SCHOOL OF GRADUATE STUDIES

JIMMA UNIVERSITY

COLLEGE OF NATURAL SCIENCES

DEPARTMENT OF CHEMISTRY

M.Sc. THESIS

PHYTOCHEMICAL INVESTIGATION AND ANTIMICROBIAL EVALUATON OF SOLVENT EXTRACTS AND ESSENTIAL OILS FROM *PIPER CAPENSE L.F.*

BY

AMIN ZAKIR MAHAMMUD

November, 2022

Jimma, Ethiopia

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A THESIS SUBMITTED TO SCHOOL OF GRADUATE STUDIES JIMMA
UNIVERSITY IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FORTHE DEGREE OF MASTER OF SCIENCE IN CHEMISTRY (General)

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AMIN ZAKIR MAHAMMUD

ADVISORS:
YINEBEB TARIKU (M.Sc. Asst. prof.)
NEGERA ABDISA (PhD,Assot. prof.)

November, 2022

Jimma, Ethiopia

DECLARATION

The data included in this thesis are the result of my investigation and I hereby declare that this thesis is my original work and has not been presented for award of any degree or diploma in any university

Amin Zakir

This M.Sc. thesis has been submitted with our approval as supervisors:

Yinebeb Tariku (MSc.,Asst. Prof)

College of Natural Sciences, Department of Chemistry, Jimma University

NegeraAbdisa (PhD, Assot. Prof)

College of Natural Sciences, Department of Chemistry, Jimma University

November, 2022

Jimma, Ethiopia

Acknowledgment

I sincerely appreciate almighty Lord for his faithfulness, strength and love that gave me from the early academic life up to this master level. I am extremely grateful to my advisors: Mr. Yinebeb Tariku(M.sc., Ass. Prof.) and NegeraAbdisa (PhD)for their valuable comments, guidance, continuous support and patience during my masters' study. It's great pleasure to thank the Department Of Chemistry, College Of Natural Science, Jimma Univesityfor material support for this research work and giving me such a chance.

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Acronyms/Abbreviations

^{13}CNMR	Carbon-13 Nuclear Magnetic Resonance
^{1}HNMR	Proton Nuclear Magnetic Resonance
DEPT	Distortionless Enhancement by Polarization Transfer
DMSO	Dimethylsulfoxide
DPPH	2,2-Diphenyl-1-picrylhydrazyl
FTIRS	Fourier Transform Infrared Spectrophotometer
GC-MS	Gas-Chromatography-Mass Spectroscopy
ROS	Reactive Oxygen Species
TMS	Tetramethysilane
UV	Ultraviolet

Abstract

Medicinal plants are good resources of Lead compounds for drug development. Thus, the aim of this study was to obtain essential oil and crude extracts from different part of *P. capense* and characterize compounds isolated from the crude extracts. The antimicrobial activities of essential oils, crude extracts and isolated compounds were also evaluated against selected bacteria (*S. aureus, E. coli, S. typhi, B. cereu*) and fungi (*C. albicans*) using the disk diffusion method. Gradient extracts (with solvents Pet-ether, chloroform, acetone and methanol sequentially), crude chloroform extracts (only from fruits) and essential oils were obtained using soxhlet, maceration and hydro-distillation methods respectively. Identification of compounds was made using GC/MS data (for essential oils) and spectral methods (NMR for the isolated compounds).The crude chloroform extracts of fruit had a yield of 106 g (13.42%) while the oils yields were 0.5% - 3% from different parts of the plant.35 - 88 compounds were identified in essential oil of different parts of *P. capense*. Oxygenated sesquiterpenes were the most abundant components in all the oils analyzed accounting to 33.76 - 48.53%. Column chromatographic analysis gave 54 fractions, which upon decantation offered two crystalline compounds characterized as Capentin (Compound 1) and 5-hydroxy-7,4'-dimethoxyflavone (compound-2). The antimicrobial activities of essential oil and crude extracts were in the range of 12 mm (fruit chloroform extracts) to 7 mm (in some parts of extract) with the highest and lowest results for bacterial and fungal strains respectively. The essential oils of fresh fruit, dry leaf and fresh root of *P.capense* and methanol extracts of stem bark are responsible for the antifungal activity. The antimicrobial activity of compound-1was found to be 9 mm for both (*S. typhi* and *B. cereus*)while that of compound-2 was found to be 7 mm for *S. typhi*. The present study indicates that fresh fruit, dry leaf and fresh root of *P.capense* Can be a source of antibiotic agents. The study provides scientific support for the ethno-medical use of different parts of *P. capense* as an antimicrobial agent.

Key word: Phytochemical, Antimicrobial activity, 5-hydroxy-7,4'-dimethoxyflavone, Capentin, *P. capense*

1. INTRODUCTION

1.1 Background of the study

Natural products, such as plants extract, open a new horizon for the discovery of new therapeutic agents [1].The use of traditional medicine and medicinal plants in most developing countries, as a normative basis for the maintenance of good health, has been widely observed and about 80% of the world's population relies on herbal medicines [2]. Plants contain a wide range of chemical compounds that can be used to treat chronic as well as infectious diseases [3]. Medicinal plants are used by the people as: anticancer, antimicrobial, antioxidant, antifungal antidiarrheal, anti-inflammatory, antiviral, analgesic and wound healing activity. Additionally, these compounds have been used to treat constipation, salmonella infection, ulcers, stomachache, dyspepsia, gastritis, syphilis, skin infection and gonorrhea [4].

Medicines derived from plants have used by people since antiquity [5]. Excluding the combinations with chemically defined active substances or isolated constituents, the World Health Organization (WHO) defines herbal medicines as containing plant material in crude or processed state as active ingredients, which may include recipients. Synthetic drugs dominated the market with the development of the pharmaceutical industry [6]. Even from these synthetic drugs, around 25% of currently prescribed medicine derived from higher plant compounds [7, 8].

The major concern of infectious diseases in Africa [9] that described as the primary cause of premature death of almost 50,000 people each days are claiming the lives [10]. Due to the emergence of strains resistant to currently available antimicrobial agents, the therapy of microbial infection is a problem[11]. One such example is the Methicillin-resistant *Staphylococcus aureus* (MRSA), which is often linked with nosocomial infections [12]. An antibiotic that reserved as a treatment of last resort, there have been reports where these organisms were resistant even to vancomycin [12]. Because of their remarkable genetic plasticity, resistant strains have emerged, as well as the heavy selective pressures of antimicrobial use that compel them to mutate [13]. Due to the mobility of the world populations, these resistant microorganisms are able to spread easily [13]. Against certain pathogenic bacteria, an alarmingly narrow range of antimicrobials is still effective and has a negative impact on both human health and the economy[7].

An important starting point to obtain new knowledge regarding plant compounds and their bioactivities were traditional medicinal use of plants. This may contribute to modern medicine. An aid in the search for novel products against various human diseases were the knowledge of traditional medicine practice [7]. The present study used to add values to the modern medicine and traditional medicinal use of plants.

1.2 Statement of the problem

*P. capense*is named “timiz” in Amharic, thatrefers to its special shape. Timiz (*P. Capense*) known as long black pepper. It is an endemic plant of East Africa found in wet highlands, *P. capence*produced traditionally for human consumption (as spice) and medical use. It linked to Ethiopian culture in several different aspects: it is gathered and transformed in traditional ways, the fact that it used in many national dishes. The Piper species have high commercial, economical and medicinal value.It is used traditionally to cure both human and animal diseases like ‘curtomat’ (pins and needles in one's legs) ‘wugat’(breathing problems) ‘kurtat’ (digestive problems) [14]. But most people used antibiotic that are produced in pharmaceutical industry, which have side effects.The previous study aimed to essential oil analysis of the fruit, leaf and stem of *P. capense*and root-bark and fruit extracts from *P. capense*. There are no studies reported in determining the antimicrobial activity of fruit extracts of *P. capense*that geographically located in a given area. Thus, the present study aims to perform the extraction of essential oil,fractionation of the Crude extracts of fruit (maceration)andsoxhlet extract of different partsof *P. capense.* And identifying phytochemical constituentsof fruit extracts andsubject to the microbial activity.

1.3 Objective of the study

1.3.1 General objective

- ✓ To identify chemical constituents in essential oil, crude extracts of *P. capense* andantimicrobial activities of essential oil,crude extracts(macerate and soxhlet) and isolated compounds.

1.3.2 Specific objectives

- ✓ To determine the yield of Essential oil and crude extracts from root, stem-bark, leaf and fruitof *P. capense.*
- ✓ To isolate pure compounds from crude extracts of fruit of *P. capence,* using column chromatography.
- ✓ To analyze the composition of essential oils using GC-MS
- ✓ To characterize the pure compounds isolated from crude extracts using NMR.
- ✓ To evaluate biological activities of essential oil, crude extracts and isolated compounds on selected bacteria: Gram positive (*B. cereus* and *S. aureus*) and Gram negative (*E. Coli* and *S. typhi*) strains and fungi (*C. albicans*)

1.4 Significance of the study

To identify chemical constituents in essential oil, crude extracts (by maceration and soxhlet) of *P. capense* and isolated compounds and their antimicrobial activities. Add value on the information that already exists about a variety of local plants that have specific medicinal properties an ethno-botanical study used. Secondary metabolites produced from Plants that are bioactive, which provide them with protection against external aggression from bacteria, fungi and viruses. This then suggests that plants contain biologically active substances protect humans and crop plants against microbial and other disease causing agents. The possibility of relying on plant extracts for use in medicine continually being investigated [15].

- ✓ Aware the society to use the *P.capense*as medicinal plant for the microbial infections for both plant and animals.
- ✓ For scientific society use as the base line for production of some chemical and medicine in chemical industrial.

2. LITERATUREREVIEW

1.5 Botanical Aspects of *P. capense*

2.1.1 The Family*Piperaceae*

The *Piperaceae* family is assigned in the order of *Piperales* and widely distributed in the tropics and subtropics regions. The family has about six genera: *zippelia, manekia, verhuellia, saururaceae, peperomia* and *piper* [16]. *Piper* and *Peperomia* contributed the most number of species in this family with the latter used as ornamental plants [17].

2.1.2 The Genus Piper

An estimated 2000 species of genus Piper are there [18]. Piper species are often shrubs, herbs or lianas commonly found in forest under growth, which distributed in the tropical regions of all the major continents [19, 20]. The Piper species found in the American tropics with the greatest diversity of an estimated 700 species, followed by an estimated 300 species found in Southern Asia [19]. Piper species are also widely distributed in the southern half of Africa [21]. Some the species are: *P. nigrum, P. guineense, P. longum, P. betle, P. cubeba, P. aduncum, P. amapaense, P. baccans, P. capitarianum, P. cyrtopodum, P. dilatatum, P. erectipillum, P. hostmannianum, P. tuberculatum P. peltata, p. sarmentosum, P. capense*andetc. From the numerous different species of piper known in Africa, four of them are located in Ethiopia: *P. umbellatum P. guineense P. nigrum*and *P. capense* [14].

2.1.3 *Piper capense*

In Ethiopia, *P. capense* known by different vernacular names including Tunjo (Afan Oromo) Turko (Kefinya) and Timiz (Amharic) which indicates a wide distribution of the plant in different parts of Ethiopia. In Ethiopia, the fruits of *P. capense* are used as a spice in many national dishes and known by its sweet aroma. Mostly cultivated and found in natural forest of some localized areas of Ethiopia including Jimma Zone and Kefa(Bongo*)*of the SNNPR [22].

Figure 1.The picture of *P. capense*.

(A) In its natural habitat (B) A closer look on different parts of *P. capence*(C)Leaves andimmature fruit (D) matured fruit (E) Dried fruit

2.2 Traditional medicinal use of *P.capense*

The fruit and other parts of *P. capens*are used for the treatment of various diseases. The therapeutic uses and preparations of *P. capense* in traditional medicine stated bellow (table 1).

Table 1 Reported therapeutic uses and preparations of *P. capense* in traditional medicine.

Therapeutic use	Part used and Preparation	Country [14, 23]
Sore throat, chest complaints, tongue ulcers, venereal disease	Bark - maceration is drunk	NS
Wounds and vaginal discharge	Bark –powdered and applied externally with petroleum jelly	NS
Sterility	Root – Decoction	NS
Paralysis caused by cerebral hemorrhage	Root – ointment made and applied to soles of the feet	NS
Sexual stimulant	Root - eat raw or cooked	Shambala, Pare
Stomach, heart and kidney	Fruit - water infusion is drunk	Europe, Africa
Cough remedy	Fruit – NS	India, East Africa
“Wugat”, “kurtat”	Fruit	Ethiopia
Urinary disorder	powdered leaves and stem barks	N. Ethiopia
Poliomyelitis	Leaves – extracts is drunk	NS
Fever and Stomach-ache	Aerial parts	N. Ethiopia

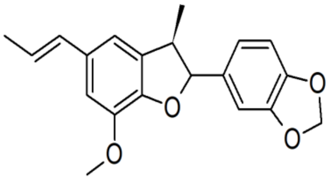
NS = Not specified

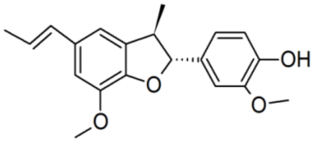
2.3 Phytochemical constituents of*P.capens*.

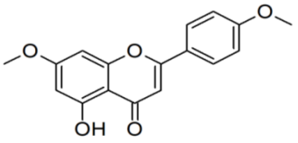
From the Piper species, a number of physiologically active compounds have been isolated: alkaloids/amides, piperolides, propenylphenols, lignans, dihydrochalcones, neolignans, terpenes, steroids, chalcones, flavones and flavanones [20]. Phytochemical analyses have shown that phenolics are the most frequently isolated compounds found in *P. capense*[10]

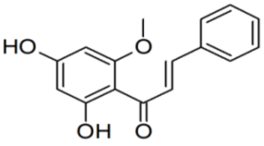
The phytochemical study of *P.capense* resulted in the isolation of different compounds. The identified compounds included three neolignans: licarin B(**1**), licarin A(**2**), and 7-(1,3-benzodioxol-5-yl)-7,8-dihydro-8-methyl-5-(2-propenyl)-furo[3,2-e]-1,3-benzodioxole(**3**); one benzophenanthridine alkaloid, nitidineisocyanate(**4**); two flavonoids amongst which were one flavone, 5-hydroxy-7,4′ -dimethoxyflavone(**5**)and one chalcone, cardamomin(**6**); three steroids amongst which were two sterols, β-sitosterol(**7**) and stigmasterol(**8**) and one glycoside, β-sitosterol 3-O-β-D-glucopyranoside(**9**),sesquiterpenoid: capentin (**10**),and two triterpenes: oleanolic acid (**11**)and lupeol(**12**)[24].

The essential oils from the fruits of *P. capense*, grown in Cameroon were rich in α-pinene (5.6 - 12.3%) and β-pinene (6.7 - 59.3%). Theessential oil obtained from the leaves of *P. capense* was largely composed of α-pinene (12.8%), β -pinene (50.1%) and β-caryophyllene (12.4%). Theoil from the stems of *P. capense* contained mostly α-pinene (14.3%) and β-pinene (61.4%) [25].









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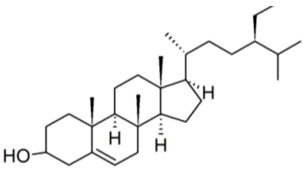

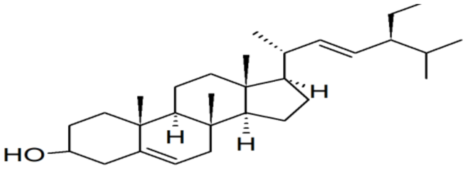

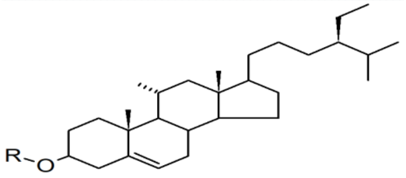

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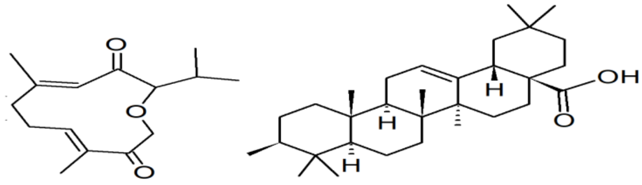

10 11 12

Figure 2 Structural of some chemicals extracted from *Piper capense*

2.4 Biological activity of *piper* species

2.4.1 Antibacterial activities

The leaf methanol extracts in disc diffusion assay have exhibited antibacterial activity against two Gram positive strains, multi-resistant *S. aureus,* and three Gram negative strains, *K. pneumoniae, P. aeruginosa* and *E. coli* [26]. Leaf extracts prepared in 95% ethanol have antibacterial activity against *S. aureus* and *B. subtilis* (zones of inhibition ranging 8–12 mm), whereas such extracts have not shown activity against *E. coliW*, *E. coli M* and *P. aeruginosa* [27, 28]. Leaf oil and 95% leaf ethanol extracts are inactive against *S. mutans, Lactobacillus sp., Aggregati bacteria ctinomycetemcomitan*s and *C. albicans*[29]. Hexane extract of fruit (pellitorine, sarmentine, 1-piperettyl pyrrolidine, pellitorine, guineensine, brachyamide B and 1-(3,4-methylenedioxyphenyl)-1Etetradecene) have exhibited antimycobacterial activity [30].

2.4.2 Antifungal activity

Theethanol extract of spices, including *P. sarmentosum*, *in vitro* tested antifungal activity against *Aspergillus niger, Aspergillus. oryzae* and *Penicilliumsp*.The obtained results demonstrated that ethanol extractof *P. sarmentosum* only showed antifungal activity against A. niger, but not *A. oryzae* and *Penicilliumsp* [31]. The ethanol extract including *P. sarmentosum*tested against selected organisms such as *Aspergillus fumigatus* and *C. albicans*. The result indicated that the ethanol extract *P. sarmentosum* was active against *C. albicans* and A. fumigates [27].

2.4.3 Antioxidant activities

Aqueous and ethanol extracts of root, stem, leave and fruit have been investigated for antioxidant activity using two in vitro models, β-carotene linoleate model and DPPH model, and these studies have shown that ethanol extracts of leaves and fruit are having good antioxidant activity

[32]. Aqueous extracts of the plant have also exhibited ferric reducing antioxidant power due to their high phenolic contents [33]. In vivo models: Aqueous extract of whole of the plant found to be effective in relieving streptozotocin-induced oxidative stress in 28-day orally treated rats with a dose, 0.125 g/kg/day [34]. Ethanol extracts of leaves and fruit in two dose strengths, 250 and 500 mg/ kg/oral, have shown preservation of antioxidant activity against CCl_4-induced oxidative stress in rats [35].

The antioxidant activity of the ethyl acetate extract, essential oil and 5-Hydroxy-7,4'-dimethoxyflavone of the fruits of *P. capense* were measured by bleaching of the purple-colored solution of 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) at four different concentrations (100, 50, 25 and 12.5 µg mL-1). The DPPH assay indicated that the ethyl acetate extract and 5-hydroxy-7,4'-dimethoxyflavone displayed pronounceable free radical scavenging activity. The essential oil also showed significant radical scavenging activity with percent inhibition of 70, 58, 50 and 45% at 100, 50, 25 and 12.5 µg mL- , respectively. As revealed from the results, 5-hydroxy-7,4'-dimethoxyflavone had the highest (84%) radical scavenging activity which turned out to be comparable with ascorbic acid (90%) used a positive control. This is most likely due to its strong ability of donating an electron and phenolic hydrogen to DPPH radical, which was visualized by immediate discoloration of the purple DPPH solution to yellow compared to the essential oil and the ethyl acetate extract of the fruits of *P. capense*. Therefore the antioxidant activity of the ethyl acetate extract partly accounts to the presence of 5-hydroxy-7,4'-dimethoxyflavone [36].

2.4.4 Insecticidal activity

Leaf oil has shown inhibitory activity against *Artemiasalina* (LC_{50} 35.2 µg/mL) and 100% mortality in subterranean termite (*Coptotermes sp*.) within 2 days, at 1% concentration. Moreover, the three isolated compounds such as *caryophyllene*, *myristicin* and an unidentified one from the oil have shown significant inhibitory activity against subterranean termite, *Coptotermes sp*. [37].

The high mosquitocidal activity of DCM fraction of O. africana is consistent with the report of Broussalis et al. for the dichloromethane extracts of *Tagetes erecta L*. (Fabaceae/Compositae) which showed a significant pesticidal activity against *Sitophilus oryzae*. In the current study, the higher activity of DCM fraction of *O. africana* and EtOAc fraction of *P. capense* may be due to the presence of bioactive components against adult stage of An. arabiensis. In line with a study by Asghari et al., DCM is a semi-polar solvent that had the ability to dissolve polar and non-polar compounds in the extract of *O. africana*. This indicates that the bioactive components in this plant had adulticidal properties against An. arabiensis and were better soluble in DCM than in other solvents [38].

2.4.5 Anticancer activity

P. capense is a source of potent cytotoxic botanicals and phytochemicals acting on various types of cancer cells including MDR phenotypes. The active constituents of *P. capense* include licarin B, licarin A, nitidine isocyanate, 5-hydroxy-7,4′-dimethoxyflavone or cardamomin. PCFb and neolignan 2 induced apoptosis in CCRF-CEM cells mediated by caspase activation, MMP alteration and increased ROS production[39]

The methanol extract of this plant has pre-viously shown good cytotoxic effects towards a panel of cancer cell lines, including HL60, CCRF-CEM and HL60AR leukemia cells, MDA-MB231 and MDA-MB231/BCRP breast adenocarcinoma cells, HCT116 p53+/+ and HCT116 p53− /− colon adenocarcinoma cells, U87.MG and U87. MGΔEGFR glioblastoma cells, and HepG2 hepatocarcinoma cells[40].

3. MATERIALS AND METHODS

3.1 Chemicals and Apparatus

Chemicals such as Water(for hydro-distillation), Chloroform, Acetone, Petroleum Ether, Methanol, Ethyl acetate and n-hexane(for Soxhletextraction), anhydrous Sodium-sulfate(to remove water from the essential oils). Gentamycine,Clotrimazole andDMSO(as positive and negative control for antimicrobial activities),silica gel 60-120 mm mesh size(for column chromatography),Mueller Hinton agar and nutrient broth as culture media(for antimicrobial test). Apparatus such as Clevenger apparatus, refrigerator, Digital Weight Balance, pestle and mortar, condenser, stand with base, evacuated rotary evaporator(HeidolphGermany, laboratory 4000) (to concentrate the extracts), round bottom flax, beaker, test tube, Uv chamber(254 and 365nm), TLC, gas jar, pitter dish, oven(N5OC GENLAB WIDNES, England), incubator(Gene lab incubator) (for antimicrobial test), hood, glass column chromatography, NMR spectra(Bruker advance 400MHz)(for the characterization of isolated compound)and GC-MS(Agilent 8890 GC system connected with 5977B GC/MSD) (to analyzethe composition of essential oils).

3.2 Plant material collection and sample preparation

The all part (fruit, leaf, stem bark and root) of *p. capense*was collected separately from GerimaGudakebele, DedoWereda, Jimma Zone, Oromia Regional state, south west of Ethiopia. Plant material was inspected for any contamination and some of the freshly collected parts(fruit, leaf, stem bark and root) of *p. capense* used for essential oil extraction.Someparts (fruit, leaf) are air-dry at room temperatureand ground to a fine powder. Some ofdried fruit and leaf were also used for essential oil and the rest was stored in air tight plastic bags in a dark area until further extracts was prepared.

3.3 Extraction of plant materials

3.3.1 Essential oil extraction

A mass of 300 g each (fruits, leaf, stem bark, roots) and 100 g of each dried (fruit and leaf)of *p. capense*was cleaned and placed in a round bottom flask fitted with condenser and then hydro distilled using Clevenger apparatus for 3 h. The residual water was removed by addition of anhydrous $Na_2SO_4$and the yields (v/w %) were calculated. Then, the essential oils were packed in a test tube covered with aluminum foil and stored in a cool dry place until they were analyzed by the GC-MS separation technique.

3.3.2 Crude extracts

A mass of 50 -55 g of each powdered plant material was sequentially extracted with 220 - 250mL of each:Petroleum ether, chloroform, acetone and methanol using Soxhlet extraction method. In each case, the extraction continued until the siphon became colorless. The extractswere filtered and concentrated under rotary evaporator at 40℃ and kept for antimicrobial activities.

A mass of 795g of powdered fruit of *P. capence* was macerated with 1.5L of chloroform for 24 hr and the solvent filtered and concentrated under rotary evaporator at 40 ℃. The extractionsrepeated three times and the extract was kept for the column chromatographic separation.

3.4 Isolation of compounds

Isolation of pure compounds was carried out on chromatographic column (500 ml), packed with 200 g silica gel. A 40 g portion of the crude chloroform extract was adsorbed on 40 g silica gel (60-120 mesh size) and loaded in to Chromatographic column and eluted with 50 ml of different ratio of petroleum ether and ethyl acetate (that is 100%:0%, 99%:1%, 98%:2% to 50%:50%) and a total of 54 fractions were collected. Selection of solvent system for elution of the column was determined after carrying out preliminary TLC analysis of the extract using various binary solvent combinations that show best separation and distinct spots were used for elution of column chromatographic separation. Under UV chamber (at 365 and 245 nm) visualization of colored spots of fractions were analyzed with TLC using naked eye. Then fractions that showed the same TLC profiles (with best matching color and Rf values) were combined and concentrated to dryness under reduced pressure using rotary evaporator.

3.5 GC-MS Analysis

GC was performed on Agilent 8890 GC system using HP-5 fused silica capillary column (30 m × 0.25 mm i.d.).GC-MS analysis was carried out using an Agilent 8890 GC system connected with Agilent 5977B GC/MSD mass selective detector at 70 eV. The oven was programed at 60-

280ºC at a rate of 10ºC/min using helium as the carrier gas), injector and detector (FID) temperature were 220ºC-270ºC, respectively and GC parameters were the same as above

GC-MS analysis was carried out using an Agilent 8890 GC system connected with Agilent 5977B GC/MSD mass selective detector at 70 eV. ionization energy HP-5 column (30 m × 0.25 mm, 0.25μm thickness) and flow rate of 1 mL $\min^{-1}$. Injector and detector temperatures were 270℃and 300℃ respectively. Oven temperature programmed from 60 to 280℃ (at a rate of 10℃ $\min^{-1}$) helium as the carrier gas and the initial temperature was detained at 60℃ for 1 min and increased at 10℃ $\min^{-1}$ to 180℃, then ramped at 20 ℃ $\min^{-1}$ to 280℃ and held there for 15 min [25]. Each crude essential oil (essential oils of flesh fruit, leaves, stem bark and root of *P. capence*and dry fruit and leaf of *P. capence*) were mixed with n-hexane (1:100 v/v). Then, injected using a split less ratio (1:10). The constituent of samples determined by comparison of the relative retention time and the mass spectra of samples compositions with retention times indicated by the literature and mass spectra available in the data library. Percentage of relative abundance of each component was calculated as:

$$\text{Relative abundance} = \frac{\text{pick area of compound}}{\text{total sum of pick area}} \text{ x } 100$$

3.6 Characterization of isolated compounds

The isolated compounds were characterized by one-dimensional NMR spectroscopy techniques usingdeutrated chloroform as a solvent. The NMR spectroscopy technique was done at Addis Ababa University.

3.7 Antimicrobial Activity Test

The antimicrobial activity test was carried using Agar disc diffusion method. The prepared paper discs from Whatman no -4 paper were approximately 6 mm in diameter. Most of the solutions prepared were 300 mg/ml chloroform extract of crude of *P. capence*, soxhlet extracts of fruit, leaf, stem bark and root of *P. capence*and 100 mg/ml of isolated compounds and 100 μl/ml of essential oil were evaluated against four bacterial strains Gram positive (*B. cerneus and S. aureus*) and Gram negative (*E. Coli and S. typhi*) and one fungal strain (*C. albicans*). 100 μl/ml of standard drugs, antibiotic (Gentamicine) and antifungal (Clotrimazole) were used as positive control while negative controls was set using disc impregnated with DMSO solvent. The discs were immersed in the solutions and thenplaced on the inoculated agar surface to allow diffusion.

The microbial strains were selected based on their availability, previous use reported in similar studies and traditional medicinal uses of the plant concerning microbial infection. Both bacterial and fungal strains were obtained from Jimma University Biology Department Microbiology Laboratory. The *in vitro* antimicrobial activities of essential oil, crude Soxhlet extracts of different parts of *P. capence*, chloroform extract of fruit of*P. capence,* compound 1and compound 2 evaluated against selected bacterial strains and fungal strain. The incubation of both bacteria and fungus strains was performed for 24 hrs to obtain fresh growing of the two microbial strains. The plates were then inverted and incubated at 37℃ for 24 hrs for both strains. After incubation period, inhibition zone diameter measured in millimeter.

4. RESULTS AND DISCUSSION

4.1 Percentage yieldof extracts

4.1.1 Essential oil

The cleaned Fresh fruit, dried fruits, Fresh leaf, dry leaf, stem bark and root of *P. capence*powdered and placed in a round bottom flask fitted with condenser and then hydro distilled using Clevenger apparatus for 3 h. The amounts of the extracted essential oils and their percentage yield of different parts of the plant are shown below (Table 2).

Table 2 . Percentage yields of Essential oils from different parts of *P. capence*

Plant material Used	Total Mass of plant material(g)	Total Volume of oil extracted (ml)	%yield (v/w)	Color
Fresh fruit	300	3	1	Light Yellow
Dry fruit	100	3	3	Y. green
Fresh leaf	300	2.5	0.83	Light Green
Dry leaf	100	2	2	Green
Stem bark	300	1.5	0.5	Y. green
Root	300	1.5	0.5	Greenish

Even though, the same extraction conditions (solvent, contact time, date of collection, date of the distillation and site of collection) were used for the hydro-distillation of different parts of *P. capence*, the fresh fruit and dry fruit yielded greater amount of essentials oil than the rest. More over the essential oil yield shows different colors for fresh and dried samples. In comparison to the previous study, the present study gave more yield for fruit, leaf and stem of *P. capense*. Similar studies carried in Ethiopia on fruits of the same plant gave variable results, some with comparable yield (2.6-2,8 %)[36] while some are with too lower yield (0.64)[25].This may be due to difference in geographic location Crude extracts

The different parts of *P. capense*(fruit, leaf, stem bark and root) were extracted sequentially Soxhlet extraction. The extract obtained then concentrated with Rota evaporator and dried. The percentage yield of Soxhlet extract were Shown below (Table 3)

Table 3 Percentage yields of crude gradient extracts of *P. capense* obtained by Soxhlet method.

Part used	Amount used (g)	Extraction yield (%)			
		PetroleumEther	Chloroform	Acetone	Methanol
Dry Fruit	54	15.2	4.7	3.7	6.7
Dry Leaf	54	9.68	15.15	4.26	10.74
Dry Stem bark	44	3.18	2.27	0.45	5.9
Dry Root	50	0.48	0.78	0.48	1

As shown In Table 3 above, the highest yields obtained were 15.2% (for Petroleum etherextract of fruit) , 15.15% (from Chloroform extract of leaf) and 10.74% (for Methanol extract of the leaf). Moreover, the leaves are the richest source of organic solvent extractable components (39.83 %) while the roots were the poorest (2.74%).

The crude chloroform extracts obtained from fruit of *P. capense*by macerations then concentrated with Rota evaporator and dried and yielded 106 g (13.42%).

In comparison the chloroform extract gave more yield thann-hexane and ethyl acetate extract. In the previous study on fruit of *P. capense*,n-hexane and ethyl acetate extract bymaceration technique yield 1.3% and 2.3%, respectively [41], but in the present study chloroform extract of the fruit of *P. capense*yielded 13.42%.

4.1 GC-MS Analysis

The oil was analyzed by GC/MS analysis and the constituents were identified based on their pick areas and retention time (Rt) values. The oil analysis result from fresh fruit, dried fruit, fresh leaves, dried leaves, stem bark (fresh) and roots(fresh) of *P. capense*enables identification of 41 (79.63%), 36 (79.39%), 37 (63.26%), 88 (81.58%) , 36 (75.93%) and 35 (76.0%) compounds respectively (Table 4).

Table 4 summery of GC MS data for essential oil from different parts of *P. capense*

SN	Name	RT	PCFF %	PCDF %	PCFL %	PCDL %	PCSB %	PCR %
1	2(10)-Pinene	6.49	-	-	-	-	-	5.37
2	3-Carene	6.51	1.43	3.43	2.54	-	1.71	11.31

3	(3E)-3,7-dimethylocta-1,3,7-triene	6.99	-	-	-	-	-	3.23
4	1-Methylene-4-(1-methylethenyl) cyclohexane	7.93	4.02	6.02	6.04	-	5.60	5.50
5	alpha.-Fenchylacetate	8.51	-	-	-	-	-	-
6	alpha-Phellandrene	9.01	1.59	3.81	-	1.22	-	-
7	(1R,4S,4aR,5S,8aS)-5-(dioxidanyl)-1,6-dimethyl-4-propan-2-yl-1,2,3,4,4a,5,8,8a-octahydronaphthalene	10.14	-	-	-	2.88	-	-
8	Eucalyptol	10.14	-	-	1.76	-	1.77	3.38
9	Bicyclo[3.1.0]hexan-2-ol, 2-methyl-5-(1-methylethyl)	10.18	7.52	6.35	-	-	-	-
10	2-Amylbuta-2,3-dienylcyclopentane	10.98	-	-	-	-	-	0.70
11	gamma-Elemene	13.57	-	-	-	0.97	2.21	-
12	Linalool	13.59	1.41	1.04	0.74	-	-	1.55
13	Terpinen-4-ol	17.83	0.54	-	-	-	-	0.98
14	L-alpha-Terpineol	18.71	2.02	1.64	0.58	-	-	0.90
15	2-Isopropyl-5-methylcyclohex-3-en-1-one,	22.42	1.53	0.97	-	-	-	-
17	Safrole	24.46	0.72	0.80	-	-	-	-
18	2-Methylbicyclo[4.3.0]non-1(6)-ene	24.54	-	-	-	-	-	7.55
19	exo-2-Hydroxycineole	25.93	0.56	-	-	-	-	-
20	(1S,2E,6E,10R)-3,7,11,11-Tetramethylbicyclo[8.1.0]undeca-2,6-diene	26.89	1.46	-	-	2.70	-	-
21	2-Norbornanol, 1,3,3-trimethyl-	26.89	-	-	-	-	-	1.53
22	(2R,3R,4aR,5S,8aS)-2-Hydroxy-4a,5-dimethyl-3-(prop-1-en-2-yl)octahydronaphthalen-1(2H)-one	26.92	-	-	-	2.40	-	-
23	1,3-Cyclohexadiene, 1-methyl-4- (1-methylethyl)-	27.42	-	-	-	0.62		2.26
24	[(1S,2S,5R)-6,6,7,7-tetramethyl-4-oxidanylidene-3-oxabicyclo[3.2.0] heptan-2-yl]methyl 2,2,2-tris(fluoranyl)ethanoate	27.99	-	-	-	-	-	1.25
25	1,3-Benzodioxole, 5-propyl-	28.45	-	-	-	0.65		
26	Copaene	28.47	-	-	0.53	-	-	-
27	(-)-beta.-Bourbonene	28.80	-	-	0.74	8.09	-	-
28	1-Vinyl-8-oxabicyclo[3.2.1]oct-6-en-3-one	29.03	-	-	-	-	-	0.53
29	(1R,2S,6S,7S,8S)-8-Isopropyl-1-methyl-3-methylenetricyclo [4.4.0.02,7] decane	29.04	-	0.82	0.79	4.42	0.51	
30	gamma.-Selinene	29.05						

31	2-Cyclohexen-1-one, 6-(4-hydroxy-1-methylbutyl)-3-methyl-, (R*,R*)-	29.12	-	-	-	-	-	0.60
32	1-Methyl-1-ethenyl-2,4-bis(1'-methylethenyl)cyclohexane	29.13	0.77	0.61	1.15	-	0.99	-
33	Bicyclo[5.2.0]nonane, 2-methylene-4,8,8-trimethyl-4-vinyl-	30.07	1.35	1.24	2.78	-	-	-
34	(1R,3E,7E,11R)-1,5,5,8-Tetramethyl-12-oxabicyclo[9.1.0]dodeca-3,7-diene	30.09	-	-	-	2.89	-	-
35	Isocaryophyllene	30.09	-	-	-	-	2.70	-
36	cis-p-Mentha-2,8-dien-1-ol	30.39						0.63
37	1,2,4-Metheno-1H-indene, octahydro-1,7a-dimethyl-5-(1-methylethyl)-, [1S-(1.alpha.,2.alpha.,3a.beta.,4.alpha.,5.alpha.,7a.beta.,8S*)]-	30.40	-	-	-	0.78	0.54	-
38	2,7,7-trimethylnorbornan-2-ol	30.90	-	-	-	-	-	0.83
39	(1R,3aS,8aS)-7-Isopropyl-1,4-dimethyl-1,2,3,3a,6,8a-hexahydroazulene	30.92	-	-	1.06	3.30	0.85	-
40	1,4,7,-Cycloundecatriene, 1,5,9,9-tetramethyl-, Z,Z,Z-	31.23	-	-	0.92	0.55		-
41	(1R,1aR,2aS,6R,6aS,7aS)-1,6,6a-Trimethyldecahydro-1,2a-methanocyclopropa[b]naphthalene	31.46	1.62	1.47	2.23	4.82	1.99	
42	Fumaric acid, di(2-methylcyclohex-1-enylmethyl) ester	31.46	-	-	-	-	-	1.45
43	(1S,4S,4aS)-1-Isopropyl-4,7-dimethyl-1,2,3,4,4a,5-hexahydronaphthalene	31.47	-	-	-	2.61	-	-
44	5-Azulenemethanol, 1,2,3,4,5,6,7,8-octahydro-alpha., alpha.,3,8-tetramethyl-, acetate, [3S-(3.alpha.,5.alpha.,8.alpha.)]-	32.00	1.30	-	-	-	-	-
45	Naphthalene, decahydro-4a-methyl-1-methylene-7-(1-methylethylidene)-, (4aR-trans)-	32.00	-	1.25	-	-	-	-
46	1,6-dimethyl-4-propan-2-yl-1,2,3,7,8,8a-hexahydronaphthalene	32.02	-	-	-	-	2.11	-
47	4(10)-Thujen-3-ol, (1S,3R,5S)-(+)-	32.11	-	-	-	-	-	1.38
48	(1S,4R)-1-methyl-4-propan-2-yl-1-cyclohex-2-enol	32.16	-	-	-	2.67	-	-
49	gamma.-Cadinene	32.19	-	-	-	-	-	-
50	(3E,6E)-Nona-3,6-dienyl 2,2,3,3,3-pentafluoropropanoate	32.26	-	-	-	-	-	0.73
51	beta.-Selinene	32.28	1.07	1.20	1.56	1.10	1.55	
52	(3E,5E)-2,6-Dimethylocta-3,5,7-trien-2-ol	32.43	-	-	-	-	-	0.81
53	Bicyclosesquiphellandrene	32.44	0.56	-	1.00	-	0.84	-
54	1,2-Dimethoxy-4-propylbenzene	32.44				0.71		-

55	Cedrol	32.60	4.88	3.79	5.21	-	5.45	-
56	5-Isopropyl-2-methylbicyclo[3.1.0]hexan-2-ol	32.71	-	-	-	-	-	2.31
57	gamma.-Terpinene	32.72	-	-	-	0.88	-	2.87
58	alpha.-Muurolene	32.74	0.53	-	0.62	1.26	1.02	-
59	Bicyclo[3.1.0]hexan-3-ol, 4-methylene-1-(1-methylethyl)-, [1S-(1.alpha.,3.beta.,5.alpha.)]-	32.76	-	-	-	-	-	0.97
60	1,3-Cyclohexadiene, 2-methyl-5-(1-methylethyl)-	33.22	-	-	-	-	-	4.40
61	(2S,3S,6S)-6-Isopropyl-3-methyl-2-(prop-1-en-2-yl)-3-vinylcyclohexanone	33.27	5.96	-	-	2.19	5.42	-
62	(-)-Germacrene D	33.42	-	-	-	7.42		-
63	(3R,3aR,3bR,4S,7R,7aR)-4-Isopropyl-3,7-dimethyloctahydro-1H-cyclopenta [1,3]cyclopropa[1,2]benzen-3-ol	33.42	3.95	11.74	3.12	2.11	5.95	-
64	delta.-Cadinene	33.51	3.86	2.57		1.05	4.55	-
65	Cyclohexanol, 3-ethenyl-3-methyl-2-(1-methylethenyl)-6-(1-methylethyl)-, [1R-(1.alpha.,2.alpha.,3.beta.,6.alpha.)]-	33.63	2.22	4.23	-	-	3.26	-
66	2,6-Dimethoxytoluene	33.65	-	-	-	-		0.55
67	1H-3a,7-Methanoazulene, 2,3,4,7,8,8a-hexahydro-3,6,8,8-tetramethyl-, [3R-(3.alpha.,3a.beta.,7.beta.,8a.alpha.)]-	33.70	0.50	-	-	-	0.69	-
68	Naphthalene, 1,2,3,4,4a,7-hexahydro-1,6-dimethyl-4-(1-methylethyl)-	33.74	-	-	0.70			-
69	Cyclopropanecarboxylic acid, nonyl ester	34.11	-	-	-	-	-	0.65
70	Cyclohexanemethanol, 4-ethenyl-.alpha.,.alpha.,4-trimethyl-3-(1-methylethenyl)-, [1R-(1.alpha.,3.alpha.,4.beta.)]-	34.13	0.52	0.64	0.70	-	0.73	-
71	Longifolenaldehyde	34.33	-	-	0.62	-	-	-
72	2-Pinen-10-ol	34.53	-	-	-	-	-	0.89
73	1,6,10-Dodecatrien-3-ol, 3,7,11-trimethyl-, [S-(Z)]-	34.58	1.18	0.84	2.50	-	2.11	-
74	(2E,4S,7E)-4-Isopropyl-1,7-dimethylcyclodeca-2,7-dienol	34.90	2.21	2.74	3.35	2.47	3.06	-
75	1H-Cycloprop[e]azulen-7-ol, decahydro-1,1,7-trimethyl-4-methylene-, [1ar-(1a.alpha.,4a.alpha.,7.beta.,7a.beta.,7b.alpha.)]-	34.97	1.19	-	1.10	-	1.43	-
76	(-)-Spathulenol	34.97		1.36		5.52		
77	(6,6-Dimethylbicyclo[3.1.1]hept-2-en-2-yl)methyl ethyl carbonate	34.99	-	-	-	1.76	-	-

78	1H-Cycloprop[e]azulen-4-ol, decahydro-1,1,4,7-tetramethyl-,[1aR-(1a.alpha.,4.beta.,4a.beta.,7.alpha.,7a.beta.,7b.alpha.)]-	35.09	0.77	-	0.90	-	1.41	-
79	2-((2R,4aR,8aS)-4a-Methyl-8-methylenedecahydronaphthalen-2-yl)prop-2-en-1-ol	35.09	-	0.81	-	-	-	0.57
80	1,3-Benzodioxole, 5-(2-propenyl)-	35.10	-	-	-	0.69	-	
81	2-Nonanone	35.65	-	-	-	-	-	1.91
82	Cedranoxide, 8,14-	35.67	1.97	-	2.54	-	2.69	
83	(4R,4aS,6S)-4,4a-Dimethyl-6-(prop-1-en-2-yl)-1,2,3,4,4a,5,6,7-octahydronaphthalene	35.69	-	-	-	2.01	-	-
84	beta.-Oplopenone	35.74	-	4.74	-	1.14	-	-
85	4,4-Dimethyladamantan-2-ol	35.94	-	-	-	-	0.75	-
86	Isospathulenol	35.94	-	-	0.62	-	-	-
87	2'-[(N-methylamino)methyl-d1]phenyl 4-toluene-d3-sulfonate	36.21	-	-	-	-	-	1.88
88	Dehydroxy-isocalamendiol	36.24	2.35	2.96	2.59	-	-	-
89	3a(1H)-Azulenol, 2,3,4,5,8,8a-hexahydro-6,8a-dimethyl-3-(1-methylethyl)-, [3R-(3.alpha.,3a.alpha.,8a.alpha.)]-	36.25	-	-	-	-	3.34	-
90	alpha.-Corocalene	36.44	-	-	-	1.36	-	-
91	Tricyclo[4.4.0.0(2,7)]dec-3-ene-3-methanol,1-methyl-8-(1-methylethyl)-	36.44	-	0.93	1.35	-	-	-
92	5-Hydroxymethyl-1,1,4a-trimethyl-6-methylenedecahydronaphthalen-2-ol	36.45	0.69	-	-	-	0.85	-
93	Isoborneol	36.54	-	-	-	-	-	1.26
94	alpha.-Bisabolene	36.57	-	-	-	1.70	-	-
95	tau.-Muurolol	36.58	2.06	0.69	2.13	0.82	2.66	
96	Bicyclo[2.2.1]heptan-2-one, 1,7,7-trimethyl-, (1S)-	36.65	-	-	-	-	-	1.33
97	tau.-Cadinol	36.66	0.61	-	0.68	0.88	0.99	
98	Borneol	36.86	-	-	-	-	-	1.20
99	(5S,8R,8aS)-8-ethyl-5-propyl-1,2,3,5,6,7,8,8a-octahydroindolizine	36.90	-	-	-	1.93	-	-
100	6-Isopropenyl-4,8a-dimethyl-1,2,3,5,6,7,8,8a-octahydro-naphthalen-2-ol	37.05	-	-	0.53	-	-	-
101	Tricyclo[6.3.0.0(5,7)]undecane, 1,8-epoxy-2,6,6,9-tetramethyl-	37.09	0.76	0.54	-	-	-	-
102	beta.-Guaiene	37.20	-	-	0.53	-	-	-
103	Bicyclo[3.1.1]heptan-3-one, 2,6,6-	37.49	-	-	-	-	-	1.18

	trimethyl-, (1.alpha.,2.beta.,5.alpha.)-							
104	2-Hydroxymethyl-2,6,8,8-tetramethyltricyclo[5.2.2.0(1,6)]undecane	37.50	-	1.72	-	-	1.97	-
105	1-Naphthalenol, 1,2,3,4,4a,5,6,8a-octahydro-4a,8-dimethyl-2-(2-propenyl)-	37.53	-	-	1.57	-	-	-
106	Lactaropallidin	37.53	1.54	-	-	-	-	-
107	2(3H)-Benzofuranone, 6-ethenylhexahydro-3,6-dimethyl-7-(1-methylethenyl)-, [3S-(3.alpha., 3a.alpha.,6.alpha.,7.beta.,7a.beta.)]-	37.68	0.61	-	-	-	-	-
108	3-(1,5-Dimethyl-hex-4-enyl)-2,2-dimethyl-cyclopent-3-enol	37.68	-	-	1.04	-	1.08	-
109	2,4,6-Octatriene, 2,6-dimethyl-,(E,Z)-	37.73	-	-	-	-	-	1.40
110	(3Z)-3-Hexenyl 2-methylpropanoate	37.81	-	-	-	2.10	-	-
111	6-Isopropenyl-4,8a-dimethyl-4a,5,6,7,8,8a-hexahydro-1H-naphthalen-2-one	38.54	3.64	3.23	1.22	-	2.60	-
112	7-Octylidenebicyclo[4.1.0]heptanes	38.61	-	1.56	-	-	-	
- 113	(1,5,5,8-Tetramethyl-bicyclo[4.2.1]non-9-yl)-acetic acid	38.85	0.82	-	-	-	-	-
114	1H-3a,7-Methanoazulene, octahydro-3,6,8,8-tetramethyl-, [3R-(3.alpha., 3a.beta.,6.alpha.,7.beta.,8a.alpha.)]-	38.87	-	0.54	-	-	-	-
115	Spiro[2.5]octane, 5,5-dimethyl-4-(3-oxobutyl)-	39.08	-	0.93	-	-	-	-
116	3-buten-2-one, 4-(5,5-dimethyl-1-oxaspiro[2.5]oct-4-yl)	39.55	-	0.51	-	-	-	-
117	Isolongifolol	39.56	1.35	-	-	-	-	-
118	4-(3,3-Dimethyl-but-1-ynyl)-4-hydroxy-2,6,6-trimethylcyclohex-2-enone	40.09	0.87	1.14	-	-	-	-
119	6-Isopropenyl-4,8a-dimethyl-1,2,3,5,6,7,8,8a-octahydronaphthalene-2,3-diol	40.51	-	0.77	-	-	-	-
	Number of compounds identified (% contribution)		41 (74.51)	36 (79.63)	37 (58.04)	88 (80.67)	36 (75.38)	35 (75.84)
	Monoterpene hydrocarbons		7.51	14.00	9.20	12.31	8.11	21.86
	Oxygenated monoterpenes		17.43	12.46	4.51	14.58	4.13	1.06
	Sesqueterpene hydrocarbons		14.76	14.30	23.15	29.39	24.70	16.55
	Oxygenated sesqueterpenes		43.43	40.10	35.02	41.13	48.53	33.76
	Diterpene hydrocarbons		-	-	-	-	-	-
	Oxygenated diterpenes		0.22	0.04	0.20	0.03	0.18	0.23
	Others		16.65	19.10	27.92	2.56	14.35	26.54

Key: RT= Retention time, PCFF= *P. capense*fresh fruit, PCDF = *P. capense*dry fruit, PCFL=*P. capense*fresh leaf, PCDL= *P. capense*dry leaf, PCSB= *P. capense*stem bark, , PCR= *P. capense*root

As shown in table 4, the oxygenated sesquiterpenes were the most abundant components in all the oils analyzed accounting to 33.76 - 48.53% the total compounds identified. The three major compounds identified in each oil were Bicyclo[3.1.0]hexan-2-ol, 2-methyl-5-(1-methylethyl)-, (1.alpha.,2.beta.,5.alpha.)-(7.52%), (2S,3S,6S)-6-Isopropyl-3-methyl-2-(prop-1-en-2-yl)-3-vinyl-cyclohexanone (5.96%), Cedrol (4.88%)(in fresh fruit), (3R,3aR,3bR,4S,7R,7aR)-4-Isopropyl-3,7-dimethyloctahydro-1H-cyclopenta[1,3]cyclopropa[1,2]benzen-3-ol(11.74%), Bicyclo[3.1.0]-hexan-2-ol, 2-methyl-5-(1-methylethyl)-, (1.alpha.,2.beta.,5.alpha.)-(6.35%), 1-methyl-1-ethylene-2,4-bis(1-methylethenyl)cyclohexane (6.02%) (in dried fruit), 1-methylene-4-(1-methylethenyl)cyclohexane(6.03%), gamma-Cadinene (5.22%), Cedrol (5.21%) (in fresh leaves), (-)-beta-Bourbonene (8.09%), (-)-Germacrene D (7.41%), (-)-Spathulenol (5.52%)(in dried leaves), (3R,3aR,3bR,4S,7R,7aR)-4-Isopropyl-3,7-dimethyloctahydro-1H-cyclopenta[1,3]cyclo-propa[1,2]benzen-3-ol(5.95%),1-methyl-1-ethylene-2,4-bis(1-methylethenyl)cyclohexane(5.60%), Cedrol (5.45%) (in fresh stem bark) and 3-Carene (11.30%),2-Methylbicyclo[4.3.0]non-1(6)-ene (7.55%), 1-methyl-1-ethylene-2,4-bis(1-methylethenyl)cyclohexane(5.50%)(in fresh roots) of *P. capense*. This shows that the three compounds Bicyclo[3.1.0]-hexan-2-ol,2-methyl-5-(1-methylethyl)-, (1.alpha.,2.beta.,5.alpha.), 1-methyl-1-ethylene-2,4-bis(1-methylethenyl)cyclohexane and Cedrol were contained in two or three of the oils as a major constituents (Figure 3).

In comparison, the chemical composition of fresh and dried fruit of *P. capense*fresh fruit consists more Oxygenated monoterpenes, Sesqueterpene hydrocarbons, Oxygenated sesqueterpenes, Diterpene hydrocarbons and Oxygenated diterpenesexcept the Monoterpene hydrocarbons. And of fresh and dried leaf of *P. capense*dry leaf consistsmoreMonoterpene hydrocarbons,Oxygenated monoterpenes, Sesqueterpene hydrocarbons, Oxygenated sesqueterpenes and Diterpene hydrocarbons except Oxygenated diterpenes.

In comparison, with the previous study was done onthe whole plant materials of *P. capense*andthe chemical composition wereSesqueterpene hydrocarbons (43.9%) and Monoterpene hydrocarbons(30.64%)[42]. In the present study in average thechemical composition wereOxygenated sesqueterpenes (40.3%)andSesqueterpene hydrocarbons (20.47%)

Theessential oils are rich in all classes of volatile chemical compounds and due to the plant part, geographical differences, environmental conditions and chemotypes, the composition is both inter and intra specifically variable [43, 44].According to Navickiene et al. [45] who distilled Brazilian species, fruit essential oils are dominated by monoterpenes (an average of 85.1%), leaf essential oils are dominated by sesquiterpenes (specifically by β-caryophyllene), while the rare root essential oil contains mainly monoterpenes but at lower level than fruits (an average of 66.9%). The essential oils from aerial parts of Cuban plants were particularly rich in oxygenated compounds, both monoterpenoids (50.3%) and sesquiterpenoids (29.2%), with lower amounts of monoterpene hydrocarbons (9.7%) and sesquiterpene hydrocarbons (8.2%) [46]. Amongst the most important compounds found in the essential oils there are, for monoterpene hydrocarbons: α-pinene, myrcene, limonene, α-terpinene, p-cymene, β-pinene, α-phellandrene, (Z)-β-ocimene; for oxygenated monoterpenoids: 1,8 cineole, linalool, terpinen-4-ol, borneol, camphor; for sesquiterpene hydrocarbons: β-elemene, β-sesquiphellandrene, (Z)-β-bisabolene, (Z,Z)-α-farnesene, ar-curcumene, α-zingiberene, δ-cadinene, β-caryophyllene, α-humulene, germacrene D, bicyclogermacrene, α-cubebene; for oxygenated sesquiterpenoids: spathulenol, (E)-nerolidol, caryophyllene oxide, α-cadinol, epi-α-bisabolol; and for phenylpropanoids: safrole, dillapiole, myristicin, elemicin, (Z)-asarone, eugenol, apiole, and sarisan [43].

Bicyclo[3.1.0]- hexan-2-ol, 2-methyl-5-(1-methylethyl)-

Cedrol

(3R,3aR,3bR,4S,7R,7aR)-4-Isopropyl-3,7-dimethyloctahydro-1H-cyclopenta[1,3]cyclopropa[1,2]benzen-3-ol

3-Carene

1-methyl-1-ethylene-2,4-bis(1-methylethenyl)cyclohexane(-)-

beta-Bourbonene

Figure 3Some major components of the essential oils from different parts of *P. capense*

4.2 Isolation of pure compounds

Selection of solvent (petroleum ether and ethyl acetate) system for elution of the column was determined after carrying out preliminary TLC analysis of the extracts using various binary solvent combinations and that show best separation and distinct spots. 54 fractions were collected, were pooled together to 18 fractions based on similarity. Then fractions were analyzed with TLC and the spots were analyzed using under UV chamber (at 365 and 254 nm). Then fractions that showed the same TLC profiles (with best matching color and Rf values) were combined in to 18fractions and concentrated to dryness under reduced pressure using rotary evaporator. Two pure compounds were obtained fromF8(pooled fromsub-fraction f_{20}-f_{23}) and F16(pooled fromsub-fraction f_{46}-f_{50}) as white and yellow crystalline solids respectively.

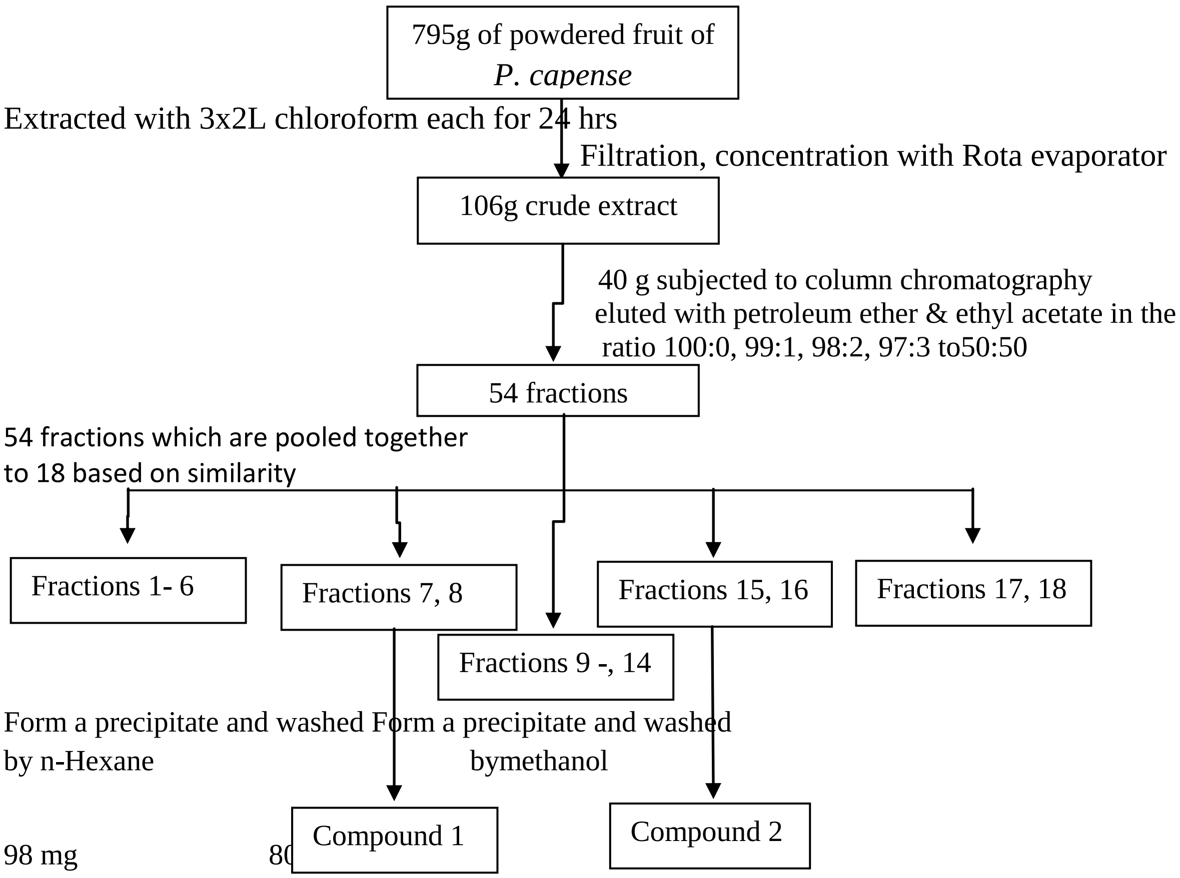

Figure 4 Extraction and isolation of compounds **1** and **2**, from the chloroform extract of the fruits of*P. capense*

4.3 Characterization of isolated compound

Compounds(**1** and **2**)were isolated as white crystalline solid (Rf= 0.7) with petroleum ether : ethyl acetate(4:1)and yellow crystalline solid (Rf = 0.6) withpetroleum ether:ethyl acetate(3:2)respectively. The^{1}H NMR,^{13}C NMRand DEPT -135 spectroscopic data wereused to characterize thesetwo compounds.

4.4.1 Characterization of compound 1

The ^{13}C-NMR spectrum(table 5, Annex-1) showed a total of 15 wellresolved carbon signals attributed to two ketone carbonyls [δ 201.72 (C-11) and δ 191.95 (C-4)], three methylenes at δ 59.60 (C-12), δ 37.53 (C-7)andδ 26.40 (C-8), four methines at δ 135.35 (C-5), δ 130.19 (C-9)δ 76.20 (C-3) and δ 29.72 (C-2), four methyls, atδ 20.59 (C-1), δ 20.50 (C-13), δ 18.76 (C-14) andδ 15.34 (C-15) andfour olefinic carbons atδ 153.02(C-6), δ135.35(C-5), δ 131.59(C-10) and δ130.19(C-9).

The 1H-NMR spectrum of compound 1(Annex 2) showed signals at δ 1.86 (3H, s), δ1.27 (3H, s), δ 0.85(3H, d) andδ0.89(3H, d) that suggest the presence of four methyl groups. The proton signals at δ 1.86 (2H, m), 2.38(2H, m) and (δ 3.07 and δ 3.44)(2H, dd) were indicate the presence three methaylene group. The ^{1}HNMR spectra at δ 2.63 (1H, d), δ 5.19 (1H, m), δ 3.81 (1H, s) and δ 5.19 (1H, m) is indicate the proton signal of four methines group.

DEPT -135 spectra (Annex 3) data showseight positive signals for CHatδ 135.35 (C-5), δ 130.19 (C-9)δ 76.20 (C-3) and δ 29.72 (C-2) and CH_3at [δ 20.59 (C-1), δ 20.50 (C-13), δ 18.76 (C-14) and δ 15.34 (C-15)], and three negative signal for CH_2at δ 59.60 (C-12), δ 37.53 (C-7) andδ 26.40 (C-8). Four signal were disappearedfrom DEPT -135 spectra data that indicate the presence of four quaternary carbon atδ 201.72 (C-11), δ 191.95 (C-4), δ 153.02 (C-6) and δ 131.59 (C-10). The spectral data generated forcompound-1 agreed well with capentin [46] available in literature shown below (Table 5).

Table 5Chemical shift data of Compound 1 and reported Literature value of ^{1}H and ^{13}C[47].

Position	δ^{1}H Experimental Multiplicity	δ^{1}H reported Multiplicity	δ^{13}C Experimental	δ^{13}C reported	DEPT-135 Experimental
1	0.89(d)	0.88(d)	20.59	20.54	20.59
2	2.63(d)	2.63(d)	29.72	29.69	29.72
3	3.81(s)	3.78(d)	76.20	76.24	76.20
4	-	-	191.95	191.83	-
5	5.95(s)	5.94(s)	135.35	135.31	135.35
6	-	-	153.02	152.90	-
7	1.86(m)	1.88(m)	37.53	37.57	37.53
8	2.38(m)	2.38(m)	26.52	26.52	26.52
9	5.19(m)	5.7(m)	130.19	130.10	130.19
10	-	-	131.59	131.63	-
11	-	-	201.72	201.56	-
12	3.07(d) 3.44(d)	3.08(d) 3.44(d)	59.62	59.60	59.62
13	0.85(d)	0.85(d)	20.50	20.60	20.50
14	1.86(s)	1.86(s)	18.76	18.76	18.76
15	1.27(s)	1.26(s)	15.34	15.36	15.34

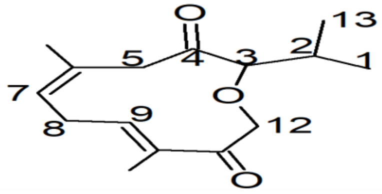

Figure5Proposed Structure of Compound-1(Capentin)

4.4.2 Characterization of compound-2

The ^{1}HNMR and ^{13}C NMR spectral data of compound-2 (Table-7) confirmed that the compound has two, two equivalent carbons and thirteen non-equivalent carbons. The compound has a total of17-carbons, which six of them oxygenated and has thirteen hydrogen atoms.

The ^{13}C-NMR spectrum of the isolated compounds-2 as showed (Annex 4). The peak for C-4' appears a singlet at δ 162.27 ppm for 4'-oxygenated ring B and the chemical shifts for the ortho carbon (C-3', 5') atδ 114.61 ppm, meta carbon (C-2', 6')) at δ 128.15 ppm and para carbon (C-l') at δ 123.64 ppm. The chemical shift appears on δ 98.16 ppm for C-6 and δ 92.72 ppm for C-8. It influenced by the effects of hydrogen bond in 'OH' at C-5. Thus, C-6 represented by signals at higher chemical shift region (downfield) than the C-8 atom. In the isolated compound 2, C-3 signal appears at δ 104.42 and δ 105.66 ppm for C-10. The chemical shifts for the signals of the carbon C-5 shifted to downfield δ 162.70 ppm, due to the influence of hydroxyl group on the C-5and the chemical shiftfor C-9 is δ 157.8 ppm. The chemical shifts showed the signal at δ 164.12 ppmfor C-2. The carbonyl carbon C-4 appeared as a less intense peak in the region 182.56 ppm. The chemical shiftremaining to downfield region at δ165.54 ppm signal ascribed to C-7.

The1H-NMR spectrum of compound 2 (Annex 5) showed signals at δ 3.90 (3H, s) and δ 3.91 (3H, s) suggestedthatthe presence of two methoxy groups. The aromatic protons at δ 6.37 (1H, d) and δ 6.49 (1H, d) indicated that the presence of Meta coupled protons on the A-ring of flavonoid. The peakat δ 6.58 (1H, s) couldindicate the proton signal on the C-ringof the flavonoid. Due to unsymmetrically para substituted B ring of flavonoid,symmetrically placed hydrogen showstheproton signals at δ 7.02 (2H, d) and δ 7.86 (2H, d). Thefirst peak is due to the protons at 3’and 5’ position while the second one is due to the protons at position 2’and 6’ of aromatic nucleus. The hydrogen of hydroxyl on carbon C-5appeared atδ 12.83 ppm.

The DEPT -135 spectra(Annex-6) data showed five positive carbon peak for CH groups and eight carbon signals were disappeared that indicate the presence of eight quaternary carbons.Thus attributed to five oxygenatedcarbons at δ 165.54 (C-7), δ 164.12 (C-2), δ 162.7 (C-5) δ 157.8 (C-9), δ 162.27(C-4’),two aromatic carbons at δ 123.64 (C-1’) and δ 105.66 (C-10), and one carbonyl carbon at δ 182.56 (C-4).The spectral data generated for compound compound-2 agreed well with 5- hydroxy-7, 4’-dimethoxyflavone [47] available in literature (Table 6).

Table 6,Chemical shift data of Compound 2 and reported Literature value of ^{1}H and ^{13}C[48].

Position	δ^{1}H Experimental Multiplicity	δ^{1}H reported Multiplicity	δ^{13}C Experimental	δ^{13}C reported	DEPT-135 Experimental
2	-	-	164.12	164.0	-
3	6.58(H,s)	6.61 (1H, s)	104.42	104.3	104.42
4	-	-	182.56	182.4	-
5	-	-	162.70	162.5	-
6	6.37 (H, d)	6.39 (1H, d, J=2.4 Hz)	98.16	98.0	98.16
7	-	-	165.54	165.4	-
8	6.49(H, d)	6.51 (1H, d, J=2.4 Hz)	92.72	92.6	92.72
9	-	-	157.8	157.7	-
10	-	-	105.66	105.5	-
1’	-	-	123.64	123.5	-
2’/6’	7.86(H, d)	7.88 (2H, d, J=9.2 Hz)	128.15	128.0	128.15
3’/5’	7.02(H, d)	7.03(2H, d, J=9.2 Hz)	114.61	114.5	114.61
4’	-	-	162.27	162.2	-
OCH_3	3.90(3H, s)	3.90 (3H, s)	55.66	55.5	-
OCH_3	3.91(3H, s)	3.91 (3H, s)	55.92	55.8	-
OH	12.83	-	-	-	-

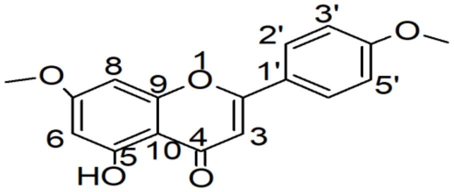

Figure 6, Proposed Structure of Compound-2, (5-hydroxy-7,4’-dimethoxyflavone)

Isolation of this compound was also reported from the fresh fruit of *P. capense* collected from Ethiopia [48] and also reported from *Combretum zeyheri* leaves were collected from Norton [49]

5-hydroxy-7,4’-dimethoxyflavoneisflavonoid have antifungal activity.Flavonoids have biological/pharmacological effects, such as their anti-inflammatory properties, vasorelaxant, antioxidant, phosphodiesterase inhibitor, antiteratogenic, hepatoprotective, lipid peroxidation inhibiting, and platelet aggregating [50, 51]. The biochemical activities of flavonoids and their metabolites are related to their chemical structure which can change according to the substitutions in the three rings, including hydrogenation, hydroxylations, methylations, sulfatations and glycosylations [52, 53]

4.5 Antibacterial Activity of isolated essential oils.

The antibacterial activity of Essential oils obtained from the different parts of *P. capense*was tested using agar disc diffusion method against Gram positive (*B. cerneus* and *S. aureus*) and Gram negative (*E. Coli* and *S. typhi*). In addition, the bioassaysconducted on fungal strain (*C. albicans*) to determine the antifungal activity of the essential oils obtained from the the different parts of *P.capense.* Theresults are displayed below (Table 7, Annex 7).

Among the six tested essential oil, root extract showed the highest inhibition zone against Gram positive bacteria (*B. Cerneus*=11) and Gram negative (*E. coli*=11,*S.typhi*=11).Fresh leaf extract showed the highest inhibition zone against Gram-positive bacteria (*S. aureus*= 11). Stem bark extract showed the highest inhibition zone against Gram positive bacteria (*S. aureus*=11) and Gram negative (*E. coli*=11). Dry fruit extract showed the highest inhibition zone against Gram-positive bacteria (*S. typhi*=11). Dry fruit and fresh leaf extract the lowestinhibition zone against (*E. coli*=7). However, all extracted essential oil shows less antibacterial activity than the observed activity for the reference drug (Gentamicin) as showed above (Table 7).

The result of antifungal test shows the fresh fruit, dry leaf and root extracts each exhibited good antifungal activities against (*C.albicans*=11) than the essential oil extracted from other parts of *P. capense*, which shows less activity on the fungal strain. However, all extracted essential oil shows less Antifungal activities than the observed activity for the reference drug (Clotrimazole).

In comparison with different parts of *P. capense*the root part show the highest activity against the bacteria (Gram positive bacteria and Gram negative bacteria) and fungal test are given above (Table 7).

Table 7 Antimicrobial activity of Essential oils from different parts of *P. capense*

Plant part	Gram positive bacteria		Gram negative bacteria		Fungal strain
	S. aureus (mm)	*B. Cereus* (mm)	*E. coli* (mm)	*S. typhi* (mm)	*C. albicans* (mm)
Fresh Fruit	10	9	8	10	11
Dry fruit	8	10	7	11	9
Fresh Leaf	9	11	7	10	8
Dry leaf	8	10	9	9	11
Stem bark	9	11	11	9	9
Root	11	8	11	11	11
Gentamicin	27	23	28	26	Nt
DMSO	NI	NI	NI	NI	NI
Clotrimazole	Nt	Nt	Nt	Nt	13

Nt = Not testedNI = No Inhibition

The disk diffusion assay employed as an indicator of antimicrobial activity. The antimicrobial activity of sequentially Soxhlet extracted by PE, CF, AC and ME from different parts of *P. capense*and the chloroform extract of fruit of*P. Capense*was tested using agar disc diffusion technique against Gram positive (*B. cerneus* and *S. aureus*), Gram negative (*E. Coli* and *S. typhi*) and fungal strain (*C. albicans*). Most common Gram-positive bacterium found in nosocomial infections [22]. Gram-negative bacteria possess a lipopolysaccharide layer which acts as a barrier, and makes them less susceptible to many antimicrobial compounds [22].Antimicrobial activity against S. aureus is of importance since S. aureus is one of the most persistent infectious microorganisms [22].The activity used to determine the antimicrobial activity of the Soxhlet extract of the different parts and the maceration extracts of the fruit of*p. capense*. The zones of inhibition for the whole parts of *P. capense* extracts are presented in Table 8.

Among the Soxhlet extract CF extract of the fruit of *P. capense*showed the highest inhibition zone against Gram-positive bacteria (*B. Cerneus*12mm). The PE extracts of the fruit and the leaf of *P. capense*showed the high inhibition zone against Gram positive bacteria (*B. Cerneus* 11mm) and Gram negative (*E. coli*11mm, *S. typhi*11mm).However, all extracted shows less activity than the control reference drug (Gentamicin). But ME extract of the stem bark of *P.capense* shows highest inhibition zone against fungal strain(*C. albicans*, 11mm).Antimicrobial activity against C. albicans has previously been reported for Methanolextracts of the stem bark of this plant [54]. In comparison, similarly the ME extract of fruit of *P. capense* in other study in West Region of

Cameroon were shows antibiotic on some of the MDR bacteria such as *E. cloacae* ECCI69 and BM47, *E. aerogenes* EA27 and EA289[55]

Table 8 Antimicrobial activity of Soxhlet extracts of different parts of *P. capense*and isolated compound from chloroform extract of fruit of *P. capense*

Part used	Extract type	Concentration (mg/ml)	Zone of inhibition (mm)				
			S. aureus	*E. coli*	*S. typhi*	*B. cereus*	*C. albicans*
Fruit	PE	300	9	9	10	11	8
	CF	300	NI	8	7	12	8
	AC	300	10	NI	7	10	7
	ME	300	NI	NI	7	11	NI
Leaves	PE	300	7	9	8	11	10
	CF	300	NI	NI	NI	NI	NI
	AC	300	NI	NI	NI	NI	NI
	ME	300	NI	NI	NI	7	NI
Stem bark	PE	300	NI	NI	NI	NI	NI
	CF	300	NI	NI	NI	NI	NI
	AC	300	NI	NI	NI	NI	7
	ME	300	NI	NI	NI	NI	11
Roots	PE	300	NI	8	10	8	8
	CF	300	NI	NI	7	8	7
	AC	300	NI	NI	NI	8	NI
	ME	300	NI	NI	NI	NI	10
Isolated compound and control							
Compound 1		100	NI	NI	9	9	8
Compound 2		100	NI	NI	7	NI	NI
DMSO		100µl	NI	NI	NI	NI	NI
G.		1µl	23	28	26	27	Nt
Ct		1µl	Nt	Nt	Nt	Nt	15

PE= petroleum ether CF = Chloroform AC=Acetone ME = Methanol G =Gentamicin
Ct= Clotrimazole NI = No Inhibition Nt= Not tested

5 CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

In this study, the essential oil extracted from the different parts of *P. capense,*different parts of *P. capense* sequentially extracted with petroleum ether, chloroform, acetone and methanol and the fruits of *P. capense* were extracted with chloroform. The essential oil of fresh fruit, dry leaf and fresh root of *P.capense*and methanol extracts of stem bark are responsible for the antifungal activity.Oxygenated sesqueterpenes was the major components of essential oil obtained from the different parts(dryfruits,dry leaf, freshfruits, fresh leaf, fresh stem bark, fresh roots)of *P. capense*.

5.2 Recommendations

The present study indicates that fresh fruit, dry leaf and fresh root of *P.capense was a* source of bioactive compounds and could be a source of antibiotic agents. The fruit extruct of *P. capense*from Gerima Guda were gave more yield therefore commercially more available. Further study is required to explore other chemical constituents of the solvent extracts of the fruits and other parts of *P. capense*. Furthermore it is also necessary to study the antioxidant of the extract of the fruits of *P. capense*.

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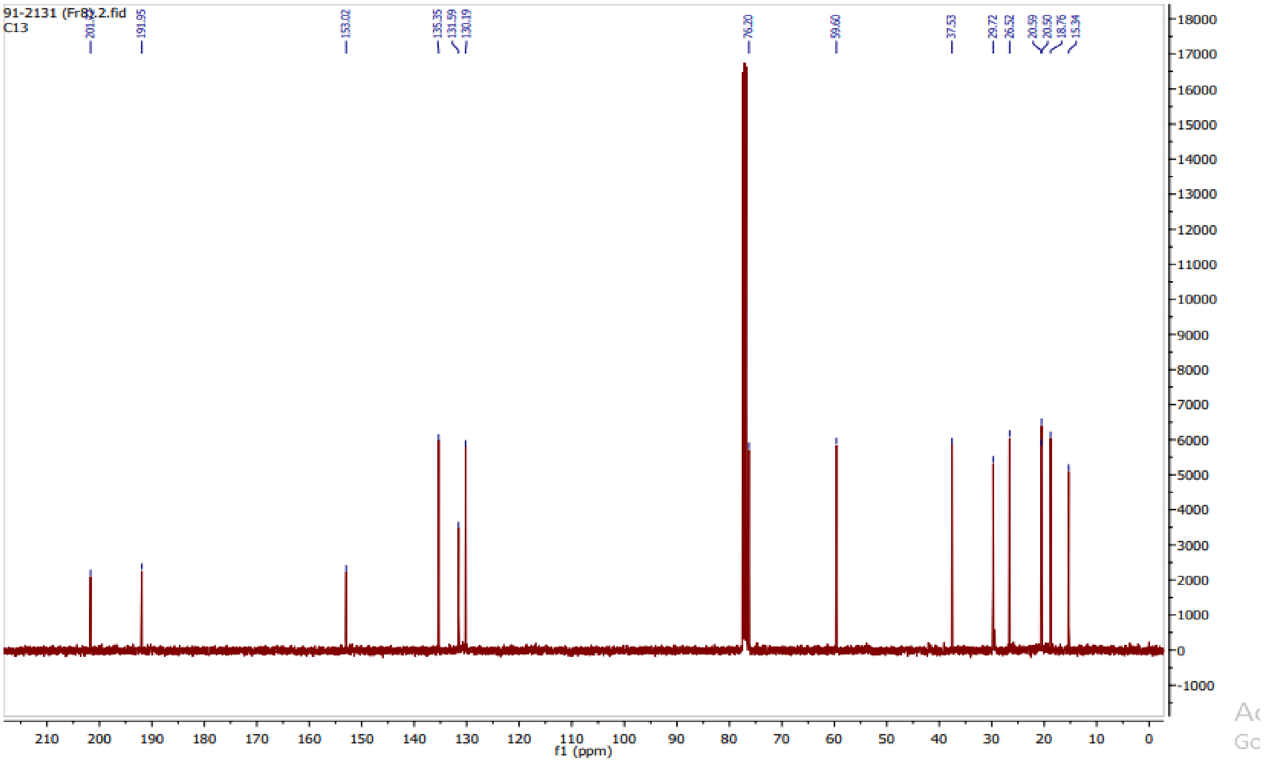
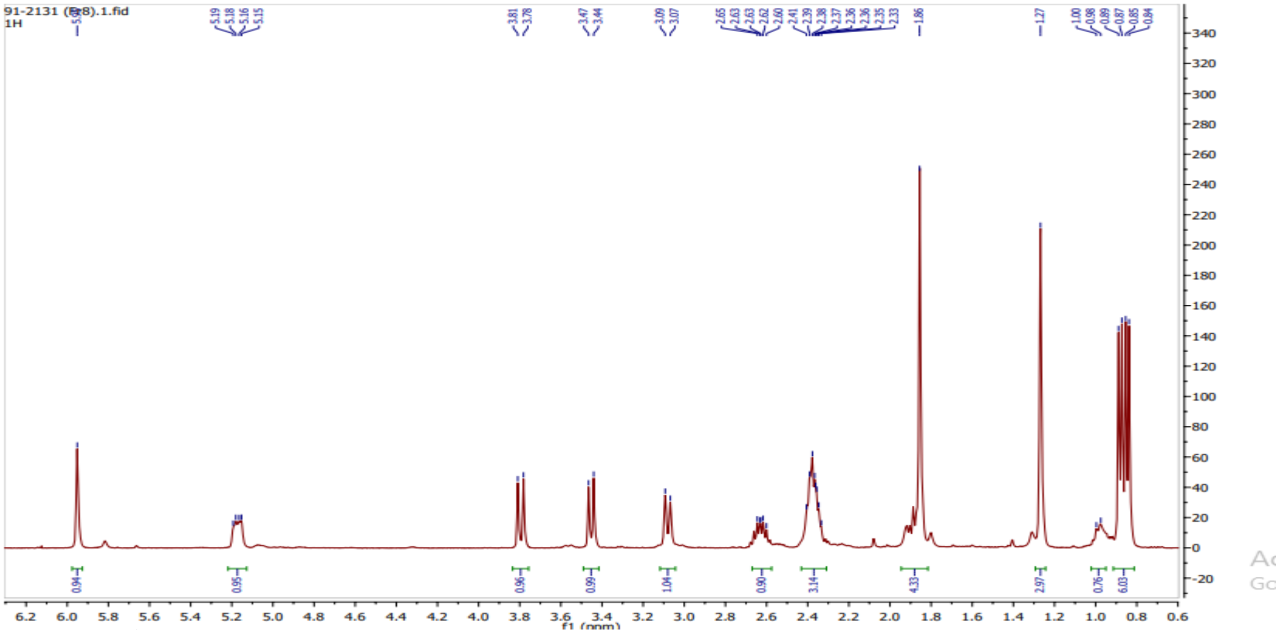
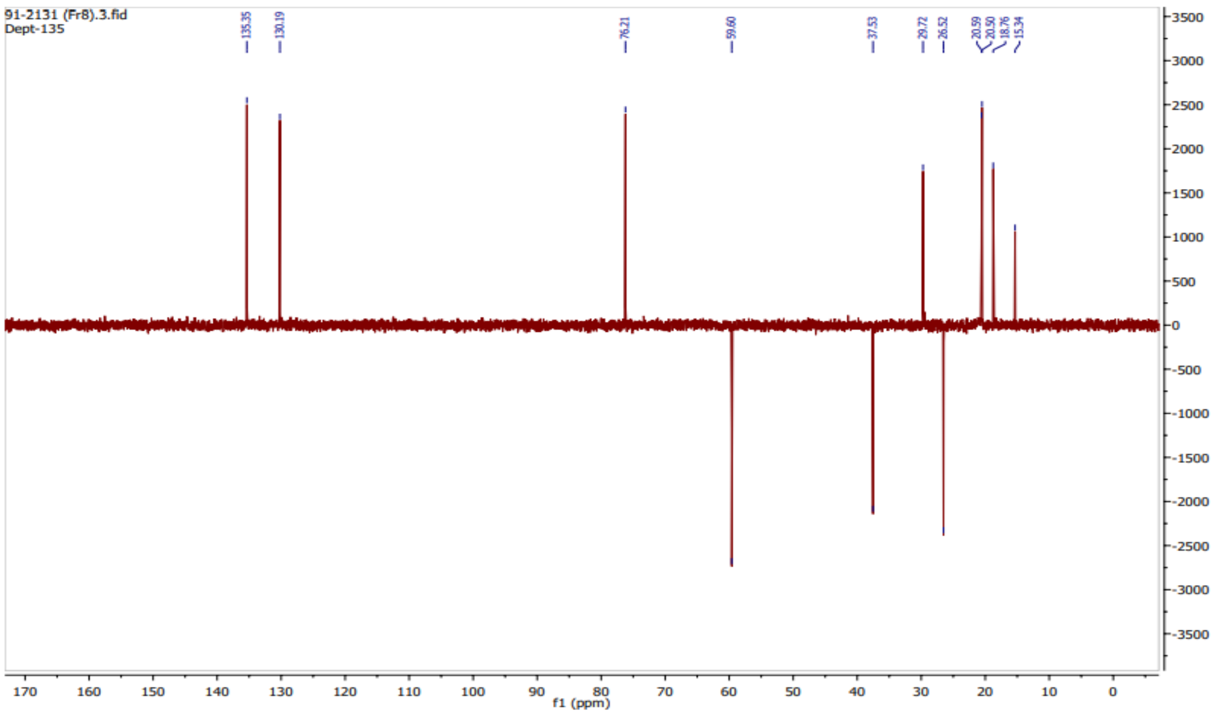
Appendices



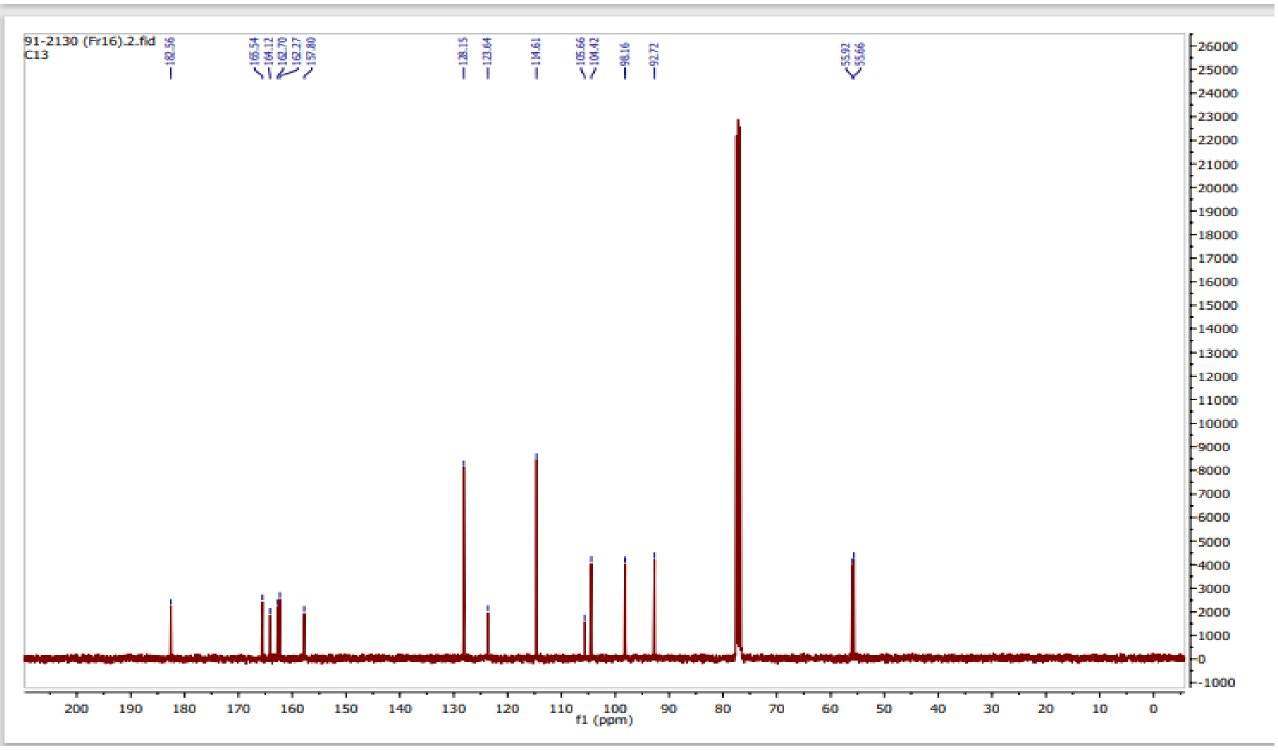
Appendix 1 ^{13}C NMR spectral data of compound 1



Appendix 2 ^{1}H NMR spectral data of compound 1



Annex 3. DEPT NMR spectral data of compound 1



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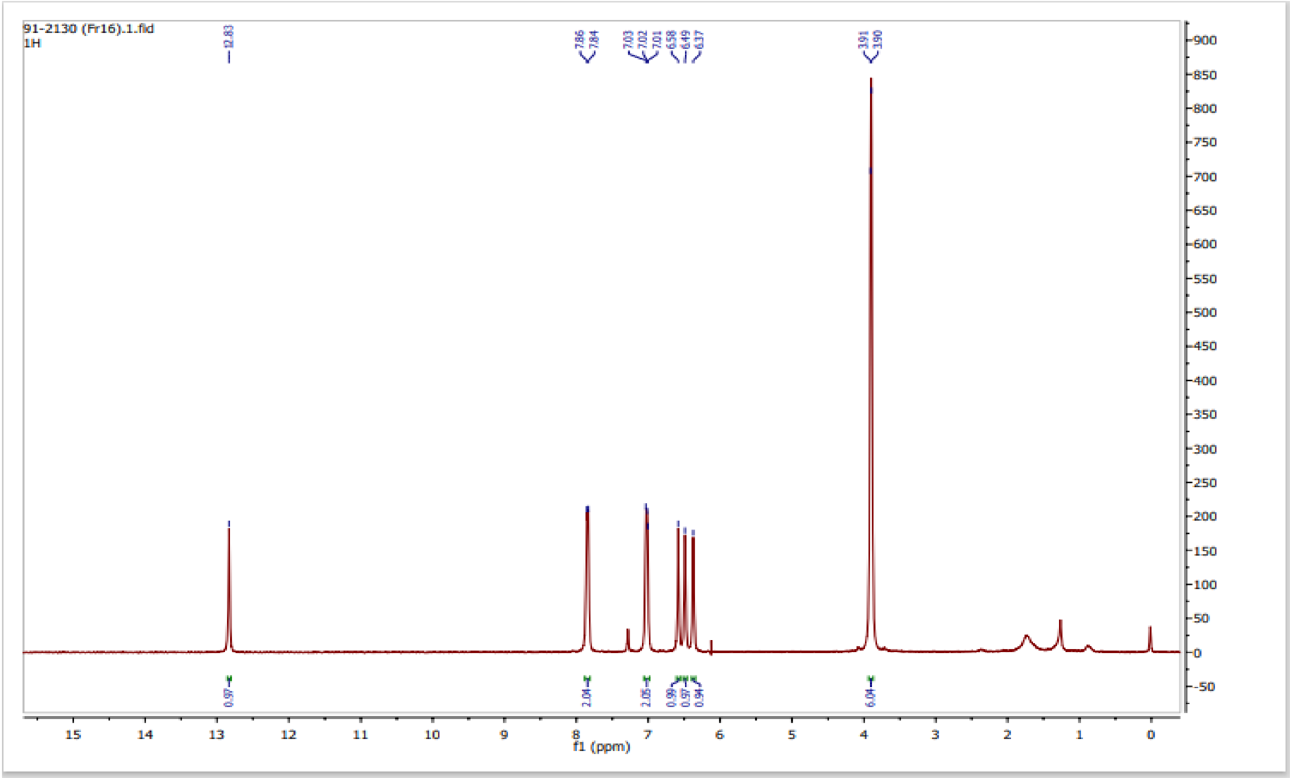
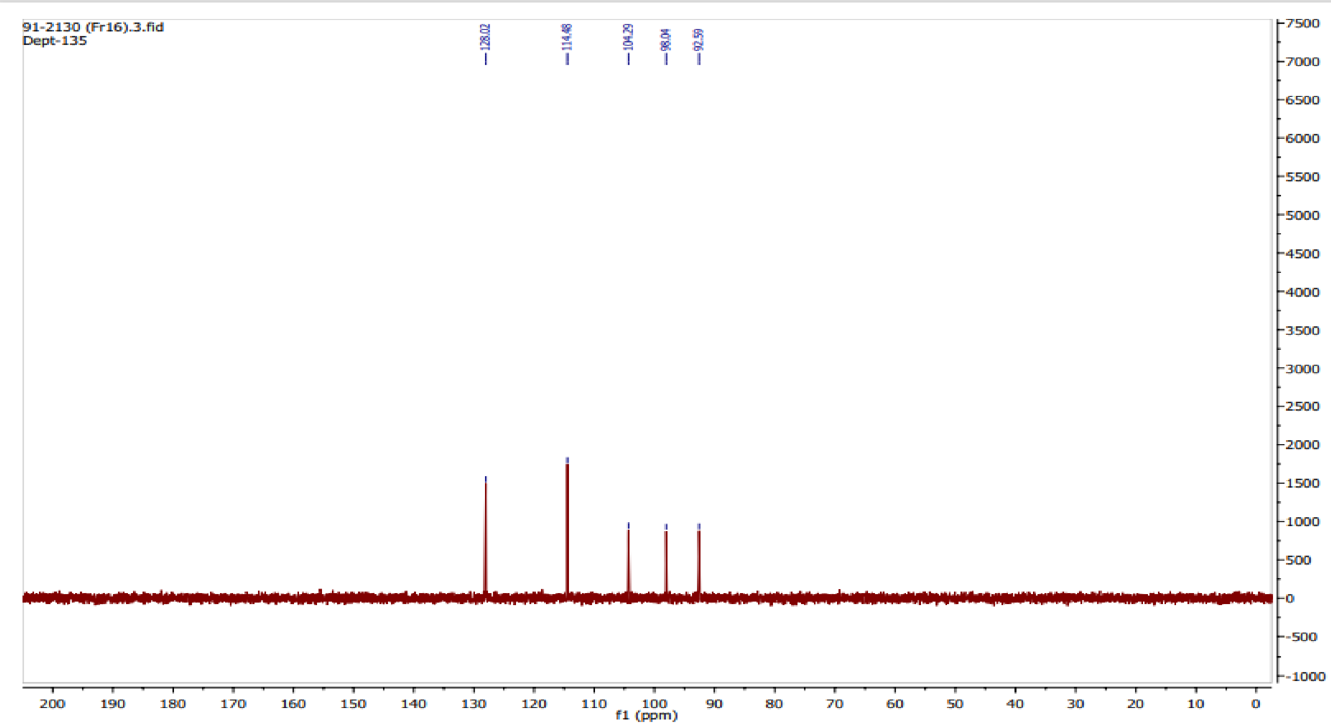
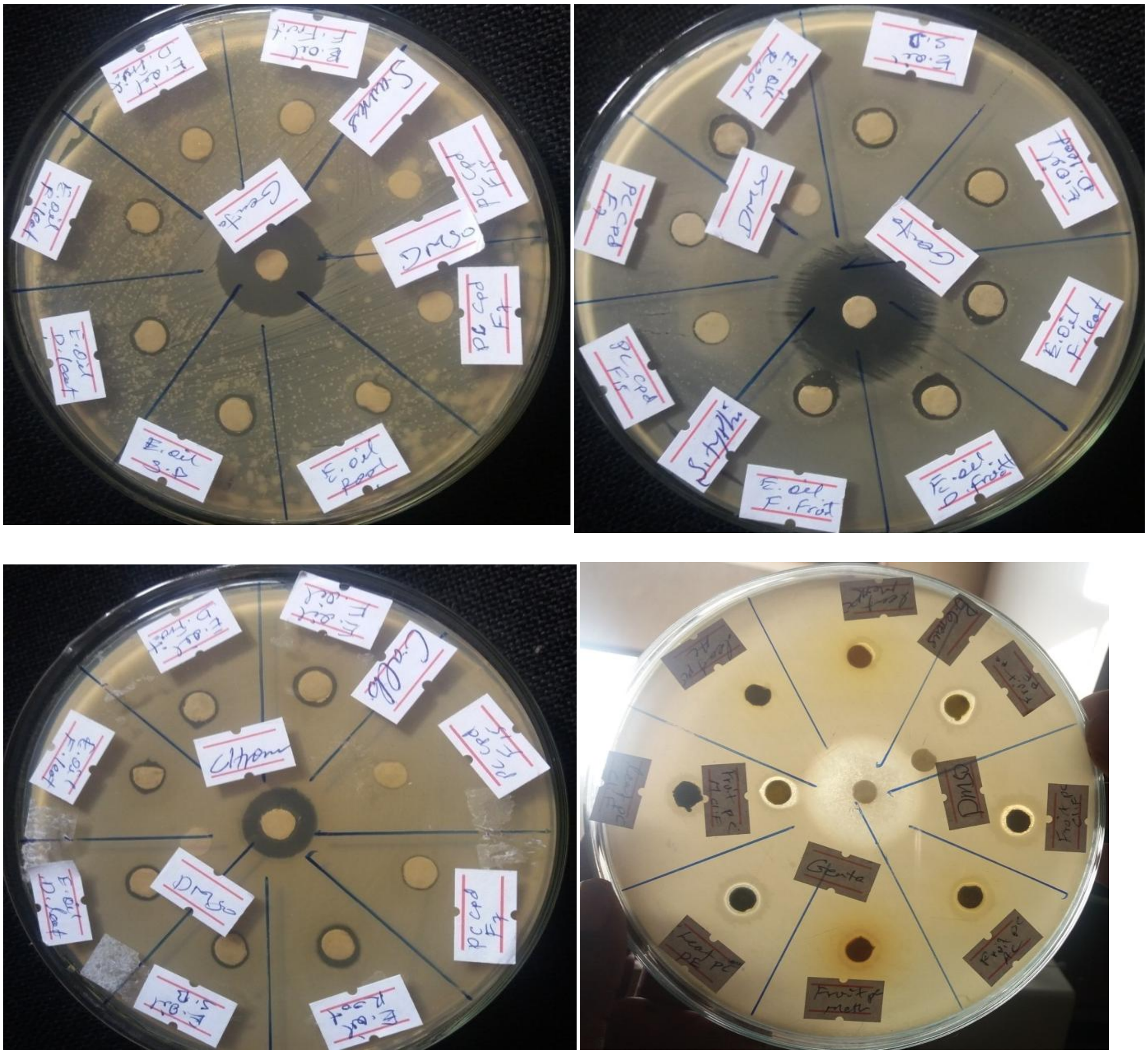


Appendix 5^{1}H NMR spectral data of compound 1



Annex 6. DEPT NMR spectral data of compound 2



Annex 7. Antimicrobial activities of essential oil, crude extracts and isolated compound