oxidation brought on by reactive oxygen species (ROS), antioxidants regulate and lessen oxidative damage in food, thereby extending the shelf life and quality of the food [3].

Ekor (2014) highlights Traditional herbal medicine has a long-standing history in many rural locations of Ethiopia. Recent review indicated that more than 80% of the world's population relied on traditional medicine for their primary healthcare needs [4].

In their systematic review, Helen et al. (2019), plants have been used as a medicinal source in Ethiopia from immemorial time to treat illness. These traditional medicines are rooted in indigenous knowledge practices. Traditional healing methods receive the majority of their care from the indigenous health care system. There are numerous different customs and religious beliefs among Ethiopians. This makes for a rich and diverse knowledge and practice of traditional medicine, including herbal treatments [5].

The medicinal properties of plants are due to some chemical substances that produce certain definite physiological action on the human body. These non-nutritional components of plants with advantageous phytochemicals can function as natural antioxidants, enhancing the body's requirements. Natural components derived from plants can be obtained from any part of the plant, including the bark, leaves, flowers, roots, fruits, and seeds. [6]. It is recognized that the products of the plants contain a variety of secondary metabolites, which may include phenols, polyphenols, terpenoids, flavonoids, tannins, alkaloids and others.

As explained in Kalia's (2005) Textbook of Industrial Pharmacognosy, the demand for plant-based investigations of medicines and other supplements are rising in both developed and developing nations. These natural products are well-known around the world since they are non-toxic, have few adverse effects, and are readily available at affordable prices [7].

According to the illustrated checklist of medicinal plants in Ethiopia by Abebe et al. (2003), peoples of Ethiopia use different parts of *Verbascum sinaiticum* for the treatment of ailments such as scabies, amoebiasis, diarrhea, epilepsy, syphilis, tumors, liver problems, stomach issues, diabetes, and asthma [8].

V. sinaiticum Benth is a part of the SCROPHULARCEAE family of medicinal plants. Having a height of up to two meters, the plant is an erect herb. The leaves of the plant are simple and widely oval oblong, and the entire plant is thickly matted and greyish [8]. The species is a perennial plant in the genus *Verbascum* (mullein), The plant has an erect stem, richly branched, densely greyish and is entirely covered with stellate hairs which are not pleasant to touch. It is not eaten by cattle, beasts and all domestic animals.

It is known by common name mullein (in English) and in Ethiopia it is called Ketetina, Yeahyajoro, Daba Keded (Amharic), Gurra Harree (Afan Oromo), which is a biennial plant growing 60-150cm in height. Various parts of this plant have folkloric uses in curing wounds (leaves), abdominal dropsy, anthrax, diarrhea and fungal infections [9].

It is native to Afghanistan, Eritrea, Ethiopia, Gulf States, Iran, Iraq, Kenya, Lebanon-Syria, Oman, Pakistan, Palestine, Saudi Arabia, Sinai, Somalia, Sudan and Yemen, and Widely distributed in all regions of Ethiopia. The present investigation presented the qualitative analysis on the roots of *V. sinaiticum*.

1.2 Statement of the problem

In Ethiopia different plants have been used as traditional medicine to treat different diseases. *V. Sinaiticum* is one of the traditional medicines in some parts of Ethiopia. There are many natural plants from which essential ingredients and components are extracted in order to cure many of the diseases that are found today [10]. *V. sinaiticum* is traditionally used for the treatment of ailments. The leaves, root, seeds and flowers of the species of the plant have been traditional medicine that encouraged the researcher to carry out the phytochemical investigation of the study. *V. sinaiticum* is traditionally used for treating various diseases, such as wound, stomachache, viral infection, cancer, sunstroke, fever, abdominal colic, diarrhea, hemorrhage, anthrax and hepatitis.

There are some reports on antimicrobial and antioxidant activities of *V. Sinaiticum* leaf extracts of preliminary tests. But phytochemical investigation on its root extract is not yet confirmed, furthermore. It seems that phytochemical studies for characterization of chemical constituents of the plant are unexploited area of research study of the constituents of the roots of *V. sinaiticum* plant for its medicinal value.

1.3 Significance of the study

Since plants provide us shelter, food, clothes, fiber and energy in the form of fuel, besides these, plants are also used for medicinal purposes for general health maintenance and wellbeing of human. Anti-bacterial and antioxidant species of plant origin have been identified from different plant extracts by developing anti-bacterial and antioxidant agents of drugs. The results of the study can help people to be aware about the plant to have the knowledge of phytochemical activity of *V. sinaiticum* (mullein) and used as a source of information for further studies.

1.4 Objectives of the study

1.4.1 General objective

The general objective of the study was to investigate the phytochemical investigation of *V. Sinaiticum* root extract.

1.4.2. Specific Objectives

- To extract root of *V. sinaiticum* using an organic solvent.
- To carry out chemical test (phytochemical screening) of the solvent extract of root and of *V. sinaiticum* in order to determine the presence of secondary plant metabolites.

CHAPTER TWO

LITERATURE REVIEW

2.1 The Family Scrophlariaceae

The family Scrophlariaceae is in the major group Angiosperms (Flowering plants). Scrophlariaceae is a family of flowering plants. The plants are annual or perennial herbs. The scrophlariaceae has a cosmopolitan distribution with 275 genera and 5000 species, with the majority found in temperate areas, including tropical mountains. The family name is based on the name of the included genus scrophularia L. Additionally, powdered *V. sinaiticum* leaves diluted with water is taken orally [11] or the filtrate is administered to treat animal trypanosomosis into the left ear and nostril [12]. Two flavonolignans, hydrocarpin and the novel sinaiticin, as well as two flavones, chrysoriol and luteolin, have been identified by analysis of *V. sinaiticum* leaves [13].

Classification of *V. sinaiticum*

Kingdom	plantae
Division	Tracheophyta
Class	Magnoliopsida
Order	Lamiales
Family	Scrophulariaceae- figworts, scrofulaires
Genus	*Verbascum* L. mullein
Species	V. sinaiticum L- wavy leaf mullein.

Figure 1 *V. sinaiticum* plant

2.2 The genus *Verbascum*

Verbascum genus includes about 360 species, distributed throughout the northern hemisphere [14]. There are several uses for the broad group of plants in the genus *Verbascum* (family

Scrophulariaceae) in traditional medicine. These plant species' aerial portions are used to treat respiratory issues such urinary tract infections, bronchial congestion, and cough. Senatore et al. (2007) investigated the growth-inhibitory and antibacterial properties of *V. sinaiticum*, these plants are used in traditional medicine because of their diverse biological qualities, which include sedative, antioxidant, anticancer, antihistaminic, analgesic, and antispasmodic properties [15].

2.2.1 Traditional use of *Verbascum* species

Utilizing *V. sinaiticum* traditionally comprises: stomachache from treating wounds [16]; cancer, viral infection [17]; sunstroke fever, diarrhea, anthrax, abdominal colic, and bleeding [12]; hepatitis treatment [18]. Because of the varied spectrum of biological activity of *Verbascum* species, they have been utilized in traditional medicine for centuries. Ethiopians used the root of *V. sinaiticum* to treat tumors [19], rheumatic pain, wound healing, ocular illnesses, mental sickness, amnesia, tapeworm, syphilis, gonorrhea, relapsing fever, elephantiasis, colds and chest infections [20]; [21]; [22]. The root is chewed for toothaches, crushed, powdered, and applied with butter to the affected area for healing wounds, and taken orally or nasally with water to treat leech infections, snake bites, and lymphadenitis. [23].

2.2.2 Previously isolated compounds (phytochemicals) from genus *Verbascum*

The genus *Verbascum* is characterized by the accumulation of steroids, glycosides, alkaloids, flavonoids, phenolic compounds, and tannins [24]. Even if numerous *Verbascum* species grow wild in various ecosystems of East Africa and Ethiopia, only a limited number of these species have been subjected to phytochemical studies. Especially endemic species of *Verbascum*, are not studied yet.

The phytochemical study from the leaves of *Verbascum* species. Among different compounds only some compounds are mentioned below.

1) Saponins
2) Monotefpene Glucosides
3) Sterol
4) Phenylethanolide Glycosides
5) Neolignan Glucosides
6) Iridoid Glycosides
7) Flavonoids
8) Steroids.

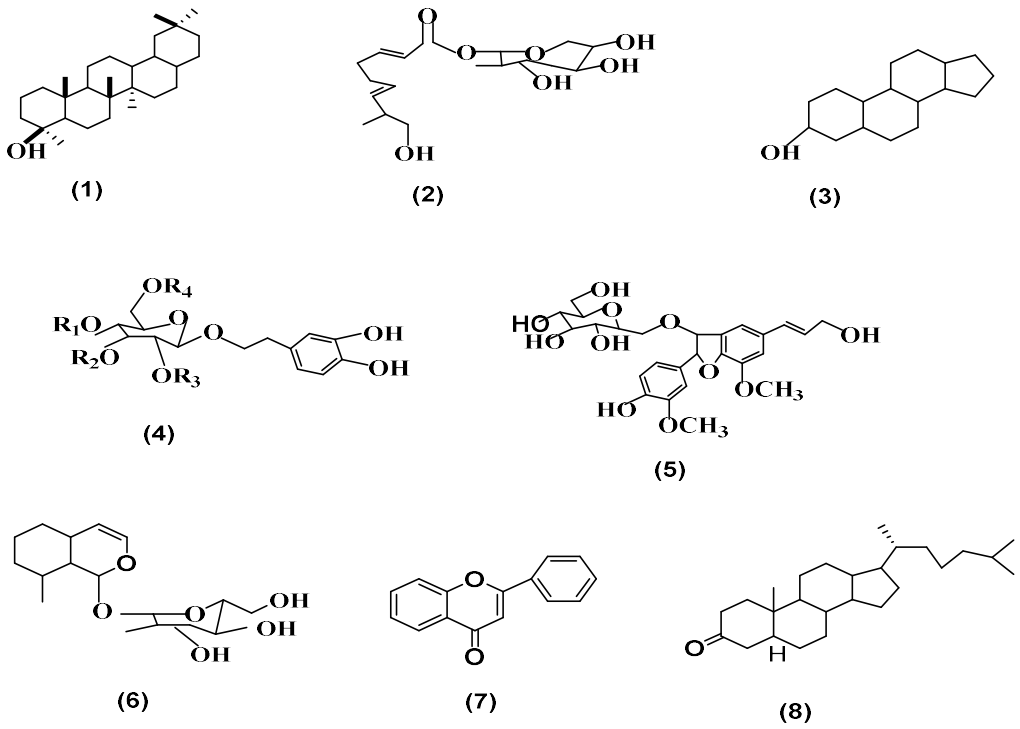


Figure 2 Some isolated compounds from genus *Verbascum*

2.3 *Verbascum sinaiticum*

The genus *Verbascum*, commonly known as "mullein'', is a widespread genus of the family scrophulariaceae which comprises more than 2500 species worldwide. The genus is represented by 233 species, 196 of which are endemic; in the flora of Turkey [25] and 8 of them are endemic plants of Ethiopia [26]. The genus *Verbascum* is characterized by biennial or perennial herbs with yellow flower [27]. Traditional medicine has included the use of *V. sinaiticum*. It is used to treat diarrhea (root), postpartum bleeding (leaves), anthrax (root and leaves), abdominal dropsy (root), and superficial fungal infections (flower and roots). [28]. While the leaf is used to cure wounds, measles, and tinea infection, the root is also used to treat mental disease, amnesia, tapeworm, syphilis, gonorrhea, relapsing fever, rheumatic pain, and elephantiasis. [29].

2.3.1. Previously Isolated compounds from V. sinaiticum

Previous studies, revealed that the phytochemical analysis from *V. sinaiticum* leaves had many chemical ingredients like alkaloids, flavonoids, phenolic compounds and tannins [30].

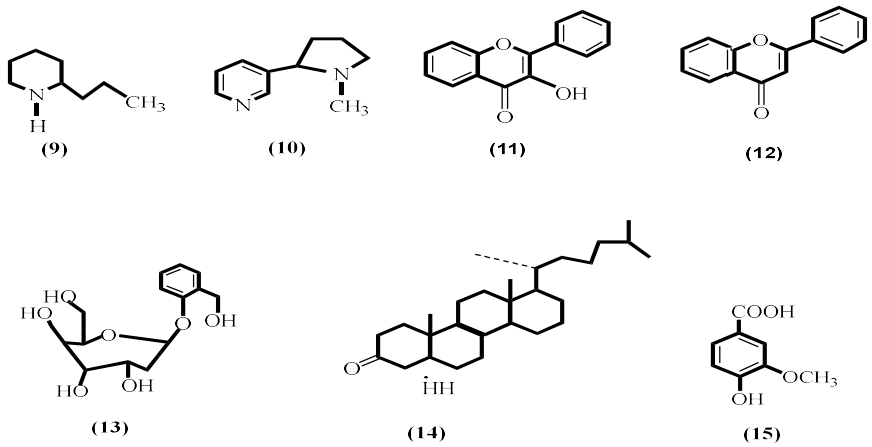


Figure 3 Compounds isolated from *V. sinaiticum* in preliminary phytochemical screening

2.4. Phytochemicals in medicinal plants

Phytochemicals are chemicals of plant origin [31]. A large variety of compounds generated from plants called phytochemicals is thought to be mainly in control of the disease prevention benefits of diets rich in fruits, vegetables, legumes, grains, and plant-based beverages like tea and wine [32]. Phytochemicals (from Greek phyto, meaning "plant") are chemicals produced by plants through primary or secondary metabolism [33]. Phytochemicals are non-nutritive chemicals found in plants. They serve as natural defense mechanisms, protecting plants from harmful microorganisms, insects, and environmental stressors. The following ten categories are used to group phytochemicals according to their chemical structure [8].

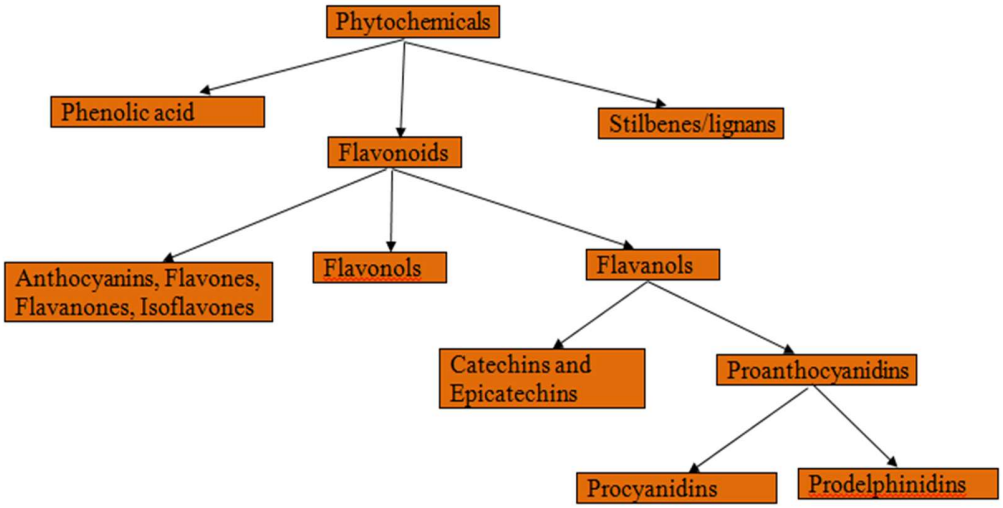


Figure 4 Classification of phytochemicals

Plant components that contain phytochemicals include stems, leaves, roots, seeds, fruits, and flowers. On the other hand, the outer layers of plant tissues contain large amounts of certain phytochemicals, particularly color pigments [34]. Previous studies have shown that phytochemicals reduce the synthesis or absorption of cholesterol and lower the risk for various ailments, including high blood pressure, diabetes, liver problems, and coronary heart disease [35]. In Europe, Asia and Northern America, several *Verbascum* species have been reported as antiseptic, astringent, demulcent, emollient, for tumors, inflammations, migraine, asthma and spasmodic coughs [36].

Depending on the function in plant metabolism, phytochemicals can be divided into two categories: primary and secondary metabolites. Plants require primary metabolites, which include lipids, proteins, amino acids, carbohydrates, purines, and pyrimidines of nucleic acids. It's possible that secondary chemicals are absent. and are not required and of secondary importance for the maintenance of life.

In contrast, the remaining plant compounds generated by cells via metabolic pathways that spread out from the primary metabolic pathways are known as secondary metabolites [37]. Recent investigations have reported that the secondary constituents are the remaining plant chemicals such as alkaloids, terpenes, flavonoids, lignin, plant steroids, curcumins, saponins, phenolic and glucosides [38].

2.4.1. Alkaloids

Alkaloids are nitrogen-containing compounds that obtained from plants [39]. They are all nitrogen-containing compounds, no general definition fits all alkaloids. Alkaloids are naturally synthesis by large numbers of organisms including animals, plants, bacteria and fungi. Alkaloids have been extensively investigated for their biological activity (e.g. anticancer, antibacterial, antiviral and nervous depressant activity in both traditional and modern medicine [40]. Alkaloids are soluble in lipids when in basic and neutral environments and soluble in water when in an acidic medium. [41]. Alkaloids have low molecular weight and are solids in nature [10]. Alkaloids are widely distributed in plants, making up about 20% of the secondary metabolites [42]. The main criteria for classifying alkaloids are their structure and place of origin. The alkaloids are divided into real alkaloids, proto alkaloids, and pseudoalkaloids based on the place they originated. Moreover, alkaloids can be divided into heterocyclic and non-heterocyclic substances based on the place of nitrogen atom is located [43]. An alkaloid with a heterocyclic ring is one that contains the nitrogen atom [44]. On the other hand, it is referred to as a non-heterocyclic alkaloid if the nitrogen atom is present but not on the heterocyclic ring (aliphatic chain) [42]. Some of important naturally occurring alkaloids are Tryptanthrin (1), nicotine(2), hygrine (3), isopelletierine(4), anabasine(5), and mysomine(6) [45].

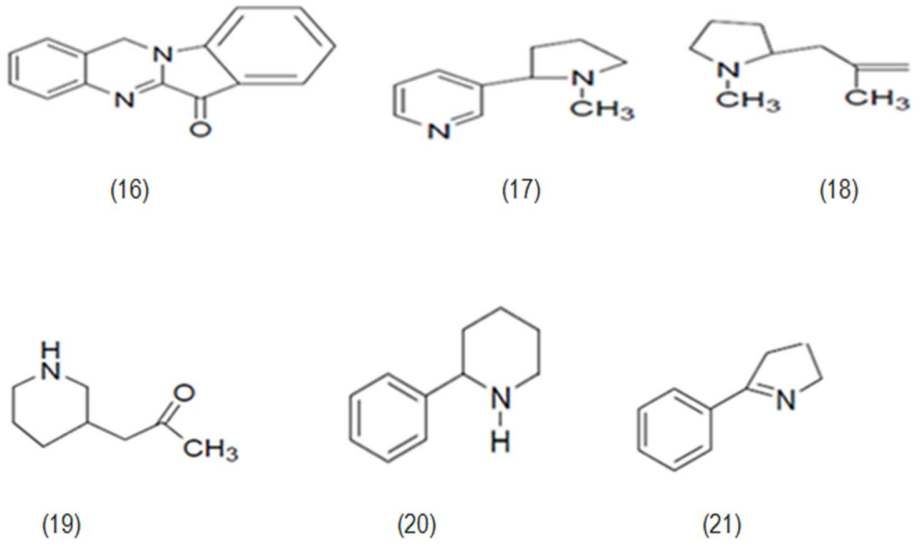


Figure 5 Structure of the important naturally occurring alkaloids.

2.4.2 Flavonoids

Flavonoids are the most diverse group of phytochemicals. They represent a structurally diverse group of polyphenolic compounds which are sythesized during plant metabolism [46]. Flavonoids are a large family of substances (more than 4000, of which several hundred are found in edible plants) and have multiple roles in plants-from attracting pollinating insects for protecting plants from environmental stressors [47]. The pattern of carbon ring substitution and the degree of oxidation vary between the different types of flavonoids. They are included in the large family of phenolic compounds or polyphenols and comprize more than 6000 different structures [48].Flavonoids are characterized by a shared skeleton of diphenylpropane, found in flavones, flavonols, flavanones, flavan-3-ols, etc., commonly present in vegetables, fruits, herbs and teas [49]. Flavonoids are used for natural dyes [50], in skin care and makeup products [51], and for skin anti-wrinkle agents [52]. Nevertheless the medical field is where these polyphenols are most prominently used. Numerous studies have identified flavonoids as anticancer agents [53], antiviral, antibacterial, and antiangiogenic [54], compounds with antimalarial, neuroprotective, antioxidant, antitumor, and anti-proliferative properties [55]. Flavonoids generally refer to the natural products of C_6-C_3-C_6 basic structures . Most of them are the chromone derivatives with the core structure of 2-phenylchromone and made up of three rings of A/B/C [56].

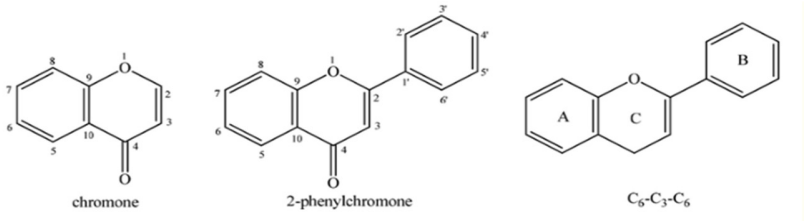


Figure 6 Basic structure of flavonoids

2.4.3 Phenolic compounds

There are more than 10,000 phenolic structures recognized in nature, ranging from simple aromatic rings to complex polymerized compounds, making the phenolic compounds one of the main and largest categories of secondary metabolites of plants [57]. The ability of phenolic compounds obtained from plant-based byproducts to scavenge free radicals in both in vivo and food systems

has been investigated [58]. Phenolic compounds act as antioxidants and contribute to overall health.

Phenolic compounds are categorized as either polymers, extensively distributed, or shortly distributed based on how widespread they are found in nature [29]. The phenolic chemicals that are found in all plants are regarded as broadly spread.

Due to phenolic compounds serve as a natural defense against microbiological illnesses and can be added to food during processing to increase the shelf life of items like catfish fillets [59].

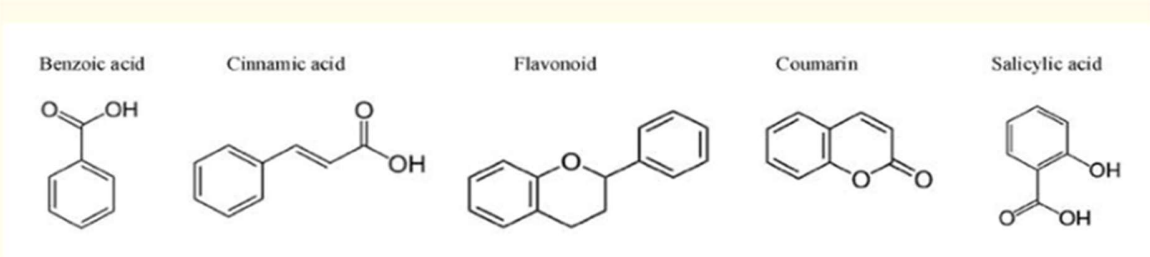


Figure 7 Examples of widely distributed phenolics.

Phenolic compounds that are shortly or less widely distributed have limited presence in plants and include simple phenols, pyrocatechol, hydroquinone, and resorcinol (figure below).

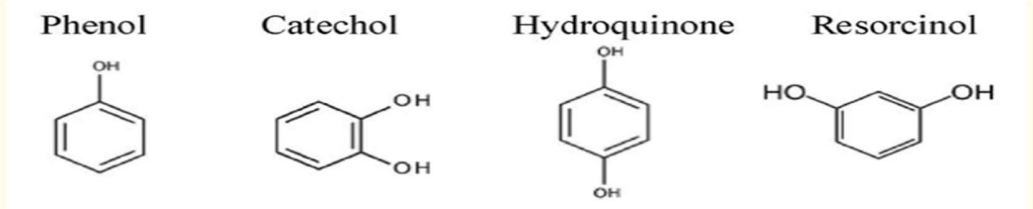


Figure 8 Examples of shortly distributed phenolics

2.4.4 Glycosides

The secondary metabolites found in plants that have non-sugar parts linked to a sugar moiety are called glycosides. It comprises the aldehyde or keto group of the sugar moiety and the hydroxyl group of non-sugar or phenolic. These substances have a variety of positive effects on both humans and animals; however, a lot of plants store these compounds in inactive form, which the body's enzymes can then activate [57]. So many plants store chemicals in the form of inactive glycosides, which can be activated by enzyme hydrolysis. Glycosides are widely distributed in plants and responsible for their medicinal value.

Many Glycosides:- are toxic, ecologically important, Serve as potential source of new drugs and starting materials for semi-synthetic drugs. Glycosides can be classified into major classes' namely cardiac glycosides, flavonoid glcosides, thiocyanate glycosides a basic structure of glycosides [72].

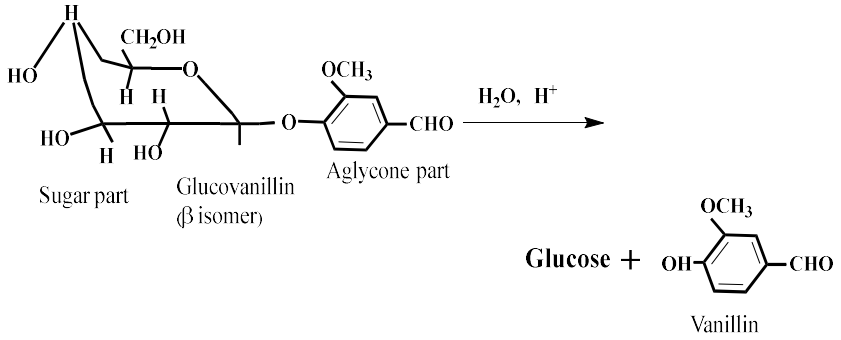


Figure 9 bonding of sugar component and aglycone component in glycosides, using glucovanillin as a specific example. The bonding is through oxygen to the carbonyl carbon. *[60]*

2.4.5 Terpenoids

Terpenoids are widely found in plants and can form cyclic structures such as steroids and sterols. The term terpenoids is used to describe terpenes (hydeocarbons) and their oxygenated, hydrogenated derivatives [61]. Terpenoids, sometimes termed isoprenoids, are a class of organic compounds that exist naturally. They are generated from the substance isoprene, which has five carbons, and its derivatives, which are referred to as terpenes, diterpenes, etc [62]. Most natural terpenes hydrocarbons have the general formula ($C_5H_8)_n$. Whereas "terpenoids" and "terpenes" are occasionally used interchangeably, terpenoids have extra functional groups, typically oxygen [63]. When terpenoids are mixed with hydrocarbon terpenes, they make up nearly 80,000 compounds [64]. The majority of known natural products are classified as secondary metabolites found in plants, accounting for over 60% of them [65].

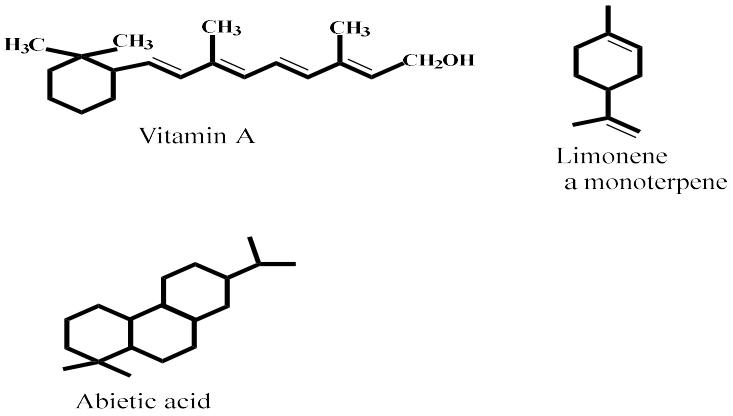


Figure 10 structure of important terpenes

2.5 Free radicals

Free radicals can be defined as molecule or molecular fragments containing one or more unpaired electrons in atomic or molecular orbital. The majority of radicals share certain characteristics in common due to the presence of an unpaired electron, including being extremely reactive and unstable. These unpaired electron(s) usually gives a considerable degree of reactivity to free radicals [66]. They can be either donate an electron or accept an electron from other molecules, therefore behaving as oxidants or reductants [67].

There are some different types of free radicals, oxygen-centered, nitrogen-centered, carbon-centered and sulphur-centered radicals are different free radical species [68].

Some reports indicate that there are numerous types of free radicals that can be formed within the body. Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are major components of the "free radical" system.They are generated by our body by different endogenous systems, exposure to conditions or pathological states. Free radicals are accountable for various disease like Cardiovascular disease, Cancer, Neurological disease, Pulmonary disease, Rheumatoid arthritis, Nephropathy, Ocular disease and Fetus [69]. Free radicals once generated through various physiological processes in living organisms, they can react with other biomolecules to attain stability.

2.6 Antioxidants

An antioxidant is a molecule stable enough to donate an electron to a rampaging free radical and neutralize it. These antioxidants' primary mechanism of action is the scavenging of free radicals, which delays or inhibits cellular damage [70], These modest molecular weight antioxidants safely

engage in a chain reaction with free radicals, stopping it before any important molecules are harmed. Among these antioxidants are uric acid, glutathione, and ubiquinol, which are created by the body during regular metabolism. [71]. There are additional, weaker antioxidants in food. Generally the role of antioxidants are to neutralize the excess of free radicals, to protect the cells against their toxic effects and to contribute to disease prevention.

CHAPTER THREE
MATERIALS AND METHOD

3.1 Materials

3.1.1 **Plant Materials Study Area**

Fresh root of *V. sinaiticum* were collected in November, 2023 from Sebeta, which is located in Sheger city, Oromia regional state.in Ethiopia that is 24 km South West of Addis Ababa.The plant material was identified and authenticated by botanist in Department of biology.

3.1.2 Chemicals and reagents

The chemicals and reagents used for this study are chloroform. ethylacetate, Methanol, cyclohexane, hydrochloric acid, sulfuric acid, ammonia solution and acetone.

3.1.3 Instruments and Apparatus

^{1}H, ^{13}C, and 2D NMR spectra were recorded on a Bruker advance 400MHz spectrometer with TMS as internal started. The necessary apparatus and instruments used for this study included electronic beam balance for mass measurement, vacuum rotary evaporator for concentrating filterate, volumetric flask, beaker, round bottom flask, UV lamp 254 nm, Whatman No.1 Filter Paper, Erlenmeyer flask, micropipette, burette, graduated cylinder, test tubes and TLC-paper are used.

3.2 Extraction and Isolation

3.2.1 Extraction

The freshly chopped *V. sinaiticum* roots were collected, cleaned by rinsing wash with running tap water to remove soil and dust particles. The roots were air dried at room temperature and ground into powder with grinder. About 10g of powdered root of *V. sinaiticum* was soaked in 1L methanol for 24 hours in Erlenmeyer conical flask. Then the mixture was filtered through Whatman No. 1 filter paper. The filterate was concentrated using a rotary evaporator under reduced pressure at 40-45°C. The remaining crude extract was refrigerated in an airtight bottle at 4°C until it was used, following solvent evaporation [72]. The remaining solvent was removed at room temperature to obtain a complete dry powdered residue (crude extract). Finally, the concentrated crude extract solution was dried and applied for coulumn chromatorgaphy.

Figure 11 Graphical representation of crude extracts and preparation process

3.2.2 Isolation

After a fine dry powder residue ground by using mortar and pistil, the dry powdered sample was adsorbed on 10 g silica gel and subjected to column preparation.The colmn was washed and rinsed with n-hexane and prepared by packing in a glass column (4 cm by 48 cm) with slurry of silica gel of 60 g in 200 ml of n-hexane.The dry powder was applied on the top of packed silica gel and fractionated using a gradient solvent system containing chloroform/cyclohexane (1:1) twice, chloroform (100%), chloroform/ethyl acetate (1:1) twice , ethyl acetate 200ml or (100%), ethyl acetate 100ml, ethyl acetate/methanol (95% to 5%), ethyl acetate/methanol (90:10), ethyl acetate/methanol (80:20) that were used to elute the components which were collected in well washed, oven dried and labeled beakers before the plant material travelled to the column neck. This successive elution was collected in 10 beakers (Table 1).

Table 1 Column chromatography of fractions of crude extracts

Fractions	Solvent system	Ratio	Volume
1	Chloroform:Cyclohexane	1:1	200ml
2	Chloroform:Cyclohexane	1:1	200ml
3	Chloroform	100%	200ml
4	Chloroform: Ethylacetate	1:1	200ml
5	Chloroform: Ethylacetate	1:1	200ml
6	Ethylacetate	100%	200ml
7	Ethylacetate	100%	100ml

The elution was stopped when the darkest part at top reached the bottom. Each fraction was monitored by TLC. Thin layer chromatography showed that the strongly fluorescence quenching at 254 nm, and to stain blue black with vanilin. Out of 10 fractions, 1-3 were colourless, fraction 4 light brown, 5 colourless, 6 colourless, 7-10 had multiple spots and colourless. Fractions 1-3 were discarded because their TLC results were joined together in invisible way, fraction 4 was prepared for column which showed three clear spots, fraction 5 was discarded because the result had no spots, fraction 6 gave a single spot on TLC with R_f value of 0.81. It was transferred in a vial labelled VSG-1.

Similarly crude extract of sample 4 which was transferred from beaker four of aqueous part was subjected to column chromatography of diameter 3 cm by 21cm height elution with solvents of Cyclo hexane/Ethyl acetate and a total of 16 fractions were collected in test tubes.

Fractions 1-4 were dicarded, because it was impure, fractions 4-6 showed the same and single spots, and fractions from 7-9 contained single spot on TLC and were the same which mixed in and showed single spot. Fractions 7-9, which were mixed in the test tube gave single spots with Rf value of 0.857 and was colourless liquid. It was transferred in a vial labelled VSG-4. After that the vials were sent for spectral analysis.

3.3 Structural Elucidation

The ^{13}C NMR, ^{1}H NMR and DEPT-135 spectra were recorded for both compound VSG-1 and compound VSG-4, the 2D-NMR (HSQC, HMBC and COSY) experiments were run for compound-VSG4. But for compound-VSG1 2D-NMR was not generated due to insufficient of sample. Therefore, this thesis constitutes characterization of compound VSG-4.

CHAPTER FOUR

RESULTS AND DISCUSSION

4. 1 Characterization of compounds isolated from the roots of *V. sinaiticum*

4.1. 1 Characterization of compound VSG-4

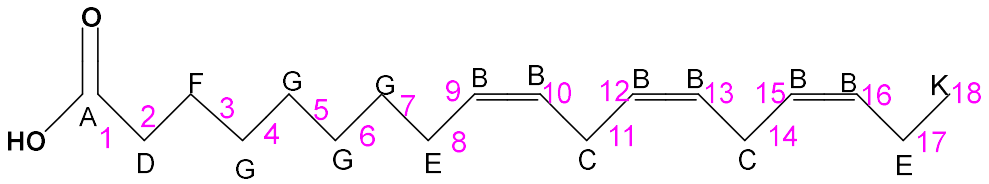


Figure 12 structure of compound VSG-4

Compound VSG-4 was isolated as a colorless liquid from Cyclohexane: Ethyl acetate (2:1) extract Rf value of 0.85 that indicating its polarity and chromatographic behavior. The complete proton and carbon assignments were determined using the combination of 1D (^{1}H NMR, ^{13}C NMR, DEPT and 2D spectra (HSQC, HMBC and COGY) NMR experiments (Appendix).

^{1}H NMR (400 MHz, Acetone-d6) δ 10.54 three double bonds at δ 5.36 from olefinic protons which contained six methine (CH) groups, and Protons attached to bis allylic carbons at δ 2.83 (multiplet) two carbons can be observed , α methylene at δ 2.29 (triplet) of protons, and Protons attached to the allylic carbons found at δ 2.07 (Quartet) signal, β methylene at a signal of δ 1.60 (multiplet), However methylene at δ 1.39 (multiplet), methylene at δ 1.32 (multiplet) and methylene at δ 1.30 overlapped, methyl at δ 1.09 (multiplet) or at δ 1.02 and δ 0.87 (multiplet) and overlapped with methylene(CH2)n[74]. (figure 4.9) and Appendix figure 2 showed that the region from δ 0.6 to δ 5.5 has seven peaks for twenty nine (29) hydrogen atoms. The peak at δ 0.87 to δ 1.02 overlapped, Additionally the proton of COOH appears in the downfield region of the NMR spectrum, around 10 -12 ppm, this is due to the electron with-drawing effect of the carbonyl group (C=O) in the carboxylic acid functional group. This effect deshielded the proton, causing it to resonate at higher frequency.

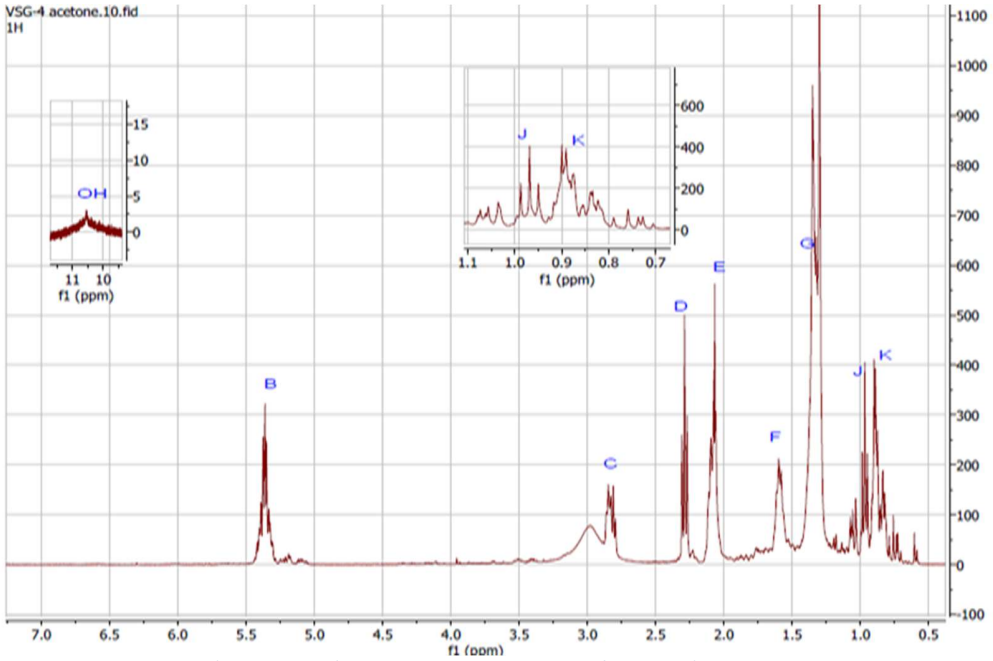


Figure 13 The H-NMR spectrum of VSG4 in Acetone

The ^{13}C NMR spectrum carbon containing one peak was the first carbon (carbonyl carbon) appeared at δ(173.77) and for alpha linolenic acid, the six olefinic carbons gave five (5) signals; δ (129.96, 129.79, 129.77, 128.08, 127.89) due to two carbons overlapped. The two carbons ; bis allylic δ (25.29, 28.82) are appeared as a single peak at δ 25.29, one carbon (δ 131.54) is alpha to the first, two carbons (δ 29.01) and δ(13.46) are allylic carbons, The other δ (20.56) is β methylene , four of δ (29.13, 29.39,33.29, 31.81) are methylene and one methylene is attached to the methyl at δ (11.72), When a methylene group (CH_2) attached to a methyl group (CH_3), the NMR signal is typically a quartet. Spin-Spin Coupling: The protons on the methylene group interact with the protons on the adjacent methyl group. This interaction is called spin-spin coupling. [74].

Peak groups are justified by DEPT-135 and HMBC. As can be seen from figure10, there were 17 peaks for 18 carbon atoms. Finally, for linolenic acid, the six olefinic carbons gave 5 (five) signals (two overlapped) and the two bis-allylic ones are slightly resolved [73]. The allylic and divinyl carbons in 13C NMR spectra can be used for determining the composition of unsaturated fatty acids. The difference in the 13C NMR and GC values was only ±5% at most. Thus, the 13C NMR technique created in this work is valid [74].

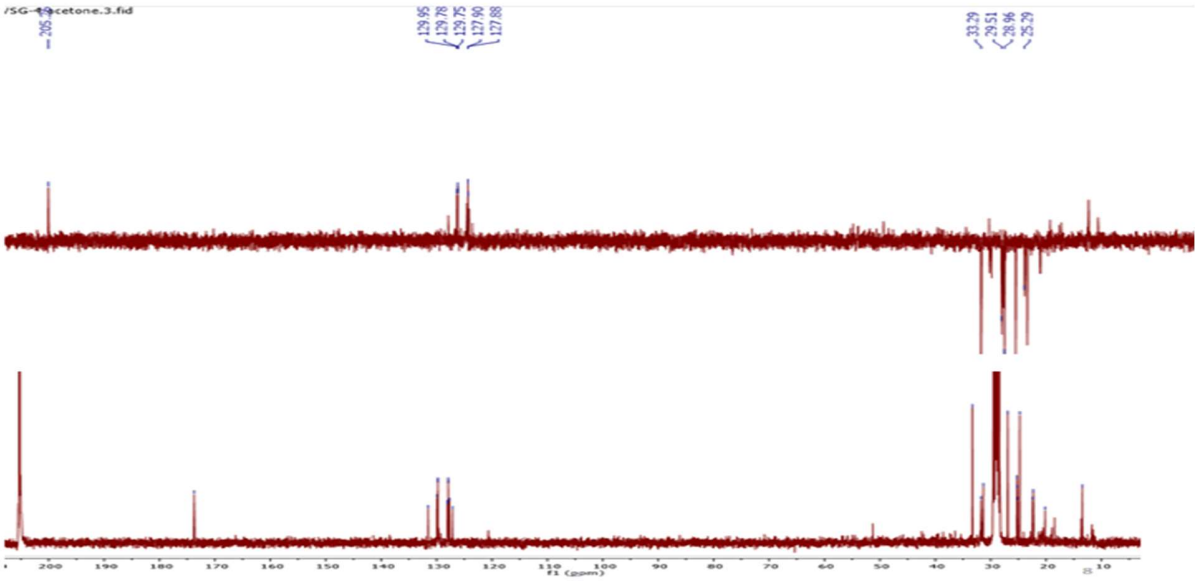


Figure 14 the 13C NMR data of the compound VSG-4

Table 2: 1H NMR (400 MHz and 13C NMR (101 MHz, Acetone) δ 205.30 spectroscopic data of 9,12,15-octadacatrienoic acid from *V. sinaiticum*

Position		VSG4 1H NMR (400 MHz, Acetone d6) **13C** NMR (101 MHz, Acetone)		Lit. 13C [8]	Lit. 1H NMR 90 MHz in $CDCl_3$ [86]
Letter	DEPT	δ_C	δ_H	δ_C	δH
D	C -2	131.5	2.29(t, J = 7.4Hz, 2H),	134.13	2.340
F	C -3	20.5	1.68 – 1.48 (m,2H),	24.69	1.61
G	C -4	29.1	1.30 (m, 4H), 1.35 – 1.29 (m, 2H), 1.43 – 1.35 (m, 2H),	29.09	1.34-1.30
G	C -5	29.4		29.13	
G	C -6	33.3		29.62	
G	C -7	29.0		29.21	
E	C 8	29.0	2.07 (qt ,J= 6.4, 3.4 Hz, 2H),	27.23	2.04 -2.07
B	C -9	129.9	5.46-526 (m, 2H),	131.85	5.17 – 5.54
B	C -10	127.7		127.16	
C	C -11	25.3	2.83 (dt , 15.0, 6.1 Hz, 2H),	25.56	2.800
B	C -12	129.8	5.46-526 (m, 2H),	128.26	5.17 – 5.54
B	C -13	128.1		128.22	
C	C -14	28.8	2.83 (dt, J = 15.0, 6.1 Hz, 2H),	25.65	2.800
B	C -15	127.9	5.46-526 (m, 2H),	127.80	5.17 – 5.54
B	C -16	129.8		130.14	
J K	C -17	13.4	1.02 – 0.67 (m, 5H).	20.56	0.973
	C -18	11.7		14.26	

Table 3: COSY Correlation of compound VSG-4

Carbon Position	Proton - Proton Correlation (COSY)
C-9 (129.86) and C-8(129.96)	H – 5.36 ⟷ H – 2.07
C-13(128.08) and C-14 (28.82)	H – 5.36 ⟷ H -2.83
C-13(128.08) and C-11(25.29)	H – 5.36 ⟷ H – 2.83
C- 12(129.79) and C – 11(33.29)	H – 5.36 ⟷ H – 2.83
C- 12(129.79) and C -14(25.29)	H – 5.36 ⟷ H - 2.83
C -2(128.08) and C -4 (29.13)	H – 2.29 ⟷ H – 1.58

The 1H-[1]H COSY spectrum (Appendix) correlations between C-9 (δ5.36) correlates with C-8(δ 2.07) identified C -9 of methine (CH) from olefinic is attached to C-8 of allylic carbon. Additionally, the proton-proton connectivity indicated in C-13 (δ 128.08) correlated with C-14 (δ 28.82) and C-11(δ 25.29), C- 12(δ 129.79) with C-11(33.29) and C -14(25.29), C -2(δ 128.08) also correlated with C -4 (δ 29.13).

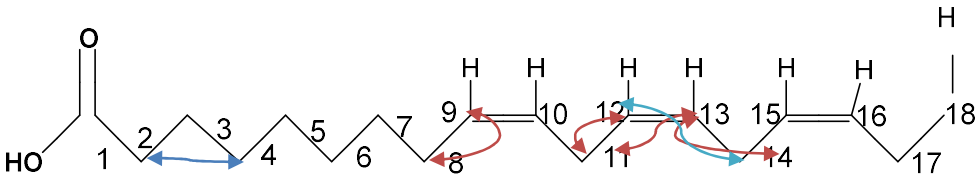


Figure 15 COSY correlations on alpha linolenic

Table 4 : the HSQC spectrum correlations of compound VSG-4

Carbon Number	δ_C	δ_H (HSQC correlation with H)	Remark
C-2	131.54	1.31 and 2.29	CH_2
C-3	20.56	1.35 and 1.12	CH^2
C-4	29.13	1.58 and 1.33	CH_2
C-5	29.39	1.58,1.45	CH_2
C-6	33.29	2.29 and 2.26	CH_2
C-7	31.81	1.97, and 2.09	CH_2
C-8	29.01	2.07 and 1.95	CH_2
C-9	129.96	5.36	CH
C-10	127.68	5.38	CH
C-11	25.29	2.83 and 2.77	CH_2
C-12	129.77	5.36	CH
C-13	128.08	5.36	CH
C-14	28.82	2.83 and 2.77	CH_2
C-15	127.89	5..36	CH
C-16	129.79	5.38	CH
C-17	13.46	1.02	CH_2
C-18	11.72	0.91, 0.91 0.88	CH_3

The HSQC correlation of compound VSG-4 of spectrum (Appendix) indicated alpha methylene proton at a signal of δ 2.29 (t, J = 7.4Hz, 2H). beta methylene correlation with carbon is at a signal of δ 1.68 – 1.48 (m,2H), correlation of four methylene groups attached with a carbon signal at δ 1.30 (m, 4H),1.35 – 1.29 (m, 2H and δ 1.43 – 1.35 (m,2H), allylic carbon correlated with signal at δ 2.07 (qt ,J= 6.4, 3.4 Hz, 2H), and olefinic carbons with three double bonds showed signal at 5.46-526 (m, 8H), finally bis allylic and ethylene (methylene , methyl) showed signals at 2.83 (dt, J = 15.0, 6.1 Hz, 4H), and 1.02 – 0.67 (m, 5H).

The HMBC spectrum (appendix 6) of compound VSG-4 showed that the correlations between protons and carbon atoms. The correlation of the methyl proton both allylic carbon and the olefinic carbon with the allylic proton and the methyl carbon and olefinic proton coupling with allylic carbon. The beta hydrogen show coupling with methylene carbon. The carbonyl carbon coupling with methylene proton.

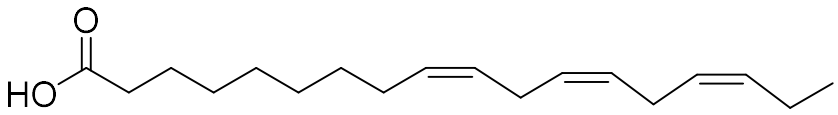
The 1H NMR spectrum revealed the presence of characteristic signals for olefinic protons, methylene groups, and methyl groups. These signals were indicative of a fatty acid structure.
The spectral data obtained for VSG-4 were compared with those of known fatty acids. The comparison suggested that VSG-4 is likely a derivative of linolenic acid, a polyunsaturated fatty acid. The presence of the characteristic peaks for the olefinic protons and the methylene groups supported this identification. To further confirm the structure of VSG-4 and the connectivity of its functional groups, the following spectroscopic techniques were employed:

COSY: The COSY spectrum revealed correlations between the olefinic protons at δ 5.36 and the adjacent methylene protons at δ 2.07, 2.83, and 2.29. These correlations confirmed the presence of olefinic group between carbons 9 and 10.

HSQC: The HSQC spectrum established the direct correlations between protons and carbons, allowing for the assignment of chemical shifts to specific carbon positions. This technique confirmed the presence of a methylene group at δ 2.29, an allylic carbon at δ 2.07, and olefinic carbons at δ 5.36.

HMBC: The HMBC spectrum revealed long-range correlations between protons and carbons, providing further evidence for the structural connectivity. For example, the correlations between the methyl protons at δ 0.87-1.02 and the olefinic carbons at δ 5.36 indicated the presence of a methylene group attached to the methyl group. Linolenic acid and its derivatives have been extensively studied for their potential health benefits.

Finally, based on the spectroscopic data described above, the isolated compound from the root extract was identified as 9, 12, 15-octadecatrienoic acid or α-linolenic acid (ALA), an essential omega-3 fatty acid known for its potential health benefits.



9, 12, 15-octadecatrienoic acid or ?-linolenic acid (ALA)

CHAPTER FIVE
CONCLUSION AND RECOMMENDATION

This study focused on the isolation and structural elucidation of secondary metabolites from the roots *V. sinaiticum*. Using different solvent system and spectroscopic method, the structure of one compound was successfully elucidated namely, 9, 12, 15-octadecatrienoic acid or α-linolenic acid. An attempt was done on another fractions of the extracts(VSF-1), However, sufficient NMR data was not able to generate due to small quantity of the isolated compound.

Based on the findings of this research, the following recommendations are proposed for future studies:

1. Isolation and Purification: Further research should focus on isolating and purifying the individual compounds identified in the root extracts of *V. sinaiticum*. This will allow for a more comprehensive evaluation of their biological activities and potential therapeutic applications.
2. Pharmacological Evaluation: The isolated compounds should be subjected to in vitro and in vivo studies to assess their pharmacological properties, including antioxidant, anti-inflammatory, antimicrobial, and anticancer activities.
3. Traditional Medicine Integration: The findings of this research can be integrated into traditional medicine practices to provide scientific evidence for the use of *V. sinaiticum* in treating various ailments.
4. Sustainable Cultivation: Research on sustainable cultivation practices for *V. sinaiticum* is essential to ensure the long-term availability of this valuable medicinal plant.
5. Comparative Studies: Comparative studies with other species of *Verbascum* can help to identify unique phytochemical profiles and potential therapeutic properties.

REFERENCES

1. World Health Organization, *WHO traditional medicine strategy: 2014-2023,* Geneva, Geneva: World Health Organization, 2013.
2. Asfaw, M., Sileshi, D., & Ashenif, T. (2017). Medicinal Plants Used for the Management of Rabies in Ethiopia. *Medicinal Chemistry*, *7*, 795-806.
3. Ames, B. N., Shigenaga, M. K., & Hagen, T. M. (1993). Oxidants, antioxidants, and the degenerative diseases of aging. *Proceedings of the National Academy of Sciences*, *90*(17), 7915-7922.
4. Ekor, M. (2014). The growing use of herbal medicines: issues relating to adverse reactions and challenges in monitoring safety. *Frontiers in pharmacology*, *4*, 177.
5. Bitew, H., Gebregergs, H., Tuem, K. B., & Yeshak, M. Y. (2019). Ethiopian medicinal plants traditionally used for wound treatment: a systematic review. *Ethiopian Journal of Health Development*, *33*(2).
6. Karavelioğullari. F. and Aytac. Z. , "Revision of the genus *Verbascum* L. (Group A) in Turkey," *Journal of the Botanical Research Institute of Texas,* vol. 1, p. 9–32, 2008.
7. A. Kalia, Textbook of industrial pharmacognosy, New Delhi, India: Oscar Publication, 2005.
8. D. Abebe, A. D. and K. U. , in *Medicinal plants and other useful plants of Ethiopia: Illustrated checklist*, Nairobi, Camerapix Publishers International, 2003, pp. 10, 102,103.
9. D. Abebe and A. Ayehu, Medicinal plants and enigmatic health practices of northern Ethiopia, B.S.P.E., 1993.
10. H. K. Thibaud, "Perfumary products from Citronella Oil," *Perfum Essent Oil Rec,* vol. 45, pp. 186-191, 1954.
11. Fullas, F. (2010). Ethiopian medicinal plants in veterinary healthcare a mini-review. *Ethiopian e-Journal for Research and Innovation Foresight (Ee-JRIF)*, *2*(1), 48-58.
12. Weldegerima, B., Abula, T., & Ragunathan, M. (2008). PHCOG MAG.: Research Article Ethno-Veterinary Use of Medicinal plants by Traditional Healers in Dabat District, Northwestern Ethiopia. *Phcog Mag*, *4*(15), 93.

13. Afifi, M. S., Ahmed, M. M., Pezzuto, J. M., & Kinghornt, A. D. (1993). Cytotoxic flavonolignans and flavones from Verbascum sinaiticum leaves. *Phytochemistry*, *34*(3), 839-841.
14. Jain, P., & Parkhe, G. (2018). An updated review on pharmacological studies of Rumex nepalensis. *J Pharm Innov*, *7*, 175-181.
15. Senatore, F., Rigano, D., Formisano, C., Grassia, A., Basile, A., & Sorbo, S. (2007). Phytogrowth-inhibitory and antibacterial activity of Verbascum sinuatum. *Fitoterapia*, *78*(3), 244-247.
16. Teklehaymanot, T., Giday, M., Medhin, G., & Mekonnen, Y. (2007). Knowledge and use of medicinal plants by people around Debre Libanos monastery in Ethiopia. *Journal of ethnopharmacology*, *111*(2), 271-283.
17. Teklehaymanot, T. (2009). Ethnobotanical study of knowledge and medicinal plants use by the people in Dek Island in Ethiopia. *Journal of ethnopharmacology*, *124*(1), 69-78.
18. Yineger, H., Kelbessa, E., Bekele, T., & Lulekal, E. (2007). Ethnoveterinary medicinal plants at bale mountains national park, Ethiopia. *Journal of ethnopharmacology*, *112*(1), 55-70.
19. Abebe, W. (2016). An overview of Ethiopian traditional medicinal plants used for cancer treatment. *European journal of medicinal plants*, *14*(4).
20. Gondal, H. Y., Zamir, R., Nisar, M., & Choudhary, M. I. (2020). Verbascum sinaiticum: a rich source of antioxidant phenylethanoid glycosides. *The Natural Products Journal*, *10*(2), 158-162.
21. Lalrinzuali, K., Vabeiryureilai, M., & Jagetia, G. C. (2016). Investigation of the anti-inflammatory and analgesic activities of ethanol extract of stem bark of Sonapatha Oroxylum indicum in vivo. *International journal of inflammation*, *2016*(1), 8247014.
22. Bileflimi, V. T. K. (2004). Chemical constituents of Verbascum L. species. *Fabad J. Pharm. Sci*, *29*, 93-107.
23. Enyew, A., Asfaw, Z., Kelbessa, E., & Nagappan, R. (2014). Ethnobotanical study of traditional medicinal plants in and around Fiche District, Central Ethiopia. *Current Research Journal of Biological Sciences*, *6*(4), 154-167.

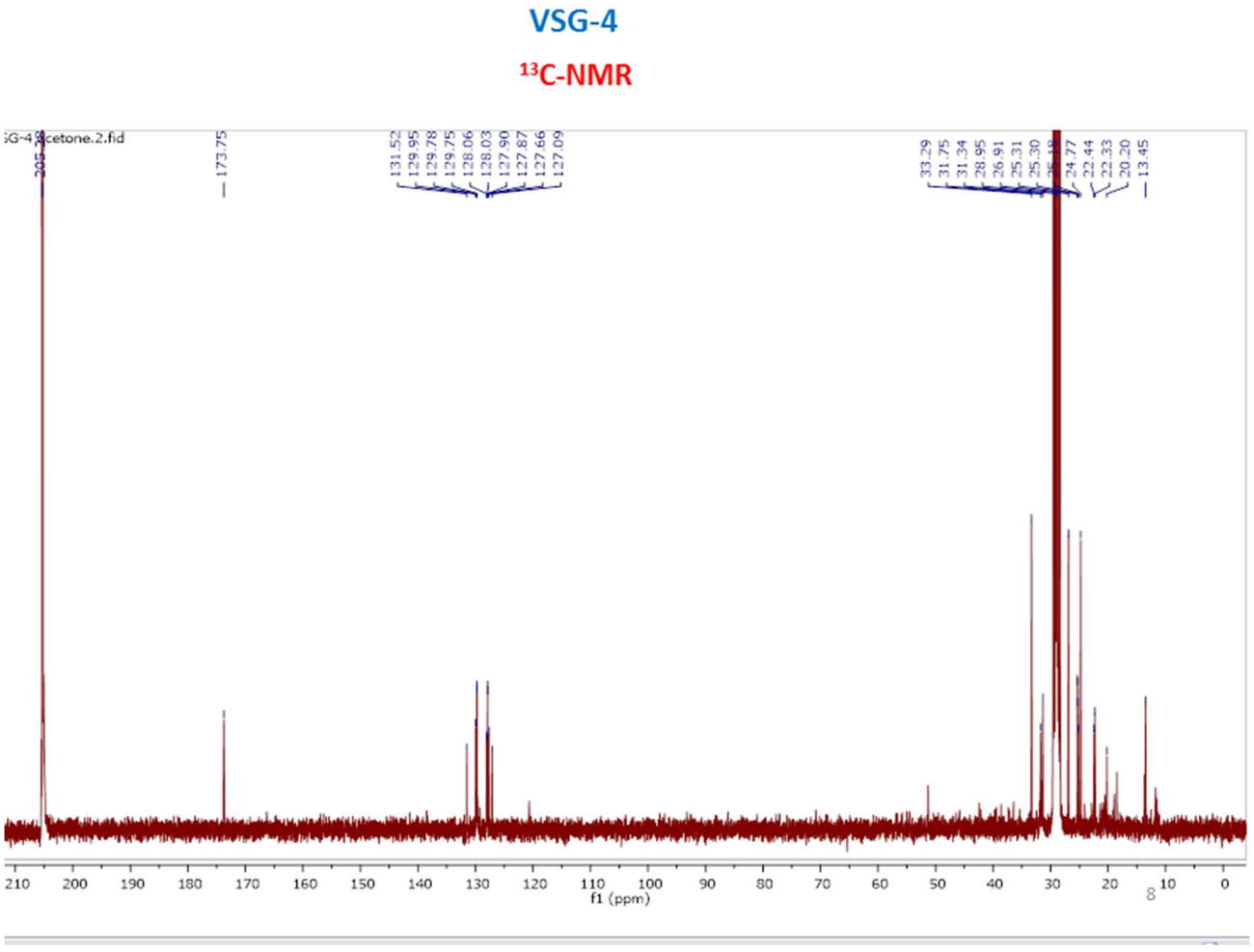
24. Tadeg, H., Mohammed, E., Asres, K., & Gebre-Mariam, T. (2005). Antimicrobial activities of some selected traditional Ethiopian medicinal plants used in the treatment of skin disorders. *Journal of ethnopharmacology*, *100*(1-2), 168-175.
25. Güner, A., Özhatay, N., Ekim, T., & Başer, K. H. C. (2000). Flora of Turkey and the East Aegean Islands, Vol. 11, Edinburgh.
26. H. I. and E. S. , Flora of Ethiopia, Addis Ababa: The National Herbarium, Addis Ababa University & Uppsala University, 1989.
27. H. I. and E. S. , Flora of Ethiopia, Addis Ababa: The National Herbarium, Addis Ababa University & Uppsala University, 1989.
28. Abebe, D., & Ayehu, A. (1993). Medicinal plants and enigmatic health practices of Northern Ethiopia.
29. Addis, G., Abebe, D., & Urga, K. (2001). A survey of traditional medicinal plants in Shirka District, Arsi Zone, Ethiopia.
30. Mergia, E., Shibeshi, W., Terefe, G., & Teklehaymanot, T. (2014). Phytochemical screening and in vitro antitrypanosomal activity of aqueous and methanol leaf extract of verbascum sinaiticum (scrophulariaceae) against trypanosoma congolense field isolate. *Lab. Anim*, *45*, 46.
31. Breslin, A. (2017). The chemical composition of green plants. *Sciencing, Leaf Group Ltd*, *76*.
32. Arts, I. C., & Hollman, P. C. (2005). Polyphenols and disease risk in epidemiologic studies. *The American journal of clinical nutrition*, *81*(1), 317S-325S.
33. Molyneux, R. J., Lee, S. T., Gardner, D. R., Panter, K. E., & James, L. F. (2007). Phytochemicals: the good, the bad and the ugly?. *Phytochemistry*, *68*(22-24), 2973-2985.
34. King, A. M. Y., & Young, G. (1999). Characteristics and occurrence of phenolic phytochemicals. *Journal of the American dietetic association*, *99*(2), 213-218.
35. Saxena, M., Saxena, J., Nema, R., Singh, D., & Gupta, A. (2013). Phytochemistry of medicinal plants. *Journal of pharmacognosy and phytochemistry*, *1*(6), 168-182.
36. G. M. A modern herbal, New York: Barnes and Noble Books, 1995.
37. Bone, K., & Mills, S. (2013). *Principles and practice of phytotherapy: modern herbal medicine*. Elsevier Health Sciences.

38. Chouhan, H. S., & Singh, S. K. (2010). Antibacterial activity of seed and flower parts of Crotalaria juncea Linn.
39. Kurek, J. (Ed.). (2019). *Alkaloids: their importance in nature and human life*. BoD–Books on Demand.
40. Thawabteh, A., Juma, S., Bader, M., Karaman, D., Scrano, L., Bufo, S. A., & Karaman, R. (2019). The biological activity of natural alkaloids against herbivores, cancerous cells and pathogens. *Toxins*, *11*(11), 656.
41. Heinrich, M., Mah, J., & Amirkia, V. (2021). Alkaloids used as medicines: Structural phytochemistry meets biodiversity—An update and forward look. *Molecules*, *26*(7), 1836.
42. Bhambhani, S., Kondhare, K. R., & Giri, A. P. (2021). Diversity in chemical structures and biological properties of plant alkaloids. *Molecules*, *26*(11), 3374.
43. Seyed Mohammad Nabavi, Mina Saeedi, Seyed Fazel Nabavi, Ana Sanches Silva, Recent Advances in Natural Product Analysis, Elsevier, 2020, pp. 505-567.
44. Xu, K., Yuan, X. L., Li, C., & Li, X. D. (2020). Recent discovery of heterocyclic alkaloids from marine-derived Aspergillus species. *Marine drugs*, *18*(1), 54.
45. Smitha, G. R., & Thondaiman, V. (2016). Reproductive biology and breeding system of Saraca asoca (Roxb.) De Wilde: a vulnerable medicinal plant. *Springerplus*, *5*(1), 2025.
46. Robards, K., Prenzler, P. D., Tucker, G., Swatsitang, P., & Glover, W. (1999). Phenolic compounds and their role in oxidative processes in fruits. *Food chemistry*, *66*(4), 401-436.
47. Kumar, S., & Pandey, A. K. (2013). Chemistry and biological activities of flavonoids: an overview. *The scientific world journal*, *2013*(1), 162750.
48. Šamec, D., Karalija, E., Šola, I., Vujčić Bok, V., & Salopek-Sondi, B. (2021). The role of polyphenols in abiotic stress response: The influence of molecular structure. Plants 2021, 10, 118.
49. Hertog, M. G., Feskens, E. J., Kromhout, D., Hollman, P. C. H., & Katan, M. B. (1993). Dietary antioxidant flavonoids and risk of coronary heart disease: the Zutphen Elderly Study. *The lancet*, *342*(8878), 1007-1011.
50. Villela, A., van Vuuren, M. S., Willemen, H. M., Derksen, G. C., & van Beek, T. A. (2019). Photo-stability of a flavonoid dye in presence of aluminium ions. *Dyes and Pigments*, *162*, 222-231.

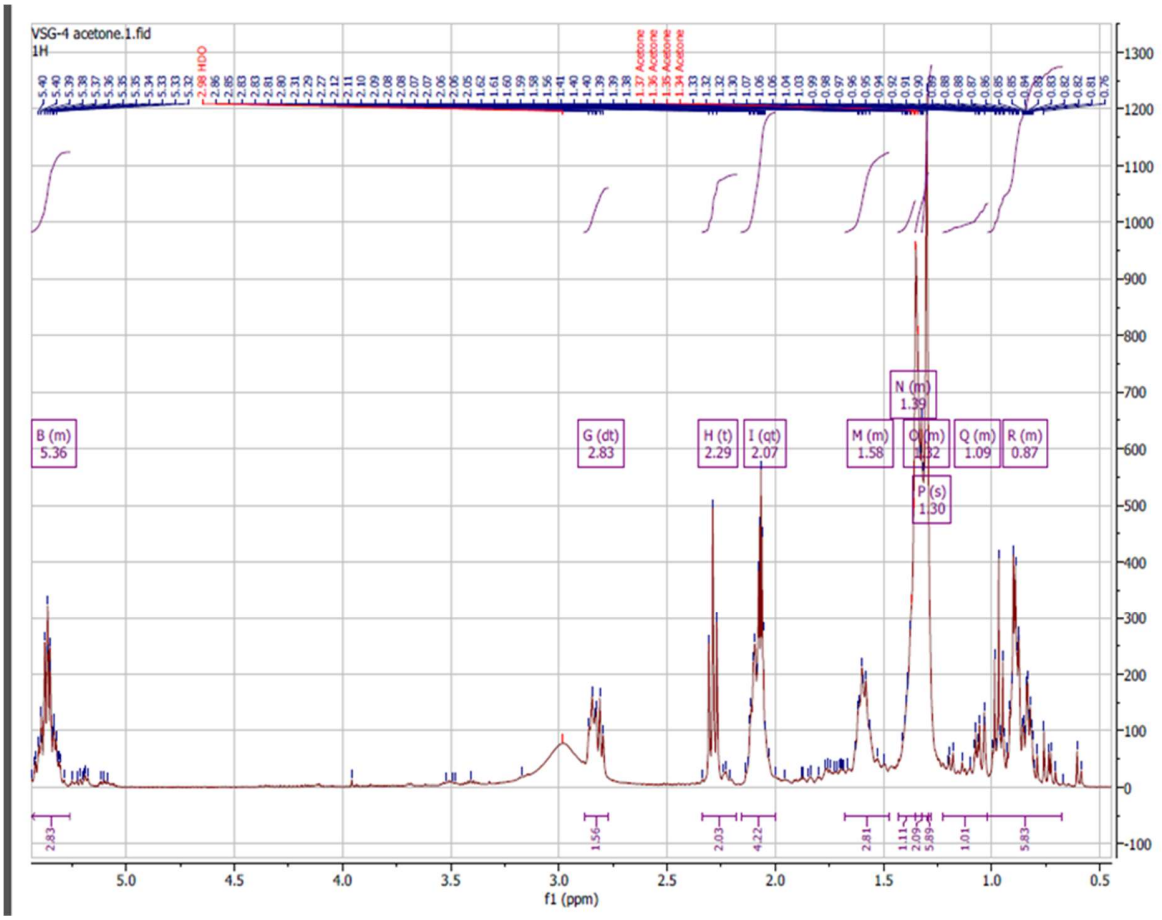
51. Lanzendörfer, G., Stäb, F., & Untiedt, S. (2002). *U.S. Patent No. 6,423,747*. Washington, DC: U.S. Patent and Trademark Office.
52. Chuarienthong, P., Lourith, N., & Leelapornpisid, P. (2010). Clinical efficacy comparison of anti-wrinkle cosmetics containing herbal flavonoids. *International journal of cosmetic science*, *32*(2), 99-106.
53. Zhao, L., Yuan, X., Wang, J., Feng, Y., Ji, F., Li, Z., & Bian, J. (2019). A review on flavones targeting serine/threonine protein kinases for potential anticancer drugs. *Bioorganic & medicinal chemistry*, *27*(5), 677-685.
54. Zhao, K., Yuan, Y., Lin, B., Miao, Z., Li, Z., Guo, Q., & Lu, N. (2018). LW-215, a newly synthesized flavonoid, exhibits potent anti-angiogenic activity in vitro and in vivo. *Gene*, *642*, 533-541.
55. Patel, K., Kumar, V., Rahman, M., Verma, A., & Patel, D. K. (2018). New insights into the medicinal importance, physiological functions and bioanalytical aspects of an important bioactive compound of foods 'Hyperin': Health benefits of the past, the present, the future. *Beni-Suef University Journal of Basic and Applied Sciences*, *7*(1), 31-42.
56. Feng, W., Hao, Z., & Li, M. (2017). Isolation and structure identification of flavonoids. *Flavonoids, from biosynthesis to human health/Ed. by Justino GC Intech Open*, 17-43.
57. Carbonell-Capella, J. M., Buniowska, M., Barba, F. J., Esteve, M. J., & Frígola, A. (2014). Analytical methods for determining bioavailability and bioaccessibility of bioactive compounds from fruits and vegetables: A review. *Comprehensive Reviews in Food Science and Food Safety*, *13*(2), 155-171.
58. Khater, M., Greco, F., & Osborn, H. M. (2020). Antiangiogenic activity of flavonoids: a systematic review and meta-analysis. *Molecules*, *25*(20), 4712.
59. Soto-Hernández, M., Tenango, M. P., & García-Mateos, R. (Eds.). (2017). *Phenolic compounds: natural sources, importance and applications*. BoD–Books on Demand.
60. "Glycoside - An overview," ScienceDirect. Available: https://www.sciencedirect.com/topics/chemical-engineering/glycoside
61. Y. A. K. *Agrophysiological traits for drought tolerance in pearl millet,* Department of Agronomy, Chaudhary Charan Singh, Haryana University, 2014.

62. Paduch, R., Kandefer-Szerszeń, M., Trytek, M., & Fiedurek, J. (2007). Terpenes: substances useful in human healthcare. *Archivum immunologiae et therapiae experimentalis*, *55*, 315-327.
63. International Union of Pure and Applied Chemistry (IUPAC), IUPAC compendium of chemical terminology, IUPAC.
64. Christianson, D. W. (2017). Structural and chemical biology of terpenoid cyclases. *Chemical reviews*, *117*(17), 11570-11648.
65. F. R. Nature's chemicals, Oxford University Press, 2010.
66. Padmanabhan, P., & Jangle, S. N. (2012). Evaluation of DPPH radical scavenging activity and reducing power of four selected medicinal plants and their combinations. *International Journal of Pharmaceutical Sciences and Drug Research*, 143-146.
67. Cheeseman, K. H., & Slater, T. F. (1993). An introduction to free radical biochemistry. *British medical bulletin*, *49*(3), 481-493.
68. Agarwal, A., Prabakaran, S., & Allamaneni, S. (2006). What an andrologist/urologist should know about free radicals and why. *Urology*, *67*(1), 2-8.
69. Pham-Huy, L. A., He, H., & Pham-Huy, C. (2008). Free radicals, antioxidants in disease and health. *International journal of biomedical science: IJBS*, *4*(2), 89.
70. Halliwell, B. (1995, November). How to characterize an antioxidant: an update. In *Biochemical Society Symposia* (Vol. 61, pp. 73-101). Portland Press Limited.
71. Shi, H., Noguchi, N., & Niki, E. (1999). Comparative study on dynamics of antioxidative action of α-tocopheryl hydroquinone, ubiquinol, and α-tocopherol against lipid peroxidation. *Free Radical Biology and Medicine*, *27*(3-4), 334-346.
72. Ani, O. N., Akpata, E. I., & Ujah, I. I (2017) Vitamin, Bioactive Compound Contents and Antioxidant Activity of Ficus capensis Fruit. *South Asian Research Journal of Natural Products,* pp. 1-8,
73. Characterizing fatty acids with advanced multinuclear NMR methods, *Magritek,* 2018. https://magritek.com/wp-content/uploads/2020/03/Application-Note-Characterizing-Fatty-Acids-with-multinuclear-NMR-Magritek-060418-back.pdf
74. Miyake, Y., Yokomizo, K., & Matsuzaki, N. (1998). Determination of unsaturated fatty acid composition by high-resolution nuclear magnetic resonance spectroscopy. *Journal of the American Oil Chemists' Society*, *75*(12), 1091-1094.

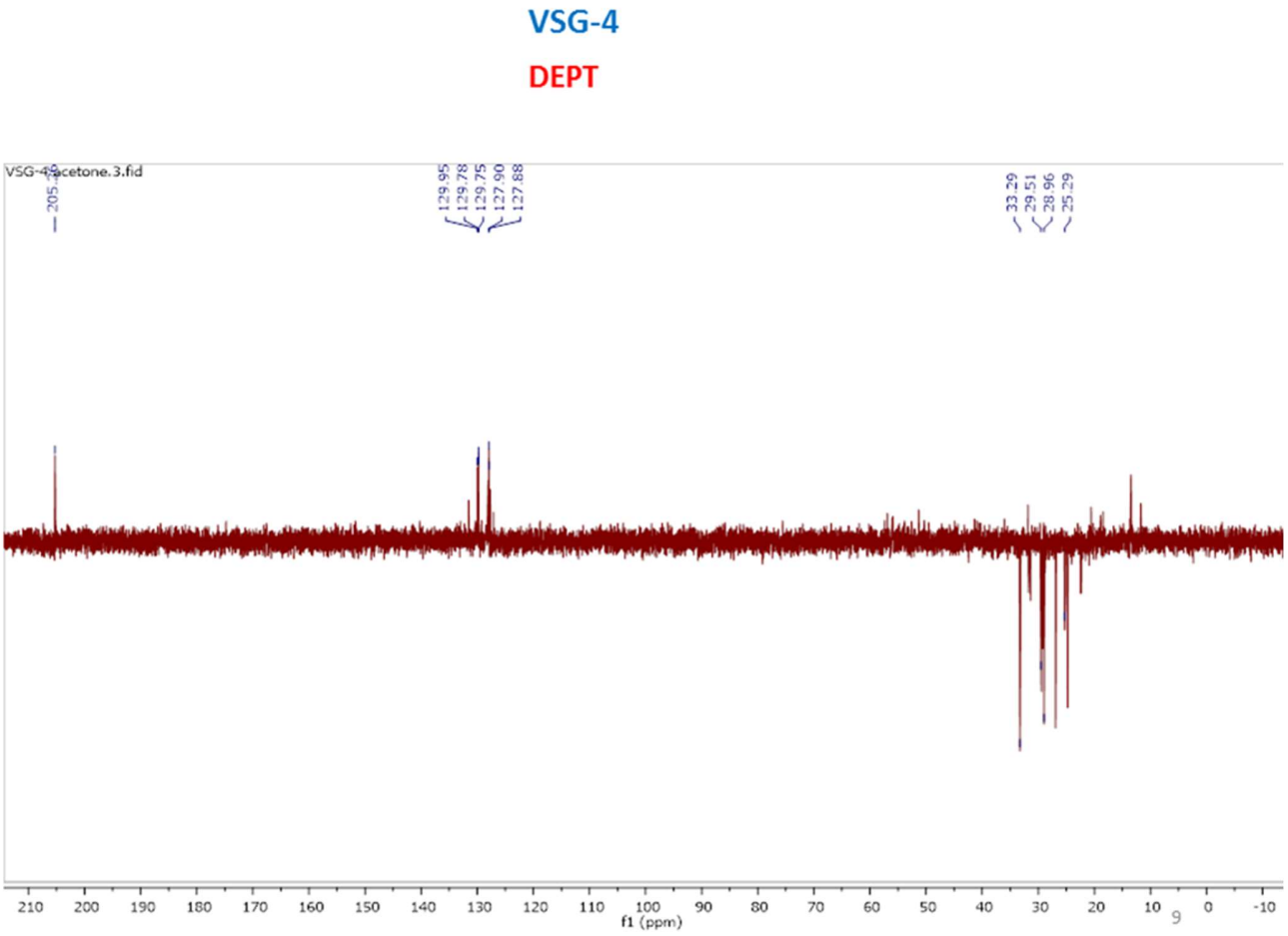
Appendix 1: 13C NMR Spectrum of VSG-4 in Acetone



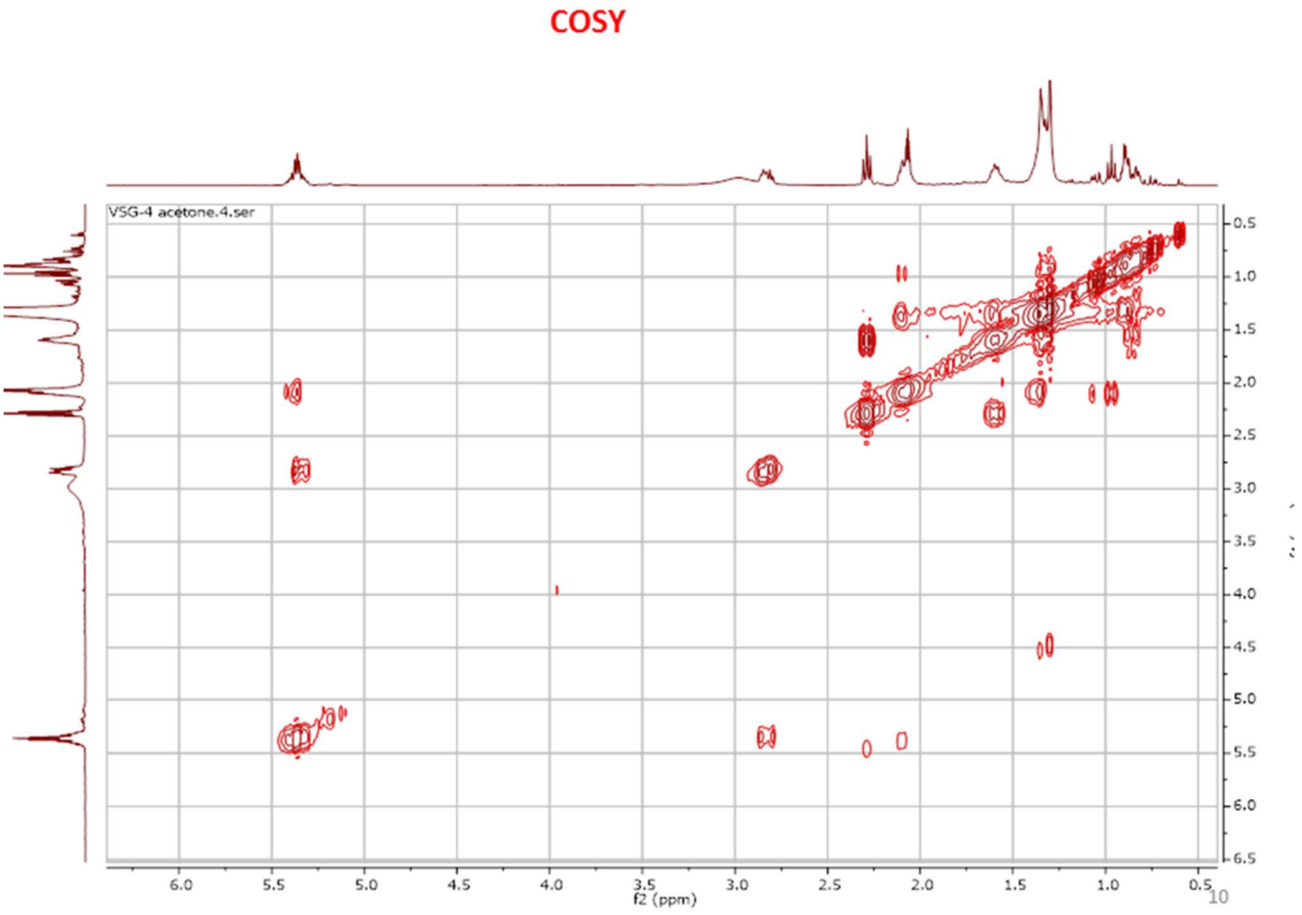
Appendix 2: 1H NMR Spectrum of VSG-4 in Acetone



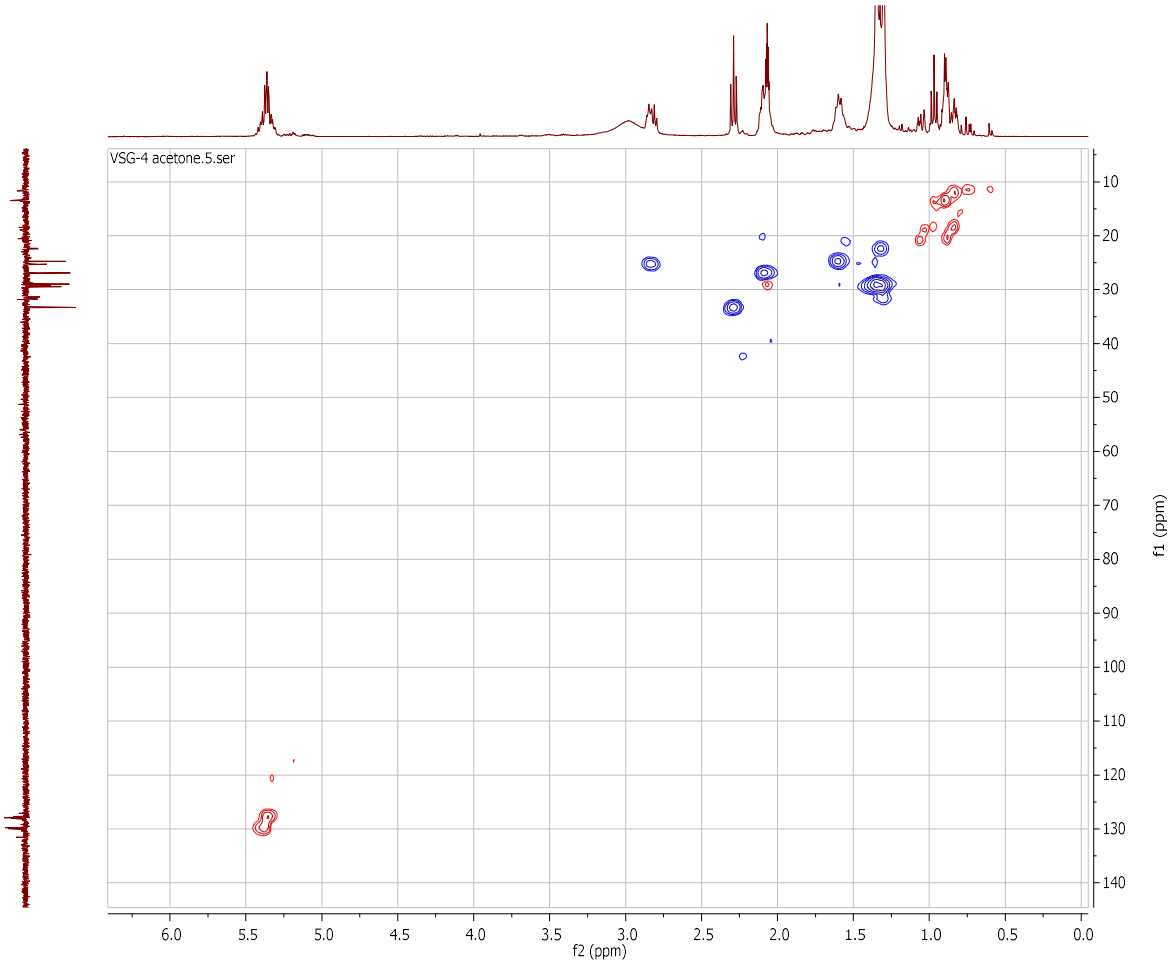
Appendix 3: DEPT Spectrum of VSG-4 in Acetone



Appendix 4: 1H-1H COSY Spectrum of VSG-4 in Acetone



Appendix 5: ^{1}HSQC Spectrum of VSG-4 in Acetone



Appendix 6: ^{1}HMBC Spectrum of VSG-4 in Acetone