ADAMA SCIENCE AND TECHNOLOGY UNIVERSITY SCHOOL OF APPLIED NATURAL SCIENCE

Research Final Report

Chemical, Nutritional and Some Biological Studies of the Tubers Extracts of *Plectranthus edulis*

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ABSTRACT

Plectranthus edulis is a tuberous crop found in some parts of Ethiopia. Though this plant is common, the nutritional and medicinal potential has not been systematically investigated. In view of this, the tubers were successively extracted with hexane, EtOAc and MeOH to furnish 0.3, 0.7 and 2.5% yield, respectively. The yellowish oil hexane extract was analyzed by GC-MS after conversion to fatty acid methyl esters using BF_3-MeOH. Results revealed the presence of palmitic, stearic, oleic and linoleic acid with the latter accounts for 42%. The hexane and EtOAc extracts after silica gel column chromatography afforded three compounds namely methyl linoleate, α-spinasterol and 3,5,7-trihydroxy-6,4'-dimethoxyflavone. The study also demonstrates that the EtOAc (76%) and MeOH (84%) extracts showed strong antioxidative activity in both ferric thiocyanate and DPPH assay. This is likely attributed to the presence of 3,5,7-trihydroxy-6,4'-dimethoxyflavone which scavenge the DPPH radical by 88%. The extracts were also examined for their antibacterial activity against S. aurous, E. coli, P. mirabilis and K. pneumonia using disc diffusion method. The EtOAc extract displayed modest activity (inhibition zone of 11 mm at 100 mg/mL) compared with ciprofloxacin used as standard drug. The proximate compositions were determined and had ash, crude fat, crude fiber, moisture, protein and carbohydrate contents ranged from 1.5 to 1.8%, 1.1 to 1.8%, 4.46 to 5.99%, 69.00 to 75.00%, 6.65 to 10.24% and 6.17 to 11.99%, respectively. The protein, crude fat, carbohydrate and fiber contents of the tuber of P. edulis were superior to those values reported for sweet potato. The mineral composition including Cd, Cr, Cu, Ni, Pb and Zn were analyzed using AAS while the Ca, K and Na contents were determined using flame photometer. The level of Cr, Cu, Ni, Pb, Zn, Ca, K and Na were found to be in the range of 0.008-0.030, 0.100-0.191, 0.330-1.065, 0.016-0.023, 0.135-0.357, 13.800-22.800, 325.00-502.50 and 12.80-13.50 mg/100g on the dry weight basis, respectively. Therefore, the tuber can contribute enormously to the supply of both macro and micronutrients in our diet. The ratio of Na/K in the tuber is less than 1 indicating the usefulness of this plant as nutraceutical against hypertension. Therefore, the nutritional profile and biological activities recorded in the present study demonstrated the usefulness of this plant as natural food and medicine.

***Key words**: P. edulis, proximate composition, α-spinasterol, 3,5,7-trihydroxy-6,4'-dimethoxyflavone, antioxidant, antibacterial*

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LIST OF ABBREVIATIONS

AAS	Atomic Absorption Spectrometer
DEPT	Distortionless Enhancement Polarization by Transfer
DMSO	Dimethylsulfoxide
DPPH	Diphenylpicrylhydrazyl
FAME	Fatty Acid Methyl Esters
GC-MS	Gas Chromatography Mass Spectromerty
IR	Infra Red
NMR	Nuclear Magnetic Resonance
UV-VIS	Ultra Violet Visible

1. INTRODUCTION

1.1. Background

Human beings have used plants as food and medicine since time immemorial. Plants serve as a reservoir of many complex organic compounds [1], many of which appear to have no direct function for the growth and development of the plant. These compounds, now known as secondary metabolites, are produced either as a result of the organism adapting to its surrounding environment or for its own survival and defense against predators. These secondary metabolites played a central role in the history of mankind and possess well defined biological functions. Recently, an attention was given to isolate these secondary metabolites from medicinal plants which are used for their healing properties in addition to their use for the production of plant derived modern drugs [2]. This is evident from the report that, 50% of all drugs in clinical use today are from natural products [3]. Few of these are aspirin from willow bark, digoxin from foxglove, quinine from cinchona bark, artemisinin from *Artemisia annua* and morphine from opium poppy, etc. Though medicinal plants are a rich source of drugs, only a relatively small percentage has been systematically investigated for the presence of bioactive compounds.

Ethiopia is home for many plants which have been used as medicine to treat a number of human and animal ailments. Some of these medicinal plants are endemic and others are indigenous with enormous economic, nutritional, cultural and medicinal benefits [4-7]. Crop plants such as coffee (*Coffea arabica*), safflower (*Carthamus tinctorius*), tef (*Eragrostis tef*), noug (*Guizotia abyssinica*), enset (*Ensete ventricosum*), are known to have originated in Ethiopia.

Despite the fact that Ethiopia's climatic condition is favorable for the growth of nutritionally significant plants, there is still shortage of food in some parts of the country. This may be partly because of the fact that the majority of Ethiopian population depends mainly on cereal crops as a food source [8]. An integration of cereal crops with root and tuberous crops into the food system of the people may partly contributes to the house hold food security in Ethiopia. In this regard, a vast majority of peoples particularly those living in the western and southern part of Ethiopia depend on tuberous plants as a source food.

Plectranthus edulis (Lamiaceae) [9] is among nutritionally significant plant widely used by peoples living in the western and southern parts of Ethiopia. *P. edulis*, locally called Dinicha Oromo in

Oromia/ Ye Walayta Dinich" in southern Ethiopia, is one of the economically important tuber crop of the genus *Plectranthus* [8]. It is known in Ethiopia due to its horticultural uses since it is fast-growing, produce lovely flowers and are resistant to most pests and plant diseases. *P. edulis* is an indigenous plant which has been cultivated for its edible tuber in highly localized areas of south and west Ethiopia. To some extent, also, the leaves are used as a cooked vegetable in some western Ethiopian regions. It is particularly important in local diets mainly between September and November since other food crops will not be ready for consumption. Beside its nutritional value, this plant is also used as a source of income in regions of Ethiopia where it is cultivated. Furthermore, the tubers are traditionally claimed to have good for people with asthma. In some areas the tuber is also used as an appetizer and against bacteria. Though the plant has significant application, the attention given to the plant in Ethiopia is very low.

Despite the significant contributions of tuberous crops towards food security and income generation, the nutrtional and medicinal potential of this crop has not yet fully been exploited and utilized. Furthermore there is limited scientific information dealing with the nutritional, biological and phytochemical content of the tuber of *P. edulis*. Hence the main purpose of this project is to study the chemical, nutritional and biological studies of the tubers of *P. edulis*.

1.2. Statement of the Problem

Ethiopia is one of the world centers of origin and diversity of medicinal and crop plants. Although attempts have been made by the government to satisfy basic needs of the population, the country is still among those with the problem of house hold food security. This may be in part because of the fact that many peoples in most parts of Ethiopia relied mainly on cereal crops [8]. Integration of cereals with tuberous crops for food can play a pivotal role to solve this problem. However, consumption of underutilized edible tuberous plants has often been looked as a sign of poverty which is largely a reflection of lack of knowledge on their nutritional and medicinal benefits. *P. edulis* is among underused indigenous tuberous crops in Ethiopia owing to poor consumer awareness of its nutritional and medicinal value. Therefore it is necessary to evaluate the nutritional profile of the tuber of this plant species. Furthermore there has been a growing interest in evaluating the antioxidant activities of plants that are used as food as they have significant impact on the status of human health and disease prevention. To the best of our knowledge no effort has been made to assess the antioxidant activity and chemical constituents of tuber of *P. edulis*. The tuber of *P. edulis* is

traditionally claimed to have antibacterial activity in addition to its uses as appetizer. This was, however, not supported by scientific investigation.

1.3. Objectives

1.3.1. General Objective

To study the chemical, nutritional and some biological profiles of the tuber extracts of *P. edulis*

1.3.2. Specific Objectives

The specific objectives of this work is to

- successively extract the tuber of *P. edulis* using hexane, EtOAc and MeOH
- determine fatty acids profile of the hexane extract of the tuber of *P. edulis*
- isolate compounds from the extracts of the tuber of *P. edulis*
- elucidate the structures of isolated compounds using spectroscopic methods including UV-Vis, IR and NMR
- evaluate the antioxidant activities of the extracts and isolated compounds
- assess the antibacterial activities of the extracts and isolated compounds
- determine proximate composition including crude protein, oil content, crude carbohydrate, crude fibre, moisture and ash content
- determine the metal composition of the tuber of *P. edulis*

1.4. Significance of the Study

The study made on *P. edulis* is believed to give an insight into the chemical constituents of the tuber of this species. Moreover, the already huge work done in different parts of the world on closer taxonomic relatives of this species increased the likely hood of the investigation to come up with some worthy findings. So, investigations of the antibacterial and antioxidant activities on such plant could serve as sources of lead compounds for further plant derived drug development. The results may increases awareness of peoples regarding the potential of this tuber both as food and medicine. This may enhances the popularity of the plant and hence contributes in solving the economic status of the peoples where the plant is grown.

2. LITERATURE REVIEW

2.1. The Family Lamiaceae

Lamiaceae is an important family of flowering plants known for the wealth of species with various medicinal properties [10]. It comprises of about 250 genera and 7,200 species [11]. Some of the genera in this family include sage (*Salvia*), basil (*Ocimum*) and mint (*Mentha*), all of which were found to have a rich diversity of ethnobotanical uses. Another important genus in this family is *Plectranthus*.

2.2. The genus *Plectranthus*

Plectranthus is a large genus containing about 300 species distributed in Africa, Asia and Australia [12]. Of these, 32 species are registered in the flora of Ethiopia [12]. Botanically, the species in the genus *Plectranthus* are erect or decumbent herbs growing up to 90 cm. tall, with edible tubers.

2.2.1. Pharmacological Uses

Medicinally various species of the genus *Plectranthus* are used for treating digestive disorders of the digestive system. *Plectranthus barbatus*, *Plectranthus amboinicus*, *Plectranthus laxiflorus*, *Plectranthus esculentus*, *Plectranthus vettiveroides* are used to treat stomach pain, nausea, vomiting, diarrhoea, mouth infections and as antihelmintics [13]. The first two species, *Plectranthus barbatus* and *Plectranthus amboinicus*, are the most frequently used plants for the treatment of burns, wounds, insect bites and allergies [13]. In Kenya and the Democratic Republic of Congo, *Plectranthus barbatus* is used in the treatment of ringworms [14]. *Plectranthus barbatus* is also used against a vast array of complaints including throat, tonsillitis, fevers, malaria and gastro-intestinal infections [15]. Previous pharmacological studies showed that *P. amboinicus* possesses antiepileptic, antioxidant and antimicrobial properties [16]. Moreover, the antibacterial, antiviral and antifungal activities of *Plectranthus barbatus* is also reported [17]

2.2.2. Horticultural Uses

Some species in the genus *Plectranthus* are known all over the world due to their horticultural uses since they are fast-growing, produce lovely flowers and are resistant to most pests and plant diseases [18]. They are planted either for their colored and attractive foliage or for their beautiful flowers hence

grown for their ornamental purposes. Many species of *Plectranthus* are resistant to diseases, and can also survive in dry conditions. Some species of *Plectranthus* planted as ornamentals in Africa, Asia, America and Australia include *Plectranthus oertendahlii*, *Plectranthus parvifolius*, *Plectranthus argentatus*, *Plectranthus amboinicus*, *Plectranthus fruticosus*, *Plectranthus madagascariensis*, *Plectranthus ciliatus*, *Plectranthus barbatus*, *Plectranthus ecklonii*, *Plectranthus zuluensis* and *Plectranthus saccatus* [19, 20].

2.2.3. As Food and Food Additives

Plectranthus amboinicus, *Plectranthus esculentus* and *Plectranthus crassus* are used as food additives. The leaves of *Plectranthus amboinicus* are used in food stuffing for flavoring and preserving beef and chicken [21]. The tubers of *Plectranthus esculentus* are not only used to mask odor of strong smells associated with goat and fish but also used to spice dishes containing tomato sauces. The leaves of *Plectranthus mollis* and *Plectranthus barbatus* and the tuber of *Plectranthus crassus* are eaten after cooking like vegetables [22, 23]. In India, the fruits of *Plectranthus parvifolius* are also eaten [24]. In Hungary, the leaves of *Plectranthus crassus* are used for culinary purposes. The tubers of *Plectranthus punctatus* and *Plectranthus edulis* are eaten in Kenya and Ethiopia, respectively. Tubers of *Plectranthus rotundifolius* are a popular food in South Africa, whereas in Tropical Asia, the tubers of *Plectranthus parviflorus* are well known [24].

2.2.4. Chemistry of the genus *Plectranthus*

Phytochemical investigation done by different scholars showed that many species in the genus *Plectranthus* contain compounds belonging to diverse groups of secondary metabolites such as terpenoids (di and triterpenoids), steroids and flavonoids.

Diterpenoids

The majority of the chemical constituents so far reported from this genus are diterpenoids, indicating the wide distribution of this class of compounds in various species of *Plectranthus*. Three diterpenes were identified from the extracts of *Plectranthus ernstii* [25] including the pimaranes 15,16-epoxy-7α-hydroxypimar-8,14-ene (**1**) and 15,16-epoxy-7-oxopimar-8,14-ene (**2**), and the labdane 1,11-dihydroxy-8,13-epoxylabd-14-ene (**3**) (Figure 1). Other diterpenes namely plectrornatin A (**4**) [37, 38], plectrornatins B (**5**) and C (**6**) [26, 27] were reported from *P. ornatus* (Figure 1).

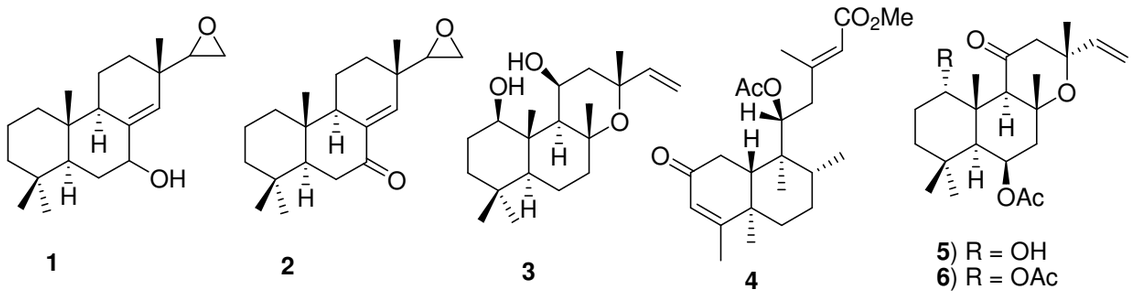


Figure 1: Diterpenes reported from *Plectranthus ernstii* and *P. ornatus*

Two known abietanes, parvifloron D (**7**) and parvifloron F (**8**), were isolated from the ethyl acetate extract of the *P. ecklonii* [28] (Figure 2). Sugiol (**9**) was also reported from the acetone extract of the same species [29].

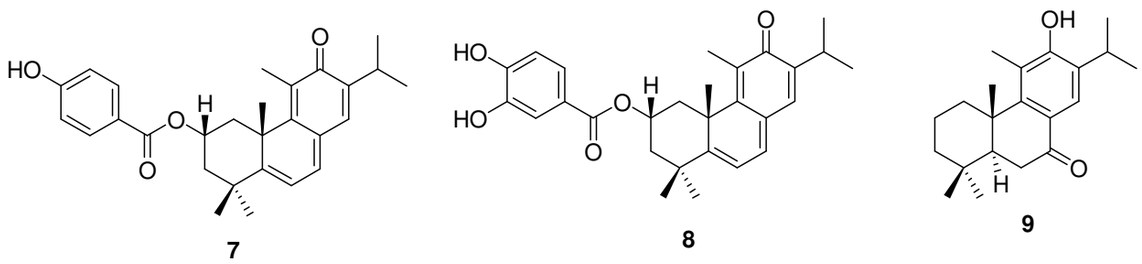


Figure 2: Diterpenes reported from *P. ecklonii*

Royleanones are abietane-12-hydroxy-11,14-dione diterpenes which have frequently been identified from *Plectranthus* plant extracts. Horminone (**10**) (Figure 3) is an abietane isolated as antibacterial agent from *Plectranthus* spp. [30]. Its 16-*O*-acetoxy derivative (**11**) and 7α-Acetoxy-6β-hydroxyroyleanone (**12**) (Figure 3) were isolated from *P. hereroensis* and *P. grandidentatus,* respectively [31-33]. Other royleanones including 6*β*-hydroxy-7α-formyloxyroyleanone (**13**), 6*β*-hydroxy-7*α*-acetoxyroyleanone (**14**), 6*β*-formyloxy-7*α*-hydroxyroyleanone (**15**), *6β*,7*α*-dihydroxyroyleanone (**16**) and coleon-U-quinone (**17**) from *P. argentatus* [34] and Plectranthone J (**18**) from *P. barbatus* were also reported [35].

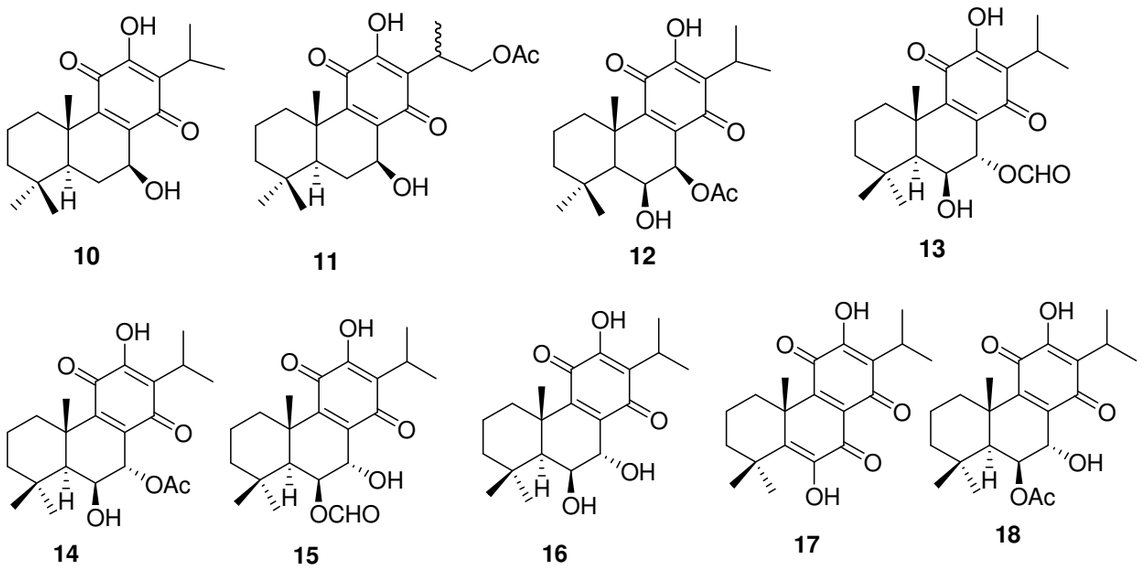


Figure 3: Some royleanones reported from the genus *Plectranthus*

Spirocoleons are another classes of diterpenoids isolated from the genus *Plectranthus*. In view of this, spirocoleons **19-26** (Figure 4) were reported from the leaves of *P. edulis* [36]

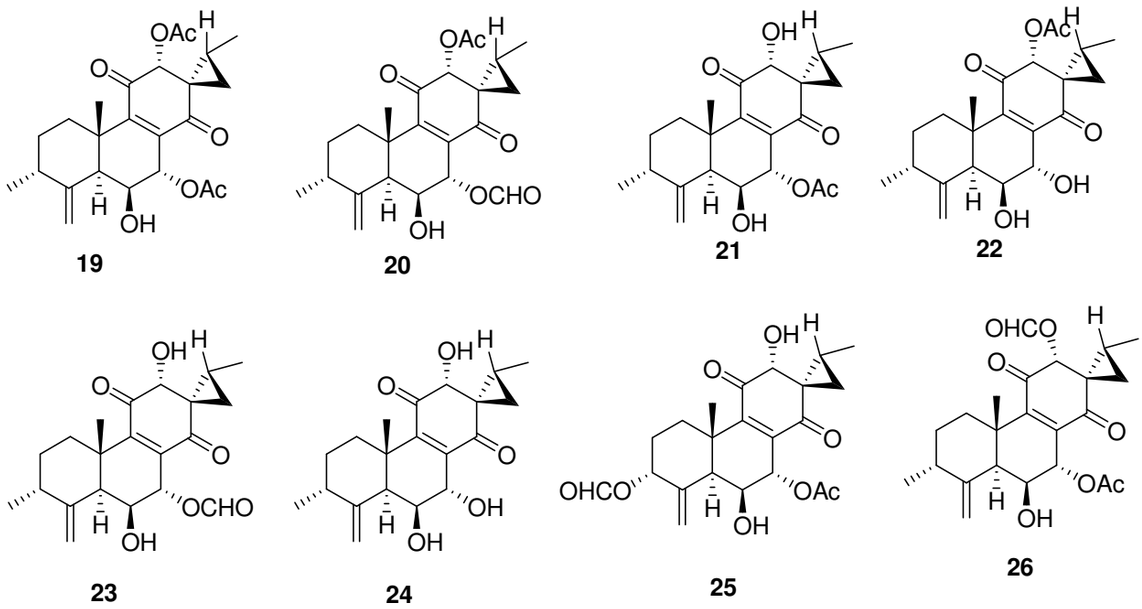


Figure 4: Spirocoleons reported from the leaves of *P. edulis*

(15S)-Lanugone O (**27**), 14-hydroxytaxodione (**28**), lanugone P (**29**), coleon F (**30**) and (16S)-coleon E (**31**) were some vinylogous quinones previously isolated (Figure 5) from various species of the genus *Plectranthus* [37].

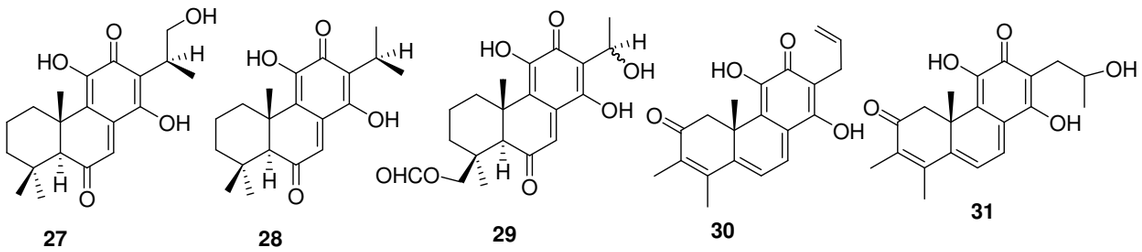


Figure 5: Some vinylogous quinones reported from the genus *Plectranthus*

Ent-kaurenes including plecostonol (**32**), coestinol (**33**), coetsidin B (**34**), coetsidin C (**35**), coetsidin D (**36**), coetsidin E (**37**), coetsidin F (**38**) and coetsidin G (**39**) (Figure 6) were reported from the genus *Plectranthus* [37]

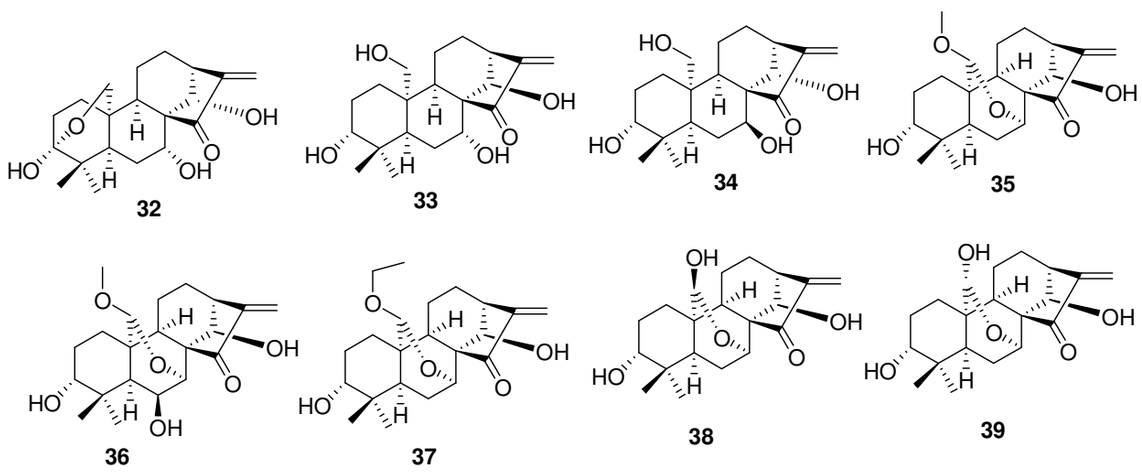


Figure 6: Ent-kaurenes reported from the genus *Plectranthus*

Triterpenoids

Triterpenoids namely plectranthoic acid (**40**), acetylplectranthoic acid (**41**), plectranthadiol (**42**), plectranthoic acid A (**43**), plectranthoic acid B (**44**), oleanolic acid (**45**), ursolic acid (**46**) and betulin (**47**) were isolated from *P. rugosus* [38, 39] (Figure 7). Furthermore *β*-sitosterol (**48**) was also reported from *P. rugosus*.

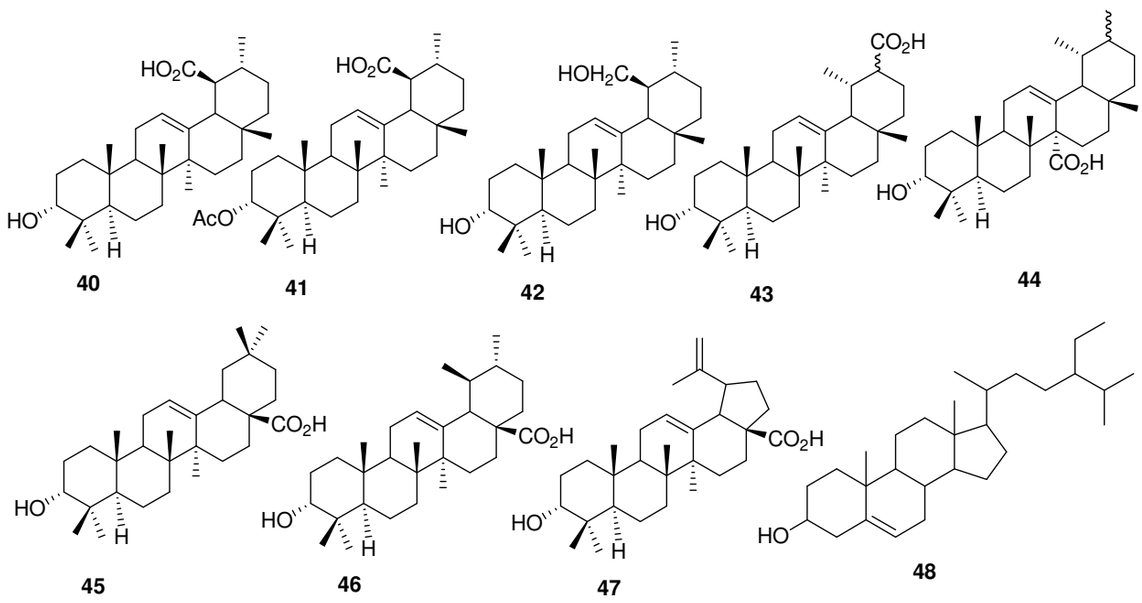


Figure 7: Triterpenoids and *β*-sitosterol (**48**) from *P. rugosus*

Flavonoids

Flavonoids seem to be rare in *Plectranthus*. Four flavonoids were identified 4',7-dimethoxy- 5,6-dihydroxyflavone (**49**) from *P. ambiguus* and chrysosplenetin (**50**) from *P. marruboides* [38]. Other flavonoids including 5,4'-dihydroxy-6,7-dimethoxyflavanone (**51**) and 5,4'-dihydroxy-6,7-dimethoxyflavone (**52**) were also reported from *Plectranthus* (Figure 8) [37]

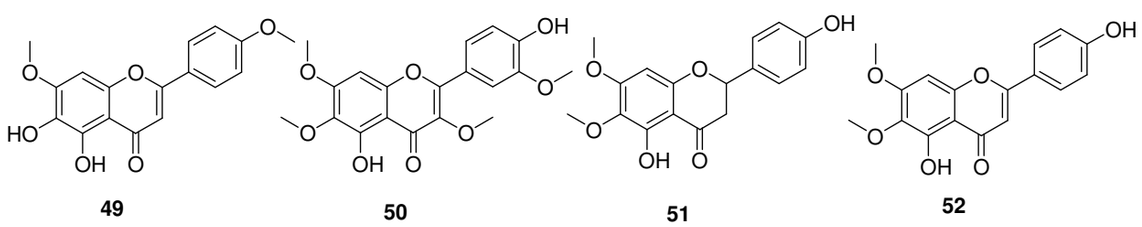


Figure 8: Flavonoids so far reported from the genus *Plectranthus*

2.3. *Plectranthus edulis*

2.3.1. Botanical Description

P. edulis (Vatke) Agnew is large, erect, aromatic herb growing to about 1 m high with hirsute, decumbent and glandular stems, and leaves of lanceolate to elliptic and root of swollen nodes which produce edible underground potato-like tubers. It occurs mainly in marshy areas and growing in different mid and high altitude areas [40].

A B

Figure 9: *P. edulis* aerial part (A) and tuber (B) (Picture taken by Tolessa Duguma).

2.3.2. Ethnobotanical Uses

Plectranthus edulis is among the four economically important tuberous crops of the genus *Plectranthus* [41], together with *P. esculentus* (Livingstone potato), *P. parviflorus* (Sudan potato) and *P. rotundifolius* (Madagascar potato) [42]. It is a popular hedge plant in East Africa and Democratic Republic of Congo. It is an indigenous plant to Ethiopia known due to its horticultural uses since it is fast-growing, produce lovely flowers and are resistant to most pests and plant diseases. *P. edulis* is cultivated in Ethiopia mainly for its edible tuber. In the western part of Ethiopia, the leaves are also eaten after cooking like vegetables. It is particularly important in local diets mainly between September and November since other food crops are not ready for consumption. This plant is also used as a source of income in regions of Ethiopia where it is cultivated. Furthermore, the tubers are

traditionally claimed to have good for people with asthma. They are also claimed to be used against bacteria and as an appetizer.

2.3.3. Reported Chemical Constituents from *Plectranthus edulis*

Efforts have been made by many scholars to isolate and identify the chemical constituents from the leaves of *P. edulis* which has led to the isolation of spirocoleons **19-26** [36]

3. METHODOLOGY

3.1. General

Melting point was determined in capillary tube with a digital electrothermal melting point apparatus. Analytical TLC was run on a 0.25 mm thick layer of silica gel GF254 (Merck) on aluminum plate. Spots were detected by observation under UV light (254 nm). Visualizing agents used were vanillin/H_2SO_4 and iodine. Column chromatography was performed using silica gel (230-400 mesh) Merck. Samples were applied on column after adsorbing the samples on silica gel. Solvents were removed using rotary evaporator (model R1001-VN, Zhengzhou Great Wall s&l&T Co.LTD). The UV-Vis spectral measurements were done using UV-Vis on T 60 U spectrophotometer (PG instruments, UK) equipped with deuterium and tungsten lamps. NMR spectra were recorded using Bruker Avance 400 spectrometer operating at 400 MHz. The IR spectra of compounds were recorded using a Perkin-Elmer BX Spectrometer (400-4000 cm^{-1}) as KBr pellets.

Gas Chromatography-Mass Spectrometer: GC-MS analysis were performed using Agilent Technologies 7820A gas chromatograph system equipped with HP-5 capillary column (30mx0.25; coating thickness, 0.25µm) and Agilent technologies 5977E mass spectroscopy ion trap detector. Analytical conditions were as follows: Injector and transfer line temperature are 220 and 260^oC, respectively; oven temperature programmed from 60^oC to 240^oC at 3^oC/min; carrier gas, helium at 1mL/min; injection 5µL; split ratio, 1:30. Identification of the constituents was based on search through mass\hunter\library\NIST11.Land mass\hunter\library\W9N11.

3.2. Plant Materials Collections and Identifications

The tubers of *P. edulis* were collected from Bako (Oromia), Guliso (Oromia) and Wolyata Sodo (southern Ethiopia). The plant material was identified by Mr. Melaku Wondafrash of the National Herbarium of Addis Ababa University (Ethiopia), where voucher specimen YD005 is deposited. The

collected plant material (Figure 9) was immediately brought to Chemistry Laboratory of Adama Science and Technology University and was allowed to dry at room temperature under shade. The dried tubers were then pulverized using an electric grinder (IKA WERK GmbH & co.KG, Type M20, Germany) and was passed in sieve (Standard sieved ISO 9001:200 quality, minimum aperture 0.7mm, Shanghai) for further analysis.

3.3 Extraction

The powdered tubers of *P. edulis* (600 g) were soaked in hexane (3 L) for 72 hrs. It was filtered and concentrated under reduced pressure using rotary evaporator at 40^{o}C to yield 2 g (0.3%). The marc was soaked in EtOAc (3 L) for 72 hrs, filtered and concentrated to furnish 4 g (0.7%). The marc after extraction with EtOAc was extracted with MeOH (3 L), filtered and concentrated to give 15 g (2.5%). Each extracts were analyzed with TLC.

3.4. Qualitative Analysis of the Chemical Constituents of Crude Extracts

The qualitative preliminary phytochemical screening of the ethyl acetate and methanol extracts of the tubers of *P. edulis* were carried out according to standard protocols previously reported in the literature [43-47].

Alkaloids

Wagner's Reagent: Potassium iodide (2 g) and iodine (1.27 g) were dissolved in distilled water (7 mL) and the solution was diluted to 100 mL with distilled water. Few drops of this solution were added to the extracts (1 mg/mL). The formation of a brown/reddish colored precipitate indicates the presence of alkaloids [43].

Terpenoids

Salkowski test: The crude extracts (1 mg) were shaken with chloroform (2 mL) in the test tube followed by the addition of concentrated sulfuric acid (3 mL) along the side of the test tube using dropper, immediate formation of a reddish brown coloration at the interface indicates the presence of terpenoids [43].

Saponins

Froth test: Each extracts (1 mg) were shaken vigorously to dissolve in 5 mL distilled water. Formation of stable persistent froth shows the presence of saponins [43].

Tannins

Potassium hydroxide (KOH) test: To 5 mg of each extracts in test tube, 2 mL of 10% potassium hydroxide (KOH) were added and shaken to dissolve. Formation of dirty precipitate indicates the presence of tannin [43].

Cardiac glycosides

Kellar-Kiliani test: Each extract (2 mg/mL) was treated with 1 mL of glacial acetic acid in a test tube and a drop of ferric chloride solution was added to it. This was carefully underlayed with 1 mL concentrated sulphuric acid. A brown ring at the interface indicated the presence of deoxysugar characteristic of cardenolides. A violet ring may appear below the ring while in the acetic acid layer, a greenish ring may form [44].

Antraquinone glycosides

Borntrager's test: To the extract solution (1 mg/mL) in the first test tube, 5% H_2SO_4 (1 mL) was added. The mixture was boiled in a water bath and then filtered in the second test tube. Filtrate was then shaken with equal volume of chloroform and kept to stand for 5 min. Then lower layer of chloroform was shaken with half of its volume with dilute ammonia. The formation of rose pink to red color of the ammoniacal layer indicates the presence of anthraquinone glycosides [45].

Phenols

Ferric chloride test: Each extracts (1 mg) was dissolved in 3 mL of distilled water and added few drops of 10% $FeCl_3$. Appearance of blue or green colour indicates presence of phenols [46].

Flavonoids

Shinoda test: Four pieces of magnesium fillings (ribbon) are added to the extract (1 mg/mL) in the beaker followed by few drops of concentrated hydrochloric acid. A pink or red color indicates the presence of flavonoids. Colors varying from orange to red indicate flavones, red to crimson indicates flavonoids and crimson to magenta indicates flavonones [47].

Steroids

Salkowski test: Each extracts (5 mg) were separately dissolved in 5 mL of chloroform. Sulfuric acid is then carefully added by dropper to form a lower layer. A reddish-brown color after standing at the interface indicates the presence of a steroidal ring [45].

3.5. Isolation of Compounds

3.5.1. Column Chromatographic Fractionation of the Hexane Extract

The hexane extract (1 g) was adsorbed and fractionated over silica gel (60 g) column chromatography. The column was eluted with hexane:EtOAc of increasing polarities to furnish 15 fractions. A volume of 50 mL each was collected. The first four fractions were collected with hexane. Fraction 2 and 3 were identified as compound **53** (400 mg). Fractions 5-8 were eluted with hexane:EtOAc (9:1) to give 100 mg white solid identified using NMR as a mixture of fatty acids. Hexane:EtOAc (4:1) was used as eluent for collecting fraction 9-11. The next four fractions were collected with hexane:EtOAc (1:1). All fractions were analyzed with TLC.

3.5.2 Column Chromatographic Fractionation of the Ethyl acetate Extract

The EtOAc extract (4 g) was adsorbed and fractionated over silica gel (150 g) column chromatography. The column was eluted with hexane:EtOAc:MeOH of increasing polarities to afford twenty fractions. A volume of 100 mL each was collected. The first three fractions were collected with 100% hexane. Fraction 4 and 5 were collected with hexane:EtOAc (9:1). Fraction 6 and 7 were collected with hexane:EtOAc 4:1 and 3:2, respectively. Hexane:EtOAc (1:1) was used as eluent for collecting fraction 8 and 9. The next two fractions were collected with hexane:EtOAc 2:3 and 1:4, respectively. EtOAc was employed to collect fractions 12 and 13. Fraction 14, 15, 16, 17, 18, 19 and 20 were collected with EtOAc:MeOH 9:1, 4:1, 3:2, 1:1, 2:3, 1:4, 1:9, respectively. Fraction 5 was applied to preparative TLC with hexane:ethyl acetate (9:1) as eluent. The band at Rf value of 0.67 was scratched with a spatula, transferred to the flask, extracted with chloroform, filtered and concentrated to furnish compound **54** (13 mg). Likewise fraction 10 of the EtOAc extract was also purified using PTLC with hexane:EtOAc (2:3) to give compound **55** (8 mg)

3.5.3. Column Chromatographic Fractionation of the Methanol Extract

The methanol extract (10 g) was adsorbed and subjected to silica gel (180 g) column chromatography. It was eluted with EtOAc:MeOH of increasing polarities. Totally 40 fractions (each 50 mL) were collected. Based on the color of fraction and TLC profiles, fractions 1-5, 6-10, 11-12, 13-14, 15-16, 21-22, 23-27, 33-35, 36-37, and 38-40 were combined. Fractions 1-5 and 6-10 were mixed and applied on PTLC using solvent ethyl acetate:methanol (7:3). The band observed at Rf value 0.65 was scratched with a spatula, transferred to the flask and extracted with methanol, filtered and concentrated to give compound **5**. It gives no signal in an NMR spectrum.

3.6. Preparation of Fatty Acid Methyl Esters (FAME)

P. edulis oil (2 g) was placed in 25 mL round bottom flask which contained hexane (6 mL) to which 4 mL BF_3-MeOH solution was added. The reaction mixture was refluxed in water bath for 30 min. Then it was cooled to room temperature. To the cooled mixture, 5 mL of water was added with vigorous shaking and two layers were formed. The upper layer was separated by using separatory funnel, dried over anhydrous Na_2SO_4, filtered and concentrated to afford 500 mg (25%). The methylated fatty acids was dissolved in hexane and analyzed using GC-MS

3.7. Antioxidant Activity

3.7.1. Diphenylpicrylhydrazyl radical (DPPH) Assay

The radical scavenging activity of the methanol extract of the tuber of *P. edulis* was done using DPPH radical scavenging assay [48]. Serial dilutions were carried out with the stock solutions (1 mg mL^{-1}) of the extract to obtain concentrations of 500, 250, 125, and 65 µg mL^{-1}. The DPPH and sample solutions were prepared using methanol as solvent. Diluted solutions (1 mL each) of the samples were mixed with 4 mL of 2,2-diphenyl-1-picryl hydrazyl (0.04% DPPH in MeOH) in a brown vials. After an incubation period of 30 min at 37^{o}C in an oven, the absorbance was determined against a blank at 517 nm. This was repeated for the EtOAc extract and isolated compounds. The percent of DPPH discoloration of the sample was calculated according to the formula:

$$(\%)\ \text{inhibition} = \frac{(\text{A control} - \text{A sample})}{\text{A control}} \times 100$$

Where A control was the absorbance of the DPPH solution and A sample was the absorbance in the presence of plant extract. The IC_{50} value, defined as the concentration of a substrate that causes 50% loss of the DPPH activity, was calculated by linear regression plots of the percentage inhibition against the concentration of the tested samples. Samples were analyzed in triplicate. Ascorbic acid was used as positive control.

3.7.2. Ferric thiocyanate Method

The anti-lipid peroxidation potential of the MeOH extract of the tuber of *P. edulis* was evaluated using thiocyanate method [49]. Each 0.1 mg EtOH extract of tuber of *P. edulis*, 100 µL of linoleic acid, EtOH (5 mL) and phosphate buffer (5 mL, 0.05 M, pH = 7) in water were separately added in to

a vial and incubated at 40^{o}C in an oven. After 24 h, 0.1 mL from each were taken and added in to a vial containing 75% aqueous EtOH (7 mL), 30% of NH_4SCN (0.15 mL) and 0.15 mL of 0.02M $FeCl_2$ in 3.5% HCl. Each was then subjected to UV-Vis spectrophotometery to record the absorbance at 500 nm. Absorbance of the blank and ascorbic acid were done in the same fashion. The percentage inhibition using ferric thiocyante method was calculated employing the following formula.

$$\text{Percentage inhibition} = 100 - \left(\frac{As}{Ab} \times 100\right)\%,$$

where As is absorbance of the sample and Ab is absorbance of the blank [50]

Likewise, the EtOAc extract was also evaluated following the procedure described in section 3.7.2.

3.8. Antibacterial Activity of the Crude Extracts

The effects of the crude extracts of the tubers of *P. edulis* were evaluated on four selected pathogenic bacteria such as *Staphylococcus aurous, Escherichia coli, Protens mirabilis* and *Klebsiella pneumonia* using disc diffusion method following previously developed procedure with slight modification [51]. This was done in Oromia Public Health Research, Capacity Building and Quality Assurance Laboratory Center, Adama, Ethiopia.

Media Preparation

Mueller Hinton agar was prepared by dissolving the solid media in distilled water. The solution was then sterilized in autoclave at 121^{o}C for 15 minutes, cooled then poured in Petri dishes. The solution was then left to solidify.

Inoculation and Incubation

Antibacterial activity was done on Mueller Hinton Agar. 1 mL of bacteria suspension was uniformly spread on the sterile Mueller Hinton Agar Petri dish. The ethyl acetate extract (700 mg) of the tuber of *P. edulis* was dissolved in DMSO (7 mL) to give 100 mg/mL. This was serially diluted with DMSO to furnish 100, 10, 1 and 0.1 mg/mL and each were evaluated for their antibacterial activities. The methanol extract and isolated compounds were prepared and evaluated following similar procedure. 6 mm-diameter wells were cut from the agar using a sterile cork-borer and all the sample concentrations (each 100, 10, 1, and 0.1 mg/mL of the EtOAc, MeOH extract and isolated compounds) were placed in the wells. The Petri dish was then placed in an incubator for 24 hours at 37^{o}C. At the end of incubation period, the inhibition diameter was measured and expressed in millimeters. DMSO was

used as negative control and ciprofloxacin was used as positive control. Antibacterial activity was determined by measuring the inhibition zone diameter (mm) against each test organism.

3.9. Analysis of Proximate Composition

Oven drying method was employed for the determination of moisture contents [52]. In this regards the sample was dried at 100-105^{o}C for 6-12 h until a constant weight was obtained. Total ash (muffle furnace at 550^{o}C for 8 h) [52], crude protein (Kjeldahl method, %N x 6.25), crude fat (Soxhlet extraction system) and fiber were determined in accordance with standard procedure [52,53]. The total percentage carbohydrate content in the sample was determined by adding the total values of crude protein, lipid, crude fiber, moisture and ash contents of the sample and subtracting it from 100 [54]. The energy value of the samples was determined by multiplying the protein content by 4, carbohydrate content by 4 and fat content by 9 [54]. All tests were carried out in triplicates.

3.10. Determination of Mineral Composition

The ground dried tuber of *P. edulis* was defatted with petrol using Soxhlet extraction to afford yellowish oil (3%). The marc (5.0 g) was incinerated in a furnace at 500°C for 4 hours and the residue was then dissolved in 2.5% HNO_3 solution (50 mL). The metal contents (Cd, Cr, Cu, Ni, Pb and Zn) were determined using atomic absorption spectrophotometer (analytik jena:ZEEnit 700p, Germany) while the Ca, K and Na contents were determined using Flame Photometer (FP 902, PG Instrument, England) following standard procedure [55]. A calibration curve was prepared using standard metal solutions to test the linearity that is determined by calculating regression line (r^2). Recovery study of the macronutrients (Ca, K and K) was made by using standard addition method.

The efficiency of the method was checked by adding known concentration of each metal in 5 g sample of *P. edulis* with the procedure shown as follows: 500 µg of 1000 mg/L Ca was spiked at once into 5 g of powdered tuber of *P. edulis* and incinerated in muffle furnace at 500℃ for four hours in the same way as the sample was treated. The incinerated sample was digested with 50 mL of 2.5% HNO_3 and analyzed by using Flame Photometer [56]. The above procedure was repeated for K and Na. The experiment was done in triplicate.

4. RESULTS AND DISCUSSION

4.1 Extraction Yield

The tubers of *P. edulis* were successively extracted with n-hexane, EtOAc and MeOH to give 2 g (0.3%), 4 g (0.7%) and 15 g (2.5%), respectively. The amount extracted using MeOH was comparatively higher than the extract obtained with hexane and EtOAc. This indicates that the secondary metabolites present in the tuber of *P. edulis* are mainly polar.

4.2. TLC Analysis of the Extracts

The hexane, EtOAc and MeOH extracts were analyzed with analytical TLC and the results were depicted in Figure 10. The mobile phases used for n-hexane, EtOAc and MeOH extracts were n-hexane:ethyl acetate (4:1), n-hexane:ethyl acetate (1:1), and ethyl acetate:methanol (1:1), respectively.

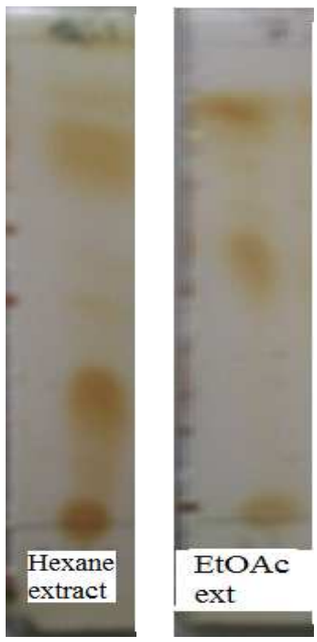


Figure 10: TLC profile of the hexane and EtOAc extract of the tuber of *P. edulis*

As revealed from the TLC profiles (Figure 10), both the hexane and EtOAc extracts contain spots which were visualized after dipping in iodine indicating the presence of secondary metabolites in the tuber of *P. edulis.*

4.3. Qualitative Analysis of the Chemical Constituents of Crude Extracts

The preliminary phytochemical screening of the methanol and EtOAc extracts of the tuber of *P. edulis* revealed the presence of terpenoids, flavonoids, phenolics, and cardiac glycoside (Table 1) while alkaloids, tannins and antraquinone glycosides were not detected. Steroids were detected only in ethyl acetate extract.

Table 1: Phytochemical screening results of the EtOAc and MeOH extracts of the tuber of *P. edulis*

Constituents	**Tests/Reagents**	**EtOAc extract**	**MeOH extract**
Alkaloids	Wagner's	-	-
Cardiac glycosides	Kellar-Kiliani	+	+
Flavonoids	Shinoda	+	+
Steroids	Salkowski	+	-
Phenols	Ferric chloride	+	+
Tannins	Potassium hydroxide	-	-
Terpenoids	Salkowski	+	+
Antraquinone glycosides	Borntrager's	-	-

- indicates absence of phytoconstituents and + indicates the presence of phytoconstituents

The presence of phenols, terpenoids, cardiac glycosides and flavonoids (Table 1) recorded in the present study is known to have some positive effects on health. For instance, the presence of plant polyphenols in *P. edulis* is important as this class of compounds is reported to decrease the incidence of some cancers [57]. The hydroxyl groups in flavonoids were reported to show antioxidant activities by scavenging free radicals or by chelating metal ions [58]. Therefore, the presence of flavonoids in the tuber of *P. edulis* may helps in the prevention of radical generation that damages the biomolecules leading to oxidative stress and early ageing.

4.4. Structure Elucidation of Isolated Compounds

In the course of this work one compound from the hexane and two compounds from the ethyl acetate extract of the tuber of *P. edulis* were isolated and characterized. This section presents the description of the physical and spectral data that led to the determination of the structures of isolated compounds.

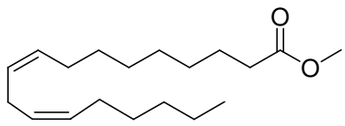
Compound 53

Compound **53** was obtained as a white solid from the column chromatographic fractionation of the hexane extract of the tuber of *P. edulis*. The compound displayed spot at Rf value 0.75 using hexane:EtOAc (9:1) as a mobile phase (Figure 11). The spot was visualized after dipping in iodine.

Figure 11: TLC profile of compound **53**

The ^{1}H-NMR spectrum (Appendix 1) of compound **53** displayed signals characteristics of olefinic protons at δ 5.36 (4H, *m*). The presence of one methoxy group is evident at 3.62 (3H, *s*). The signal at δ 2.80 (2H) accounts for the presence of diallylic methylene protons. The spectrum also showed signal at δ 1.30 (12H, bro. s) characteristics of overlapping signals of protons on methylene carbons. The presence of terminal methyl group in the alkyl chain is evident at δ 0.88 (3H, *t*).

The proton decoupled ^{13}C-NMR spectrum (Appendix 2) of compound **53** with the aid of DEPT-135 (Appendix 3) demonstrated carbon resonances of 19 carbon atoms of which one is due to quaternary, four methines, two methyls and twelve methylenes. The carbon signal at δ 173.1 is evident for the presence of ester carbonyl. The compound exhibited four carbon signals in the olefinic region at δ 129.8, 129.7, 127.9 and 127.8 justifying the presence of two double bonds in the compound. This agrees very well with the ^{1}H-NMR spectrum of compound **53** which only showed the presence of four olefinic protons in the olefinic region. The ^{13}C-NMR spectrum also showed signal due to methoxy group at δ 50.0. This, in combination with the signal at δ 173.1, clearly indicated as the compound is methyl ester. The signals characteristics of aliphatic carbons were observed in the region between δ 33.4 to 22.4. Furthermore, the most upfield carbon signal at δ 13.4 in the ^{13}C-NMR spectrum of compound **53** account for the presence of terminal methyl group in the alkyl chain. The NMR spectral analysis mentioned above indicated that compound **53** is in agreement with methyl linoleate whose structure is shown in Figure 12.



53

Figure 12: Chemical structure of methyl linoleate (**53**)

Compound 54

Compound **54** was obtained as a white solid after silica gel column chromatography of the ethyl extract of the tuber of *P. edulis*. Its melting point was 144-145^{o}C. TLC (hexane:EtOAc, 9:1) gave rise to a spot at Rf = 0.67, visualized after dipping in iodine. The UV-Vis spectrum (methanol) of compound **54** (Appendix 4) demonstrated the absence of conjugated chromophore in the compound. The IR spectrum (Appendix 5) showed absorption band at 3391 cm^{-1} indicating the presence of O-H (hydroxyl) stretching. The presence of aliphatic C-H stretching is evident at 2922 cm^{-1}. The presence of olefinic C=C and C-O stretching were at 1629 and 1075 cm^{-1}, respectively.

The ^{1}H-NMR spectrum ($CDCl_3$) of compound **54** (Appendix 6) revealed the presence of six methyls at δ 0.57 (3H, *s*), δ 0.80-0.81 (9H, *m*), δ 0.87 (3H, *d*, *J* = 6.40 Hz) and δ 1.13 (3H, *d*, *J* = 6.80 Hz) characteristics of sterols. The ^{1}H-NMR spectrum also displayed signal at δ 3.60 (1H, *m*) corresponding to a proton on oxygenated methine. The presence of three olefinic proton signals were evident at δ 5.07 (1H, *dd*, *J* = 8.8 and 15.2 Hz), 5.17 (1H, *m*) and 5.18 (1H, *dd*, *J* = 8.4 and 15.2 Hz) which is characteristics of compounds containing two double bonds. The remaining proton signals integrating for 25 hydrogens were observed in the range δ 2.30 to 1.00

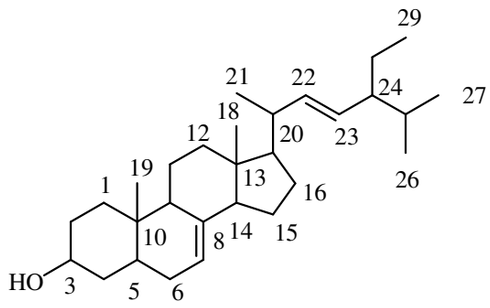
The proton decoupled ^{13}C-NMR (Appendix 7) and DEPT-135 spectra (Appendix 8) of compound **54** revealed the presence of 29 well resolved carbon signals including six methyl, nine methylene, eleven methine, and three quaternary carbons. The three quaternary carbons were resonated at δ 139.6, 43.3 and 34.2. The earlier signal is evident for the presence of olefinic quaternary carbon while the latter two are accounted to the presence of aliphatic quaternary carbons. The carbon signal for an oxygenated carbon was observed at δ 71.1. The presences of two double bonds were evident from the appearance of three olefinic methine carbon signals at δ 117.5, 129.5 and 138.1. This agrees very well with the ^{1}H-NMR spectrum which showed the presence of three olefinic proton resonances. The remaining 22 carbon resonances were detected in the region between δ 55.9 to 12.0. Close inspection of the NMR spectral data (^{1}H, ^{13}C and DEPT-135) indicated that compound **54** is α-spinasterol

(Figure 13). This was confirmed by comparing the ^{13}C-NMR spectral data of compound **54** with those reported in the literature for *α*-spinasterol and the results are presented in Table 2 [59].

Table 2: ^{13}C-NMR spectral data of compound **54** ($CDCl_3$) along with literature reports for *α*-spinasterol ($CDCl_3$) [59]

Position	^{13}C data of **54**	Lit. for *α*-spinasterol [59]	Position	^{13}C data of **54**	Lit. for *α*-spinasterol [59]
1	37.1	37.1	16	28.5	28.5
2	31.5	31.4	17	55.9	55.8
3	71.0	71.0	18	12.0	12.0
4	38.0	37.9	19	13.0	13.0
5	40.3	40.2	20	40.8	40.8
6	29.6	29.6	21	21.3	21.3
7	117.4	117.4	22	138.2	138.1
8	139.6	139.5	23	129.4	129.4
9	49.4	49.4	24	51.2	51.2
10	34.2	34.2	25	31.8	31.9
11	21.5	21.5	26	21.0	21.0
12	39.4	39.5	27	19.0	19.0
13	43.3	43.2	28	25.4	25.4
14	55.1	55.1	29	12.2	12.2
15	23.0	23.0			

The NMR spectral data of compound **54** are in close agreement with the literature reported for *α*-spinasterol (**54**) (Table 2) [59] whose structure is given in Figure 13.



54

Figure 13: Chemical structure of *α*-spinasterol

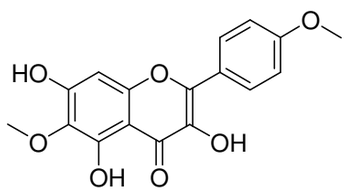
Many literature reports showed that *α*-spinasterol exhibits anti-tumor property in breast, ovarian and cervical cancer cells [60]. It was also reported to have anti-inflammatory activity [61].

Compound **55**

Compound **55** was obtained as a yellow solid from ethyl acetate extract of the tubers of *P. edulis*. Its melting point was 226-228^{o}C. The UV-Vis spectrum (methanol) of compound **55** (Appendix 9) showed absorption bands at 271 (band II) and 338 nm (band I) suggesting the presence of flavonoids skeleton in the compound. The absorption maxima of band II in the UV-Vis spectrum of flavonoid containing 6-methoxy are reported to be below 279 nm while those with 6-hydroxy exhibit absorption maxima beyond 279 nm [62]. Hence the UV-Vis spectrum of compound **55** clearly indicated the presence of methoxy group on C-6. The IR spectrum of compound **55** (Appendix 10) displayed absorption band at 1690 cm^{-1} attributable to an *α,β*-unsaturated carbonyl carbon. The compound also displayed hydroxyl stretching at 3375 cm^{-1}. The presence of C-O and C-H stretching were evident from the observed absorption bands at 1027 and 2962 cm^{-1}, respectively.

The proton NMR spectrum of compound **55** (Appendix 11) suggested the presence of two methoxy groups at *δ* 3.65 (3H, *s*) and 3.76 (3H, *s*). A pair of doublets at 6.89 (2H, *d*, *J* = 8.80 Hz) and 7.92 (2H, *d*, *J* = 8.80 Hz) were characteristic of H-2'/H-6' and H-3'/H-5', respectively of the 1,4-disubstituted aromatic ring system. The proton NMR spectrum also displayed a singlet signal at *δ* 6.30 (1H, *s*) typical of proton on C-8 of flavonoids.

The proton decoupled ^{13}C-NMR spectrum of compound **55 (Appendix 12)** showed the presence of two methoxy groups at δ 56.2 and 59.9. The methine carbon signals observed at δ 113.8 (C-3', 5') and 129.9 (C-2', 6') were due to symmetrically placed aromatic carbons on unsymmetrically *para* substituted B-ring of flavonoids. The carbon signal at δ 93.5 is characteristics of C-8. The diagnostic signal in the ^{13}C-NMR spectrum at δ 178.7 is suggestive for the presence of α,β-unsaturated carbonyl carbon. The compound exhibited oxygenated aromatic quaternary carbon signals at δ 161.7 (C-4'), 155.8 (C-2), 152.8 (C-7), 149.7 (C-9), 146.7 (C-5), 138.3 (C-3) and 128.5 (C-6). Also observed quaternary carbon signals were at δ 122.7 and 105.2 due to C-1' and C-10, respectively. The spectral data generated for compound **55** was in agreement with 3,5,7-trihydroxy-6,4'-dimethoxyflavone (**55**) whose structure is depicted in Figure 14



55

Figure 14: Structure of 3,5,7-trihydroxy-6,4'-dimethoxyflavone

4.5. Fatty Acid Profile of the Tuber of *P. edulis*

The fatty acid compositions of the oil of tuber of *P. edulis* were determined using gas chromatography-mass spectrometry (GC-MS), with the chromatogram depicted in Figure 15.

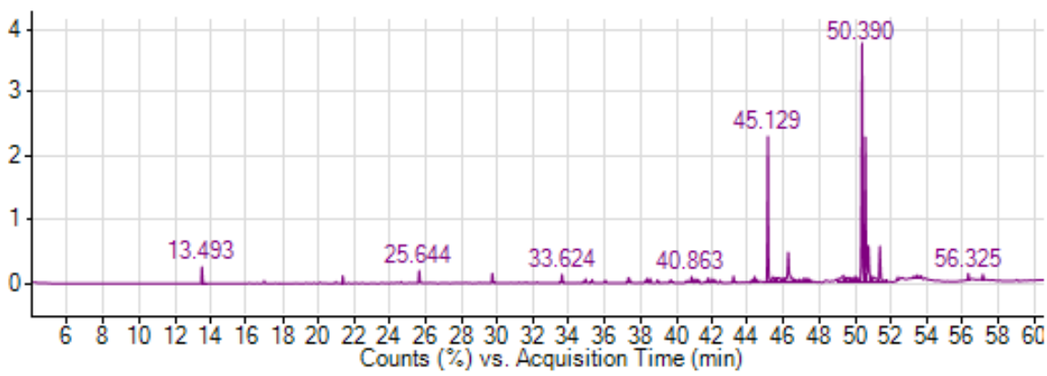
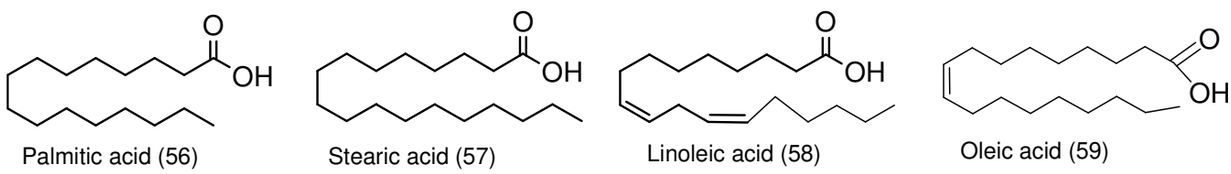


Figure 15: GC chromatogram of fatty acids of the oil of the tuber of *P. edulis*

The results obtained from GC-MS showed that *P. edulis* oil contains a large proportion of double bond containing fatty acids. The dominant unsaturated fatty acid, obtained at retention time of 50.39 min, was found to be linoleic acid (**58**) which accounts for the total of 42% of the fatty acids. The spectrum also showed other fatty acids at retention time of 45.12, 50.56 and 50.74 min which were due to palmitic (31%), oleic (20%) and stearic (7%) acid, respectively (Figure 16). These fatty acids are typical fatty acids found in various vegetable oils. The low values of the saturated fatty acids recorded in the present study shows that the oil can be used by people with hypertension.



Palmitic acid (56) Stearic acid (57) Linoleic acid (58) Oleic acid (59)

Figure 16: Fatty acids obtained from the tuber of *P. edulis*.

The fatty acid methyl esters identifications were made by comparing the spectra of the components with the database of the spectrum of known components stored in the GC-MS library. The mass spectrum of the peak observed at retention time of 50.39 min, belonging to linoleic acid, is as depicted in Figure 17. The mass spectrum (Figure 17) clearly showed the molecular ion of the fatty acid methyl ester was observed at m/z 294.3.

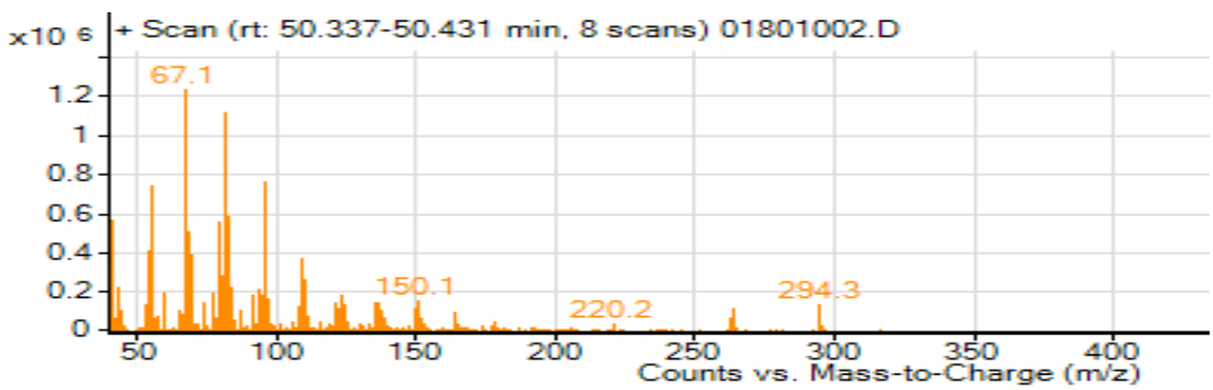


Figure 17: Mass spectrum of linoleic acid (Peak at 50.39 ppm)

Linoleic acid is one of the two families of essential fatty acids that human body cannot synthesize it from other components. This fatty acid is used in the biosynthesis of prostaglandins and cell

membranes. The fatty acid plays role for maintaining epidermal water barrier of the skin, maintain cell fluidity, improved immune and inflammatory responses.

4.6. Antioxidant Activities

4.6.1. DPPH Radical Scavenging Assay

DPPH radical scavenging assay is among a simple method widely used as an index to evaluate the antioxidant activities of extracts or pure compounds. The scavenging activity was expressed in terms of percent inhibition and $IC_{50,}$ the amount of active extracts necessary to decrease the initial DPPH absorbance by 50%. Results showed that the methanol and EtOAc extracts of the tuber of *P. edulis* changed the purple colored DPPH solution into yellow. This is a clear indication of the radical scavenging potential of the extract. The percent inhibition of the methanol and EtOAc extract of the tuber of *P. edulis* were found to be 84 and 76% at 100 µg/mL, respectively. The results were close to the percent inhibition displayed by ascorbic acid (90% at 100 µg/mL) used as positive control indicating strong activity of the extracts of the tuber of *P. edulis* as radical inhibitor. It was also observed that the radical scavenging activities of the extract increased with increasing concentration (Table 3).

Table 3: DPPH radical scavenging activity of MeOH extract of *P. edulis*

Samples tested	Concentration in µg/mL	%DPPH inhibition	IC_{50}	Remark
MeOH extract of *P. edulis*	100	84±0.92	12	
	50	70±1.20		
	25	62±1.30		
	12.5	50±0.89		
EtOAc extract of *P. edulis*	100	76±0.85	17	
	50	65±1.10		
	25	54±0.98		
	12.5	45±0.73		

3,5,7-trihydroxy-6,4'-dimethoxyflavone	100	88±0.42	7.5
	50	70±1.00	
	25	62±1.20	
	12.5	47±0.76	
α-spinasterol	100	15	-
Ascorbic acid	100	90±0.45	3.1

Values are mean ± standard deviation of triplicate analysis

The presence of phenolics and flavonoids received much attention as potential natural antioxidant in terms of their ability to act as efficient radical scavengers, hydrogen donors, singlet oxygen quenchers and metal chelators [63-64]. The high DPPH radical scavenging activity displayed by the tuber of *P. edulis* is likely ascribed to the presence of phenolics and flavonoids. This is in agreement with the chemical screening test which showed the presence of phenolics in the tuber of *P. edulis.*

As a follow up to the antioxidant activities shown by the extracts of the tuber of *P. edulis*, the radical scavenging tests were done for compounds isolated from this species, namely, 3,5,7-trihydroxy-6,4'-dimethoxyflavone and *α*-spinasterol. The best radical scavenging activity was displayed by 3,5,7-trihydroxy-6,4'-dimethoxyflavone (Table 3) with its percent radical scavenging activity (88% at 100 µg/mL) and IC50 value (7.5 µg/mL) close to ascorbic acid used as positive control. This is most likely because of the fact that this compound has phenolic hydroxyl groups for free radical scavenging activity. The presences of this compound in the EtOAc extract of the tuber of *P. edulis* likely accounts for the antioxidant activity displayed by this species.

4.6.2. Ferric thiocyanate method

The degree of lipid per-oxidation can be used to assess the antioxidant potential of compounds or extracts. As depicted in Table 4, the MeOH and EtOAc extracts of the tuber of *P. edulis* inhibited primary peroxide formation by 78 and 75% at 100 µg/mL, respectively. The result was turned out to

be comparable with ascorbic acid, demonstrating the potential of the tuber in preventing the formation of lipid peroxides.

Table 4: Anti-lipid peroxidation activities of the MeOH extract of *P. edulis*

Sample name	Absorbance at 500 nm	%inhibition	Remark
Blank	0.56	-	
Ascorbic acid	0.11	80	
P. edulis MeOH extract	0.12	78	
P. edulis EtOAc extract	0.14	75	

Ascorbic acid was used as positive control; values are mean of triplicate analysis

Flavonoids are able to reduce highly oxidizing free radicals such as superoxide, peroxyl, alkoxyl, and hydroxyl radicals by hydrogen atom donation [65]. Because of their capacity to chelate metal ions (iron, copper, etc.), flavonoids also inhibit free radical generation [65]. Flavonoids protect lipids against oxidative damage. Therefore the strong anti-lipid peroxidation activity displayed by the tuber of *P. edulis* indicates the potential of the plant as natural antioxidants. Hence, the presence of flavonoids in this plant can improve the health status of its users

4.7. Antibacterial Activity of the Crude Extracts

The EtOAc and MeOH extracts were assessed for their *in vitro* antibacterial activities using disc diffusion method. The results of inhibition zone in diameter against the four tested pathogens (*Staphylococcus aurous, Escherichia coli, Protens mirabilis* and *Klebsiella pneumonia*) and the controls are summarized in Table 5.

Table 5: Antibacterial activities of the extracts of the tuber of *P. edulis*

Extracts	**Con. (mg/mL)**	**Inhibition zone in mm**					
EtOAc extract		*S. aurous*	*E. coli*	*Protens mirabils*	*Klebsiella pneumonia*	DMSO	Ciprofloxacin
	100	8	11	8	7	6	17
	10	6	9	8	6	6	15
	1	6	6	6	6	6	15
	0.1	6	6	6	6	6	15
MeOH extract	100	6	6	8	6	6	18
	10	6	6	8	6	6	18
	1	6	6	6	6	6	17
	0.1	6	6	6	6	6	16

Results showed that none of the bacterial strains had shown positive result at the sample concentration from 0.1 to 1 mg/mL. The ethyl acetate extract displayed maximum zone of inhibition (11 mm) at 100 mg/mL against *E. coli* compared with other bacterial strains tested in this study. The result is significant compared with negative control which showed no inhibition zone but modest compared with ciprofloxacin, the standard drug used as positive control. However, the EtOAc extract showed little activity against the other strains tested. *α*-spinasterol and 3,5,7-trihydroxy-6,4'-dimethoxyflavone were also assessed for their antibacterial activities. The two compounds were found active against *E. coli*

4.8. Analysis of Proximate Composition

This study was done to evaluate the nutritive value of three samples of the tubers of *P. edulis* grown in different parts of Ethiopia. Using standard procedures, the proximate composition of the three samples were determined and presented in Table 6. The results (Table 6) showed that the tuber of *P. edulis*

contained low values of ash and crude fat. In common with other roots and tubers [66], the tuber of *P. edulis* has high moisture content resulting in relatively low dry matter content. The moisture content is an index of water activity. The level is a measure of its susceptibility to microbial contamination [54]. The high moisture content displayed by the tuber of *P. edulis* reveals that the sample need proper care for preservation as they will be prone to deterioration. On the other hand, the high moisture content provides for greater activity of water soluble enzymes and co-enzymes [67]. The high water content is also significant as the body does not need to use some of its own water to digest them. Hence the body uses less energy to digest and can then assimilate all the nutrients much faster.

Table 6: Proximate composition of the tuber of *P. edulis* grown in Ethiopia

Parameters	%composition of *P. edulis* from			Sweet potato [66]
	Bakko	Guliso	Wolayta Sodo	
Moisture	74.0±1.24	69.0±1.43	75.0±1.80	64.34± 0.42
Ash	1.80±0.03	1.7±0.04	1.50±0.03	0.40 ± 0.02
Crude protein	9.02±0.12	6.65±0.00	10.24±0.13	0.81 ± 0.09
Crude fat	1.80±0.04	1.6±0.06	1.10±0.02	
Crude fiber	5.16%±0.05	4.46%±0.04	5.99%±007	0.12 ±0.01
Nitrogen free extract	8.22±0.09	11.19±0.12	6.17±0.04	2.04 ±0.01
Nitrogen content	1.44±0.11	1.06±0.0	1.64±0.12	0.13 ±0.01
Energy value	85.08Kcal/100g	85.76Kcal/100g	75.54Kcal/100g	

Values are mean ± standard deviation for three replications

Many literature report showed as excess intake of crude fat has some established health implications especially for the overweight [54]. The consumption of excess amounts of fats has been recognized as the most important dietary factor aiding increased level of cholesterol. In this regard, the low fat content displayed by the three samples of the tuber of *P. edulis* may reduce the risk of coronary heart disease and lower the risk of hypertension. This low value of fat observed in this study is also significant as it is safe for consumption by mankind in the era where obesity poses a serious health problem. The tuber of *P. edulis* comprised of significant amount of protein. The higher protein contents of the tuber of *P. edulis* studied indicates that its intake can contribute to the formation of hormones which controls various body functions including growth, repair and maintenance of body. In addition, it may be taken as a preferred option to animal proteins for diabetics as the later tend to be high in saturated fats. This confirms that the tuber of *P. edulis* is an energy-giving food [51]. The

protein content of the tubers of *P. edulis* is superior to the values reported for the tuber of sweet potato (0.91%) [66] and *Dioscorea rotundata,* yam (0.7%) [68].

The presence of such significant amount of carbohydrate in the tuber of *P. edulis* has beneficial effects on human health. It can serve as a good source of carbohydrate which constitutes a major class of naturally occurring organic compounds that are essential for the maintenance of animal life. In addition, these carbohydrates may serve as substrates for the production of aromatic amino acids and phenolic compounds. Thus the carbohydrate levels of the studied samples suggest its usefulness as alternative source of glucose. The energy value of the tuber of *P. edulis* from Bako and Guliso were turned out to be higher energy value than Wollyata Sodo (Table 6).

The presence of fiber in foods is useful in the management of cardiovascular disease [69], diabetes mellitus, colorectal cancers and weight reduction in obsessed individuals [51]. Though excess amount of food fiber reduces absorption of nutrients, and result in insufficient energy for growth in children, they have also some positive attributes. The presence of significant amount of fiber in the tubers of *P. edulis* is significant as it is effective in combating many life threatening diseases. It is also needed in the diet to aid digestion and absorption of glucose and fat.

4.9. Mineral Composition

Minerals are important component of diet because of their physiological and metabolic function in the body. They are used by human to perform certain chemical reactions which are essential for the normal functioning of our body. In the present work the levels of the micronutrients and macronutrients of the tuber of *P. edulis* collected from Bako, Guliso and Wolayta Sodo (WS) were analyzed and the results are presented in Table 7. The cadmium content of the tuber of *P. edulis* was below detection limits. Many literature reports showed that cadmium has no known nutritional value, and it is highly toxic to both plants and animals [70]. The seriousness of the acute cadmium poisoning in humans including kidney damage, destruction of testicular tissue and destruction of red blood cells were well established [71]. Because of the chemical similarity, cadmium may also replace zinc in some enzymes, thereby altering the stereostructure of the enzyme and impairing its catalytic activity. The very low value of cadmium in the tuber of *P. edulis* can be taken as one positive aspects of this plant as food.

The level of chromium content was in the range between 0.008 to 0.030 mg/100g. The value was found relatively superior in samples collected from Wolayta Sodo compared with those samples collected from Guliso and Bakko. Chromium is among metals reported to potentiate insulin action and restores normal glucose tolerance. The estimated safe and adequate daily dietary intake recommendations are 0.050 to 0.200 mg/day for adult [72]. The copper content of the analyzed sample was 0.100-0.191 mg/100g. The ability of copper to promote the development of connective tissues that allow for efficient bones, cartilage and blood vessels through all bodily systems is reported [72]. The level of this metal in the tuber of *P. edulis* is turned out to be comparable with the values reported for potato tuber (0.1 to 0.3 mg/100 g) [73].

Table 7: Mineral composition of *P. edulis* tuber analyzed by AAS and flame photometer

Sample source		Mineral composition of the tuber of *P. edulis* (mg/100g)							
	Cd	Cr	Cu	Ni	Pb	Zn	Ca	K	Na
Wolayta Sodo	BDL	0.030	0.100	0.335	0.016	0.135	22.8±0.1	502.5±0.7	12.8±0.2
Bakko	BDL	0.020	0.191	0.697	0.022	0.130	15.1±1.1	490±1.0	13.5±0.7
Guliso	0.0005	0.008	0.134	1.065	0.023	0.357	13.8±0.1	325±2.0	13±1.0
Values reported from other sources			0.1-0.3[a]			0.23 to 0.27[b]	23.04-29.97[b],1.3 to 27.8[a]	115-203[b]; 239-694[a]	23-28[b]
Recommended in take (in mg/day) for humans	-	0.050-0.200		0.025-0.035	0.015-0.100	3-30	210 to 1300		120-1500

BDL = below detection limit; a = in mg/100g from potatoes; b = in mg/100g from sweet potato; samples for analysis of Na, Ca and K were done in triplicate

The level of Ni in the tuber was from 0.335 mg/100g for Wolayta Sodo and 1.065 mg/100g for Guliso. Nickel plays a major role in helping the body absorb the iron it needs [72]. It assists in breaking down glucose, helps in creating energy for daily use and even contributes to the production of certain enzymes that initiate important chemical reactions such as the development of nucleic acids. Hence, the significant amount of Ni observed from the tuber of *P. edulis* has some health implications. Also analyzed in this study was the content of Pb which ranges from 0.016 to 0.023 mg /100g. The level was found higher for samples collected from Bakko and Guliso while comparatively lower value is recorded for sample from WS. The Pb content obtained in the present study was found to be in the range of the typical daily dietary intake of lead which is 0.015 to 0.100 mg [73]. The tuber *P. edulis* had 0.130 to 0.357 mg/100g of zinc. The level was superior for samples collected from Guliso. The values were close to those values reported in the literature for sweet potato (0.23 to 0.27 mg/100g) [74]. Reports showed that zinc can boosts the immune system and diminish symptoms associated with common cold or flu more quickly in those individuals who consume regularly, or take supplements to fight off these infections and viruses [73]. Zinc works with about 200 different types of enzymes in the body in an effort to maintain normal growth and development patterns.

Flame photometer was employed to determine the level of Ca, K and Na. Results showed that the level of Ca were from 13.8 to 22.8 mg/100g (Table 7). The value was inferior for sample collected from Guliso. The calcium content obtained in the present study is therefore comparable with the values reported for sweet potato and potato [74,75], the recommended daily intake of calcium is 210 to 1300 mg/day. Therefore, the tuber under study can be used as food that can be used as source of calcium. Furthermore the presence of Ca in the tuber of *P. edulis* helps to support bone structure of human beings. Potassium content (325 to 502.5mg/100g) in the tuber of *P. edulis* was relatively high in all samples analyzed in the present study. It was also found that the level of K obtained in the present study is superior than the value reported for sweet potato (308.67 to 328.67 mg/100g) and potato [74-75]

Sodium is an important mineral that assist in the regulation of body fluid and in the maintenance of electric potential in the body tissue [68]. Compared to other major elements evaluated in this

study, Na was found in low amounts with values ranging from 12.8 to 13.5 mg/100g. The values were inferior to those reported for sweet potato [76]. The recommended intake of sodium with diet ranges from 120 to 1500 mg/day [72]. Na/K ratio of the tubers of *P. edulis* studied varies from 0.025 to 0.04. Various report showed that [74], a food source having Na/K ratio of less than 1 has impact on lowering blood pressure since Na/K ratio is known for help in controlling high blood pressure. Thus, it can be concluded that all the three samples of the tuber of *P. edulis* are good food that have impact on lowering blood pressure. *P. edulis* consumption could be recommended as useful nutraceutical therapy for hypertensive individuals. The result obtained from the analysis revealed that the tuber can be used as food.

The flame photometric method was validated by assessing its accuracy which was done using recovery method. The recovery of the flame photometric method was on average found to be 99% which indicates excellent accuracy of the results. The atomic absorption spectrometric method was validated by using its linearity. The calibration plot obtained from this result exhibited a correlation coefficient of 0.999 indicating a high degree of correlation and a good linearity of the method.

5. CONCLUSIONS AND RECOMMENDATIONS

The preliminary phytochemical screening of the methanol and EtOAc extracts showed the presence of terpenoids, flavonoids, phenolics and cardiac glycosides which have some reported positive effects on health. Silica gel column chromatographic fractionation of the hexane and EtOAc extracts of tuber of *P. edulis* furnished three compounds, namely, methyl linoleate, α-spinasterol and 3,5,7-trihydroxy-6,4'-dimethoxyflavone. To the best of our knowledge, the latter two compounds were not yet been reported before from this genus. The yellowish oil hexane extract analyzed with GC-MS after transforming to fatty acid methyl esters showed the presence of a large proportion of unsaturated fatty acids (62%). Hence the oil may be used by people with hypertension.

The radical scavenging activities and anti-lipid peroxidation potential of the methanol and EtOAc extracts of the tubers of *P. edulis* were comparable with ascorbic acid, a natural antioxidant used as positive control. The activity displayed by the EtOAc extract is likely accounted to the presence of 3,5,7-trihydroxy-6,4'-dimethoxyflavone which inhibit DPPH radical by 88% at 100 µg/mL. The antioxidant activity displayed by the tuber of *P. edulis* indicates the potential of the plant as natural antioxidants.

The EtOAc and MeOH extracts were also assessed for their *in vitro* antibacterial activities using disc diffusion against *Staphylococcus aurous, Escherichia coli, Protens mirabilis* and *Klebsiella pneumonia*. The ethyl acetate extract displayed maximum zone of inhibition (11 mm) at 100 mg/mL against *E. coli* compared with other bacterial strains tested in this study. The result is significant compared with negative control which showed no inhibition zone but modest compared ciprofloxacin, the standard drug used as positive control.

The protein, crude fat, carbohydrate and fiber contents displayed by the tuber of *P. edulis* were superior to those values reported for the tuber of sweet potato indicating the potential of this plant as food. The level of Cr, Cu, Ni, Pb, Zn, Ca, K and Na were found to be in the range of 0.008-0.030, 0.100-0.191, 0.330-1.065, 0.016-0.023, 0.135-0.357, 13.800-22.800, 325.00-502.50 and 12.80-13.50 mg/100g on the dry weight basis, respectively. Therefore, the tuber can contribute enormously to the supply of both macro and micronutrients in our diet. The ratio of Na/K obtained in the present study is less than 1 making the tuber of *P. edulis* as useful nutraceutical against hypertension. Therefore, the nutritional profile and biological activities recorded in the present study demonstrated the usefulness of this plant as natural food and medicine.

Further chemical work is necessary to isolate minor constituents from the polar solvent extracts of the tuber of *P. edulis* using preparative HPLC. It is also necessary to conduct further antibacterial activities of the extracts and minor constituents on other strains of bacterial pathogens. One of the major constraints in the cultivation of *P. edulis* as raised by the farmers during an interview made while sample collections was shortage of planting materials to use for

conventional propagation methods using tubers. Therefore, it is necessary to start *in vitro* propagation of *P. edulis* using plant tissue culture techniques.

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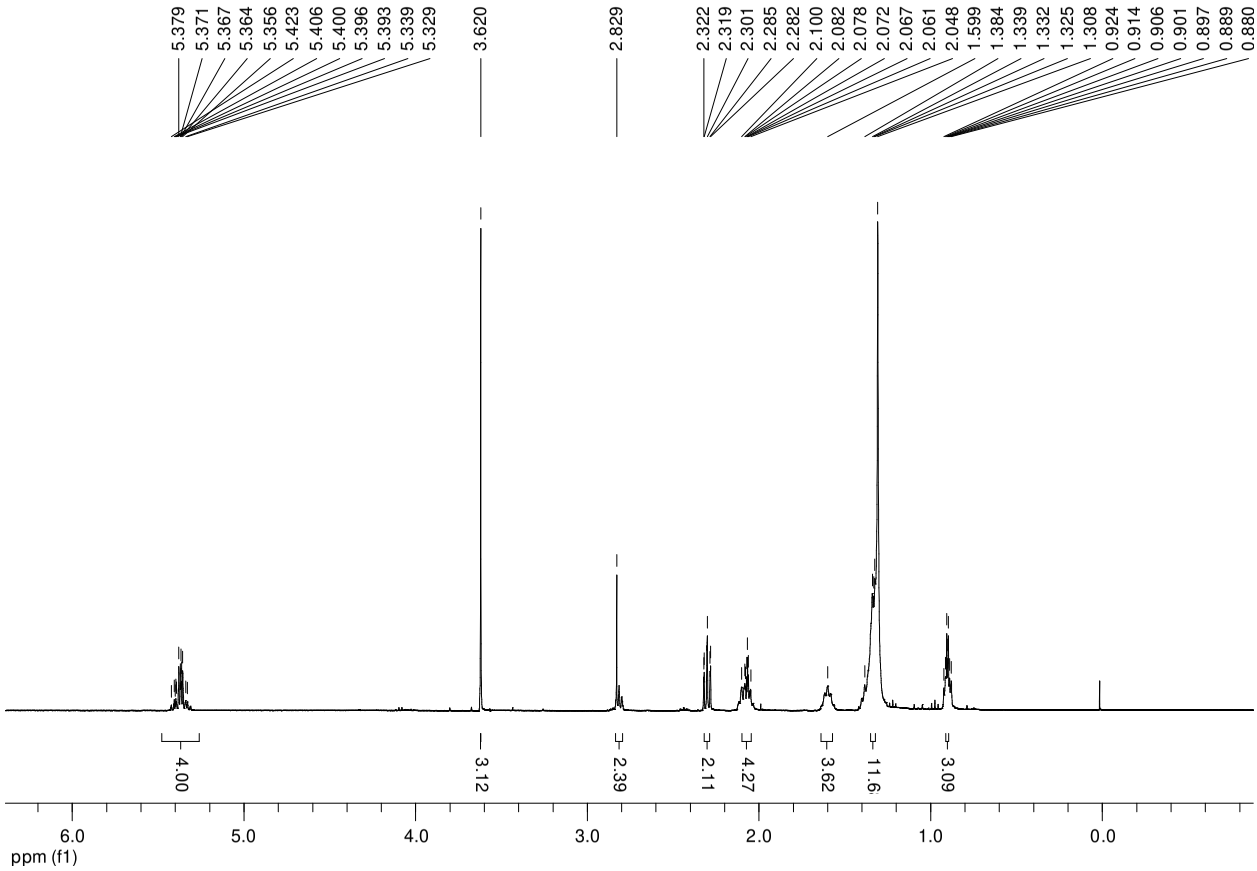
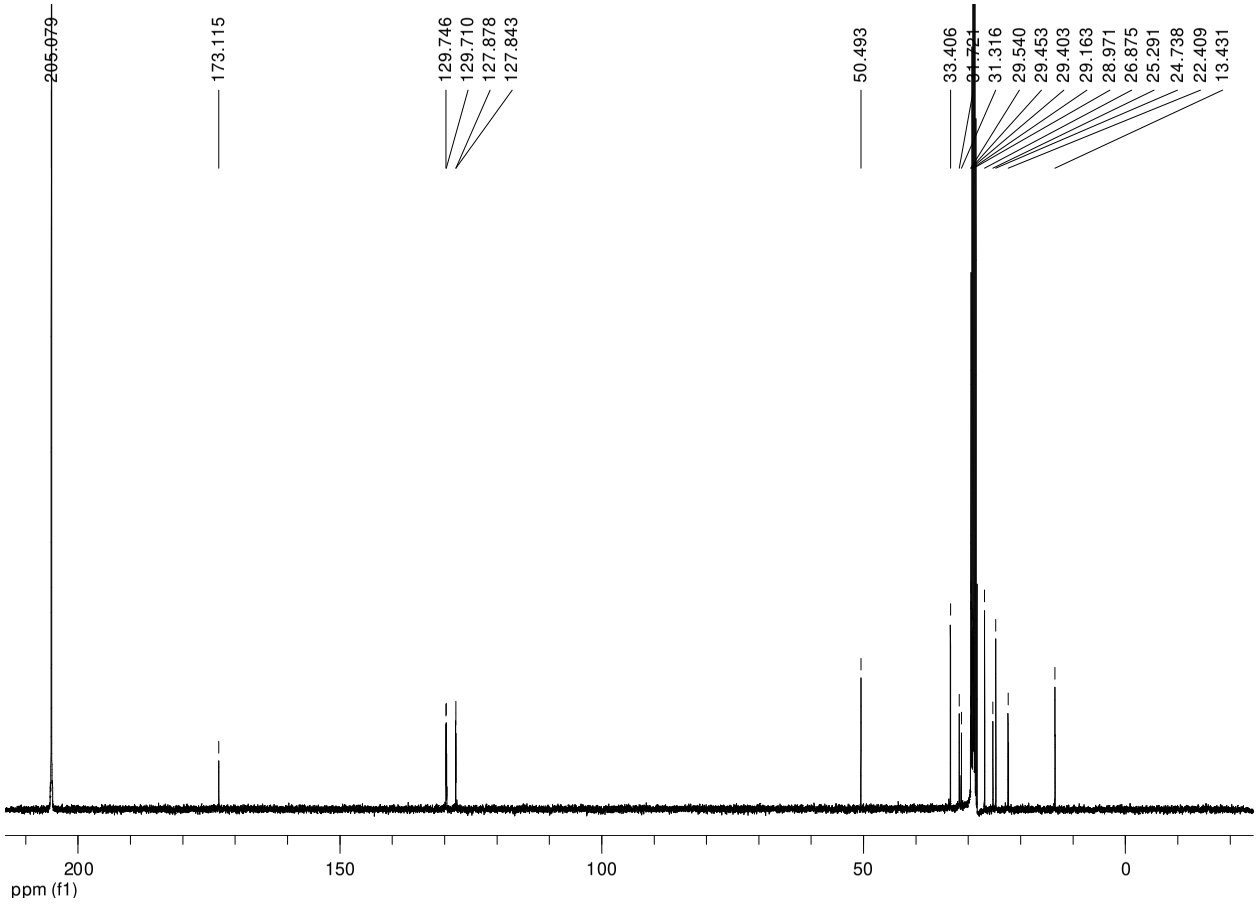
Appendices

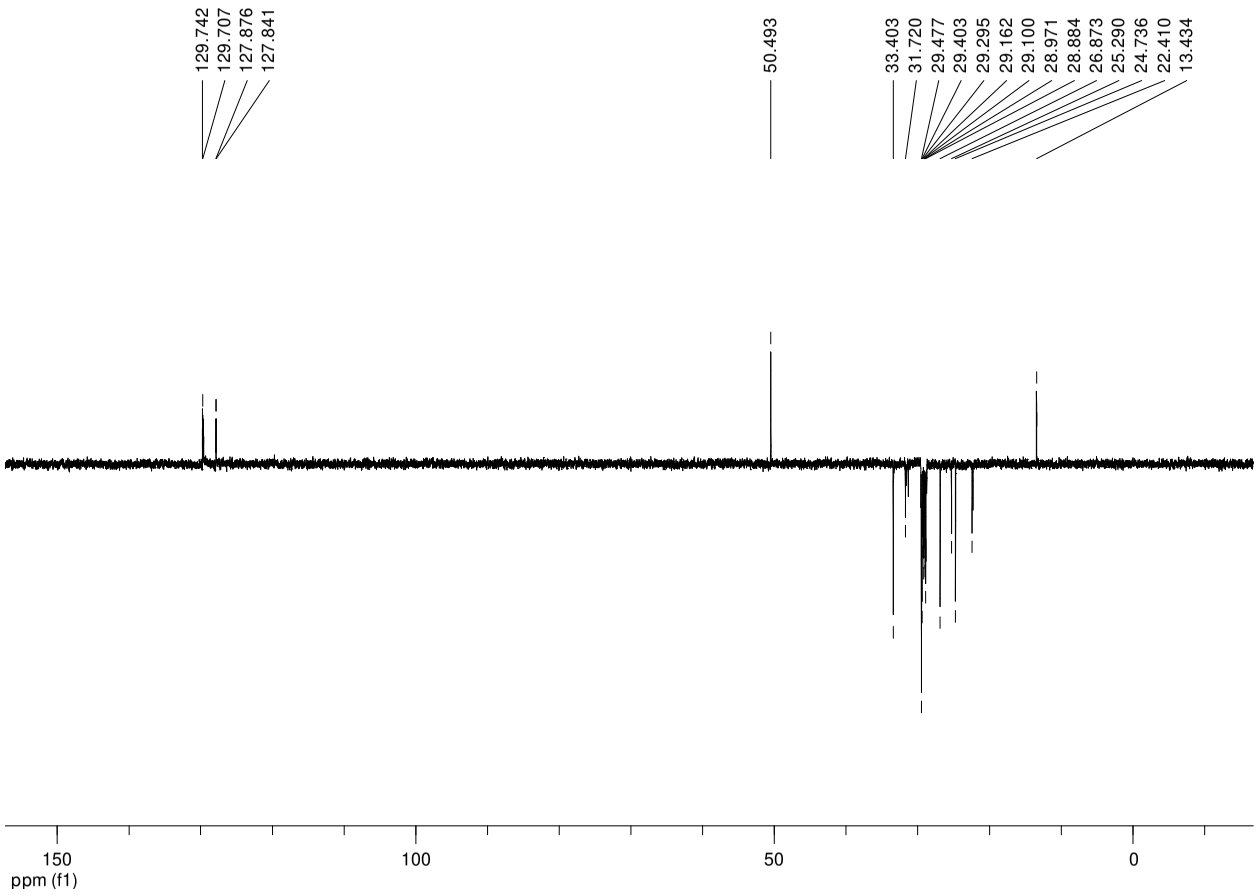
Appendix 1: ^{1}H-NMR spectrum ($CDCl_3$) of methyl linoleate



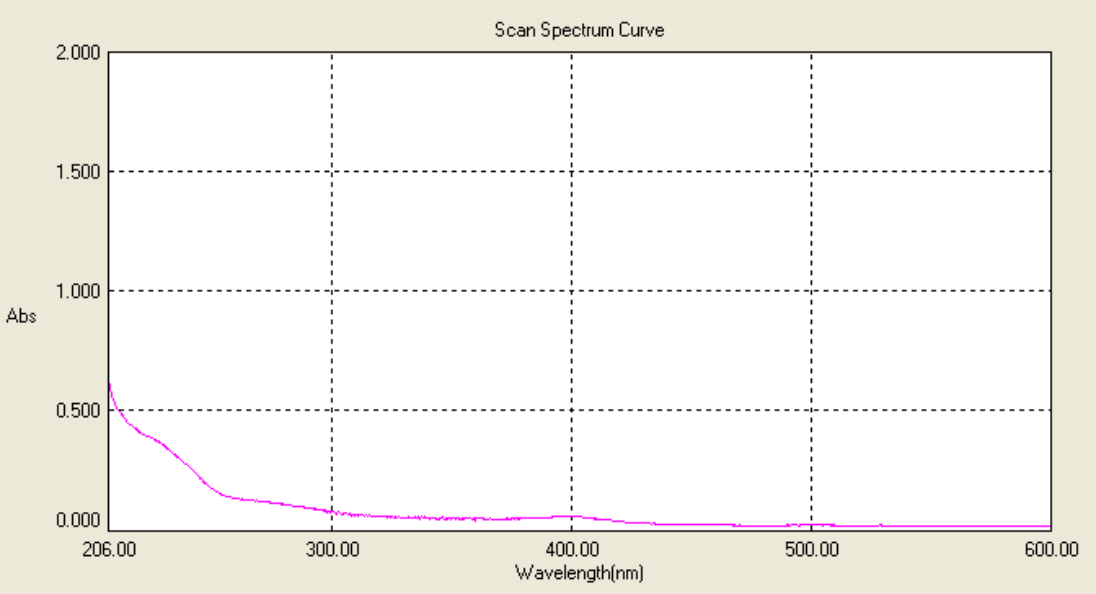
Appendix 2: ^{13}C-NMR spectrum ($CDCl_3$) of methyl linoleate



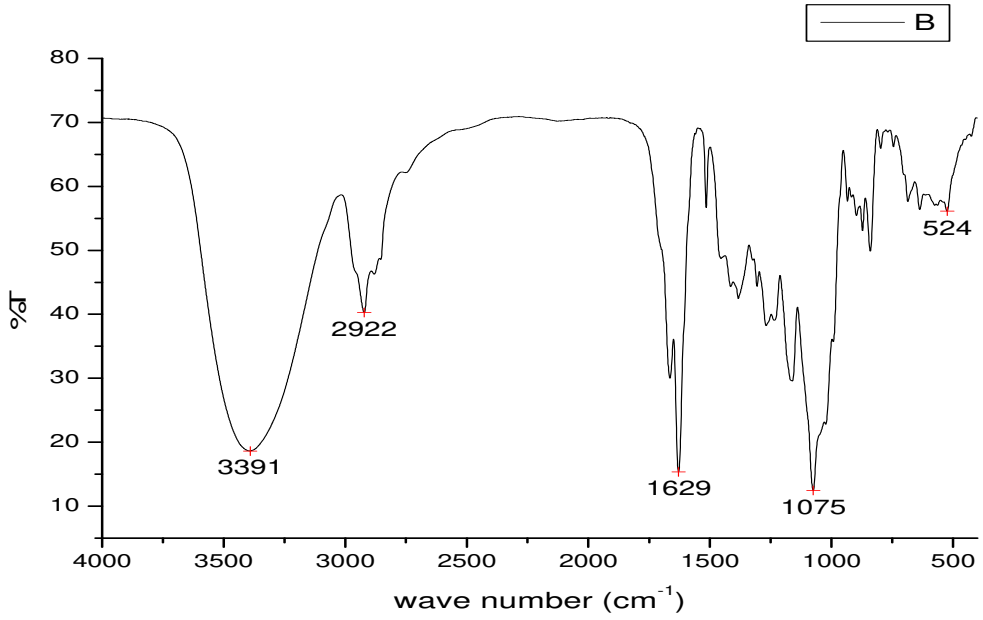
Appendix 3: DEPT-135 NMR spectrum ($CDCl_3$) of methyl linoleate



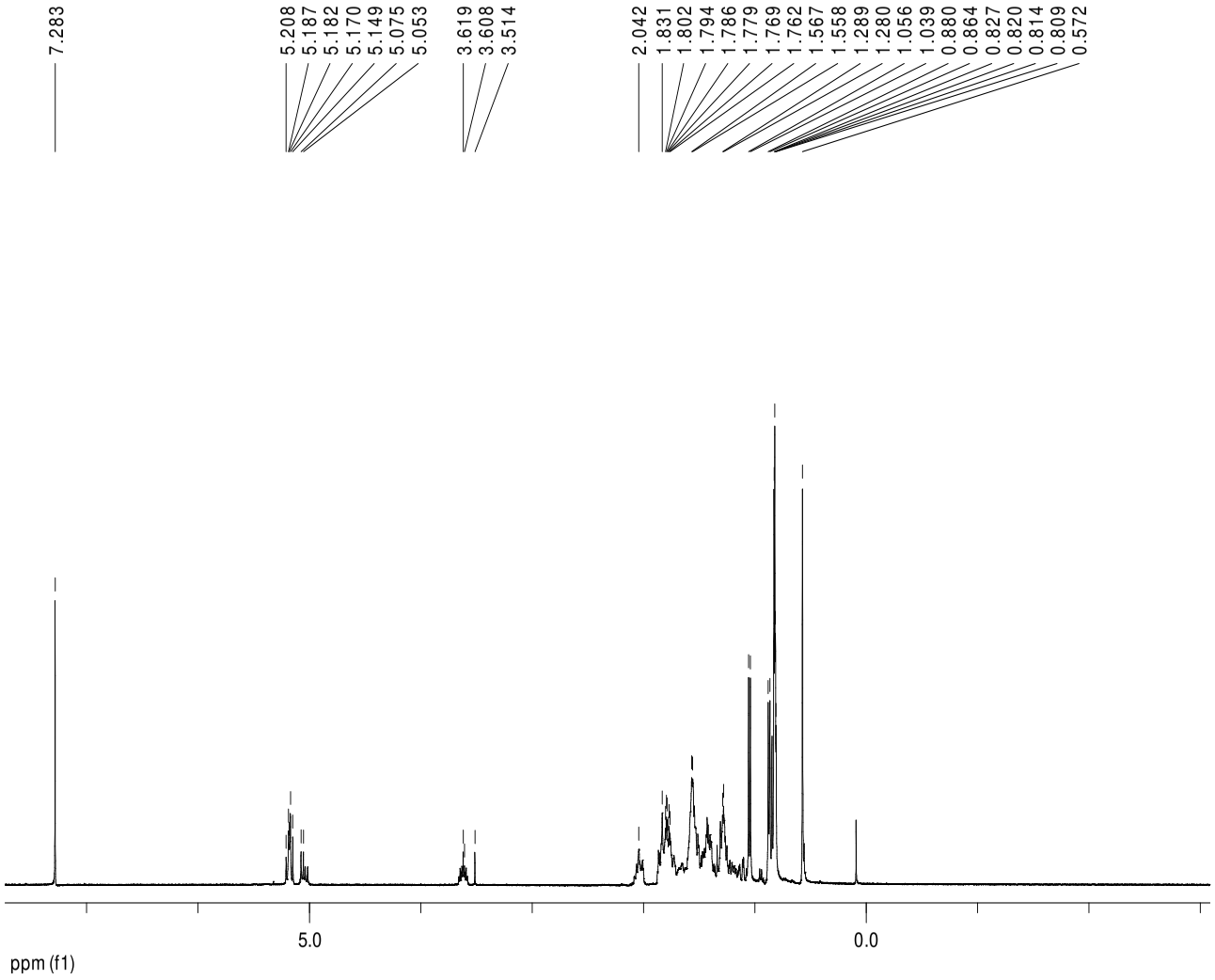
Appendix 4: UV-Vis spectrum of α-spinasterol



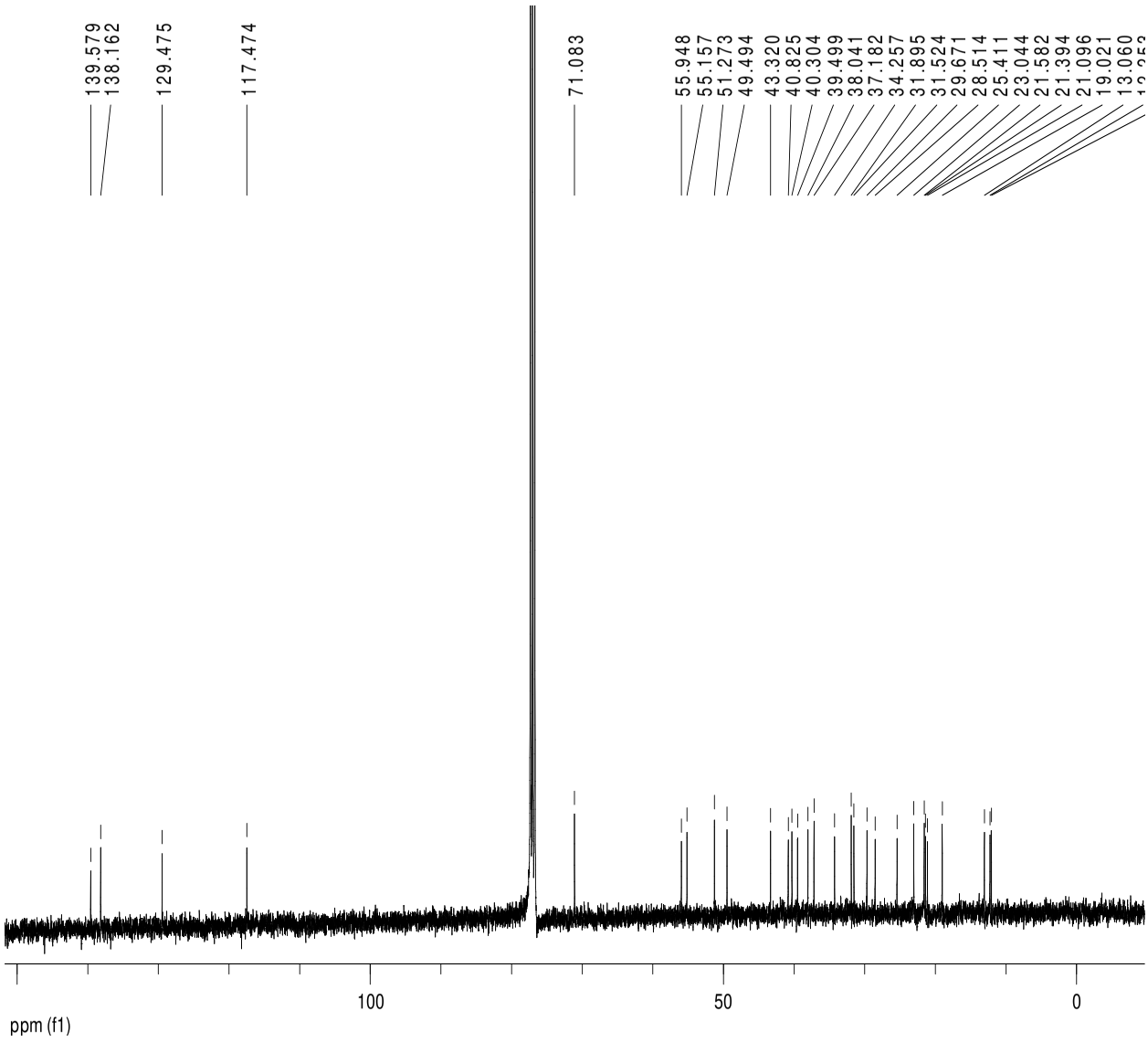
Appendix 5: IR (KBr) spectrum of α-spinasterol



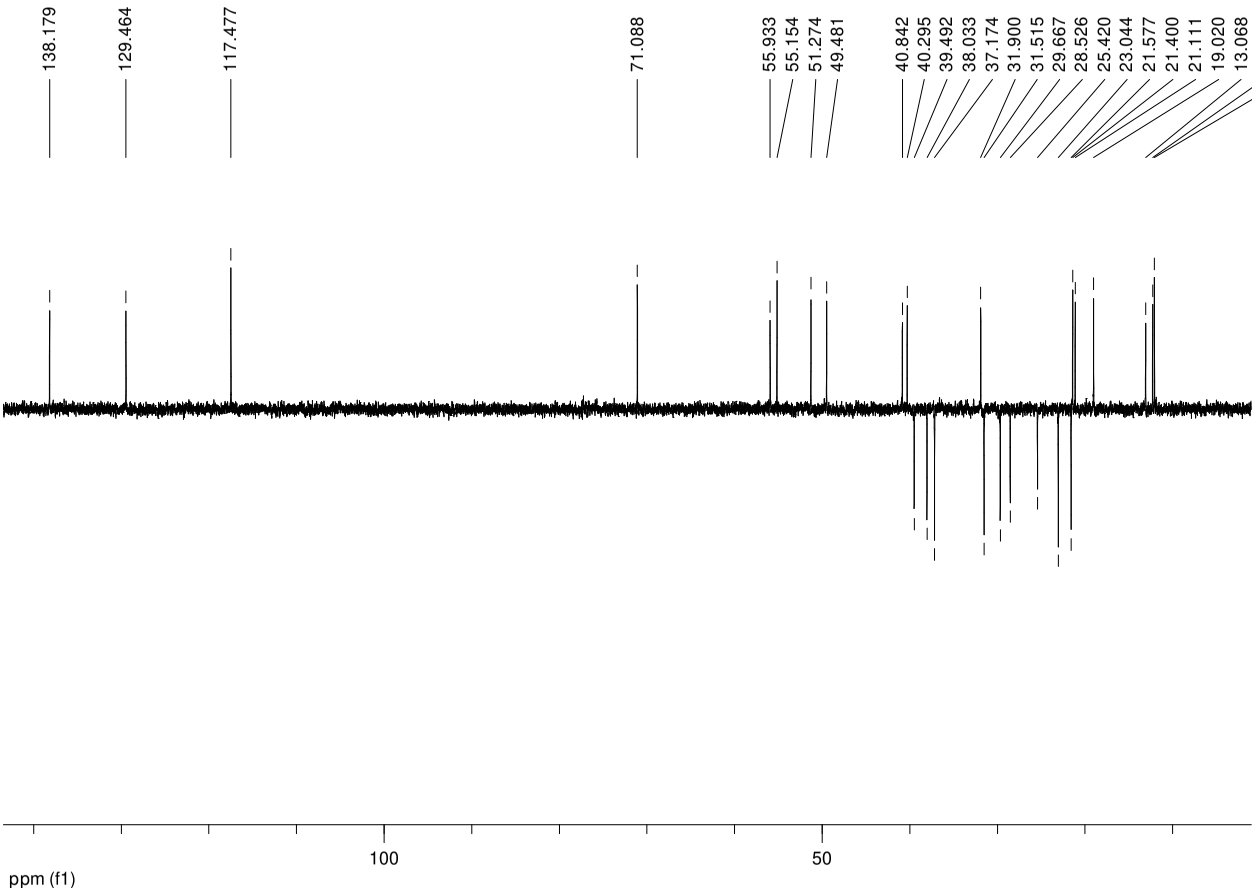
Appendix 6: ^{1}H-NMR spectrum ($CDCl_3$) of α-spinasterol



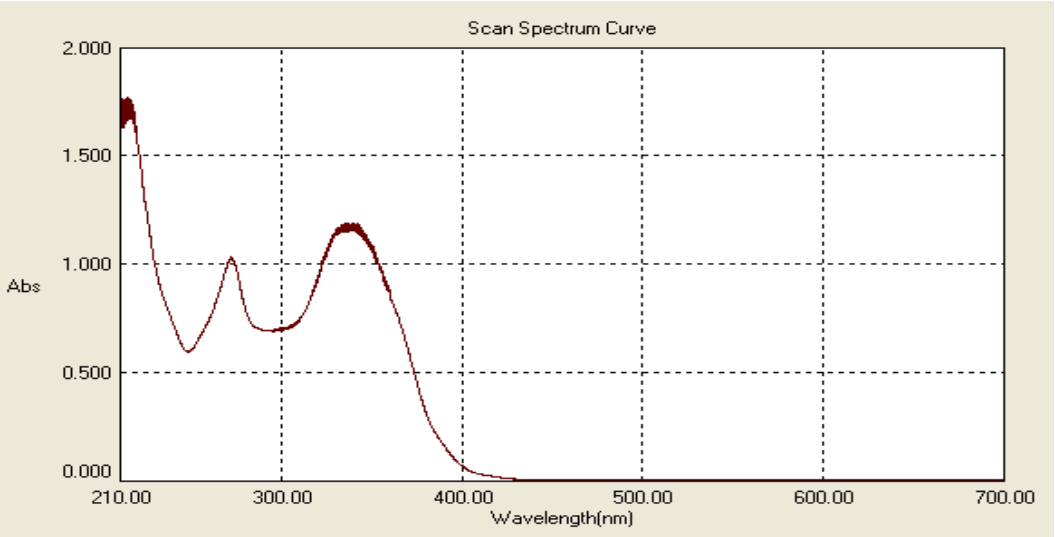
Appendix 7: ^{13}C-NMR spectrum ($CDCl_3$) of α-spinasterol



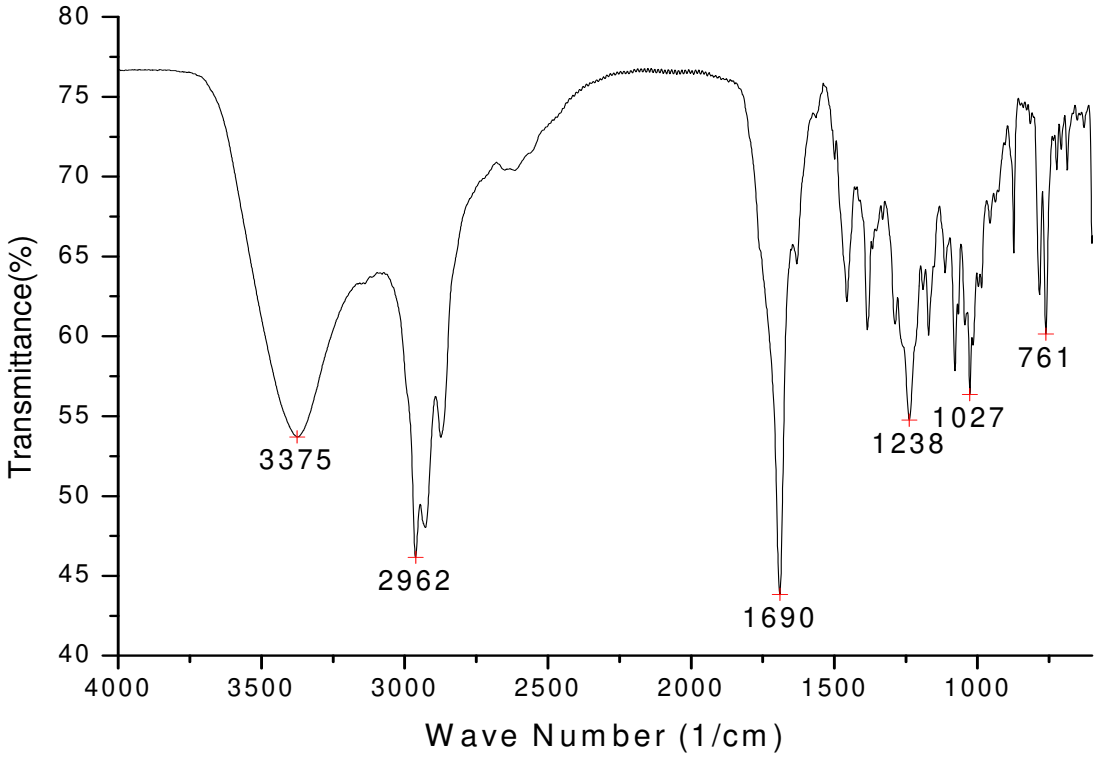
Appendix 8: DEPT-135 NMR spectrum ($CDCl_3$) of α-spinasterol



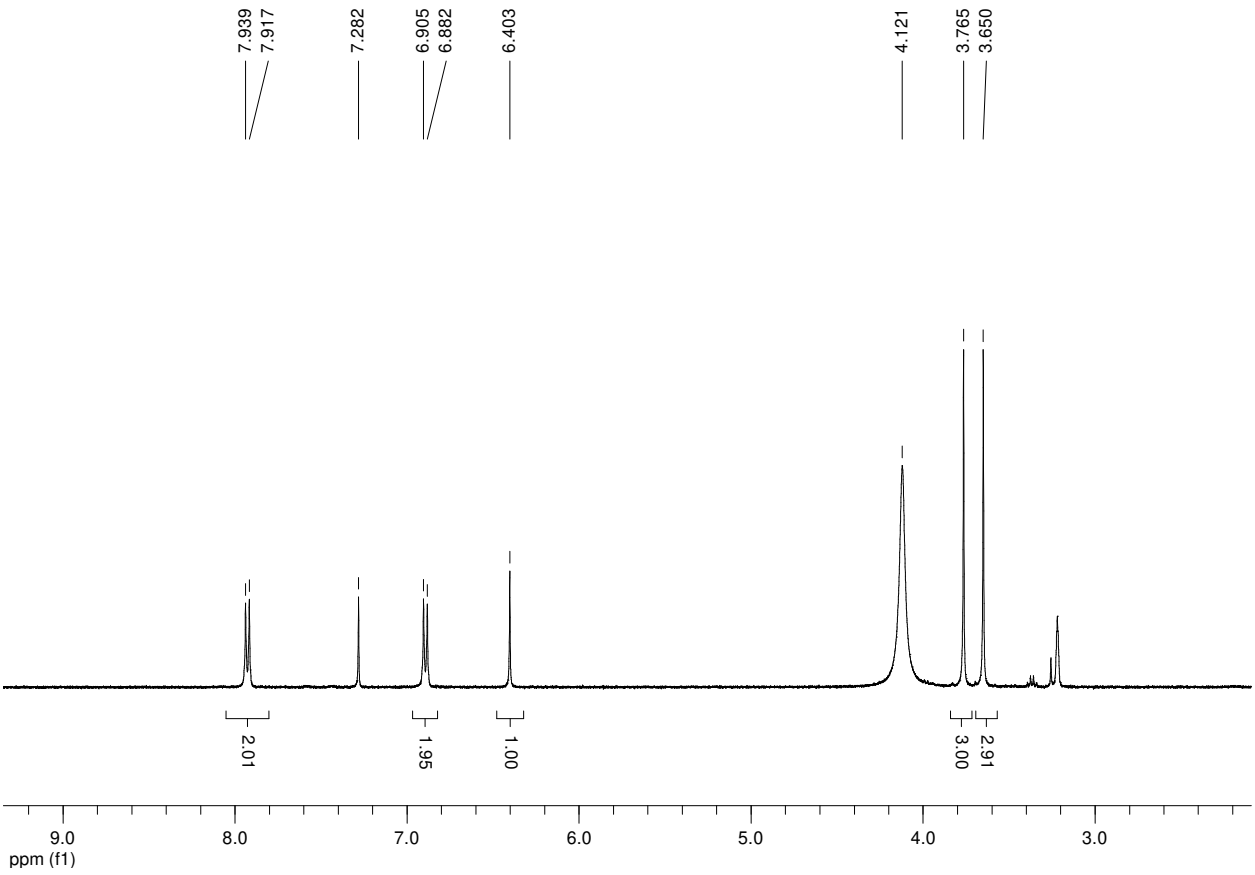
Appendix 9: UV-Vis spectrum of 3,5,7-trihydroxy-6,4'-dimethoxyflavone



Appendix 10: IR spectrum of 3,5,7-trihydroxy-6,4'-dimethoxyflavone



Appendix 11: ^{1}H-NMR spectrum of 3,5,7-trihydroxy-6,4'-dimethoxyflavone



Appendix 12: ^{13}C-NMR spectrum of 3,5,7-trihydroxy-6,4'-dimethoxyflavone