Chemical Composition and Antimicrobial Activity Of Essential Oils Extracted From Eucalyptus Camaldulensis Leaves Grown In Sudan

Mohamed A. Diriye[1,*], Mahmoud Mohamed Ali[2], Omer A. Ishag[3]

Mukhtar Abdi Mohamed[4]

[1,2.3.4] Faculty of Pure and Applied Science, Department of Applied and Industrial Chemistry,

International University of Africa,Sudan

[a]mamd86@hotmail.com, [b]mahmoudy11@yahoo.com, [c]oaisaack@hotmail.com, [c]mukhtaar522@gmail.com.

Abstract

The essential oil content, determined via hydrodistillation using Clevenger apparatus, of the leaves of *Eucalyptus camaldulensis* collected from single location in the garden of the International university of Africa(IUA) (Khartoum area) was found to be (1.6%). The identification of the oil constituents and their percentage composition were investigated using gas chromatography-mass spectrometry (GC/MS) analysis. 34 constituents were identified in the oil of *E.camaldulensis*. The composition of the major constituent of the essential oil of the *E.camaldulensis* was found to be rich in eucalyptol (72.95%). the minor constituents in the oil were Alpha-pinene (6.34%), D-lemonene (4.46%), 2(10)-pinen-3-ol (3.17%), p-cymene (2.79%). The essential oils obtained were tested for their biological activities namely antimicrobial activity. Against bacteria (*E. coli*, *Staphylococcus aureus*, *Salmonella typhi, Klebsiella pneumoniae.)* and fungals *(Candida albicans and Aspergillusniger).*

Keywords: *Eucalyptus camaldulensis*, Myrtaceae, essential oil composition, gas chromatography, antibacterial activity.

Introduction:

The Eucalyptus, a native genus from Australia, belongs to the Myrtaceae family and comprises about 800 species [1]. More than 300 species of this genus contain volatile oils in their leaves, although fewer than 20 of these have ever been exploited commercially for the production of essential oils rich in 1,8-cineole (more than 70%) which are used in the pharmaceutical and cosmetic industries [2]. Leaf extracts of Eucalyptus have also been approved as food additives [3]. In fact, for many years there has been intense interest in essential oils as a source of natural products. They have been screened for their potential uses as alternative remedies for the treatment of many infections and as natural food

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preservatives [4,5]. A number of studies have demonstrated the antimicrobial properties of Eucalyptus species essential oils against a wide range of microorganisms. The most studied were those from E. globulus [6,7], E. camaldulensis, E. tereticornis and E. citriodora [6]. Only a few studies have investigated their activity against pathogenic and food spoilage bacteria [8,9]. Eucalyptus essential oils rich in 1,8-cineole (70%) have been used by inhalation in pulmonary infections [10]. E.camaldulensis is an important ethno medicinal plant belonging to the family of Myrtaceae [11]. It is a large evergreen tree up to 20 m in the Sahel and neighboring Sudanese regions. Calcifuges this species gets its Latin name of the city Camaldule Australia. It is located in the east of the continent, especially near rivers. We are called River Gun. This tree easily reaches 40m high and it is diverging branches are often spread [16]. E.camaldulensis is used as a remedy for sore throat and other bacterial infection of the respiratory and urinary tracts. Essential oils of the leaves are used in the treatment of lung diseases while the volatile oils are used as expectorants [11]. Topical ointments containing eucalyptus oil have also been used in traditional Aboriginal medicines to heal wounds and fungal infections. Eucalyptus oil obtained by steam distillation and rectification of the fresh leaves has Eucalyptol (1,8-cineole) as its active ingredient and this is responsible for its various pharmacological actions[12]. The antimicrobial activities of the methanolic extracts of E.camaldulensis have also been reported [13,14]. The emergence of bacterial resistance to the currently available antimicrobial drugs necessitates further research in the discovery of new safe and effective antibacterial agents [15]. Investigation of certain indigenous plants for their antimicrobial activity is therefore of utmost importance. This study is aimed at investgating the antimicrobial activity of Eucalyptus camaldulensis aganist some bacteria and fungals thereby

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establishing it as a potential antimicrobial agent.

Materials and methods:

Collection of the plant material: Samples of clean mature leaves of the *Eucalyptus camaldulensis* were collected from single location in the garden at the department of applied and industrial chemistry, International university of Africa on the 5th day of March 2016. The fresh plant material were shade dried at room temperature over three days.

Extraction of essential oils: 500 gram of dried samples of Eucalyptus camaldulensis were subjected to Hydro-distillation using a Clevenger apparatus for 4 hours Using 500 ml of distilled water. The volume of essential oils was determined from a calibrated trap. The essential oils in the distillate were dried over anhydrous sodium sulfate (drying agent) and kept in the freezer.

GC-MS analysis: GC-MS analysis was carried out by injection (0.1 µL) of the leaf oil on a QP-2010 instrument with a mass selective HP 597A detector fitted with Ulbon HR-1 capillary column (50 m x 0.25 mm, film thickness 0.25 µm). GC-MS operation condition split mode: carrier gas helium at a flow rate of 1.5 mL/min; temperature programme 60-300ºC (100 C/min), injector temperature 300ºC and detector temperature 280ºC. The mass spectrometry conditioned was as follows: ionization voltage, 70 eV; emission current, 40 mA; mass range 0–400 Da, ion source temperature, 200ºC.

Testing of Extracts for Antibacterial Activity on standard bacteria: The cup-plate agar diffusion method was adopted with minor modifications[17]. To assess the antibacterial activity of the prepared extract. 0.01 ml of each standardized bacterial stock suspension colony- forming units per ml had been thoroughly mixed with 20 ml of sterile nutrient agar. 20 ml of the inoculated nutrient agar will be distributed into sterile Petri dishes. The agars plate will be left to set and in each of these plates 1 cup, 10 mm in diameter, was cut using a sterile cork borer No. 4 and the agar discs removed. The cup had been filled with 0.1ml of the extract using micro titer-pipette and allowed to diffuse at room temperature for two hours. plates were incubated in the upright

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position at 37ºC for 18 hours. Methanol was used as a dissolving chemical and had been carried out as control. After incubation the diameters of the results and growth inhibition zones were measured and tabulated. Bacteria tested in this way were four: (*E. coli*, *Staphylococcus aureus*, *Salmonella typhi*, *Klebsiella pneumoniae.)*

Testing for anti-fungal activity: The same method of the bacteria were adopted. Instead of nutrient agar, Sabouraud-dextrose agar had been used. The inoculated mediawere incubated at 25ºC for one day for the *Candida albicans and Aspergillusniger.*

Result and discussion:

Chemical composition of the essential oil isolated from leaves of E. *camaldulensis:*

The yield of essential oil from *E.camaldulensis* after hydrodistillation was 1.6%. The chemical composition of the essential oil is listed in Table(1) By GC-MS components were identified representing 100% of the total oil; most abundant constituents were eucalyptol (72.95%) [Figure.1], Alpha-pinene (6.34%), D-lemonene (4.46%), 2(10)-pinen-3-ol (3.17%), and p-cymene (2.79%). Chemical constituents of the essential oils of E.camaldulensis contain 19 oxygenated terpenoids, and 15 hydrocarbon terpenoids compounds.

Table 1. Chemical composition of the essential oil isolated from leaves of *E. camaldulensis*

Peak#	Component	Area %
1	Beta-pinene	0.12
2	Thujene	0.06
3	Alpha-pinene	6.34
4	Camphene	0.18
5	2,4(10)-thujadiene	0.02
6	Furandione	0.06
7	l-beta.-pinene	0.25
8	Beta-myrcene	0.5
9	Alpha-phellandrene	0.25

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10	Eucalyptol	72.95
11	D-lemonene	4.46
12	(+)-2-Carene	0.06
13	p-cymene	2.79
14	Gamma-terpinene	0.54
15	Terpinolene	0.34
16	Iso-amyl 2-methylbutanoate	0.10
17	Isopentyle pentanoate	0.40
18	Exo-fenchol	0.26
19	Alpha-campholenal	0.05
20	2(10)-pinen-3-ol	3.17
21	Pinocarvone	0.93
22	Endo-borneol	0.32
23	Terpinen-4-ol	0.82
24	Trans-p-mentha-1(7),8-dien-2-ol	0.86
25	Alph-terpineol	0.50
26	2-pinen-10-ol	0.16
27	Cis-carveol	0.16
28	Iso-carveol	0.49
29	2-isopropylidene-3-methylhexa-3,5-dienal	0.28
30	(-)-carvone	0.10
31	Alpha-cubebene	0.03
32	Alloaromadendrene	0.94
33	Gama-eudesmol	0.13
34	Eudesm-4-(14)-en-11-ol	0.84
Total		100

The composition of the essential oil from *E.camaldulensis*, especially from the leaves, has been widely studied. By surveying the data reported we found a great diversity of oil composition, which was effected by many factors such as: geographical origin, tissue explored, date of harvest, genetic factors etc. [18, 19]. Two groups of *E. camaldulensis* essential oils can be distinguished: those that contain 1,8-cineole (eucalyptol) as the main compound, like the oil examined here which include *E. camaldulensis* from Mali,

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Mozambique, Nigeria, Egypt and Iran [20-21] and those that contain spathulenol, p - cymene and cryptone as main compounds, and small quantities of 1,8-cineol, and which are similar to E. camaldulensis from the south of Florida, Jerusalem and Greece [22, 23, 18]. As already mentioned, the essential oil of eucalyptus species is of great commercial value. In the south-eastern countries such as Thailand, E. camaldulensis is mainly planted for the use as a pulpwood. Evidently, during the process of paper making, a large amount of waste such as leaves is disposed. Therefore, the possibility of exploiting the leaves as a source of oil production is being extensively investigated [24].

Figure(1) 1,8-Cineole (Eucalyptol)

antimicrobial activities of E.*camaldulensis* :

Antimicrobial activity is useful in natural product research especially for the treatment of dermatological disease. The essential oils of the leaf of *E.camadulensis* were investigated for their antimicrobial activity against four bacterial and two fungal, the results shown in tables numbered (2,3).

Table (2): The antibacterial Activities of *E.camaldulensis* essential oil.

Bacteria	inhibition zones mm	Activity
E. coli	21	+ + +
Staphylococcus aureus	24	+ + +
Salmonella typhi	23	+ + +
Klebsiella pneumonia	18	+ +

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Table (3): The antifungal Activities of *E.camaldulensis* essential oil.

Fungal	*inhibition zones mm*	Activity
Candida albicans	30	+ + +
Aspergillusniger	18	+ +

The Activity:

The essential oils were tested at 10 μL per disc for their putative antimicrobial activity against four bacterial species and two fungals, the results are shown in Tables (2,3) According to the zone diameter inhibition (zdi) values expressed in mm, results were ranked as follows: not sensitive (–) for zone diameters equal to 8 mm or below; sensitive (+) for zone diameters between 8 and 14 mm, very sensitive (++) for zone diameters between 14 and 20 mm and extremely sensitive (+++) for zone diameters equal or larger than 20 mm [25-26]. The essential oils of the leaf of E.camaldulensis were investigated for their antimicrobial activity against four bacteria and two fungal. Obtained results of antimicrobial experiment showed that the essential oils of E.camaldulensis have high activity to prevent the growth of E.coli, S.aureus, S.typhi(bacteria) and Candida albicans (fungi) ranged between (21-30) mm. Antimicrobial activity of essential oil is due to the presence of a mixture of monoterpenes and oxygenated monoterpenes (most of the antimicrobial activity in the oils has been attributed to the oxygeated monoterpenes); identification of such compounds with a wide biological activity is critical for the mankind as it helps in the search for chemical structures that should assist in designing new drugs as therapeautic agents against human pathogens. The antimicrobial activity of the essential oil from Eucalyptus leaves can be attributed to the presence of high concentration of 1,8-cineole (15% -78%) [27], which has been reported to stimulate respiration, relieve coughing, helps to expel mucus, relax the respiratory muscles, and thus it is used for the management of bronchitis, asthma, catarrh, sinusitis and throat infections [28]. It has been found to have relatively strong antimicrobial properties

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against many important pathogens and spoilage organisms including S.aureus and E. coli [29]. In addition, other compounds suchas α-Pinene, p-cymene, β-caryophyllene, β -pinene, spathulenol and carvacrol, which have relatively strong antibacterial properties may be responsible for this activity [30,31,32-33]. The minor compounds such as borneol, pulegone, thujone, γ-terpinene and nerolidol already are known to exhibit an antibacterial activity [31,34-35]. Therefore, the synergistic effects of these active chemicals with other constituents of the essential oil should be taken into consideration for the antimicrobial activity [63,29,34].

Conclusion:

E.camaldulensis is a potential source of essential oil and Hydro-distillation is the method used to for extract the eucalyptus oil, and The main constituent of the oil is Eucalyptol (72.95%). The present study has revealed that essential oil of E.camaldulensis leaves have significant antimicrobial activity against E.coli, S.aureus (bacteria) and Candida albicans (fungi). This confirms traditional medicine use of essential oil of Eucalyptus leaves as an antibacterial agent. The results of this study, therefore, form a good basis for selection of essential oil of E.camaldulensis leaves for their use as a natural antimicrobial agent for the treatment of several infectious diseases caused by E. coli, S. aureus and Canida albicans which have developed resistance to antibiotics.

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