Applied Biological Research 26(2): 219-224; (2024)
DOI: 10.48165/abr.2024.26.01.25

CHEMICAL COMPOSITION OF ESSENTIAL OILS EXTRACTED FROM *Melaleuca citrina* (Curtis) Dum. Cours. LEAVES

Abdulrahman Mahmoud Dogara, Harmand A. Hama and Dogan Ozdemir
Biology Education Department, Tishk International University, Erbil (Iraq)
*****e-mail: *abdulrahman.mahmud@tiu.edu.iq*

(Received 30 December, 2023; accepted 25 April, 2024)

ABSTRACT

The medicinal properties of plants have been known for centuries, making them an invaluable resource for the people in need of treatment. Humans on every continent have long relied on the healing properties of plants, a practice that may be traced back to prehistoric times. *Melaleuca citrina* is a plant with substantial therapeutic potential. To the best of our knowledge, no published work has characterized the chemical composition of *M. citrina* in Nigeria. This study aimed to analyse the bioactive chemical constituents of essential oil extracted from the leaves of *M. citrina* in Nigeria. A Clevenger-style apparatus was used to hydro-distillate the freshly chopped leaves of *M. citrina* for a duration of two to three hours. After the extraction process, the essential oils were separated and dried using anhydrous sodium sulphate. Then, the oil composition was examined using gas chromatography-mass spectrometry. Here are the main compounds: (-) Spothulenol 8.6%, Cryptomeridiol 5.5%, Selinene gamma 8%, Pinocarveol cis-> 15%, Cymene para 10%, Selinene delta 10%, and globulol 27.3%. Astringent, antibacterial, anti-inflammatory, and antiseptic are just a few of the fascinating biological characteristics exhibited by globulol, the main component of essential oils. Essential oils extracted from the leaves of Melaleuca citrina possess strong natural qualities that can work as anti-inflammatory, antibacterial, antiseptic, and astringent agents.

Keywords: Aromatic, antimicrobial, essential oil, Nigeria

INTRODUCTION

Plants have been recognized as a significant reservoir of medicinal substances. The use of plants for medicinal purposes can be traced back to prehistoric times, with ancient traditions practiced by humans on all continents. Plants possess a significant amount of bioactive chemicals, essential for the management of chronic and infectious diseases, so have been extensively exploited to treat a wide range of diseases (Gogoi *et al.*, 2021). Plants produce various primary and secondary metabolites which are classified based on their chemical composition and the process by which they are synthesized (Cours, 2020). Essential oils have been used as preservatives and medicinal substances due to their inclusion among secondary metabolites. Furthermore, these oils have the ability to enhance flavor and aroma in many food items (Edris, 2007). Essential oils derived from plants have a measurable physiological effect on the human body (Packiyalakshmi *et al.*, 2017).

Essential oils are extracted from plants and are defined by their volatility and unique sensory properties (Ferreira *et al.,* 2022). The volatile chemicals and essential oils found in aromatic plants are widely valued for their medicinal and culinary applications (Mahmoud *et al*., 2019). Essential oils extracted from plants have become increasingly popular in recent years, because of their use in food and beverage, cosmetics, and other industries. Essential oils (EO) and their constituent chemicals can be useful for use in food items due to their antifungal, antibacterial, and antioxidant characteristics (Salem *et al.*, 2018). EOs have a wide range of effects, from mild to strong, and are used in the food sector as preservatives and antioxidants (Ferreira *et al.*, 2022).

Melaleuca is a genus of trees and shrubs that contains about 100 different species (Myrtaceae). These plants are known as tea trees. There are many volatile oils in the genus Melaleuca. Their main uses are in the manufacture of cosmetics, antiseptics, and disinfectants. They are also used as carminatives and to treat a variety of ailments (Farag *et al.*, 2004). *Melaleuca citrina* (Curtis) Dum. Cours, commonly known as the red bottle brush or 'Callistemon', is an aesthetically pleasing perennial tree that taxonomically falls into the Myrtaceae family and has significant medicinal value (Cours, 2020). The plant is renowned in traditional medicine for its properties that alleviate coughs and bronchitis, as well as its ability to repel insects. Additionally, its volatile oil has been utilized for its antibacterial and antifungal properties. There is a dearth of information regarding the GC-MS analysis of phytoconstituents found in the essential oil derived from the leaves of *M. citrina* in Nigeria. This study aimed to analyze the bioactive chemical constituents of essential oil extracted from the leaves of *M. citrina* in Nigeria.

MATERIALS AND METHODS

Study area

The state of Kaduna, located in northern Nigeria at coordinates of 10 ° 35'N, 7 ° 19'E, has a population of 6,066,562 individuals residing within an area spanning 46,056 km^2. The region experiences two unique weather patterns: arid conditions prevailing from October to May and a wet climate prevailing from June to August, but the weather exhibits seasonal variations.

Plants material

The leaves of *M. citrina* (Fig. 1) were collected in the wild in Kaduna state, Nigeria. Herbarium specimens of medicinal plants collected in the field were identified by an expert from Ahmadu Bello University (ABU Zaria (ABU02516).

Hydro-distillation of essential oils

Freshly chopped *Melaleuca citrina* leaves of 1 to 2 cm were hydrodistilated for 2-3 h in Clevenger-style equipment (Mahmoud *et al.*, 2019). After extraction, essential oils were collected individually, dried over anhydrous sodium sulfate, and kept in airtight vials at 4 ° C until use.

Fig. 1: The plant of *Melaleuca citrina*

Gas chromatography-mass spectrometry (GC-MS) analysis

The Shimadzu GC-MS QP2010 Ultra model and gas chromatograph interfaced to a mass spectrometer (GC-MS) equipped with an Rxi-5Sil MS, fused silica capillary column (30 mL × 0.25 mm ID × 1× df, composed of 100% dimethyl polysiloxane1,4-bis (dimethylsiloxy) phenylene dimethyl polysiloxane) were used for GC-MS analysis of extracted essential oils (Cours, 2020). An electron ionization device with an ionizing energy of 70 eV was used for GC/MS detection. With an injection volume of 1 µL and a split ratio of 50:1, helium gas (99.999%) was utilized as the carrier gas at a constant flow rate of 1 mL min^{-1}. The injector temperature was 250 ° C and the ion source temperature was 280 ° C. Starting at 100 ° C (isothermal for 2 min), the oven temperature was designed to increase by 5°C min^{-1} to 200°C, then by 10°C min^{-1} to 280°C, concluding with an isothermal 2 min at 280 ° C (Cours, 2020). The GC ran for 32 min, with mass spectra recorded at 70eV, scan intervals of 0.3 sec, and fragments ranging from 40 to 800 Da. Lab Solutions software was used to handle mass spectra and chromatograms (Cours, 2020).

Identification of compounds

The identification of constituents present in essential oil was determined through a comparative analysis of their retention indices and fragmentation patterns by mass spectra (Mahmoud *et al.*, 2019). This comparison was made with a database from a National Institute of Standards and Technology (NIST) computer library (Mahmoud *et al.,* 2019).

RESULTS AND DISCUSSION

Phytochemicals are bioactive molecules derived from plants that possess therapeutic properties and have the potential to mitigate and prevent the onset of various ailments (Dogara, 2023). The production of herbal goods or modern drugs requires the use of authentic and safe plant resources. Quality control plays a crucial role in the field of ethno pharmacology, as it serves to assess the integrity and reliability of plant materials utilized in the development of contemporary pharmaceuticals or herbal supplements. Currently, there is a growing demand for herbal drugs, which has resulted in the occurrence of drug replacement and adulteration (Kumar *et al.,* 2023). Expansive exploitation of plant resources is being carried out to meet the requirements and desires of herbal industries, resulting in depletion of plant resources (Kumar *et al.,* 2023). Therefore, it is imperative to thoroughly document, authenticate and validate plant resources to maintain the quality standards of herbal products. Therefore, scientists use gas chromatography-mass spectrometry (GC-MS), a hybrid analytical approach that combines the separation capabilities of gas-liquid chromatography with the detection capabilities of mass spectrometry, to evaluate the presence of numerous substances in each sample (Neves *et al.,* 2022).

The study on a fresh weight basis revealed a distilled oil yield of 1.9% from the leaves of *Melaleuca citrina*. The recovery content of the following studies (1.9%) differs significantly from the other studies (Dogara, 2023). The essential oil yield was found to be higher compared to the essential oil yield of certain species within the same family (Moraes *et al.,* 2022). The variation in the yields of the EO fractions could be attributed to the extraction process utilized. Several researchers investigating different plant species have observed that the choice of extraction methods can have a significant impact on both the mass yields and chemical compositions obtained at the end of the extraction process (Ferreira *et al.,* 2020). Variation in extract yield of different parts of medicinal plants is significantly influenced by factors such as extraction solvent, the presence of compounds, and the polarity of the metabolites (Dogara, 2023). The plant is an excellent source of essential oil, as evidenced by the recovery of oil from the leaf.

A total of 23 compounds were qualitatively identified (Fig. 2), comprising 98.8% of the total composition. Monoterpenes made up 32. 8% of the total compounds, sesquiterpenoids account for 23%, while the remaining compounds make up 43.3% (Table 1; Fig. 2). The main compounds, in terms of their respective percentages, were (-)-globulol (27.3%), Pinocarveol<cis-> (15%), Cymene<para-> (10.9%), Selinene<delta-> (10.8%), Spathulenol (8.6%) and Cryptomeridiol (5.5%) (Fig. 3). Minor compounds are defined as any other compound with a % less than 5 (Table 1). Compounds below 1% are considered traced compounds (Table 1).

The essential oil of the leaves exhibited a high abundance of (-)-Globulol. The findings suggest that globulol may serve as a primary antibacterial component within the crude ethanol extract derived from *Eucalyptus* globulus fruits (Tan *et al.,* 2008). It is highly probable that (-)-

Table 1: Chemical composition of *Melaleuca citrina*

S. No.	Retention time (min)	Chemical compounds identified	Area (%)	Molecular formula
1.	6.531	Terpinene<gamma->	1.4	$C_{10}H_{16}$
2.	7.447	Terpinolene	2.5	$C_{10}H_{16}$
3.	8.336	Dodecanal	0.6	$C_{12}H_{24}O$
4.	9.543	Cymene<para->	10.9	$C_{10}H_{14}$
5.	10.221	Ocimene<(E)-beta->	0.3	$C_{10}H_{16}$
6.	12.301	Phellandrene<beta->	1.6	$C_{10}H_{16}$
7.	13.338	Mentha-2,4(8)-diene<para->	0.8	$C_{10}H_{16}$
8.	14.465	Pinocarveol<cis->	15.0	$C_{10}H_{16}O$
9.	15.224	Hepten-2-ol<6-methyl-5->	0.7	$C_8H_{14}O$
10.	16.354	(-)-Globulol	27.3	$C_{15}H_{26}O$
11.	17.001	4,8-dimethyl-2-isopropylazulene	0.5	$C_{15}H_{18}$
12.	18.341	Terpinen-4-ol acetate	1.2	$C_{32}H_{46}O_2$
13.	19.321	Spathulenol	8.6	$C_{15}H_{24}O$
14.	20.324	Muurolene<gamma->	0.8	$C_{15}H_{24}$
15.	21.631	Cedren-13-ol acetate<8->	2.3	$C_{17}H_{26}O_2$
16.	22.321	Eudesmol<beta->	3.1	$C_{15}H_{26}O$
17.	22.741	Cryptomeridiol	5.5	$C_{21}H_{38}O_6$
18.	23.457	Caryophyllene<9-epi-(E)->	1.1	$C_{15}H_{24}$
19.	24.678	Viridiflorol	0.2	$C_{15}H_{26}O$
20.	25.897	Isovalencenol<(E)->	1.5	$C_{15}H_{24}O$
21.	26.321	Selinene<delta->	10.8	$C_{15}H_{24}$
22.	27.346	Alaskene<alpha->	0.2	$C_{15}H_{24}$
23.	28. 981	Guaiene<alpha->	1.9	$C_{15}H_{24}$
	Monoterpenese compound		32.5 %	-
	Sesquiterpenes compound		23.0 %	-
	Other compounds		43.3 %	-
	Total		98.8 %	-

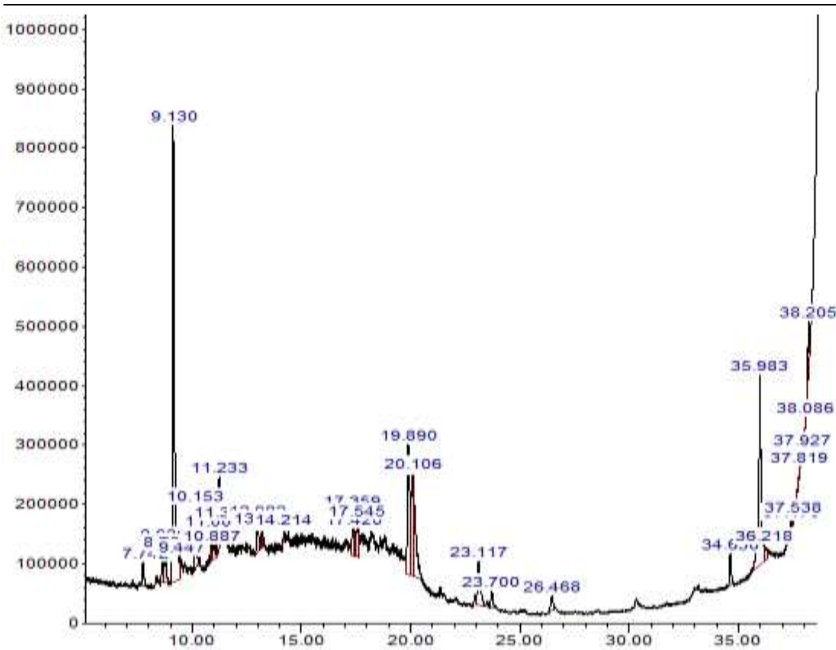

Fig. 2: Chromatogram of essential oil from *Melaleuca citrina*

Globulol served as the primary active component in the activity of essential oil. Eucalyptol was the primary constituent of *Melaleuca citrina* samples collected from India (Cours, 2020). *Melaleuca citrina* oils have varying chemical compositions based on factors such as geographical location, harvest season, and leaf age. Variations in the atmospheric and environmental conditions surrounding the plants, including factors like sun exposure and rainfall patterns, have an impact

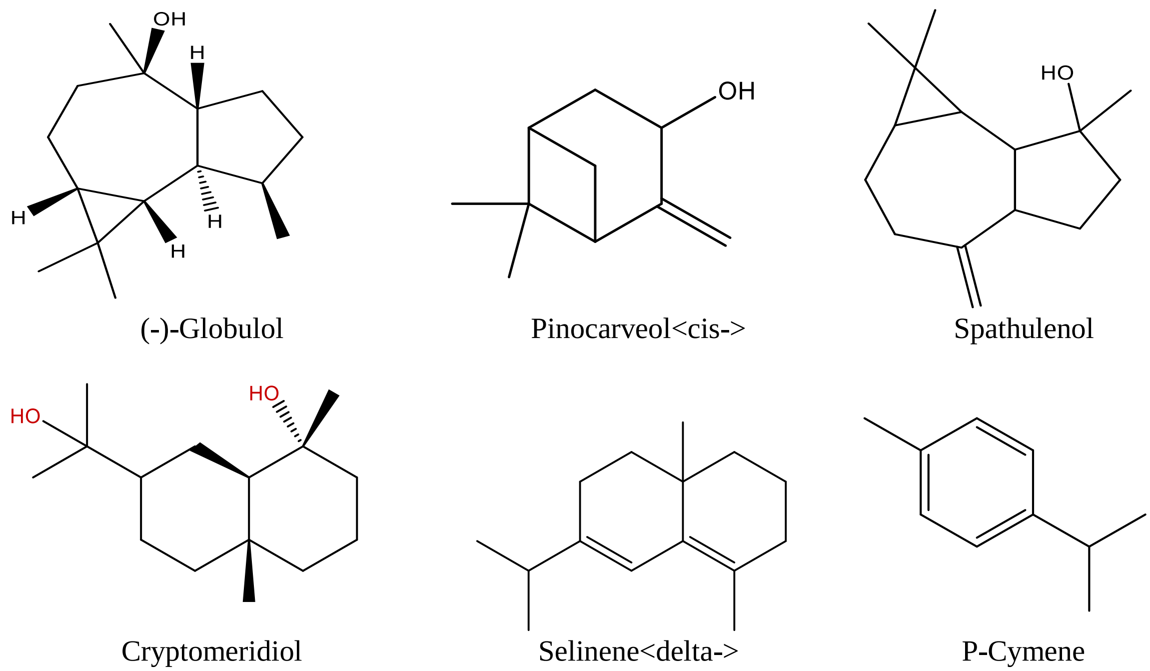

Fig. 3: Major compounds of essential oil found in *Melaleuca citrina*

on the composition of plant extracts. The chemical compositions of essential oils from specific species vary depending on factors such as harvesting seasons and geographical sources. The leaf extract of the plant can exhibit several biological properties, including antifungal, antibacterial, antioxidant, anti-inflammatory, and antitumor activities. These effects can be attributed to the presence of specific phyto-chemical components, which validates the traditional therapeutic usage of the plant. Therefore, based on the available information, there is a lack of scholarly research related to the chemical composition of *M. citrina* specifically within the geographical context of Nigeria. The present investigation was prompted by a lack of knowledge about the constituents of the plant oil, given its historical use in the treatment and control of cancer, malaria, and typhoid fever. The research encompasses a comprehensive examination of the chemical composition of essential oil derived from the leaves of *M. citrina*. Hence, this work establishes a fundamental basis for future investigations of the beneficial properties of plants.

Conclusion: Traditional medicines place high priority on the use of plants, and many ancient communities around the world, especially those in Africa, are significantly dependent on plant life for survival. For thousands of years, people have used aromatic and medicinal herbs to cure a variety of illnesses. The literature has not described the chemical composition of *M. citrina* in Nigeria. Typhoid fever, cancer, malaria, and many other illnesses have been treated with the plant for a long time. In terms of percentages, (-)-globulol (27.3%) was the most abundant compound present in plant leaves. This study serves to establish a foundational reference point for future evaluations of these crucial oils, while also helping to identify potential instances of counterfeit or adulterated products from Nigeria.

REFERENCES

Cours, D. 2020. GC-MS analysis of phytoconstituents of the essential oil from the leaves of *Melaleuca citrina* (Curtis). *Advances in Zoology and Botany*, **8**: 87-98.

Dogara, A.M. 2023. Chemical composition of corymbia citriodora. *Nusantara Bioscience*, **15**: 172-178.

Edris, A.E. 2007. Pharmaceutical and therapeutic potentials of essential oils and their individual volatile constituents: A review. *Phytotherapy Research*: *An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives*, **21**: 308-323.

Farag, R., Shalaby, A., El-Baroty, G., Ibrahim, N., Ali, M. and Hassan, E. 2004. Chemical and biological evaluation of the essential oils of different *Melaleuca* species. *Phytotherapy Research,* **18**: 30-35.

Ferreira, O.O, Cruz J.N., De Moraes Â.A.B., De Jesus P.F.C., Lima R.R., Anjos T.O.D., Siqueira G.M., Nascimento, L.D.D., Cascaes, M.M. and De Oliveira, M.S. 2022. Essential oil of the plants growing in the brazilian amazon: Chemical composition, antioxidants, and biological applications. *Molecules*, **27**(14): 4373. [*10.3390/molecules27144373*].

Ferreira, O.O., Da Cruz, J.N., Franco, C.D.J.P., Silva, S.G., Da Costa, W.A., De Oliveira, M.S.and Andrade, E.H.D.A. 2020. First report on yield and chemical composition of essential oil extracted from *Myrcia eximia* Dc (myrtaceae) from the Brazilian amazon. *Molecules*, **25**: 783. *https://doi.org/10.3390/molecules25040783*.

Gogoi, R., Begum, T., Sarma, N., Kumar, P.S. and Lal, M. 2021. Chemical composition of *Callistemon citrinus* (Curtis) skeels aerial part essential oil and its pharmacological applications, neurodegenerative inhibitory, and genotoxic efficiencies. *Journal of Food Biochemistry,* **45**: e13767. [*https://doi.org/10.1111/jfbc.13767*].

Kumar, A., Jangid, P.P., Marimuthu, S., Gurav, A.M., Srikanth, N., Mangal, A.K., Venkateshwarlu B. and Shiddamallayya, N. 2023. Identification and authentication of *Agnimantha* plant species used in ayurveda on the basis of anatomical and molecular phylogenetic analysis. *Plant Science Today,* **10**: 26-38.

Mahmoud, A.D., Ali, A.M., Khandaker, M.M., Fatihah, H.N.N., Awang, N.A. and Mat, N. 2019. Discrimination of *Syzygium polyanthum* cultivars (wight) walp based on essential oil composition. *Journal of Agrobiotechnology,* **10**: 1-9.

Moraes, Â.A.B., De Jesus P.F.C, Ferreira, O.O., Varela, E.L.P., Do Nascimento, L.D, Cascaes, M.M., Da Silva, D.R.P., Percário, S., De Oliveira, M.S., De Aguiar and Andrade, E.H. 2022. *Myrcia paivae* O. Berg (Myrtaceae) essential oil, first study of the chemical composition and antioxidant potential. *Molecules*, **27**: 5460. [*https://doi.org/10.3390/molecules27175460*].

Neves, N.C.V., De Mello, M.P., Smith, S.M., Boylan, F., Caliari, M.V. and Castilho, R.O. 2022. Chemical composition and *in vitro* anti-helicobacter pylori activity of *Campomanesia lineatifolia* Ruiz & Pavón (Myrtaceae) essential oil. *Plants*, **11**: 1945. [*https://doi.org/10.3390/plants11151945*].

Packiyalakshmi, D., Athilakshmi, P., Gayathri, S., Karthiga, P., Thiri, B.R. and Manikandan, G. 2017. Antimicrobial potential of different solvents leaf extract of *Millettia peguensis* against selected pathogens. *The Pharma Innovation Journal*, **6**: 119-124.

Salem, M.Z., Elansary, H.O., Ali, H.M., El-Settawy A.A., Elshikh, M.S., Abdel-Salam, E.M. and Skalicka, W.K. 2018. Bioactivity of essential oils extracted from *Cupressus macrocarpa* branchlets and *Corymbia citriodora* leaves grown in Egypt. *BMC Complementary and Alternative Medicine,* 18: 1-7.

Tan, M., Zhou, L., Huang, Y., Wang, Y., Hao, X. and Wang, J. 2008. Antimicrobial activity of globulol isolated from the fruits of *Eucalyptus globulus* Labill. *Natural Product Research,* **22**: 569-575.