ISSN 2063-5346

METABOLITES ANALYSES OF *EUCALYPTUS GLOBULUS BY* GC-MS AND ANTIBACTERIAL ACTIVITY OF EXTRACT

R.D. Barde and M. M. V. Baig

Article History: Received: 01.02.2023 Revised: 07.03.2023 Accepted: 10.04.2023

Abstract

The leaves of *Eucalyptus globulus* were steam distilled to isolate the essential oil and the characterization was done by GC–MS. The identification of thirty-two compounds was confirmed that represented 100% of the oil. The key components identified were 1,8-Cineole (80.20%) α-Pinene (8.5%), β-Eudesmol (3.44%) α-Phellandrene (1.09%). The antibacterial activity of the oil was tested by the disc diffusion and micro-broth dilution methods against two bacterial isolates. The oil exhibited moderate growth suppression against both the Gram-negative bacteria with inhibition zones of with inhibition zones of 16 to 19 mm and MIC values of 125 to 500 µg/mL. 1,8-Cineole and β-Eudesmol were found to be the active antibacterial compounds present in the oil.

Keywords: *Eucalyptus globulus*, GC-MS, Antibacterial activity.

Department of Zoology, SGB College, Tq. Purna, Dist. Parbhani

Department of Botany and Department of Biotechnology, Yeshwant Mahavidyalaya, Nanded 431602, e-mail: mmvbaig@gmail.com

DOI:10.31838/ecb/2023.12.s1-B.340

Introduction

The plant contains different molecules: macromolecules such as proteins, DNAs and RNAs; and small molecules called metabolites. The primary metabolites are involved in the mechanisms essential to the life of the plant such as growth, development, reproduction (Baig et al., 2002). It contains amino acids, sugars and lipids. Secondary metabolites are synthesized in defence mechanisms against biotic (herbivores, insects, viruses, etc.) or abiotic (drought, UV radiation, etc.) attacks. They participate in the phenomena of attraction of pollinators such as pigments essential oils. The plant being a independent organism, therefore, it is constantly forced to adapt to its environment. It therefore sets up many metabolic pathways to produce thousands of different molecules necessary for its survival (Shulaev *et al.*, 2008.).

Metabolomics is the qualitative and quantitative measurement of all metabolites in cells, tissues, organisms. At present, there is no analytical method allowing the analysis of the entire metabolome. Metabolomes are extremely complex. There are several hundred thousand different metabolites in plants. Some of them have similar structures (isomers). Then the metabolites are present at different concentrations within the metabolome (Idle and Gonzalez, 2007).

There are therefore several approaches in metabolomics, used independently or in a complementary manner, depending on the biological question posed. The goal of Metabolic fingerprinting is to detect as many metabolites as possible without identifying them. This approach is used when we want to observe metabolic differences between two populations of individuals e.g. wild or mutant or the search for biomarkers e.g. in healthy and diseased organism. The Metabolic profiling focus on several families of compounds, without necessarily identifying all the metabolites (de Falco *et al.*, 2022.).

Eucalyptus is one of the significant plants distinguished by its aromatic aroma owing to an essential oil that is mostly found in the leaves. It is a member of the Myrtaceae family and a worldwide dispersed genus that is significant as one of the three species used most frequently for pulpwood plantations. Numerous members of the Eucalyptus genus are used in folk medicine to cure a variety of illnesses, including the common cold, the flu, fever, and bronchial infections. Traditional treatments for respiratory infection and dental symptoms include the use of eucalyptus leaves and the vapour produced when dried leaves are boiled with water. Recent research supports this oil's analgesic, anti-inflammatory, and antipyretic effects (Amri *et al.*, 2023).

Eucalyptus globulus Labill. is a very large tree (up to 50m tall) native to Australia. The interest of the Eucalyptus genus are its leaves rich in essential oils (between 5 and 35mL per kg) traditionally used against benign acute bronchial affections. The phytochemical profile of the essential oil is well known, but can vary greatly from one chemotype to another. The metabolite profile also depends upon the extraction process and solvent used (Harkat-Madouri *et al.*, 2015). The purpose of this study was to analyse the chemical makeup of *E. globulus* essential oil as well as its antibacterial properties and ability to combat some bacteria that causes diseases in the fishes.

Material and General Methods:

Plant material

Fresh leaves of *E. globulus* were collected from Yeshwant Mahavidyalaya, Nanded Campus located in the State of Maharashtra, India. The leaves were dried for two weeks in the shade at ambient temperature (25–30 ◦C) and then crushed to obtain a powder. This product was stored in plastic bags at 4 ◦C following the procedure described earlier (More, and Baig, 2013.).

Extraction process:

The essential oil was extracted from dried E. globulus leaves by steam distillation for 4 h in a laboratory steam distillation apparatus. The dried powder was also extracted with different solvent viz. ethanol, ethyl acetate and petroleum ether. The extraction of fresh leaves was done by immersing fresh leaves in hot ethanol and then the leaf material was macerated in 70% ethanol. (Kothari and Baig, 2013)

Microbial culture

Two bacterial isolates viz. *Pseudomonas fluorescens* and *Aeromonas hydrophila* were used in the present work were isolated from diseased fishes from earlier studies. The culture were isolated, identified and characterised following standard methods (Barde, 2021).

Antibacterial activity:

The antibacterial test was carried out according to methods given earlier. Discs containing 15 µL of the oil dissolved in DMSO were placed on the inoculated plates with test microorganisms. Growth inhibition zones (including disc diameter of 5 mm) were measured after 24 h of incubation at 37°C. Tetracycline was used as positive controls. The Minimum inhibitory concentration (MIC) values were measured by the microdilution broth susceptibility assay recommended by CLSI and as reported earlier. Data are expressed as the means three independent experiments (Baig, 2022).

GC MS Analysis

GC–MS analysis of the essential oils was done using a GCMS-2010 Shimadzu Instrument. GC–MS analysis of *Eucalyptus globulus* essential oil was performed following reported protocols. The oil constituents were identified by comparing MS data with NIST mass spectral library and published mass spectra (Andleeb et al. 2022).

Result and discussion

Chemical composition

The essential oil obtained by steam distillation from dried leaves of *E. globulus* was light yellow in colour and had a distinct, pungent odour. The oil was analysed by GC/MS. The components were identified following to their Retention indices and mass spectra used standard injected references and NIST database. Thirty-two compounds were identified, representing 97.34% of the total oil components (Table 1). About 97% of the extract comprised of Monoterpene hydrocarbons, Oxygenated monoterpenes, Sesquiterpenes and Others compunds. Among this wide range of compounds detected in the oil, the major components were 1,8-cineole (80.20%) followed by α-Pinene (8.50%), β-Eudesmol (3.44%) and α-Phellandrene (1.09%) and many compound were present in leas than 1% and large number were present as traces below 0.05%. The results showed variation in composition with respect to data in the literature isolated from different location for the essential oil derived from the same plant species (Joshi et al., 2016). In an earlier study, thirty-five compounds were identified and quantified in the essential oil, the major ones being 1,8-cineole (69.53%)followed by α-pinene (11.94%) and trans-pinocarveol (4.81%) (Bendaoud et al., 2009)

In the present study, much higher concentrations of 1,8-cineole (80.20%) followed by α-Pinene (8.50%) were found. These differences can be attributed to the effect of geographical and climatic factors (Joshi et al., 2016). The main molecules of the essential oil are 1,8-cineole, α-pinene, α-phellandrene and β-Eudesmol. Similar composition of essential oil of of *Eucalyptus globulus* was reported (Čmiková et al., 2023)(Figure 2).

Table 1. Chemical composition of E. globulus.

Sr. No.	Compound	RI	Amount %
1	α-Thujene	924	0.05
2	α-Pinene	933	8.50
3	Camphene	949	0.05
4	Sabinene	972	Tr
5	β-Pinene	975	0.58
6	β-Myrcene	988	0.65
7	α-Phellandrene	1007	1.09
8	α-Terpinene	1015	0.05
9	1,8-Cineole	1035	80.20
10	*p*-Cymene	1026	Tr
11	β-Phellandrene	1031	Tr
12	γ -Terpinene	1059	0.35
13	α-terpinolene	1088	0.12
14	*p*-Cymenene	1093	Tr
15	endo-Fenchol	1112	0.19
16	trans-Pinocarveol	1140	0.37
17	*cis*-β-Terpineol	1142	Tr
18	Camphor	1145	Tr
19	Camphene hydrate	1145	0.17
20	Borneol	1164	0.24
21	Pinocarvone	1169	0.72
22	Terpinen-4-ol	1180	Tr
23	*p*-Cymen-8-ol	1185	Tr
24	*cis*-Pinocarveol	1186	Tr
25	α-Terpineol	1191	Tr
26	*trans*-Carveol	1218	Tr
27	Carvone	1241	Tr
28	α-Gurjunene	1413	Tr
29	β-Caryophyllene	1418	0.21
30	β-Gurjunene	1434	0.06
31	β-Caryophyllene oxide	1582	0.23
32	β-Eudesmol	1651	3.44
RI: Retention indices, Tr.: Traces below 0.05 %			

A very large number of volatile molecules characteristic of eucalyptus essential oils were identified. All the molecules identified using these GC-MS methods could be extracted from eucalyptus leaves using different solvents as well. This extraction method makes it possible to obtain both a very large number of volatile molecules characteristic of eucalyptus essential oil.

Antibacterial activity

To test for antibacterial activities of *Eucalyptus globulus* leaf oil, Two Gram-negative isolates from fishes were used. The results are shown in Table 2. The leaf oil showed moderate inhibition against both bacterial isolates. Among these, it exerted inhibitory activities against both the bacterial isolates with inhibition zones of 16 to 19 mm and MIC values of 125 to 500 µg/mL. Hence, the leaf oil inhibited the Gram-negative bacteria and can be used for treatment.

Table 2: Antibacterial activity of the leaf oil and compounds from Eucalyptus globulus

	Bacterial Isolate	*Pseudomonas fluorescens*	*Aeromonas hydrophila*
Whole oil of *Eucalyptus globulus*	Zone of Inhibition (mm)	18±0.8	17±0.4
	MIC µg/mL.	375	375
α-Pinene	MIC µg/mL.	500	500
α-Phellandrene	MIC µg/mL.	500	500
1,8-Cineole	MIC µg/mL.	125	125
β-Eudesmol	MIC µg/mL.	250	250
Tetracycline (25 µg disc)	Zone of Inhibition (mm)	22±0.4	21±0.4

This study has demonstrated that the amount of 1,8-Cineole (eucalyptol) in the essential oil of *Eucalyptus globulus*, determines the oil's commercial worth. In many studies from different countries, various species of Eucalyptus oil does not contain β- Eudesmol, which is present in the considerable concentration essential oil in the present study. Studies using single or combined purified main compounds will enable a better understanding of the functions related to different bioactivities.

References

Amri, I., Khammassi, M., Ben Ayed, R., Khedhri, S., Mansour, M.B., Kochti, O., Pieracci, Y., Flamini, G., Mabrouk, Y., Gargouri, S. and Hanana, M., 2023. Essential Oils and Biological Activities of *Eucalyptus falcata, E. sideroxylon and E. citriodora* Growing in Tunisia. *Plants*, *12*(4), p.816.

Andleeb F, Asif M, Yaseen HS (2022) Exploration of molecular mechanisms responsible for anti-inflammatory and anti-angiogenic attributes of methanolic extract of Viola betonicifolia. Inflammopharmacology 30:1459–1474.

Baig, M. M. V., Baig, M. I. A., & Muley, S. M. (2002). Enhanced growth of groundnut by plant growth promoting rhizobacteria. *International Arachis Newsletter*.22; 60-62

Baig, M.M.V., 2022. Phytochemical and antimicrobial activity screening of

seeds of *Psoralea corylifolia* L. Phytomedicine Plus, 2(2), p.100278.

Barde, R. D. (2021). Bacterial Diversity Associated with *Labeo Rohita* Isolated from Cultured And Natural Water Bodies. *European Journal of Molecular & Clinical Medicine*, 7(11), 2020.

Bendaoud, H., Bouajila, J., Rhouma, A., Savagnac, A. and Romdhane, M., 2009. GC/MS analysis and antimicrobial and antioxidant activities of essential oil of Eucalyptus radiata. *Journal of the Science of Food and Agriculture*, *89*(8), pp.1292-1297.

Čmiková, N., Galovičová, L., Schwarzová, M., Vukic, M.D., Vukovic, N.L., Kowalczewski, P.Ł., Bakay, L., Kluz, M.I., Puchalski, C. and Kačániová, M., 2023. Chemical composition and biological activities of *Eucalyptus globulus* essential oil. *Plants*, *12*(5), p.1076.

de Falco, B., Grauso, L., Fiore, A., Bonanomi, G. and Lanzotti, V., 2022. Metabolomics and chemometrics of seven aromatic plants: Carob, eucalyptus, laurel, mint, myrtle, rosemary and strawberry tree. *Phytochemical Analysis*, 33(5), pp.696-709.

Harkat-Madouri, L., Asma, B., Madani, K., Said, Z.B.O.S., Rigou, P., Grenier, D., Allalou, H., Remini, H., Adjaoud, A. and Boulekbache-Makhlouf, L., 2015. Chemical composition, antibacterial and antioxidant activities of essential oil of Eucalyptus globulus from Algeria. *Industrial Crops and Products*, *78*, pp.148-153.

Idle, J.R. and Gonzalez, F.J., 2007. Metabolomics. Cell metabolism, 6(5), pp.348-351.

Joshi, A., Sharma, A., Bachheti, R.K. and Pandey, D.P., 2016. A comparative study of the chemical composition of the essential oil from Eucalyptus globulus growing in Dehradun (India) and around the world. Orient. J. Chem, 32(1), pp.331-340.

Kothari, M.N. and Baig, M.M.V., 2013. Production and characterization of extracellular polygalacturonase by *Erwinia carotovora* MTCC 1428. *Int J Adv Biotechnol Res*, *4*(1), pp.981-998.

More, D.R. and Baig, M.M.V., 2013. Fungitoxic properties of Pongamia pinnata (L) Pierre extracts against pathogenic fungi. *International Journal of Advanced Biotechnology and Research*, *4*(4), pp.560-567.

Shulaev, V., Cortes, D., Miller, G. and Mittler, R., 2008. Metabolomics for plant stress response. *Physiologia plantarum*, 132(2), pp.199-208.