Napata college

Pharmacy program

Essential Oil from *Eucalyptus camaldulensis:* GC-MS Profile, Antimicrobial and Antioxidant Properties

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الآية

قال الله تعالى:

﴿فَتَعَالَى اللَّهُ الْمَلِكُ الْحَقُّ ۗ وَلَا تَعْجَلْ بِالْقُرْآنِ مِن قَبْلِ أَن يُقْضَىٰ إِلَيْكَ وَحْيُهُ ۖ وَقُل رَّبِّ زِدْنِي عِلْمًا﴾

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Dedication

Our Fathers……

Our Mothers……..

Brothers……..

TO

Sisters and Friends……..

We dedicate this thesis……

With Our Love ;;;;;;;;;;;;;

ABSTRACT

Background: Natural plants as herbal remedies are being employed to prevent and cure several illnesses that vary in different community. Medicines had been explored from leaves, flowers and barks of plants. Continuous spread of infectious diseases is a major problem worldwide. Due to multiple and repeated issues with antibiotics efficacy, it became essential to evaluate biological properties of plants from different geographical origins. *Eucalyptus camaldulensis (*Family: *Myrtaceae)* leaves have been reported for various medicinal effects like antibacterial, anti-inflammatory, anti-viral, and anti-oxidant.

Objectives: The objective of the present study is to determine the chemical composition and evaluation of two pharmacological activities; antimicrobial and antioxidant of *E.camaldulensis* essential oil.

Methods: Essential oil was obtained by steam distillation method. And subjected to GC-MS to determine the active constituents. Antimicrobial activity applied with disc diffusion method whereas antioxidant activity was performed through DPPH assay.

Result: GC-MS results of the pale yellow essential oil revealed a total of seventeen compounds which identified and representing 99.0% of the total oils. The major constituents of the oil were; Eucalyptol (34.15 %), *P*-Cymene (22.07%), (-) Globulol(9.14%),1H-Cycloprop[e]azulene, decahydro-1,1,7-trimethyl-4-m (8.17%), gamma.-Terpinene(5.60%).

Antimicrobial activity against three pathogenic bacterial strains exhibited a good inhibition zone which ranged between 44 to 8mm at 12.5, 25 and50 30 µg/mL oil, whereas *C. albicana* obtained 40 to 12 at the same concentrations.

The antioxidant activity demonstrated a weak activity (58±**0.01**) this inhibition is lower than that obtained by the standarad Propyl gallate (92±**0.0**).

Conclusion: The important role that *E.camaldulensis* plant play in folk medicine has led us to develop this study in order to investigate the major pharmacological activities, so this plant can be used in future as a valuable source of pharmacologically active antioxidants and antimicrobial agents.

Keywords: *E.camaldulensis* (Leaves); Gas Chromatography-Mass Spectrometry (GC-MS); Antioxidant Activity; Antimicrobial Activity.

خلاصة البحث

الخلفية: تستخدم النباتات الطبيعية كعلاجات عشبية لمنع وعلاج العديد من الأمراض التي تختلف في المجتمعات المختلفة. تم استكشاف الأدوية من أوراق وأزهار ولحاء النباتات. يعد الانتشار المستمر للأمراض المعدية مشكلة رئيسية في جميع أنحاء العالم. بسبب المشكلات المتعددة والمتكررة المتعلقة بفعالية المضادات الحيوية ، أصبح من الضروري تقييم الخصائص البيولوجية للنباتات من أصول جغرافية مختلفة. تم الإبلاغ عن أوراق الكينا camaldulensis (العائلة: Myrtaceae) لتأثيرات طبية مختلفة مثل مضادات البكتيريا ومضادات الالتهابات ومضادات الفيروسات ومضادات الأكسدة.

الأهداف: الهدف من هذه الدراسة هو تحديد التركيب الكيميائي وتقييم نشاطين دوائيين ؛ مضادات الميكروبات ومضادات الأكسدة من زيت E.camaldulensis الأساسي.

الطريقة: تم الحصول على الزيت العطري بطريقة التقطير بالبخار. وتخضع لـ GC-MS لتحديد المكونات النشطة. تم تطبيق نشاط مضادات الميكروبات باستخدام طريقة نشر القرص بينما تم إجراء نشاط مضاد للأكسدة من خلال مقايسة DPPH.

النتيجة: أظهرت نتائج GC-MS للزيت العطري الأصفر الباهت إجمالي سبعة عشر مركبًا حددت وتمثل 99.0٪ من إجمالي الزيوت. المكونات الرئيسية للنفط هي: يوكاليبتول (34.15٪) ، P-Cymene (22.07٪) ، Globulol (9.14) (-)٪ (، 1 azulene [e] Prop-Cyclo-H، 1-decahydro،1،7-trimethyl-4-m (8.17٪) ، جاما .-تيربينيني (5.60٪).

أظهر نشاط مضادات الميكروبات ضد ثلاث سلالات بكتيرية ممرضة منطقة تثبيط جيدة تراوحت بين 44 إلى 8 مم عند 12.5 و 25 و 50 30 ميكروغرام / مل من الزيت ، بينما حصل C. albicana على 40 إلى 12 بنفس التركيزات.

أظهر النشاط المضاد للأكسدة نشاطًا ضعيفًا (58 ± 0.01) هذا التثبيط أقل من ذلك الذي تم الحصول عليه بواسطة Standard Propyl gallate (92 ± 0.0).

الخلاصة: إن الدور المهم الذي يلعبه نبات E.camaldulensis في الطب الشعبي قادنا إلى تطوير هذه الدراسة من أجل التحقيق في الأنشطة الدوائية الرئيسية ، لذلك يمكن استخدام هذا النبات في المستقبل كمصدر قيم لمضادات الأكسدة النشطة دوائيًا والعوامل المضادة للميكروبات.

الكلمات المفتاحية: E.camaldulensis (أوراق)؛ اللوني للغاز - مطياف الكتلة (GC-MS) ؛ النشاط المضاد للأكسدة؛ نشاط مضادات الميكروبات.

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Abbreviations	Meaning
WHO	*World Health Organization*
E.coli	*Escherichia coli*
UTI	*Urinary Tract Infection*
VOCs	*Volatile Organic Compounds*
GC-MS	*Gas Chromatograph – mass spectrometer*
DPPH assay	*2,2-diphenyl-1-picryl-hydrazyl-hydrate*
EC	*Epicatechin*
EGcg	*Epigallocatechin-3-gallate*
Ppt	Precipitate
MHA	Mueller Hinton agar
Mm	Millimeter
μM	Micrometer
μg	Microgram
Mg/ ml	Milligram/ milliliter
MDIZ	Mean diameter of growth inhibition zone in mm

CHAPTER ONE

1.1 An overview:

The world is fertile with natural and medicinal plants, which are now more focused than ever, because they have the capability of producing many benefits to society, indeed to mankind, especially in the line of medicine and pharmacology. The medicinal power of these plants lie in phytochemical constituents that cause definite pharmacological actions on the human body. [1]. *Eucalyptus camaldulensis* synonym blue gum tree (Family: *Myrtaceae*) is an evergreen tree, one of the most widely cultivated trees native to Australia, South Africa, India and Southern Europe. The bark was used against toothaches and gum aches. The leaves were used against headaches which occurred on one side of the head.

E. *globulus Labill* synonym blue gum tree possess antidiabetic, anti-inflammatory, antibacterial, antimalarial and Antioxidant properties. Eucalyptus contains several biologically active compounds such as essential oils (Terpene oxides:1,8-cineole; monoterpenes: alpha-pinene, limonene, gamma-terpinene, paracymene; sesquiterpenes: aromadendrene sesquiterpenols: globulol), flavonoids, phenol acids, and tannins.

Eucalyptol or 1,8 cineole is the majority compound with a concentration of 70 to 85%. Constituents and their antioxydative effects in Eucalypyus leaf extract used as a natural food additive [3]. Antimicrobial is destroying or inhibiting the growth of microorganisms and especially pathogenic microoganisms.

An antioxidant may be defined as ‘any substance that when present at low concentrations, compared with those of theoxidizable substrate, significantly delays or inhibits oxidation of that substrate for convenience, antioxidants have been traditionally divided into two classes, primary or chain-breaking antioxidants and secondary or preventative anti-oxidants.

(GC/MS).GC/MS is a technique that can be used to separate volatile organic compounds (VOCs). However other uses of GC or MS, combined with other separation and analytical techniques, have been developed for adionuclides, explosive compounds, Trinitrotoluene (TNT), and metals. [6]

1.2. Justification:

The emergence of antibiotic resistance in E. coli is an important problem. Finding alternative antimicrobial agents from plant extracts has received growing interest. E. *camaldulensis* is a safe, cheap beverage that has been reported to have antimicrobial and antioxidant activities against various pathogenic bacteria. Polyphenolic components of E. *camaldulensis* have antibacterial activity, due to all the above reasons, its essential to evaluate eucalyptus leaves biological properties to provide a potential source for development of new antimicrobial and antioxidant agents.

1.3. Objective:

The Aim of this study is to determine the chemical profile of E. *camaldulensis*. Leaves essential oil and its antimicrobial and antioxidant activities.

1.3.2. Specific objectives:

1. To extract the volatile oils of the plant leaves through the hydro -distillation technique.

2. To investigate the chemical composition of the leaves using GC _MS techniques.

3. To determine the antimicrobial activity against some selected pathogenic bacterial strains using disc diffusion method.

4. To evaluate the antioxidant activity through the DPPH radical scavenging assay.

CHAPTER TWO

2. Literature Review:

2.1. Taxonomy of *Eucalyptus camaldulensis*:

Kingdom: plantae

Family: *Myrtaceae*

Genus: *Eucalyptus*

Species: *Eucalyptus camaldulensis*

Order: *Myrtales*

Medicinal Plant

2.2. Plant Description:

The natural distribution of *E. camaldulensis* is confined to Tasmania, Victoria, and New South Wales between latitudes 31 and 43 degrees S. This species is most common in southeastern Tasmania, islands in the Bass Straits, the Ottway Ranges, and Wilson's Promontory in Victoria.

The leaves are leathery in texture, hang diagonally or vertically, and are studded with glands contain a fragrant volatile oil. The flower in bud are closed with a cup-like covering (hence the name of the genus, derived from the Greek eucalyptus well-covered), which is unnerved off as a lid while the flower expands. The fruit is bounded by a woody, cup shaped container and contains abundant minute seeds. [7]

2.3 Chemical constituents:

E. camaldulensis is the one of the most important species of Eucalyptus. The essential oil in the leaves is commonly used for medicinal purposes. The quantity of essential oil ranges from less than 1.5 to over 3.5%.

GC-MS analyses revealed the presence of twenty seven compounds representing 100 % of the .total oil. In these 27 compounds. The essential oil consisted mainly of oxygenated monoterpenes, monoterpenes and oxygenated sesquiterpenes. Of these, 1, 8-eucalyptus (72.71%) α- terpined (2.54%), terpiene-4-ol (0.34%), and linalool (0.24%) were the main oxygenated monoterpenes, while α-eudesmol (0.39%), (-)- globulol (2.77%), and epilobulol (0.44%) were the main sesquiterpene. Several significant compounds were α-terpineol acetate (3.1%), geranyl acetate (0.71%), L-pinocarveol (0.36%), β-sabinene (0.25%), and terpinolene (0.19%). A portion (0.26%) of the total constituents remains unidentified. [8]

2.4. Medicinal uses:

E camaldulensis It rich sources of phytochemical constituents which contain flavonoids, alkaloids, tannin and propanoids. Which are present in leafs, steam, and root of the plant. They are various properties like anti-inflammatory, antibacterial, antiseptic, astringent, anti-diabetic, anti-oxidative, antiviral, antitumor, antihistaminic, anticancer cytochrome p450 inhibitor and hepatoprotective effect have also been reported by many researchers. The present review article critically discusses about various phytochemical associated with the plant along with numerous therapeutics application exhibited by the plan. [9]

2.5. Anti-bacterial effects:

Eucalyptus oil (EO) and its major component, 1,8-cineole, have antimicrobial effects against many bacteria.

The antibacterial activities of the essential oil of *Eucalyptus globulus* (EOEG) was determined against 7 fish pathogenic bacteria (Edwardsiella tarda, Streptococcus iniae, S. parauberis, Lactococcus garviae, Vibrio harveyi, V. ichthyoenteri and Photobacterium damselae) obtained from farmed olive flounder. The inhibitory activity was evaluated by three methods: Disc diffusion method, minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC).

2.6. Antioxidant activity

Antioxidants are compounds that inhibit oxidation, a chemical reaction that can produce free radicals and chain reactions that may damage the cells of organisms. Antioxidants such as thiols or ascorbic acid may act to inhibit these reactions.

Antioxidant activity can be monitored by a variety of assays with different mechanisms, including hydrogen atom transfer (HAT), single electron transfer (ET), reducing power, and metal chelation, among others.

technical issues that limit use and compromise validity of three commonly-used assays – TEAC/ABTS•+, DPPH, and ORAC. Recommendations are made for discontinuing use of ABTS•+ and DPPH radicals for measuring radical quenching .[10]

Eucalyptus tea is high in flavonoids, which are antioxidants that may lower your risk of certa3.

2.7 Previous studies

Study [1]:

Essential oil of E. camaldulensis is tested against some bacterial strains [15], results Indicted that, the oil is active against many Gram positive (0.07–1.1%) and Gram Negative bacteria (0.01–3.2%). The antibacterial effect is confirmed for bark and leaf Extracts (conc. from0.08 µg/mLto200 mg/mL). E. camaldulensis essential oil and Extracts are among the most active against bacteria when compared with those from other Species of genus Eucalyptus. Thus the most fungal model or ganisms are sensitive to 0.125–1.0% of E. camaldulensis essential oil. The extracts are active against C. Albicans (0.2–200 mg/mL leaf extracts and 0.5 mg/mL bark extracts.

Study [2]:

The antimicrobial potential of two E. camaldulensis essential oils against multi-drug Resistant (MDR) Acinetobacter baumannii and GC-MS aredetermine [16]. Results showed That The dominant components of both essential oils were spatulenol, cryptone, pcimene, 1,8-cineole, terpinen-4-olandβ-pinene.The detected MICs for the E. camaldulensis essential oils were in range from 0.5 to 2 µl mL-1. The bio auto graphic assay confirmed antibacterial activity of polar terpene compounds.

Incombination with conventional antibiotics (ciprofloxacin, gentamicinand polymyxinB), the examined essential oils showed synergistic antibacterial effect in most of the cases, while in some even re-sensitized MDRA. Baumannii strains.The synergistic interaction was confirmed by time-kill curves for E. camaldulensis essential oil and polymyxin B combination which reduced bacterial count under detection limit very fast ,i.e. after 6 hours of incubation.

Study [3]:

The *in vitro* antimicrobial activity of acetone, methanol and water extracts of leaf, stem and bark *Eucalyptus camaldulensis* L. (*Myrtaceae*.) It is used as a remedy for sore throat and other bacterial infection of the respiratory and urinary tracts. Antimicrobial was examined against six bacterial species *Bacillus. megaterium, B.subtilis, Staphylococcus, S. aureus, Micrococcus luteus and E.coli* using the agar well diffusion method. Phytochemical screening was carried out for phenols,

flavonoids and tannins. Results showed that the plant extracts exhibited a dose-dependent inhibition of microorganisms. The acetone and methanol extracts of leaf and stem bark of *E. camaldulensis* displayed maximum antibacterial activity against all the bacterial species studied. *E. camaldulensis* extracts contained phenols, flavonoids and tannins at varying levels. The ability of the crude extracts of the test plant to inhibit the growth of bacteria is an indication of its broad spectrum. The antibacterial activity of the leaf extracts of *E. camaldulensis* can be attributed to the action of the phytochemical compounds it contains. There was no significant difference in the antimicrobial activity of the extracts on Gram-negative and Gram positive bacteria despite the differences in their cell wall components. The crude and pure methanol extract of *E. camaldulensis* has been found to be effective against *Staphylococcus aureus* (0.9cm) *and Bacillus megaterium* (3.1.cm) diameter zone of inhibition [17].

Study [4]:

The volatile oil compositions of *Eucalyptus camaldulensis* var. *brevirostris* leaves obtained by hydrodistillation (HD) and supercritical fluid extraction methods (SFE) were analysed qualitatively and quantitatively by GLC-MS. Ninety different components were separated and most of them identified. In both extracts the main constituents were found to be β-phellandrene (8.94 and 4.09%), *p*–cymene (24.01 and 10.61%), cryptone (12.71 and 9.82%) and spathulenol (14.43 and 13.14%). The yield of the monoterpene hydrocarbons in HD oil (0.288 g/100 g fresh leaves) was slightly higher compared with that in the SFE extract (0.242 g/100 g fresh leaves). The SFE extract possessed higher concentrations of the sesquiterpenes, light oxygenated compounds and heavy oxygenated compounds than the HD oil. The relationship between the antioxidant activity and chemical composition of the extracted oils was investigated. The significant amounts of *p*–cymen-7-ol and thymol are responsible for the antioxidative activity of both extracts. The concentration of both compounds, but especially that of *p*–cymen-7-ol (2.25%), is higher in the SFE extract. This corresponds with the higher antioxidative activity of the SFE compared with the HD extract. *p*–Cymen-7-ol, a compound newly identified in leaves of *Eucalyptus* species, exhibited superior antioxidant activity in comparison with that of butylated hydroxyanisole [18].

Study [5]:

The leaf essential oils (six samples) from three clones of *Eucalyptus camaldulensis* Dehnh. were characterized by gas chromatography-mass spectrometry. Radical scavenging and antioxidant properties were investigated by the 1,1-diphenyl-2-picylhydrazyl (DPPH) assay and the *β*-carotene bleaching test. It was found that the whole essential oil and its fractions had significant antioxidant effects when they were tested by each method. In the DPPH assay, the *E. camaldulensis* leaf oils showed IC_{50} inhibitory concentrations in the range of 1.75–12.62 mg/ml. In the *β*-carotene bleaching test, the IC_{50} valued were in the range of 14.30–118.55 μg/ml [19].

CHAPTER THREE

3. Materials and Methods

3.1. Study Design: Experimental study design.

3.1.1. Study Area: The research was done in Napata Collage ,University of Medical Sciences and Technology UMST (for GC-MS)and Institute of the Medicinal, Aromatic Plants, and Traditional Medicine Research (MAPTMRI).

3.1.2. Study Duration: The research was conducted between September, 2022 and December,2022.

3.1.3. Data Collection:

Primary Data Collection: From manual and computerized experiments and observations.
Secondary Data Collection: From references (journals, published articles, books, and websites).

3.1.4 Collection of the Plant:

Leaves of the *E. .camaldulensis* were collected Khartoum state in October, 2022. The plant was recognized and authenticated by one taxonomist from the Botany Department, Faculty of Science, University of Khartoum, Sudan. The plant was identified visually through the characteristics of leaves and flowers. The leaves were cut, washed, and shade dried. Plant materials were then milled using mortar and pestle.

3.2.Preparationof plant extracts

3.2.1. Methanolic extract

Twenty grams of the dry powder from the plant material will subjected to steam distillation using methanol (200 mL) as organic solvent, at room temperature.

3.3. Gas Chromatography\Mass Spectroscopy analysis (GC\MS) Gas chromatography coupled to mass spectrometry analyses will perform using QP2010- Shimadzu equipment, operating in the EI mode at 70 eV. An SLB5 column (30 m x 0.25 mm x 0.25 µm) will employ with a 20-min temperature program of 190-325°C at 5°C/min. The injector temperature was 280° C, the flow rate of the carrier gas (helium) will 0.8 mL/min, and the split ratio was 1:50.

3.4. Antioxidant activity

DPPH radical-scavenging test Antioxidant activity of extracts will estimate using *in vitro* 2,2-diphenyl-1- picrylhydrazyl (DPPH) scavenging radical method. Test samples will dissolve separately in methanol to get test solution of 1 mg/mL. Assays will perform in 96-well, microtiter plates. 140 µL of 0.6.10-6 mol/L DPPH will add to each well containing 70 µL of sample. The mixture will shake gently and leave to stand for 30 min in dark at room temperature. The absorbance will measure spectrophotometrically at 517 nm using a microtiter plate reader. Blank will have done in the same way using methanol and sample without DPPH and 4.1 g. and control was done in the same way but using DPPH and methanol without sample. Ascorbic acid was used as reference antioxidant compound. Every analysis was done in triplicate.

3.5. Bacterial strains and growth conditions

The essential oils were tested at 10 µL per disc for their putative antimicrobial activity against one bacterial specie *(E. coli)*

The antimicrobial activity of EOs was determined by using the disk agar diffusion method. The growing stock cultures were stabilized through various cycles for uniform growth. Sterilized Muller Hinton agar (Sigma-Aldrich, Taufkirchen, Germany) was cooled to 50 ″C and inoculated with 100 µL fresh culture of each one of the above mentioned bacteria (105–106 bacteria/mL), separately. The inoculated medium (15 mL) was poured into sterilized petri dish of 9 cm diameter and swirled to distribute homogenously. Disks (9 mm diameter, Whitman filter paper no. 3) injected with 20 µL either oil or standard antibiotics (see below) were applied on solid agar medium. The plates were placed at 4 ″C for 1–2 h and then incubated at 37 ″C for 24 h. The zones of inhibition (including the size of the disk as well = 0.9 mm) on the media were measured with ruler.

3.6 Testing of antimicrobial:

3.6.1 Culture media:

Muller Hinton agar dehydrated media (38g) was dissolved in liter of purified water and heated with frequently agitation. Media was sterilized at 121℃for 15 minutes and cooled to 45-50℃ and dispensed into sterile Petri dishes.

3.6.2 Preparation of reference strain of bacteria:

Bacteria stander was bringing from National public health Laboratory (stack) in Brain Heart infusion media, and then was grown on nutrient agar, incubated at 37f℃ or 24 hours, the suspended bacteria growth was harvested in sterile normal saline and the suspension ready to uses.

3.6.3 Preparation of reference strain of fungi:

Fungal was bring from stack in Brain Heart infusion media, and then was grown on nutrient agar, incubated at 25 ℃ for 24 hours, the suspended fungal growth was harvested in sterile normal saline and the suspension ready to uses.

3.6.4 Preparation of serial dilution:

Using one ml of extract (100% conc.) put in 1st tubes which contain one ml of peptone water (50% conc.), half of the tube transfer to the 2nd tube with 1 ml of peptone water (25% conc.) the process continue to the last tube (12.5% conc).

3.6.5 Antimicrobial sensitivity using Disk diffusion method:

Filter paper of 5mm diameter using Whatman no.1filter paper was prepared and sterilized by autoclaving at 121℃ for 15mintes, kept in the refrigerator for further use. The test microorganism was subculture from brain heart infusion media to sterile nutrient agar plates, then make suspension of micrograms by use sterile peptone water and compare with 0.05%McFarland reagent, then used sterile cotton swabs take from suspension and make streaking in Mueller Hinton media. 30µl of the various extract i.e. ethanol extract, petroleum ether extract was aseptically transferred to each disc at all dilutions with concentration (2000,1000,500, 250, 125). Then by flamed forceps the discs were aseptically placed over the Muller Hinton agar seeded with test microorganism. Plates wears incubated in an upright position at 37 ℃ for 24hours.after 24hours the diameter of zone of inhibition was measured in mm and result were recorded.

3.6.6 Disc diffusion susceptibility tests:

Disc diffusion techniques are used by most laboratories to test routinely for antimicrobial susceptibility. A disc of blotting paper is impregnated with a known volume and appropriate concentration of an antimicrobial, and this is placed on a plate of susceptibility testing agar uniformly inoculated with the test organism. The antimicrobial diffuses from the disc into the medium and the growth of the test organism is inhibited at a distance from the disc that is related (among other factors) to the susceptibility of the organism. Strains susceptible to the antimicrobial are inhibited at a distance from the disc whereas resistant strains have smaller zones of inhibition or grow up to edge of the disc. (Monica Cheesbrough, 2006).

CHAPTER FOUR

4. Results:

4.1. Extractive Yields and Physical Characteristic:

The weight of the plant material before drying = 100 g.
The weight of the plant material after drying = 50 g.
The yield % = 1.8%

Extractable matter percentage is one of the parameters used for the characterization of crude drugs, the average of the extracted oil is 1.8%. The color and texture of the oil was also reported (Table 1).

❖ **Table 1: Yield and Physical Characteristic of E. camaldulensis extracted oil**

Type of Extract	Yield %	Physical State	Color	Texture
	1.8%	Liquid	Pale yellow	Smooth

4.2. Gas Chromatography-Mass Spectrometry (GC-MS): -

This study evaluated the chemical composition of the essential oil from the leaves of *E. camaldulensis*, through GC-MS procedure. A total of seventeen compounds were identified representing 99.0% of the total oils. The major constituents of the leaves were; Eucalyptol (34.15 %), P-Cymene (22.07%), (-)-Globulol(9.14%),1H-Cycloprop[e]azulene, decahydro-1,1,7-trimethyl-4-m

(8.17%), gamma.-Terpinene(5.60%). The results of the GC-MS analysis of the oils are presented in Table 2and Fig 1.

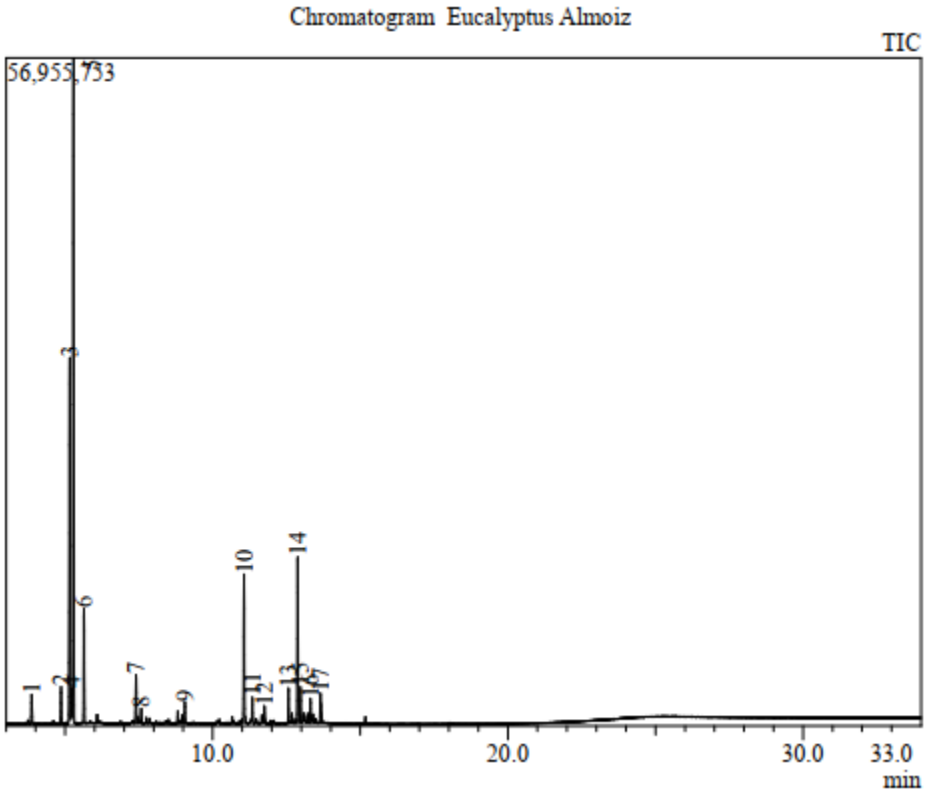
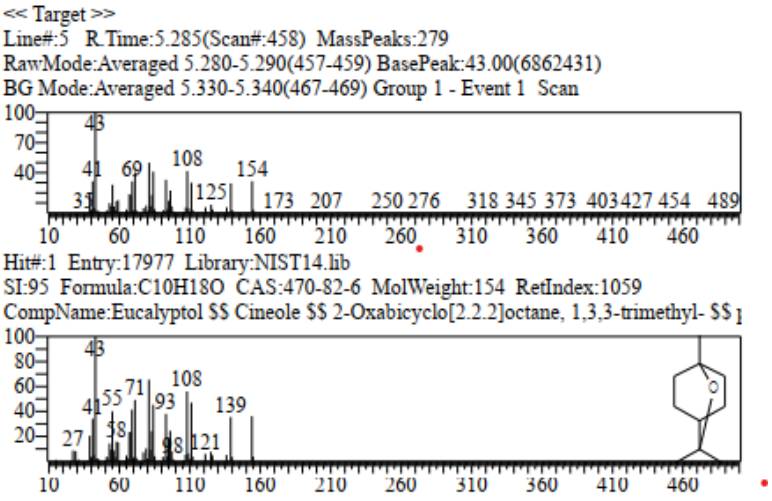


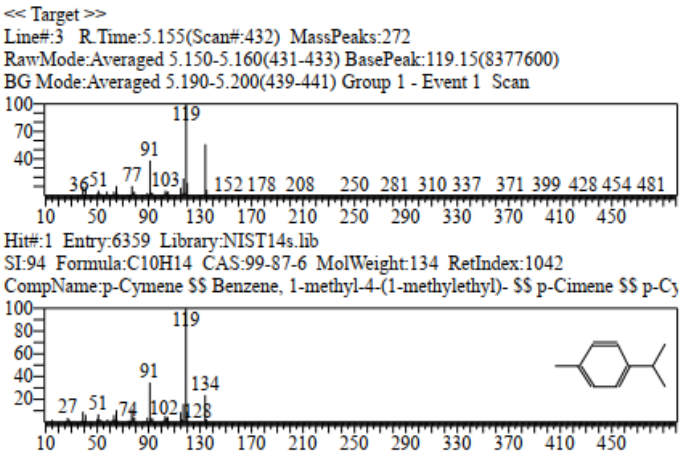
Fig 4.1: GC-MS Chromatogram of *E. camaldulensis* essential oil

Table 2: Chemical Composition of *E. camaldulensis* essential oil

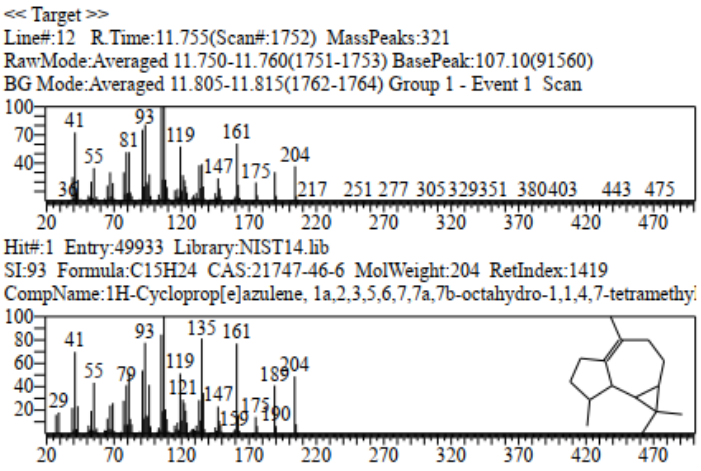
Peak #	Name	R.Time	Area	Area%
1	.alpha.-Pinene	3.867	5063215	1.91
2	.alpha.-Phellandrene	4.862	5601841	2.12
3	p-Cymene	5.156	58418869	22.07
4	D-Limonene	5.217	5572696	2.10
5	Eucalyptol	5.284	90407492	34.15
6	.gamma.-Terpinene	5.639	14817032	5.60
7	Terpinen-4-ol	7.405	7609442	2.87
8	.alpha.-Terpineol	7.580	1910024	0.72
9	Phenol, 2-methyl-5-(1-methylethyl)-	9.058	2876426	1.09
10	1H-Cycloprop[e]azulene, decahydro-1,1,7-trimethyl-4-m	11.061	21628705	8.17
11	(1R,9R,E)-4,11,11-Trimethyl-8-methylenebicyclo[7.2.0]	11.346	3150820	1.19
12	1H-Cycloprop[e]azulene, 1a,2,3,5,6,7,7a,7b-octahydro-1	11.753	2844518	1.07
13	(1aR,4S,4aR,7R,7aS,7bS)-1,1,4,7-Tetramethyldecahydr	12.570	4981379	1.88
14	(-)-Globulol	12.878	24205397	9.14
15	1H-Cycloprop[e]azulene, decahydro-1,1,7-trimethyl-4-m	12.979	5178581	1.96
16	5-Azulenemethanol, 1,2,3,3a,4,5,6,7-octahydro-.alpha.,.	13.312	3666122	1.38
17	2-(4a,8-Dimethyl-2,3,4,5,6,8a-hexahydro-1H-naphthale	13.651	6809810	2.57
264742369				100.00



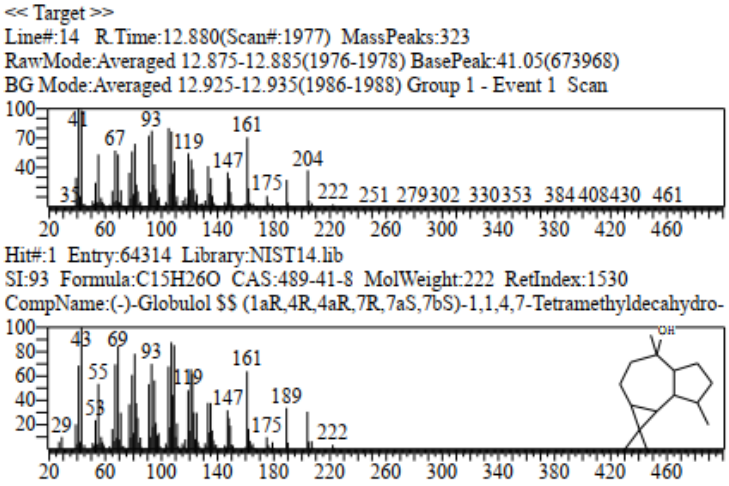
Chromatogram of Eucalyptol



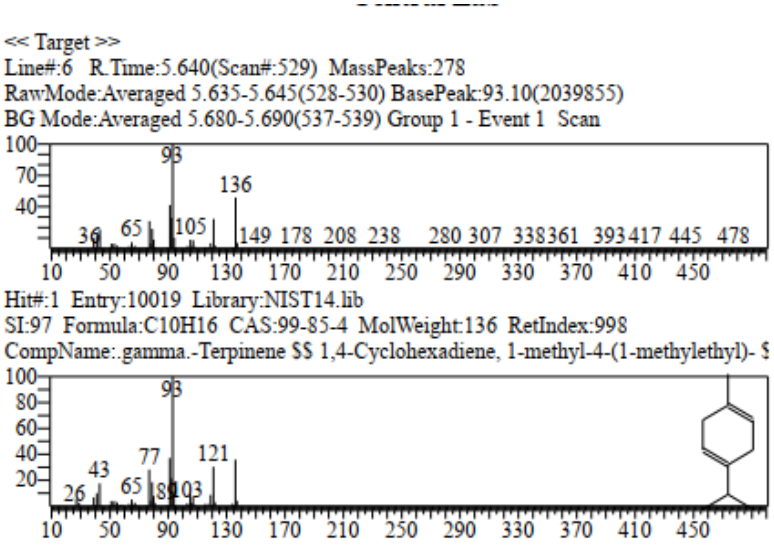
Chromatogram of p-Cymene



Chromatogram of 1H-Cycloprop[e]azulene, decahydro-1,1,7-trimethyl-4-m



Chromatogram of (-) Globulol



Chromatogram of gamma-Terpinene

Fig 4.2: GC-MS Chromatograms of the Major Identified Components of *E. camaldulensis* essential oil

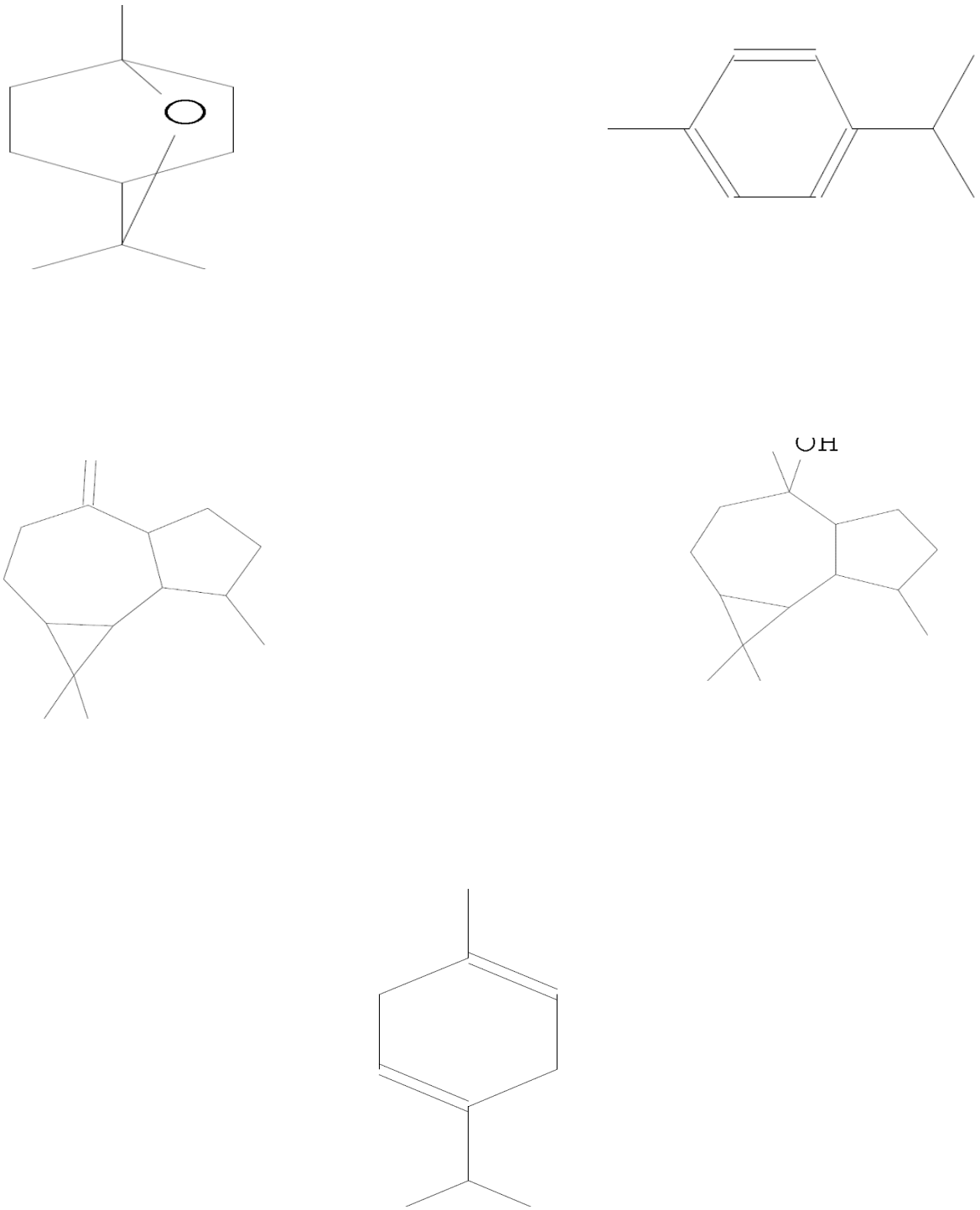


Fig 4.3: Chemical structure of the major identified compound of *E. camaldulensis* essential oil

4.3. Antimicrobial Activity

The antibacterial and antifungal activities results are summarized in Tables 3 and Fig 4. The results indicate that, the inhibition zones resulting from the antibacterial activity ranged between 44 and 8 mm at 12.5, 25 and50 30 µg/mL oil concentrations. The inhibition zones resulting from the antifungal activity against *C. albicana* ranged between 40 and 12 mm at same concentration.

Table 3: Zone of Inhibition (mm) of *E. camaldulensis* essential oil against tested bacteria

Bacterial strain	Zone of inhibition by mm		
	50%	**25%**	**12.5%**
E.coli	**44mm**	**41mm**	**20mm**
C.albicans	**40mm**	**20mm**	**12mm**
S,aureus	**34mm**	**10mm**	**No inhibition zone**
K.pneumoniae	**12mm**	**8mm**	**No inhibition zone**
P.aerginosa	**No inhibition zone**	**No inhibition zone**	**No inhibition zone**

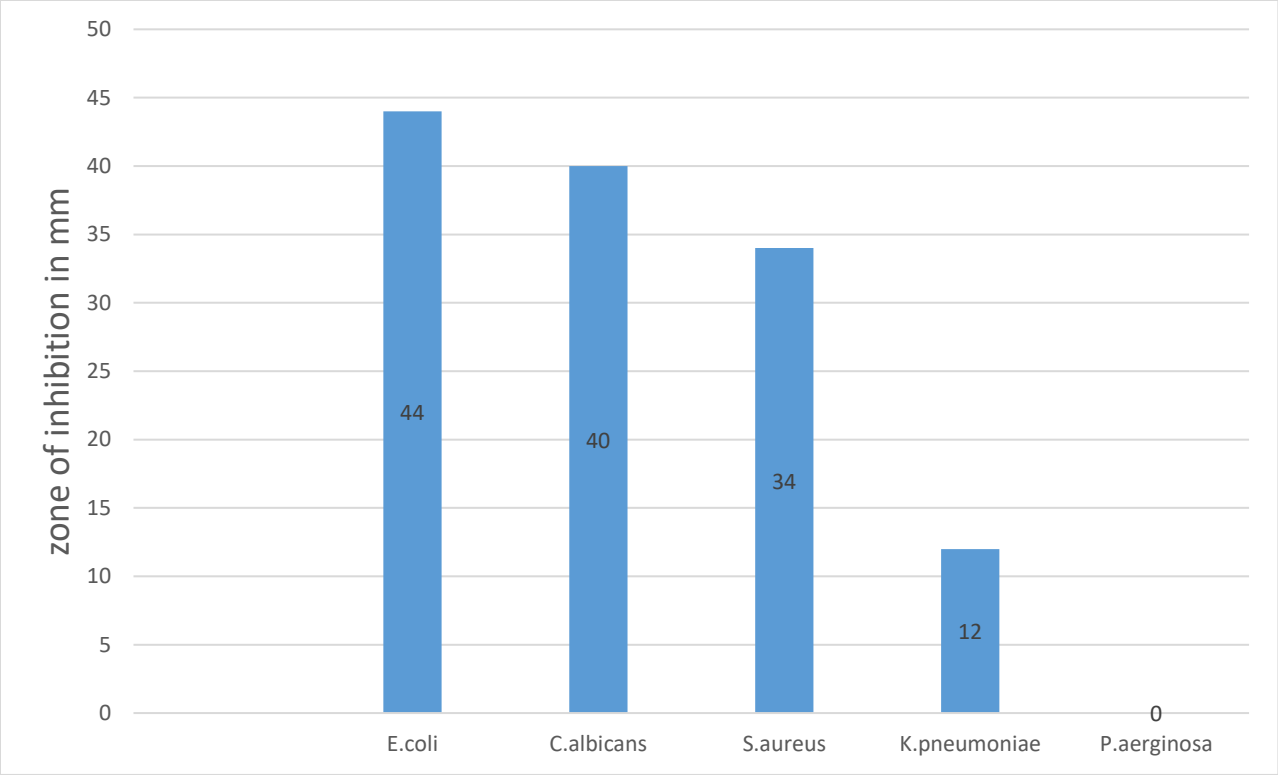


Fig 4: Zone of Inhibition (mm) of *E. camaldulensis* essential oil against tested bacteria

4.4. Antioxidant Activity

Percentage inhibition of DPPH free radical scavenging activity of *E. camaldulensis* essential oil are presented in (Table 5and Figure 8). The oil exhibited weak activity (58**±0.01**) this inhibition is lower than that obtained by Propyl gallate (92**±0.0**).

Table 5: DPPH Free Radical Scavenging Activity of *E. camaldulensis* essential oil

No.	Sample Code	%RSA ±SD (DPPH)
1	*E. camaldulensis*	58 ±0.01
Standard	Propyl gallate	92 ±0.0

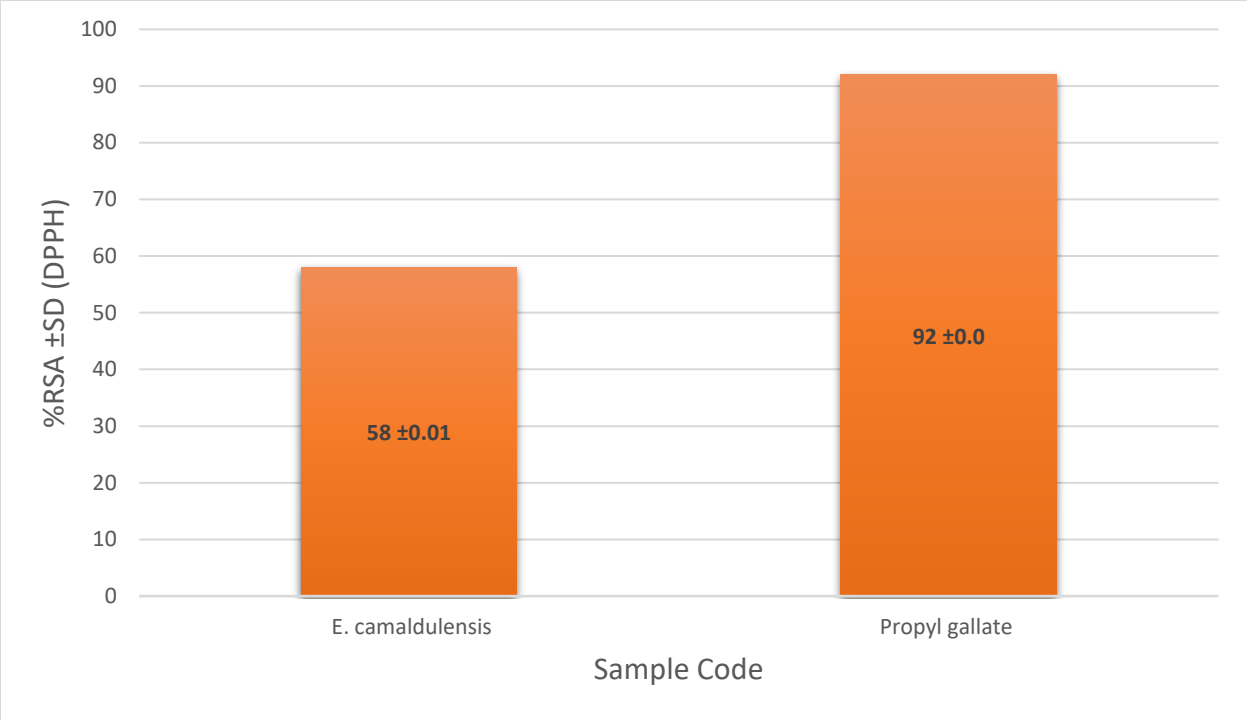


Fig 6: DPPH Free Radical Scavenging Activity of *E. camaldulensis* essential oil:

CHAPTER FIVE

5.1 Discussion:

Extraction represents the primary step in obtaining the crude mixture of compounds from plants. Quality and quantity of the extracts dependent on the target compound structures, natural sources, and type of processes. (Karacabey *et al.*, 2013), explaining the different phenolic composition in the extracts obtained with different procedures. In this study, the chemical composition, antimicrobial and antioxidant activities of ***E. camaldulensis*** essential oil were determined. The oil obtained yield percentage 1.8%. with pale yellow colour.

The analysis of oil through the GC-MS technique is revealed different number of compounds, belonging to different classes of secondary metabolite, the major are Eucalyptol (34.15 %), *p*-Cymene (22.07%), (-)-Globulol (9.14%),1H Cycloprop[e]a zulene, decahydro-1,1,7-trimethyl-4-m (8.17%), gamma.-Terpinene(5.60%).

Thus, it was previously reported that the main constituents of oil in *E. camaldulensis* were 1,8-cineole (21.75%),β-pinene (20.51%), α-pinene (15.6%) and terpineol(9.41%); spathulenol (37.46%), *p*-cymene (17.20%) and crypton (8.88%) in *E. gomphocephala*; spathulenol(18.37%), *p*-cymene (19.38%) and crypton (16.91%) in *E.camaldulensis* (Salem *et al*.2015).On the other hand ,Dogan *et al* (2017)determined the main leaf oil constituents which were; *p*-cymene (42.1%), eucalyptol (1,8-cineole)(14.1%), α-pinene (12.7%) and α-terpinol (10.7%)which is similar to our findings.

Thus our results showed that, plant oil is rich in terms of monoterpene hydrocarbons and oxygenated monoterpenes.

The study of the antibacterial, antifungal activities of essential oils of the leaves exhibited potent antimicrobial activity against three tested bacterial strains and one pathogenic fungus *C.albicans. inhibition* zome is ranged betwwen 44to 8ml/mgat different concentration.

Ghalem and Mohamed [46], demonstrated that leaf essential oils of *E. globolus* and *E.camaldulensis* exhibited inhibitory effects on *S. aureus* more than *E. coli*.

It is well demonstrated that the antimicrobial activity of an essential oil is linked to its chemical composition. The functional groups of compounds found in essential oils are associated with their antimicrobial characteristics. It was reported that the antibacterial activity of *Eucalyptus* essential oils is generally due to components such as 1,8-cineole (eucalyptol), *p*-cymene, pinene, γ-terpinene, which were detected in our plant oil. For example, several reports have shown that 1, 8-cineole has strong antimicrobial activity against many important pathogens and spoilage organisms, including *S. aureus* and *E. coli, these* reports strongly support our findings. The antimicrobial activities of *E. camaldulensis* leaf oils could be attributed to eucalyptol (1,8-cineole), *p*-cymene. (Sonboli 2006).

Antioxidant activity was also studded through DPPH assay, results values revealed that the antioxidant activity of *E. camaldulensis* essential oil was still less active than the standard Propyl gallate. This activity may be attributed primarily to the less content of phenolic components of the *E. camaldulensis* essential oil.

From the obtained results, it is obvious that the chemical composition of the essential oil has an important impact on the antioxidant activity of the oil, in relation to the presence of some substances such as α-thujone, camphor, 1,8-cineole, β-thujone and borneol which are absent in our oil.

CHAPTER SIX

6.1 Conclusion and Recommendations:

Our results showed that *E. camaldulensis* essential oil is rich in terms of monoterpene hydrocarbons and oxygenated monoterpenes. This study demonstrates the occurrence of *p*-cymene/ eucalyptol (1,8-cineole) and α-terpineol chemotype of *E. camaldulensis* in Sudan.

This study clearly indicated that, essential oils from plant leaves have antibacterial and antifungal effects on some pathogen microorganisms. This essential oils showed antimicrobial activity with inhibition zone ranged between 44to 8 mg/ml at different concentrations. The antioxidant activity express weak percent comparing to that of the standard. The major components of the essential oils, eucalyptol and *p*-cymene, could be responsible for these effects. Consequently, leaf essential oil appeared to be more effective, this effect might be because of its rich *p*-cymene content.

Recommendations:

1. Further GC-MS was performed for identification of bioactive compounds of essential oil of *E. camaldulensis***.**

2. Further research is therefore recommended to isolate, purify, and characterize these chemical constituents with a view to supplementing conventional drug development especially in developing countries.

3. Further, *in vivo* studies are required to shed light on the mode of action of this medicinally important plant and isolation of individual compounds would help in developing treatments with fewer side effects.

4. Further investigation of essential oil activity against a wider range of microorganism including multidrug resistant bacteria.

5. Conduction of cytotoxicity studies on the plant essential oil extract to evaluate its safety.

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Appendix

GC-MS Device


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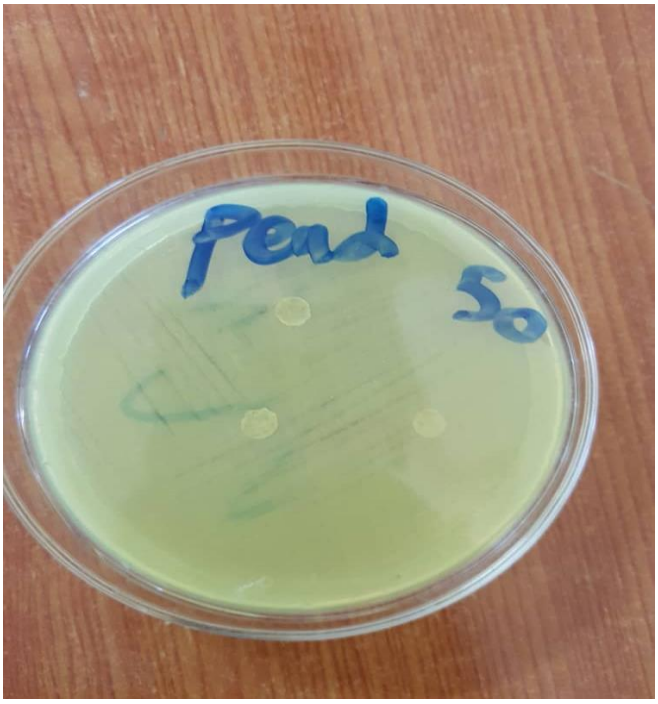
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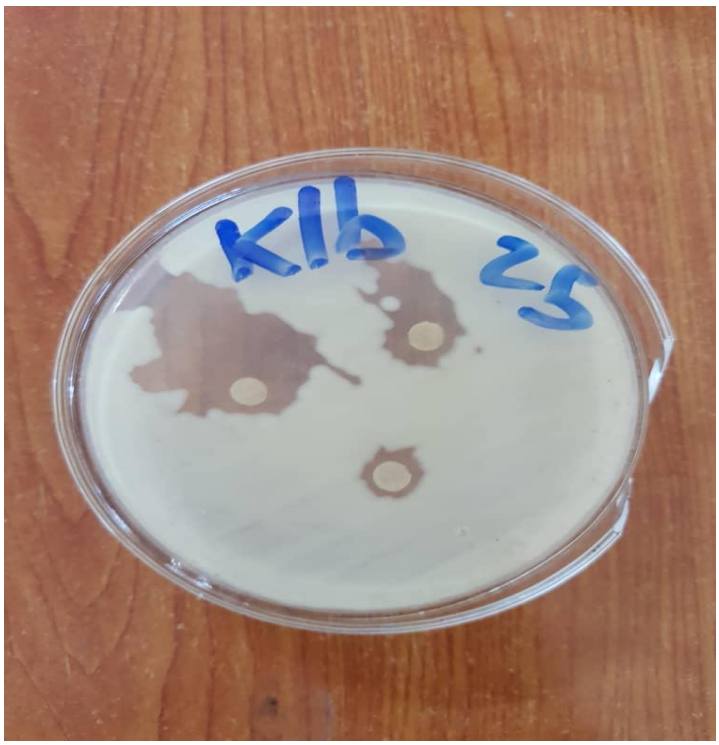
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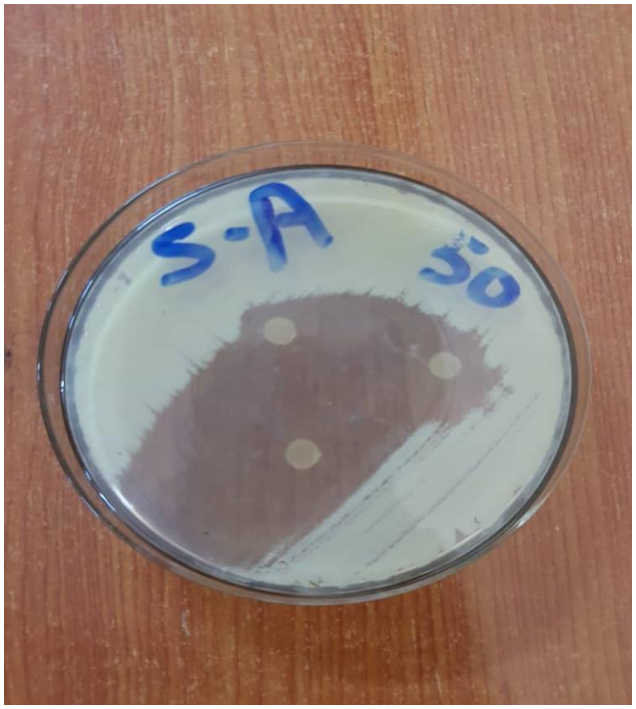
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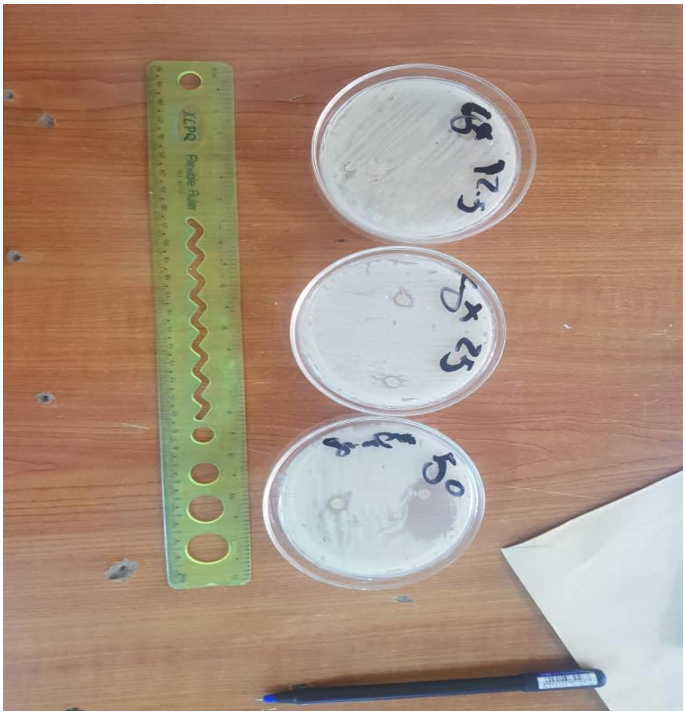
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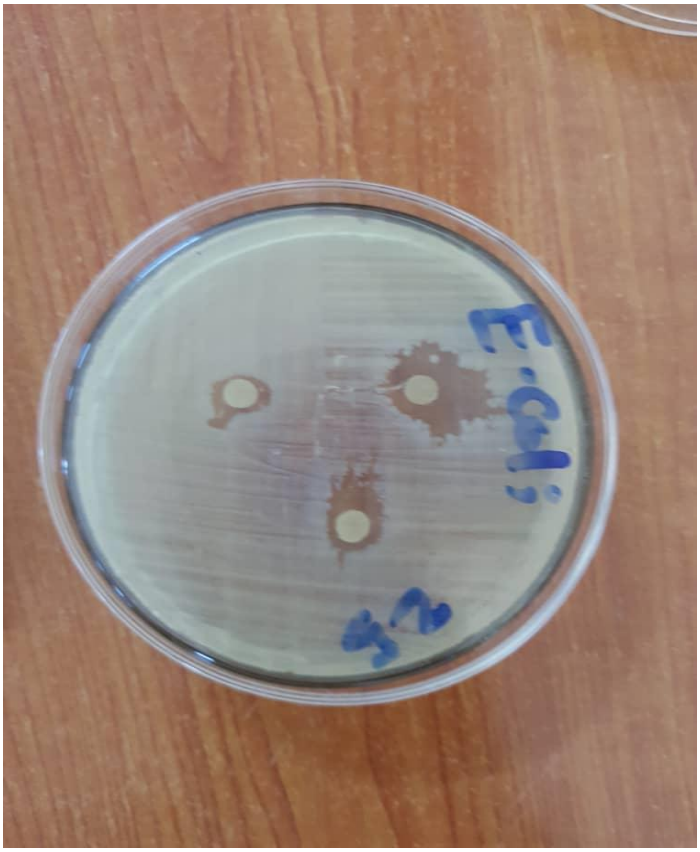
Cand 12.5
Cand 25
Cand 50


E. Coli 12.5


Pad
25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Kle
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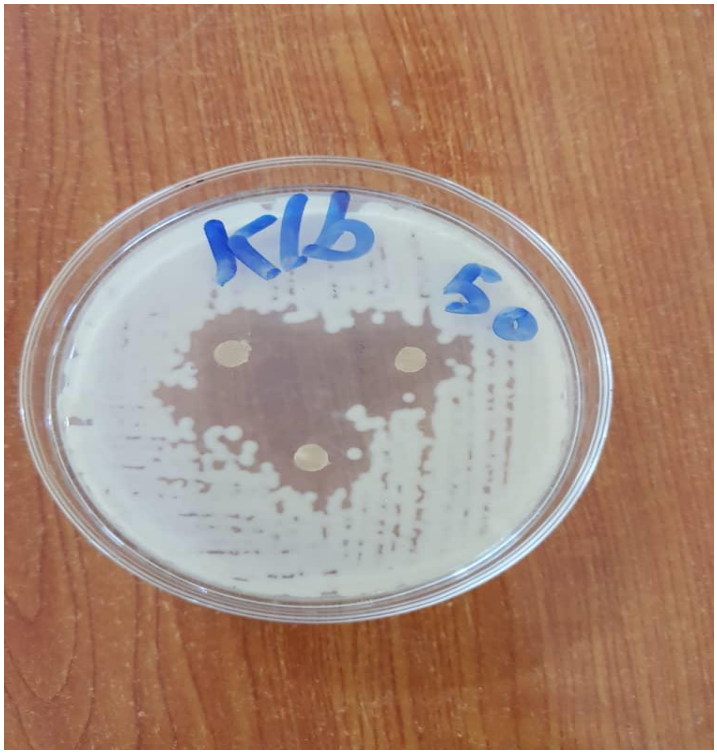
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