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Research Article

EXPLORING THE POTENTIAL OF EUCALYPTUS GLOBULUS ESSENTIAL OIL AS A NATURAL ANTIMICROBIAL AGENT: IDENTIFICATION AND CHARACTERIZATION OF BIOACTIVE TERPENOIDAL CONSTITUENTS AND THEIR SEASONAL VARIATIONS

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ABSTRACT

Eucalyptus globulus, a plant from family myrtaceae, commonly known as blue gum, grows well in different parts of world and has been known since decades because of its rich ethanomedicinal and therapeutic importance. The aim of this study is to determine the oil content variation, characterization and antimicrobial properties of the essential oils of *Eucalyptus globulus* grown in Saharanpur region (Uttar Pradesh). The leaf oil from *Eucalyptus globulus* tree obtained by hydro - distillation significantly containing their oil content from the range 0.6% to1.04% v/w. The chemical constituents of essential oil were analysed by Gas Chromatography – Mass Spectroscopy (GC - MS). Twenty compounds were characterized in the oil and the main components of the essential oil were Eucalyptol, α–Terpineol, gamma-Terpinene, Alloaromadendrene, Epiglobulol, (-)-Globulol, (-)-Isolongifolene, Nerolidol. The results of the antimicrobial activity tests revealed that the essential oil of *Eucalyptus globulus* has antimicrobial activity, especially against bacterial strain *Pseudomonas. Aeruginosa (MTCC 2642)* i.e.11.50mm 20 µL of oil/disc and fungal strain *Candida. Albicans* i.e 12mm 20 µL of oil/disc.

INTRODUCTION

The emergence of antimicrobial resistance has become a significant public health concern, leading to the need for new antimicrobial agents. Essential oils have been identified as a promising alternative to synthetic antimicrobial agents due to their natural origin, low toxicity, and potential effectiveness[1]. *Eucalyptus globulus* essential oil is one such essential oil that has shown promising antimicrobial properties against a variety of pathogenic microorganisms, including bacteria, fungi[2-3].

The potential antimicrobial activity of *Eucalyptus globulus* essential oil can be attributed to its bioactive terpenoidal constituents, including 1, 8-cineole, α- pinene, and limonene among others[2]. Previous studies have suggested that the composition of *Eucalyptus globulus* essential oil varies depending on several factors, including the season in which it is harvested[4]. Therefore, it is crucial to identify and characterize the bioactive terpenoidal constituents of *Eucalyptus globulus* essential oil and assess their potential as natural antimicrobial agents, taking into account their seasonal variations.

Seasonal effects on essential oil content (percentage) of *Eucalyptus globulus* species were investigated. Few studies have been reported on the chemical constituents of the essential oil obtained from the leaves of *Eucalyptus globules Labill*[5], and to remedy this, we choose to investigate one of the major Eucalyptus species - *Eucalyptus globules Labill* which grows in Saharanpur region (Uttar Pradesh). We report here the isolation of the water-distilled volatile oil from the leaves of *Eucalyptus globules Labill*, the qualitatively examination of the chemical constituents of this essential oil and their quantitative determination by direct comparison with results from MS databases attached to the GC-MS instruments following GC-MS analysis. This research paper aims to explore the potential of *Eucalyptus globulus* essential oil as a natural antimicrobial agent by identifying and characterizing its bioactive terpenoidal constituents and their seasonal variations. This study will contribute to a better understanding of the chemical composition of *Eucalyptus globulus* essential oil and its potential use as a natural antimicrobial agent.

In addition to its potential as an antimicrobial agent, *Eucalyptus globulus* essential oil has been reported to possess other biological activities, such as antioxidant, anti-inflammatory,

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and analgesic properties[6-7]. These properties have led to its use in traditional medicine for the treatment of various ailments, including respiratory disorders, wounds, and rheumatism[8].

METHODOLOGY

Collection of plant material

Fresh leaves of *Eucalyptus globulus* commonly known as Blue gum were collected from Saharanpur region (Uttar Pradesh) in July 2021 to June 2022. Plant was got authenticated from Indian Institute of Integrative Medicine (CSIR) Jammu 180002. All solvents and reagents were of analytical grade.

Extraction of volatile oil

The fresh mature leaves (250 g) were completely immersed in water overnight, then hydro distilled for three hours for complete extraction of essential oil, using a commercial Clevenger apparatus. The light yellowish green coloured oil was obtained. The oil samples obtained from hydro distillation were freed from moisture by adding anhydrous sodium sulphate and absolute oil samples were obtained. The resulting oils was collected, preserved in a sealed amber glass sample tube and stored at 4°C under refrigeration until analysis. The amount of oil obtained from plant material was calculated as: Oil (% v/w) = Observed volume of oil (ml)/Weight of sample (g) × 100.

Chemical analysis

Determination of the chemical composition of the extracted Eucalyptus oil was carried out by Shimadzu GC-17A Gas Chromatography (GC) and Gas Chromatography coupled with the Mass Spectroscopy QP5050column (GC/MS), respectively.

Gas chromatography analysis

The analysis of the essential oil were carried out on Nucon Gas chromatograph equipped with FID detector with EC WAX open tubular column .The oven temperature was programmed from 40°C for 5 min to 240°C at 5°C/min. Injection and detector temperature were 200°C and 240°C respectively. Nitrogen was used as the carrier gas. Diluted oil with solvent (1µl) was injected into the GC.

Gas chromatography / Mass spectroscopy analysis

GC-MS analyses of the oil were performed on Varian cpsil 8 capillary column (30 m×0.25mm×0.25µm thick film).The oven temperature was programmed at 50-250°C at the rate of 5°C/ min .Helium was used as carrier gas (1ml/min). Split ratio was 1:50. 1 µl of diluted oil in toluene was manually injected into the GC/MS.

Compound identification

The constituents were identified by comparison of their mass spectra with those of NIST02 library data for the GC-MS system. The results were further confirmed by comparison of their retention indices of the compounds with literature data.

Antimicrobial Activity

Microbial strains

The essential oils extracted from *Eucalyptus globules* leaves were tested against bacteria [Gram + ve microbial strains: *Staphylococcus aureus* (MTCC 96), *Bacillus subtilis* (MTCC 2451) and Gram - ve microbial strains: *Escherichia coli* (MTCC 729), *Pseudomonas aeruginosa* (MTCC 2642), *Salmonella typhi* (MTCC 1251)] and Fungi [*Candida. Albicans*, *Aspergillus Niger*] using paper disc diffusion method. All the bacterial, fungal strains were procured from the Microbial Type Culture Collection and gene bank (IMTECH, Chandigarh, India). The antibacterial and antifungal activity was determined by observing the zone of inhibition in comparison to standard antibiotic Ciprofloxacin disc and Amphotericin B.

Antimicrobial activity of volatile constituents of essential oils

Nutrient agar plates were prepared by pouring 20 ml of sterile medium cooled to 45°C in presterilized plates aseptically. After solidification of the medium plates were inverted and incubated at 37°C overnight to check for any contamination of the medium / plates etc. The plates were inoculated with 100µl of 12-16 hrs old culture and spread it with the help of spreader, so that a uniform lawn of culture is obtained. Petriplates were allowed to dry. Discs measuring 5 mm in diameter were prepared from whatman filter paper no.1 using cork borers. The discs were pooled in a Petri dish and sterilized by autoclaving at 15 lbs / psi for 20 min.The discs were then dried in hot air oven at 100°C for 1 hour. Using sterile forceps 3 discs were placed on the surface of agar medium of inoculated plates. Discs were in complete contact with the agar surface. On each of the discs the 20 microliter of parent oil was absorbed aseptically. Plates were incubated at 37°C for 24 hours. Plates were visually examined for the inhibition zone around the discs. Size of inhibition zone indicated the sensitivity of the culture to the parent oil.

RESULT AND DISCUSSION

From Fig.1, it can be seen that the content of essential oils in different months varied from 0.6% to 1.04%. The amount of oil obtained from plant material depends on the age of plant leaves from the young leaves more oil is extract out as compared to older branches. The amounts of the components from the volatile oil were determined by the peak area normalization method. This presence of several overlapping peaks shows the complexity of the mixture. The chemical constituents of the essential oil are listed in Table 1. The essential oil consisted mainly of oxygenated monoterpenes, and sesquiterpene hydrocarbons. Of these, 1, 8-eucalyptol (28.09 %), α-terpineol (6.81 %) were the main oxygenated monoterpenes, and (-) - globulol (10.66 %), and epiglobulol (1.87 %), alloaromadendrene (1.83 %), (-)-Isolongifolene (2.09 %) were the main sesquiterpene hydrocarbons. Several significant compounds were gamma-Terpinene (2.42 %), D-limonene (2.49 %). The yield of essential oil and the content of 1, 8-eucalyptol are not within the values reported in the literature[9] and the percentage contents of the main constituents are not similar to those given in the literature[10, 11]. Constituent, such as isolongifolene, nerolidol had no literature data[10, 11]. This possibly may be due to the fact that the *Eucalyptus globulus* trees grown in Saharanpur regions (Uttar Pradesh) is not a typical Eucalyptus species but appears to be a hybrid or mutated and requires further taxonomical studies.

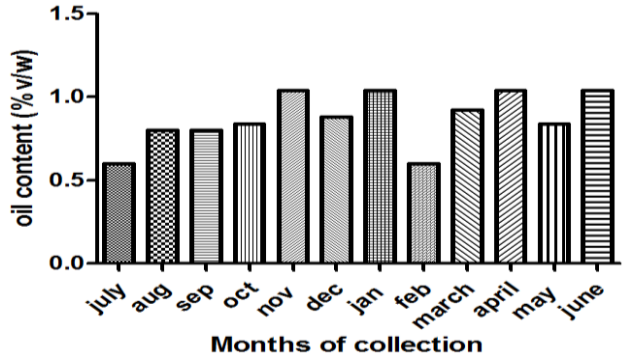

Fig 1 Shows the % age (v/w) yield of oil from the month of July to June

Table 1 Chemical constituents of the leaves of *Eucalyptus globules Labill.*

Peak #	R. Time	Area	Area %	Name	Base m/z
4	9.353	1087694	2.49	D- Limonene	68.10
5	9.415	12290623	28.09	Eucalyptol	43.05
6	9.651	1058818	2.42	Gamma – Terpinene	93.10
10	10.990	2979449	6.81	L - alpha –Terpineol	59.10
12	12.966	799153	1.83	Alloaromadendrene	161.15
14	13.802	820154	1.87	Epiglobulol	82.10
16	13.966	4663868	10.66	(-) – Globulol	43.05
17	14.032	912144	2.09	(-) – Isolongifolene	109.15

The essential oil obtained from *Eucalyptus globulus Labill*. was tested for antimicrobial activity against pathogenic fungi and bacteria. The result of antibacterial and antifungal of essential oil are shown in the Table 2 and 3. Maximum antibacterial activity is shown against *P. aeruginosa* (11.50 ± 0.707) while minimum antibacterial activity is shown against two microbial strains i.e *B. subtilis and* E. *coli* (9.0 ± 0.00). Maximum antifungal activity is shown against Candida. albicans (12 ± 0) as compared to aspergillus niger strain. But *Eucalyptus globules Labill* showed poor activity against grm + ve bacterial, grm - ve bacterial and fungal strains when compared with standard antibiotics ciprofloxacin (5 µg/disc) and Amphotericin B (10 µg/disc).

Table 2 Antibacterial activity against *Euclaptus globulus* oil

S. No	Micro-organism	*Euclaptus globulus* oil (20 µL)	Ciprofloxacin (5µg /disc)
1.	*S. aureus*	10.0 ± 1.41	32.0 ± 9.89
2.	*B. subtilis*	9.0 ± 0.00	38.0 ± 22.82
3.	*E. coli*	9.0 ± 0.00	35.0 ± 7.07
4.	*S. typhi*	10.0 ± 0.00	41.0 ± 9.192
5.	*P. aeruginosa*	11.50 ± 0.707	37.0 ±15.55

Values are mean inhibition zone (mm) ± S.D of two replicates, N.D means not determined

Table 3 Antifungal activity against *Euclaptus globulus* oil

S. No	Micro-organism	*Euclaptus globulus* oil (20 µL)	Amphotericin B (10 µg / disc)
1.	*A. Niger*	2.5 ± 0.1	18 ± 1.0
2.	*C. Albicans*	12 ± 0	14.66 ± 0.57

Values are mean inhibition zone (mm) ± S.D of two replicates, N.D means not determined

CONCLUSION

During the seasonal variation, we conclude that oil yield depends on age of plant leaves. Maximum oil is extracted out from young leaves of *Eucalyptus globules* tree. In the present work, nine constituents of the essential oil from Eucalyptus globules Labill grown in the Saharanpur regions (Uttar Pradesh) were successfully identified and determined. A comparison with literature data showed that the quantity of 1, 8-cineole was much below than 70%. Some trace or minor chemical constituents have not reported in the literature. Therefore, our present study had clearly indicated that the oils of the *Eucalyptus globules Labill* at the Saharanpur regions (Uttar Pradesh) not suitable for medicinal commodities, but can be used in cosmetics industry.

As earlier reported that essential oil having antibacterial and antifungal activity against different microbial strains due to presence of high content of eucalyptol (72.71%) as active constituents but in case of parent oil, we conclude that essential oil of *Eucalyptus globulus* having less antibacterial and antifungal activity against different microbial strains due to presence of low content of eucalyptol (28.09%).

In case of seasonal variation of major constituents of *Eucalyptus globulus*, oil was collected every month starting from July 2021to June 2022 and subjected to GC analysis. Results showed that the percentage yield of eucalyptol was maximum in the month of November- December and least in the month of March. Hence it is recommended to collect the plant during November - December to get the maximum yield of eucalyptol.

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