

Phytochemical Profiling and Bioactivity Assessment of *Melaleuca bracteata* F. Muell. from Nainital, Uttarakhand

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ABSTRACT

Background: *Melaleuca bracteata* (Genus: *Melaleuca*; Family: *Myrtaceae*) commonly known as the tea tree, is an important aromatic and medicinal plant. **Objectives:** This research aims to find out the essential oil composition, antioxidant and antibacterial activity of *M. bracteata* leaves from the bhabar region of Nainital as well as to find out the essential oil's composition of flowers of *Melaleuca bracteata*. **Materials and Methods:** Essential oil was extracted via the hydrodistillation technique through Clevenger type apparatus. GC-MS (GCMS-QP 2010 Ultra, He: 73.3 kPa, flow: 16.3 mL/min, oven: 50–270°C) identified phytochemicals. Antioxidant assays (DPPH @495 nm, ABTS @750 nm, Hydroxyl, NO) were analyzed using *GraphPad Prism 6*. Antibacterial activity (0–100 µg/mL, 630 nm) was tested on *B. subtilis*, *S. aureus*, *E. coli*, and *P. aeruginosa* using *Ciprofloxacin* (10 µg) as control. **Results:** A total of 32 compounds were identified, representing 99.95% and 99.31% of the aerial and flower oils, respectively. Methyl eugenol (90.8–77.18%) was predominant, followed by (E)-methyl cinnamate, linalool, and a terpineol. Oils showed strong antioxidants and antibacterial activity against the pathogens. **Conclusion:** The findings suggest that due to its high essential oil yield, rich chemical composition, and significant biological activities, *Melaleuca bracteata* could be cultivated as an industrial and medicinal crop.

Keywords: Antibacterial, Antioxidant, *Melaleuca bracteata*, Methyl cinnamate, Methyl eugenol, *Myrtaceae*.

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INTRODUCTION

The *Melaleuca bracteata* F. Muell. belongs to *Myrtaceae* family of plants which is diverse, with essential oils rich in various genera such as *Melaleuca*, *Eucalyptus*, *Leptospermum*, *Myrtus*, *Pimenta*, *Plinia*, *Psidium*, *Eugenia Pseudocaryophyllus* and *Syzygium* (Siddique *et al.*, 2020). *Melaleuca*, a genus with around 230 species, is widely distributed, mainly in Australia and Tasmania and shows significant phenotypic diversity in different ecosystems (Joshi *et al.*, 2021, Fatma *et al.*, 2020). Essential oils from *Melaleuca* species exhibit antibacterial, anti-inflammatory, fungicidal, acaricidal, antioxidant, and antiviral properties. Major constituents such as methyl eugenol, methyl ether, 1,8-cineole, and terpinen-4-ol have been identified in the essential oils of various *Melaleuca* species from different regions. *Melaleuca bracteata*, also known as black tea-tree or river tea-tree, is widely distributed across eastern, central, and northern Australia. It is cultivated globally for its ornamental value and medicinal properties. It is a versatile plant,

ranging from a large shrub to a medium-sized tree, typically reaching heights of up to 15 m. Its foliage consists of small, narrow, and hairy leaves measuring between 3 to 12 mm long. The plant produces small bottlebrush-like flowers, about 20 mm long, primarily at the tips of its twigs, with woody, cup-shaped capsules as fruits, measuring 2-3 mm long and 2.5-3 mm wide, appearing on branches (Babu *et al.*, 2022). Previous studies have highlighted its medicinal properties, including reported antisecretory, antiulcerogenic, anti-HIV, cytotoxic, insecticidal, antifungal, and growth-inhibition activities. The essential oil of this plant varies in composition based on geographic region. Major components include methyl eugenol, elemicin, (E)-isoelemicin, and (E)-methyl isoeugenol. The essential oil from Malaysian origin, for instance, contained 76.0% methyl eugenol with significant fruit fly attractant activity. Betulinic acid, oleanolic acid, water-soluble betaines, and triterpene, betulinic acid, have been characterized in extracts from different parts of the plant, showing anti-HIV, cytotoxic, and antifungal activities (Goswami *et al.*, 2017). Uttarakhand, known for its natural beauty in the Himalayas, is a treasure trove of plants and animals. It has different climates, soils, and landscapes that support many useful plants, both wild and grown. The region's height varies from 200 to 7817 meters above sea level, and it's divided into five parts based on its geology. These parts include areas like the Trans-Himalayas, Great Himalaya, Lesser Himalaya, and the Outer Himalaya,



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which includes the Tarai and Bhabhar. The highest parts are icy, while the lower areas are covered in forests and grasslands. Some major cities, such as Haldwani and Ramnagar, sit in a region called the Himalayan Bhabhar belt, which is a stretch of land along the Himalayan foothills (Kholiya *et al.*, 2023). The Kumaun Himalayan Bhabhar region spread over a geographical area of 51125 km², 77°34' to 81°02' E longitude and 28°43' to 31°27' N latitude (Padalia *et al.*, 2018). The flowing water from Shivalik Himalayas is submerged here due to lack of porosity nature of soils that leads to dry land surface over the area. Towards its south, the Tarai belt is running parallel to the Bhabhar that composed with wet to marshy land through the reemergence of the river and regarded as a cap of sediments, silt etc. form of deposition as well (Devi, 2020) A thorough examination of existing literature uncovered a gap in the exploration of the medicinal and aromatic properties of *M. bracteata* found in Himalayan and bhabhar belt of Kumaun Uttarakhand.

MATERIALS AND METHODS

Sample Collection

The Plant Material: Fresh plant material of *Melaleuca bracteata* including leaves and flowers were collected from, Haldwani (29.2183° N & 79.5130° E), Nainital, Uttarakhand.

Extraction Method: Essential oil was extracted from *M. bracteata* via the hydrodistillation technique through Clevenger type apparatus (Clevenger, 1928) at the Department of Chemistry, M B Govt. P. G. College Haldwani. The aerial parts, comprising stems and leaves (200 g) of the plant, were thoroughly cleaned before undergoing hydro-distillation using a Clevenger-type apparatus for approximately 4 hr. The weight of the dried samples was recorded before hydro-distillation. The essential oil contents (%) were expressed as volume of essential oil vs. weight of fresh leaves (v/w).

$$EO\ Yield = \frac{Volume\ of\ Essential\ Oil\ (mL)}{Weight\ of\ Fresh\ Leaves(gm)} \times 100$$

The obtained oil was dehydrated using anhydrous Na₂SO₄ and stored in a refrigerator until analysis by GC-FID/GC-MS.

Chemical analysis of essential oil through GC-MS

GC-MS was used to analyze and identify the phytochemical content of the essential oil using GCMS-QP 2010 Ultra equipment with helium as the carrier gas at a pressure of 73.3 kPa and a split ratio of 10:1. The overall flow rate was 16.3 mL/min during the study, with a column flow rate of 1.21 mL/min, 39.9 cm/sec linear velocity and 3 mL/min purge flow were maintained, respectively. Carrier gas saver, high-pressure injection, and splitter hold were all turned off, and the oven temperature was set to 50°C RAMP@ 3°C/min up to 220°C (isotherm for 2 min), then 6°C/min up to 270°C (isotherm for 2 min), then hold for 16 min, using Flame Thermionic Detector (FTD). The components were identified by comparison with linear Retention Indices (RI) from literature

Adams (Adams, 2007). Mass spectra with those of NIST mass spectral library or coinjection with standards.

Biological Activities

Antioxidant Activity

DPPH (2,2-diphenyl-2-picrylhydrazyl) radical scavenging activity

To measure DPPH (2,2-diphenyl-2-picrylhydrazyl) radical scavenging activity, 5 µL of various test compound stocks (ranging from 0% to 5%) were added to 0.1 mL of 0.1 mM DPPH solution in a 96-well plate. The reaction was conducted in triplicate, with duplicate blanks containing 0.2 mL DMSO/Methanol and 5 µL of different compound concentrations. The plate was incubated in the dark for 30 min. Post-incubation, decolorization was measured at 495 nm using an iMark microplate reader (BioRad) (Abubak *et al.*, 2020, Imam *et al.*, 2011). A reaction mixture with 20 µL deionized water served as the control. The scavenging activity was expressed as '% inhibition' relative to the control, and IC₅₀ was determined using Graph Pad Prism 6 software. The percentage inhibition (IC %) of free radical by DPPH was calculated using the formula:

$$DPPH\ Scavenging\ Activity = \frac{Abs\ Control - Abs\ Sample}{Abs\ Control} \times 100$$

Where, Abs control and Abs sample represent the absorbance value for control sample and test sample, respectively.

ABTS Radical Scavenging Ability: ABTS radicals were prepared by mixing 2.45 mM APS and 7 mM ABTS solution, then diluted 100-fold to create the ABTS free radical reagent. In a 96-well plate, 10 µL of various sample stocks (0 to 2.5%) were added to the standard (Ascorbic Acid, 5 mg/mL) and 200 µL of the ABTS free radical reagent. The mixture was incubated in the dark at room temperature for 10 min. After incubation, the absorbance of the decolorisation was measured at 750 nm using an iMark microplate reader (BioRad) (Cao *et al.*, 1998, Gupta *et al.*, 2009). Results were compared to the negative control, and the IC₅₀ was calculated using Graph Pad Prism 6 software. The percentage inhibition (IC%) of free radical by DPPH was calculated using the formula:

$$ABTS\ Radical\ Scavenging\ Activity = \frac{Abs\ Control - Abs\ Sample}{Abs\ Control} \times 100$$

Where, Abs control and Abs sample represent the absorbance value for control sample and test sample, respectively.

Hydroxyl Free Radical Scavenging Assay: A reagent mixture of 66 µL was prepared, consisting of 10 µL EDTA (0.5M), 24.14 mg deoxyribose, 88 µL FeCl₃ (10 mg/mL), 28 µL H₂O₂ (6%), and water up to 33 mL. To the wells of a 96-well plate, 10 µL of the sample (0–5%), 24 µL of phosphate buffer (50 mM, pH 7.4), and 10 µL of ascorbic acid were sequentially added. The mixture was then incubated at 37°C for 1 hr. Gallic Acid (0–50 µg/ml) was used as the standard. After incubation, 50 µL of 10% TCA and 50 µL

of 1% TBA were added to each well, resulting in the development of a pink chromogen. Absorbance was measured at 540 nm using a microplate reader (iMark, BioRad) (Rahman *et al.*, 2015, Hazra *et al.*, 2008). The IC₅₀ was calculated using GraphPad Prism 6 software.

$$\text{Hydroxyl Free Radical Scavenging Activity} = \frac{\text{Abs Control} - \text{Abs Sample}}{\text{Abs Control}} \times 100$$

Where, Abs control and Abs sample represent the absorbance value for control sample and test sample, respectively.

Reactive Nitrogen Oxide Scavenging Assay: A reaction mixture was prepared containing 50 µL of 10 mM sodium nitroprusside (Fisher Scientific, Cat no.-27864), 40 µL of distilled water, and 10 µL of sample/standard (Gallic Acid – SRL, Cat no.- 13142)/blank. This mixture was pre-incubated at room temperature for 15 min in the presence of light. After incubation, 100 µL of Griess reagent was added to the test and control wells, followed by an additional incubation for 5-10 min at room temperature for chromophore development and stabilization. Absorbance was measured at 540 nm and 660 nm using a microplate reader (iMark, BioRad) (Rao, 2013). The IC₅₀ was calculated using GraphPad Prism 6 software.

$$\text{Reactive Nitrogen Oxide Scavenging activity} = \frac{\text{Abs Control} - \text{Abs Sample}}{\text{Abs Control}} \times 100$$

Where, Abs control and Abs sample represent the absorbance value for control sample and test sample, respectively.

Antibacterial Activity

Four bacterial strains of significant importance were used to test the antibacterial properties of the essential oils. The antimicrobial activity was tested against two Gram positive bacteria *Bacillus subtilis* (MTCC 1133) and *Staphylococcus aureus* (MTCC 96) and two Gram negative *Escherichia coli* (MTCC 452) and *Pseudomonas aeruginosa* (MTCC 3541). 0.5 Mc Farland

Standard dilution of microbes to be used for the study. 100 µL diluted log cultures of bacteria was added to the micro centrifuge tube and added with 5 µL of prepared treatment dilutions of different concentrations 0, 6.25, 12.50, 25.00, 50.00, 100 to the defined tubes and incubated for 24 hr. After Incubation all content was transferred to the 96 well plate and turbidity reading was taken by Elisa Plate Reader (iMark Biorad) at 630 nm. Ciprofloxacin (10 µg) was used as Positive Control.

RESULTS

Essential oil composition of aerial part: A total of 32 compounds were identified, accounting for 99.95% and 99.31% of the essential oil from aerial parts and flowers respectively. The predominant compound in both oils was methyl eugenol, (90.8%-77.18%) of the total composition, followed by (E)- methyl cinnamate (6.42 %-13.26%), linalool (0.23%-2.21%) and α-terpineol (0.24%-1.20%). 4-terpineol, elemicine, spathulenol, cadin-4-en-10-ol, benzoic acid, 3,4,5-trimethoxy methyl ester, conifer aldehyde

methyl ether are the other compounds common in both essential oils. Phenylpropanoids are the major constituents in the essential oils of both the oils marking 96.97 and 90.83 of the total constituents. The percentage composition of the compounds identified in both the essential oils is given in Table 1. Collation of the current results with earlier reports from India revealed noteworthy qualitative variations.

In vitro antioxidant activity

DPPH Radical scavenging activity

In this study, the antioxidant activity of essential oils was compared with ascorbic acid, a reference standard antioxidant compound. A good inhibition action on DPPH free radicals produced in the reaction was observed at all tested concentrations of the oil. The IC₅₀ value for *Melaleuca bracteata* is 0.3831 compared to 1.773 of Ascorbic acid.

ABTS Radical Scavenging Ability

In this study, the antioxidant activity of essential oils was compared with ascorbic acid, a reference standard antioxidant compound. A good inhibition action on ABTS Radical produced in the reaction was observed at all tested concentrations of the oil. The IC₅₀ value for *Melaleuca bracteata* is 0.06937 compared to 0.8237 of Ascorbic acid.

Hydroxyl Free Radical Scavenging Assay

The antioxidant activity of essential oils was compared with Gallic Acid, a reference standard antioxidant compound. A good inhibition action on Hydroxyl free radicals produced in the reaction was observed at all tested concentrations of the oil. The IC₅₀ value for *Melaleuca bracteata* is 0.8192 compared to 14.71 of Gallic Acid.

Reactive Nitrogen Oxide Scavenging Assay

The antioxidant activity of essential oils was compared with Gallic Acid, a reference standard antioxidant compound. A good inhibition action on Nitrogen Oxide produced in the reaction was observed at all tested concentrations of the oil. The IC₅₀ value for *Melaleuca bracteata* is 2.766 compared to 12.28 of Gallic Acid. Table 2 represents IC₅₀ value of essential oil of *Melaleuca bracteata* against different radicals.

Anti-bacterial activities

The zone of inhibition diameters and Minimum Inhibitory Concentrations (MICs) of the essential oils against the tested microorganisms are presented in Table 3. In the study by Goswami *et al.*, (2017), the essential oil showed no activity against *P. aeruginosa* and *E. coli* strains. However, in our study, the essential oil was found to be effective against these two pathogens, as well as *S. aureus* and *B. subtilis*, with zone of inhibition values ranging from 6.66 to 12.00 mm.

Table 1: Essential oil composition of *Melaleuca bracteata*.

Sl. No.	Compound Name	R.T.	RI _{exp}	Percentage Concentration				
				Aerial Part	Flower	Goswami <i>et al.</i> , 2017	Joshi <i>et al.</i> , 2021	
1.	α-thujene	7.367	927	-	t	0.08	0.10	
2.	α-pinene	7.614	933	-	t	-	0.10	
3.	Sabinene	9.112	972	-	t	-	-	
4.	β-pinene	9.280	978	-	t	t	0.10	
5.	Myrcene	9.819	991	-	t	0.12	0.30	
6.	α-phellandrene	10.476	1007	-	t	0.16	1.00	
7.	p-cymene	11.281	1025	t	0.43	0.36	0.70	
8.	Limonene	11.466	1030	-	t	0.30	0.30	
9.	1,8-cineol	11.569	1030	-	0.30	0.34	0.20	
10.	α-terpinolene	13.947	1086	-	t	0.26	1.10	
11.	Linalool	14.796	1101	0.23	2.21	0.94	1.20	
12.	Citronellal	17.083	1152	-	t	-	0.10	
13.	(-)-4-terpineol	18.358	1174	-	0.17	0.40	0.20	
14.	Cymen-8-ol	18.815	1197	-	0.19	-	-	
15.	α-terpineol	19.140	1201	0.24	1.20	0.32	0.40	
16.	Citronellol	20.650	1232	-	0.64	t	0.40	
17.	Neral	21.059	1243	-	0.13	-	0.10	
18.	Geraniol	21.724	1255	-	0.13	0.07	0.10	
19.	α-citral	22.424	1271	-	0.42	-	-	
20.	Methyl geranate	24.753	1326	-	0.12	-	-	
21.	Citronellyl acetate	25.993	1353	-	0.12	-	-	
22.	Eugenol	26.218	1357	0.24	0.39	t	0.50	
23.	(E)-methyl cinnamate	27.652	1384	6.42	13.26	4.12	8.00	
24.	Methyl eugenol	28.969	1403	90.31	77.18	88.18	74.80	
25.	Germacrene D	31.376	1480	0.12	-	0.60	1.10	
26.	Cis-calamenene	33.040	1537	0.10	t	-	0.70	
27.	Elemicine	34.242	1550	0.18	0.17	0.30	0.20	
28.	Spathulenol	35.264	1576	0.57	0.24	0.22	0.30	
29.	Viridiflorol	35.581	1594	0.24	-	0.10	-	
30.	t-murrolol	37.827	1645	0.10	-	-	0.5	
31.	Cadin-4-en-10-ol	38.255	1659	0.41	0.13	0.14	0.4	
32.	Coniferaldehyde methyl ether	42.912	1777	0.51	1.78	-	0.30	
Total Composition					99.95	99.31	97.13	93.20
Monoterpene hydrocarbons					t	t	0.96	3.00
Oxygenated hydrocarbons					0.47	5.63	2.11	2.70
Phenylpropanoid					96.97	90.83	92.34	83.30
Sesquiterpene hydrocarbons					0.12	t	0.60	1.10
Oxygenated sesquiterpenes					1.50	0.54	0.76	1.40
Others					0.89	2.31	0.36	1.70

R.T.: Retention time, RI_{exp}: Experimental value of retention index, t = trace (< 0.05%).

Table 2: Antioxidant activity of essential oil of *Melaleuca bracteata*.

Oil/Standard	Antioxidant activity in terms of IC ₅₀ (µg/mL ± SD)			
	DPPH radical scavenging activity	ABTS Radical Scavenging Ability	Hydroxyl Free Radical Scavenging Assay	Nitrogen Oxide Scavenging Assay
MB (L)	0.3831 ± 0.27	0.06518 ± 0.07	0.8192 ± 0.28	2.766 ± 0.18
Ascorbic Acid	1.773 ± 0.76	0.8237 ± 0.17	-	-
Gallic Acid	-	-	14.71 ± 0.28	12.28 ± 0.19

Values are means of four replicates ± standard deviation. IC₅₀ = Half maximal inhibitory concentration; SD= Standard deviation.

Table 3: Antimicrobial potential of essential oils of *Melaleuca bracteata*.

Sl. No.	Concentration	<i>E. coli</i>	<i>S. aureus</i>	<i>P. aeruginosa</i>	<i>B. subtilis</i>
1	0	0	0	0	0
2	6.25	06.66±0.56	06.66 ± 0.57	08.66 ± 0.57	09.00 ± 0.00
3	12.50	10.00±0.00	10.05 ± 0.00	10.00 ± 0.00	10.00 ± 0.00
4	25	11.11±0.00	11.00 ± 0.00	11.00 ± 0.00	07.50 ± 0.70
5	50	11.66±0.57	11.66 ± 0.57	10.66 ± 0.57	09.50 ± 0.71
6	100	12.00±0.00	12.00 ± 0.00	08.66 ± 0.57	12.00 ± 0.00
	MIC (MB)	1000	500	500	500
	MIC (Standard)	0.06	0.4	0.02	0.05

Values are means of four replicates ± standard deviation.

DISCUSSION

The results of the current study show very high percentage of Phenylpropanoid in essential oil of *Melaleuca bracteata*. The methyl eugenol constitutes almost 90.8% of the total composition of leaves oils which is higher than the previous studies done by the Goswami *et al.*, 2017 (88.18% methyl eugenol) and Joshi *et al.*, 2021 (74.80 % methyl eugenol) from Tarai region of Kumaun, Uttarakhand and less than the study done by the Yasin *et al.*, 2021 from Faisalabad (96.02% methyl eugenol). Compared to leaves oils the flower oils have lower percentage of methyl eugenol, 77.18% but higher percentage of (E)-methyl cinnamate 13.26%.

The antioxidant assays (DPPH, ABTS, Hydroxyl, and Reactive Nitrogen Oxide) demonstrated that *M. bracteata* oil possesses a remarkable ability to neutralize free radicals across different radical systems. The IC₅₀ values obtained in all assays were substantially lower than those of standard antioxidants such as ascorbic acid and gallic acid, indicating stronger antioxidant potential. In the DPPH assay, the oil exhibited an IC₅₀ value of 0.3831 compared to 1.773 for ascorbic acid, highlighting its high hydrogen-donating capacity. Similarly, in the ABTS assay, the oil showed an exceptionally low IC₅₀ value of 0.06937, suggesting superior electron transfer ability. The hydroxyl and reactive nitrogen oxide scavenging assays further confirmed the oil's strong radical quenching efficiency, with IC₅₀ values of 0.8192 and 2.766, respectively, both notably lower than their respective standards. These findings suggest that *M. bracteata* essential oil contains

potent antioxidant constituents capable of combating oxidative stress, possibly due to the presence of phenolic or terpenoid compounds reported in previous studies (e.g., Goswami *et al.*, 2017; Adekunle *et al.*, 2019).

The antibacterial results also underscore the broad-spectrum efficacy of *M. bracteata* essential oil. Contrary to the observations by Goswami *et al.*, 2017 who reported no activity against *Pseudomonas aeruginosa* and *Escherichia coli*, our study demonstrated measurable inhibition zones against these strains, along with *Staphylococcus aureus* and *Bacillus subtilis*. The zone of inhibition values ranging from 6.66 to 12.00 mm indicates that the oil possesses effective antibacterial components, possibly acting through disruption of microbial cell membranes or inhibition of essential enzymatic processes. The observed differences may be attributed to variations in the chemical composition of essential oils due to geographical, seasonal, and environmental factors influencing the plant's secondary metabolite profile.

CONCLUSION

This investigation into the essential oil derived from the aerial parts and flowers of *Melaleuca bracteata* in the Bhabar belt of Uttarakhand reveals a distinctive chemical profile, predominantly featuring Phenylpropanoid. The pronounced antioxidant activity demonstrated by the oil, effectively scavenging DPPH, ABTS, hydroxyl, and nitrogen oxide radicals, underscores its potential as a natural antioxidant agent. Additionally, the oil exhibits

significant antimicrobial properties against various bacterial strains, aligning with findings from other *Melaleuca* species.

Given these bioactive properties, the *Melaleuca* oil from this region holds promising applications in the medical and cosmetic industries. Its antioxidants and antimicrobial activities suggest potential uses in developing therapeutic agents and natural preservatives. Furthermore, the high methyl eugenol content offers opportunities for its incorporation into fragrances and skincare products, catering to the growing consumer preference for natural ingredients.

Future research should focus on comprehensive toxicological assessments and clinical evaluations to ensure the safety and efficacy of this oil in various applications. Additionally, exploring sustainable harvesting and production methods will be crucial to meet industrial demands while preserving the ecological balance of the region.

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ABBREVIATIONS

Abs control: Absorbance value for control sample; **Abs sample:** Absorbance value for control test sample; **ABTS:** 2,2-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid; **B. subtilis:** *Bacillus subtilis*; **DPPH:** 2,2-Diphenyl-1-Picrylhydrazyl; **E. coli:** *Escherichia coli*; **EDTA:** Ethylenediamine tetraacetic acid; **EO:** Essential oil; **IC50:** Half Maximal Inhibitory Concentration; **M. bracteata & MB:** *Melaleuca bracteata*; **MIC:** Minimum Inhibitory Concentration; **MTCC:** Microbial Type Culture Collection and Gene Bank; **NIST:** National institute of standards and Technology; **P. aeruginosa:** *Pseudomonas aeruginosa*; **S. aureus:** *Staphylococcus aureus*; **TBA:** Thiobarbituric acid; **TCA:** Trichloroacetic Acid.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

SUMMARY

The present study investigated the essential oil composition, antioxidant and antibacterial activity of *M. bracteata* leaves from the bhabar region of Nainital, Uttarakhand. Essential oils were extracted from the leaves and flowers using the hydrodistillation method and analyzed by GCMS, which identified 32 compounds

representing more than 99% of the total oil content. Methyl eugenol was found to be the major constituent, followed by (E)-methyl cinnamate, linalool, and α -terpineol.

The antioxidant potential of the oils was evaluated through DPPH, ABTS, Hydroxyl, and Nitric oxide assays, showing strong radical scavenging activity, even higher than standard compounds like ascorbic and gallic acids. The antibacterial tests revealed that the oils were effective against both Gram-positive (*Bacillus subtilis*, *Staphylococcus aureus*) and Gram-negative (*Escherichia coli*, *Pseudomonas aeruginosa*) bacteria.

Overall, the study highlights that *Melaleuca bracteata* possesses rich chemical constituents and remarkable antioxidant and antibacterial activities. These findings suggest that this plant holds great promise for use in pharmaceutical, cosmetic, and food industries and could be developed as a valuable industrial and medicinal crop.

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