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# Methyl Eugenol, 1,8-Cineole and Nerolidol Rich Essential Oils with their Biological Activities from three *Melaleuca* Species Growing in Tarai Region of North India

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**HIGHLIGHTS**

- E-nerolidol, 1,8-cineol and methyl eugenol rich species of Melaleuca were identified in this study.
- Essential oils exhibited strong antioxidant,antibacterial and *Invitro* anti inflammatory activity.
- These marker constitutents impart valuable place of this crop in agriculture.

**Abstract:** The essential oils from the fresh leaves of three Melalecua spp. viz; *Melaleuca leucadendron* (L.) *Melaleuca linariifolia* Sm. and *Melaleuca bracteata* F. Muell. growing in Tarai region of North India were analyzed by a combination of gas chromatography/mass spectrometry. The analysis revealed the presence of several constituents of industrial and pharmacological importance. *M. leucadendron* essential oil was found to be dominated by E-nerolidol (85.7%) rich chemotype. 1,8-cineole (61.1%) along with significant presence of α-terpineol (12.3%), α-pinene (4.0%), β-myrcene (3.8%), and E-caryophyllene (1.7%) were identified in the essential oil from *M. linariifolia* Similarly *M. bracteata* was dominated by the presence of phenylpropanoids viz; methyl eugenol (74.8%) and methyl cinnamate (8.0%). The essential oils were studied for their i*n-vitro* antioxidant, anti-inflammatory and antimicrobial potential. All the oils revealed potential antioxidant activity with maximum in *M. bracteata* essential oil. All the oils exhibited significant antibacterial activity against *Bacillus megaterium*, *Staphylococcus aureus*, *Escherichia coli*,*Salmonella typhimurium* and anti-fungal activity against phytopathogenic fungi *Fusarium oxysporum*, *Sclerotinia sclerotiorum*, *Exserohilum turcicum* and *Curvularia lunata*. The observations from present study suggest further cultivation of Melaleucas and its commercialization as industrial crops.

**Keywords:** Methyl eugenol; 1,8-cineole; (E)-nerolidol; Melaleuca Antioxidant; Antifungal; antimicrobial.

## INTRODUCTION

The genus *Melaleuca* is an aromatic and medicinal shrub. The shrubs are generally found in open forest, woodland or shrubland, particularly along water courses and the edges of swamps [1]. The *Melaleuca* genus belongs to the Melaleucae tribe, subfamily Myrtiodeae [2] and predominantly occurs in Australia. It comprises approximately 230 species of worldwide occurrence with 220 species endemic to Australia and Tasmania, but also occurring in Indonesia and New Papua Guinea [3,4]. The family Myrtaceae is well known for economically important timber trees, especially *Eucalyptus* spp., oils (e.g., *Eucalyptus* spp., *Melaleuca* spp.), and cultivated as ornamentals plants such as *Callistemon* (bottlebrush), *Chamelaucium* (wax-flower), *Eucalyptus* spp., *Leptospermum* (tea tree), and *Myrtus* (myrtle) [5]. The fleshy-fruited species include many economically important food plants, agricultural crops, and ornamentals, including the Mediterranean genus *Myrtus* (myrtle), spices such as clove (*Syzygium aromaticum* /L./ Merr. & L.M. Perry), allspice (*Pimenta dioica* /L./ Merr.), and bay rum (*Pimenta racemosa* / Mill./ J. W. Moore), and the fruits of *Psidium* (guavas), *Myrciaria*, *Eugenia*, *Syzygium*, *Plinia* and *Luma*[6]. The plants of genus *Melaleuca* are commonly known as tea tree and are rich source of commercially valuable volatile oils [7]. The essential oils of various *Melaleuca* species have been reported to show extensive compositional variability under different geographic and ecological conditions. Phenylpropanoids (methyl eugenol, (*E*)-methylisoeugenol), monoterpenoids (mainly, 1,8-cineole, terpinen-4-ol, terpinolene, along with p-cymene, α-terpinene, α-terpineol, α-pinene), and sesquiterpenoids ((*E*)-nerolidol, viridiflorol, ledol, β-caryophyllene) have been reported as marker constituents in the essential oils from most of the *Melaleuca* species[8-15]. Commercially useful essential oils have been sourced from the broad leaved *M.quinquenervia* (niaouli oil) and *M. cajuputi* (cajuput oil) and the small-leaved *M. alternifolia-M. linariifolia* complex[16]. These oil have been used mainly in the manufacture of cosmetics, germicides and as antiseptic agents. Studies also revealed the broad spectrum antimicrobial potential of essential oils [17,18]. The leaves and stem of several *Melaleuca* species are source of essential oils with strong aroma for medicinal application, with potential use for cancer treatment alongwith its use in traditional medicine against many pathological conditions like acne, wounds, sores, dandruff, and skin lesions [19-21]. Previous studies also suggest the allelopathic properties of *Melaleuca* species resulting in an inhibition of other species in the same ecosystem. The bare ground in *Melaleuca* forests was an excellent example of allelopathy in this genus [22].

Literature survey revealed no pre-existing comparative study of Melaleuca species from different climatic zones of India. In present scenario people are again relying on natural resources for pharmaceutical and neutraceutical practices. Hence its need of time to screen these indigenous essential oil resources for their judicious exploitation, cultivation and commercialization. The present study was carried out to investigate the compositional diversity among the essential oils of three Melaleuca species viz; *Melaleuca bracteata, Melaleuca linariifolia* and *Melaleuca leucadendron* grown in Tarai region of North India. The bioactivity of these oils as antifungal, antimicrobial, and antioxidant agents was also assessed to evaluate their pharmaceutical and neutraceutical values.

## MATERIAL AND METHODS

### Plant materials and isolation of essential oil

The fresh aerial parts of *Melaleuca* species were collected from Medicinal Plant Research and Development Centre (MRDC) of the G.B.Pant University, Pantnagar. (*M.bracteata* and *M.linariifolia*) and Forest Training Institute, Lalkuan (*M.leucadendra*) (Nainital, Uttarakhand). Voucher specimen and herbarium record of the plant have been retained in Herbarium. Freshly collected leaves were hydro-distilled separately in a Clevenger apparatus for 3-4 h for extraction of essential oils. The essential oils were collected, dehydrated over anhydrous $Na_2SO_4$ and stored in an amber color vials at 4 $^0$C in refrigerator for further analysis.

### Analysis and identification of essential oil constituents

The GC/MS analysis was carried out using Agilent 6890 gas chromatograph fitted with Mass Selective Detector 5973 and Autosampler 7683. Column HP-5 (30 m× 0.25 mm I.D.; 0.25 µm of film thickness) was used. The injector temperature was 250 $^0$C. Helium was used as carrier gas with a flow rate of 1.0 mL/min.

Detector temperature was 150 $^0$C, source temperature was 230 $^0$C, ionization energy was 70 eV. Chromatograms were recorded in a programmed regime of linear temperature which increase from 50 $^0$C to 320 $^0$C at the rate of 3 $^0$C/min. Compounds of the essential oil and relative percentage composition are compiled. Linear retention indices (RI) were calculated using a homologous series of *n*-alkanes ($C_8$-$C_{25}$) under the same temperature-programmed conditions. The compounds were identified with the help of NIST 14 and Wiley 9 mass spectra libraries and by comparing the experimental RI with those from literature [23].

## Antioxidant activity

### DPPH Radical scavenging activity

2,2-diphenylpicrylhydrazil (DPPH) free radical scavenging activity was performed by following the reported protocols with modifications [24,25]. Briefly the reaction mixture contained 5 mL of 0.004% methanol solution of DPPH and different amount ( 5- 25 µg/mL) of essential oils. The solutions were rapidly mixed and scavenging capacity was measured spectrophotometrically(Thermo Scientific Evolution 201) by monitoring the decrease in absorbance at 517 nm. BHT was used as positive control while reaction mixture (DPPH radical solution) minus essential oil solution was taken as control. Inhibition of free radicals (IC %) was calculated by using the formula:

$$IC\ \% = A_0\text{-}A_s/\ A_0 \times 100 \quad (1)$$

Where, $A_0$ = Absorbance value of Control sample $A_s$ = Absorbance value of Test sample IC = Inhibitory concentration.Percent inhibition was plotted against concentration and the standard curve was drawn using standard antioxidant (BHT) to calculate the $IC_{50}$ values for standard and essential oils.

### Metal chelating activity

The metal chelating activity of essential oils were examined by the reported methods [26]. It was based on the principle of the $Fe^{+2}$ chelating ability where the absorbance of the $Fe^{+2}$ - ferrozine complex was measured at 562 nm. 0.1 mL of 2 mM $FeCl_2$. $4H_2O$, 0.2 mL of 5 mM ferrozine and 4.7 mL of methanol was added to different amounts (5 - 25 µg/mL) of essential oils. After incubation, the absorbance of test samples were measured at 562 nm. The IC% was calculated as:

$$IC\ \% = A_0\text{-}A_s/\ A_0 \times 100 \quad (2)$$

Where, $A_0$ = Absorbance value of Control sample $A_s$ = Absorbance value of Test sample IC = Inhibitory concentration. Percent inhibition was plotted against concentration and the standard curve was drawn using standard antioxidant (citric acid) to calculate the $IC_{50}$ values for standard and essential oils.

### Reducing power

The reducing power of essential oils were analyzed by the method developed earlier and recently being used [27]. In brief varying amount (5-25 µg/mL) of essential oils were mixed with 2.5 mL of phosphate buffer (200 mM, pH= 6.6) and 2.5 mL of 1% potassium ferricyanide, $K_3[Fe(CN)_6]$. After 20 minute incubation at 50±1ºC, 2.5mL of trichloroacetic acid was added to the mixtures, followed by centrifugation at 650 RPM for 10 min. The upper layer (1.0 mL) was mixed with 5.0 mL of distilled water and 1 mL of 0.1% ferric chloride.The resultant solutions were measured at 700 nm using UV spectrophotometer. All the readings were taken in triplicate and BHT was used as the standard.

The reducing power of samples was calculated using the formula given below:

$$\text{Reducing activity}\ \% = A_0\text{-}A_s/\ A_0 \times 100. \quad (3)$$

Where, $A_0$ = Absorbance value of Control sample $A_s$ = Absorbance value of test sample.Percent inhibition was plotted against concentrations and the standard curve was drawn using standard antioxidant (BHT) to calculate the $RP_{50}$ values for standard and essential oils.

### Nitric oxide scavenging activity

Sodium nitroprusside (SNP) was used to generate nitric oxide and was measured by the Griess reagent. It is based on the principle that SNP in aqueous solution at physiological pH spontaneously generates NO, which interacts with oxygen to produce nitrite ions that can be estimated by the use of Griess reagent. Scavengers of NO compete with oxygen leading to reduced production of NO. 2 ml of SNP

(10 mM) in phosphate buffer saline (PBS) pH 7.4 was mixed with different amount (5–25 µg /mL) of essential oil and incubated at 25°C for two and half hours. The above were reacted with 1 mL of Griess reagent (1% sulphanilamide, 0.1% naphthylethylenediamine dichloride and 2 mL orthophosphoric acid). Finally absorbance was measured at 546nm. Ascorbic acid was taken as standard [28].

### Super oxide radical scavenging activity

1 mL of nitroblue terazolium (156 mM), 1.0 mL nicotinamide adenine dinucleotide (reduced) (468 mM) and 0.1 mL of phenazine methosulphate solution (PMS) in 0.1 M of phosphate buffer solution (pH 7.4) were added to different amounts (5- 25 µg/mL), of essential oils followed by incubation at 25 °C for 5 min and the absorbance was read at 560 nm against blank, containing all reagent except PMS. Ascorbic acid was taken as standard [29].

Super oxide radical scavenging activity was calculated by following equation.

$$\text{Superoxide radical scavenged (\%)} = \text{IC \%} = A_0\text{-}A_s/\ A_0 \times 100. \quad (4)$$

Where, $A_0$= Absorbance value of Control sample A = Absorbance value of Test sample IC = Inhibitory concentration. Percent inhibition was plotted against concentration. The standard curve was drawn using standard antioxidant (ascorbic acid) to calculate the $IC_{50}$ values for standard and essential oil.

### Antifungal activity

### Plant pathogenic fungi

The soil borne pathogenic fungi *Fusarium oxysporum, Sclerotinia sclerotiorum, Curvularia lunata and Exserohilum turcicum* were procured from the Department of Plant Pathology, College of Agriculture, G.B.P.U.A&T, Pantnagar, India. Cultures of each fungal species were maintained on Potato Dextrose Agar (PDA) and stored at $4^0$ C.

### In-vitro antifungal activity

Antifungal activity of the oils was tested by the poisoned food technique using potato dextrose agar (PDA) medium against the test fungi. The essential oil samples were prepared by dissolving the requisite amounts in 10% DMSO and then added into 20 mL PDA to obtain different final concentrations [30,31]. Mycelial plugs (2.0 mm in diameter) from the edges of each culture were incubated in the center of each PDA plate (85 mm diameter). The control sets were prepared using equal amounts of 10% DMSO in place of the oil. The prepared plates were inoculated aseptically with assay discs of the test fungi and incubated at 26 ± 2 $^0$C for 3-7 days until the growth in the control plates reached the edge of the plates. Growth inhibition of each fungal strain was calculated as the percentage inhibition of radial growth relative to the control. The plates were used in triplicate for each treatment [32]. $IC_{50}$ values of constituents were graphically obtained from the dose response curves based on measurement at five different concentrations.

### Antibacterial activity

### Bacterial strain

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 megaterium* and *Staphylococcus aureus* and two Gram negative *Escherichia coli* and *Salmonella typhimurium*. These strains were obtained from the Department of Veterinary Public Health, College of Veterinary, G.B.P.U.A.T, Pantnagar, India. All the strains were stored in the appropriate medium before use. The antibacterial activity was studied using standard protocol [33].

### Determination of the minimum inhibitory concentration (MIC)

The MIC was determined using standard protocol with the serial dilution of 5µg/mL,10 µg/mL,15 µg/mL, 20 µg/mL, 25 µg/mL. A 100 µL suspension containing $1 \times 10^6$ CFU/mL of bacteria were spread on nutrient agar plates [34]. The wells were filled with 50µL of essential oil solution in the inoculated nutrient agar plates. The bacterial plates were incubated at 37 ± 2°C for 24 hours. The MIC was defined as the minimum

concentration of the oil inhibiting the visible growth of each bacterium on the agar plate, so the lowest concentration of each essential oil showing a clear zone of inhibition was taken as the MIC. DMSO was used as the negative control, while refamycin was used as positive control. Each experiment was performed in triplicate.

### Determination of *in-vitro* anti-inflammatory activity

### Inhibition of albumin denaturation

*In vitro* anti-inflammatory activity was performed by the method generally being practiced [35]. The reaction mixture (5.0 mL) consisted of 0.2 mL of egg albumin (hen's fresh egg), 2.8 mL of phosphate buffered saline (PBS, pH 6.4) and 2 mL of varying amounts (250-1000 ppm) of essential oils. Similar volume of double-distilled water was used as control. The mixtures were incubated at (37±2 °C) in an incubator for 15 min followed by heating at 70 °C for 5 min. After cooling, the absorbance was measured at 660 nm by using distilled water as blank. Diclofenac sodium was used as reference drug and treated similarly for determination of absorbance. The percentage inhibition of protein denaturation was calculated by using the following formula:

$$\% \text{ inhibition} = 100 \times (Vt / Vc - 1). \quad (5)$$

Where, $V_t$ = absorbance of test sample, $V_c$ = absorbance of control. The oil concentration for 50% inhibition ($IC_{50}$) was determined by plotting percentage inhibition with respect to control against treatment concentration.

### Statistical analysis

The mean values and standard deviations were calculated for all tests. The data were analyzed using SPSS 16.0 statistical software. The results were calculated by Analysis of Variance (ANOVA). The means were compared by Duncan tests at a level of significance of $p < 0.05$.

## RESULTS

### Chemical constituents of essential oils

The chemical composition of essential oils led to the presence of marker constituents previously recorded from *Melaleuca* species with slight qualitative and quantitative differences. GC–MS analysis of the essential oils led to the identification of over 90 constituents from three species. The oil yield for *M.leucadendron* was highest (1.02%) followed by *M.bracteata* (0.62%) and *M.linariifolia* (0.60%).The detailed composition has been presented in Table 1.

**Table 1.** Comparative Chemical compositions of *Melaleuca species*

| S.N. | Compounds | RI | % Composition | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | Present Study | | | Padalia et.al [36,37,49] | | |
| | | | ***M. leucadendron*** | ***M. linariifola*** | ***M. bracteata*** | ***M. leucadendron* (2015)** | ***M. linariifola* (2015)** | ***M. bracteata* (2017)** |
| 1. | Methyl 2-methylbutanoate | 780 | 0.1 | 0.1 | t | - | - | - |
| 2. | α-thujene | 924 | - | 0.1 | 0.1 | - | t | 0.08 |
| 3. | α-pinene | 932 | 0.4 | 4.0 | 0.1 | t | 1.99 | - |
| 4. | camphene | 948 | t | 0.1 | - | 0.10 | t | - |
| 5. | benzaldehyde | 960 | 1.2 | - | t | - | - | - |
| 6. | sabinene | 969 | | | | 0.64 | | |
| 7. | β-pinene | 975 | t | 1.6 | 0.1 | 0.50 | 0.85 | 0.04 |
| 8. | 6-methyl-5-hepten-2-one | 985 | 0.1 | - | - | - | | - |
| 9. | β-myrcene | 990 | t | 3.8 | 0.3 | 0.10 | 1.78 | 0.12 |
| 10. | pseudolimonene | 1002 | - | 0.2 | - | - | - | - |
| 11. | α-phellandrene | 1002 | - | - | 1.0 | - | - | 0.16 |
| 12. | p-mentha -1(7),8-diene | 1002 | | | | | 0.14 | |
| 13. | α-terpinene | 1014 | - | 0.3 | 0.1 | - | - | - |
| 14. | p-cymene | 1021 | - | - | 0.7 | - | 0.21 | 0.36 |
| 15. | limonene | 1025 | 0.4 | - | 0.3 | - | 0.15 | 0.30 |
| 16. | **1,8-cineole** | 1030 | 0.1 | **61.1** | 0.2 | 0.30 | **77.40** | 0.34 |
| 17. | E-ocimene | 1046 | t | 0.1 | 0.1 | | - | - |
| 18. | γ-terpinene | 1056 | t | 0.8 | 0.1 | t | 0.41 | - |
| 19. | α-terpinolene | 1086 | 0.1 | 0.2 | 1.1 | - | - | 0.26 |
| 20. | Methyl benzoate | 1093 | t | 0.1 | t | - | - | - |
| 21. | linalool | 1099 | 0.7 | 0.2 | 1.2 | 0.52 | 0.19 | 0.94 |
| 22. | exo-fenchol | 1120 | | | | | 0.10 | |
| 23. | camphor | 1141 | | | | 0.38 | | |
| 24. | isopulegol | 1142 | 0.4 | - | t | - | - | - |
| 25. | citronellal | 1152 | - | - | 0.1 | - | - | - |
| 26. | δ-terpineol | 1165 | - | 0.6 | - | - | 0.46 | - |
| 27. | Terpin-4-ol | 1174 | t | 1.1 | 0.2 | t | 0.76 | 0.40 |
| 28. | p-cymen-8-ol | 1183 | - | - | 0.1 | - | - | - |
| 29. | α-terpineol | 1190 | 0.1 | 12.3 | 0.8 | 0.10 | 7.72 | 0.54 |
| 30. | Methyl chavicol | 1197 | - | - | 0.4 | - | - | 0.32 |
| 31. | citronellol | 1228 | 0.2 | - | 0.4 | - | - | 0.04 |
| 32. | neral | 1239 | - | - | 0.1 | 0.22 | - | - |
| 33. | E-geraniol | 1253 | t | - | 0.1 | - | - | 0.07 |
| 34. | linalyl acetate | 1254 | | | | - | | |
| 35. | Methyl citronellate | 1260 | - | - | 0.1 | - | - | - |
| 36. | α-citral | 1269 | - | - | 0.2 | - | - | - |
| 37. | geranial | 1270 | | | | | | 0.12 |
| 38. | bornyl acetate | 1284 | | | | 0.11 | | |
| 39. | S-methyl thiobenzoate | 1290 | 0.1 | - | - | - | - | - |
| 40. | Z-methyl cinnamate | 1304 | - | - | 0.1 | - | - | - |
| 41. | Methyl geranate | 1323 | - | - | 0.1 | - | - | - |
| 42. | δ-elemene | 1335 | | | | - | | |
| 43. | α-terpinyl acetate | 1346 | | | | 0.53 | | |
| 44. | α-cubebene | 1348 | - | - | 0.1 | - | - | - |
| 45. | Citronellyl acetate | 1353 | - | - | 0.2 | - | - | - |
| 46. | eugenol | 1356 | 0.1 | 0.3 | 0.5 | - | t | 0.04 |
| 47. | Neryl acetate | 1364 | - | 0.1 | - | - | t | - |
| 48. | α-ylangene | 1369 | | | | | t | |
| 49. | isoledene | 1371 | - | 0.1 | - | - | - | - |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 50. | α-copaene | 1374 | - | - | 0.1 | - | t | - |
| 51. | E-methyl cinnamate | 1383 | - | - | 8.0 | - | - | 4.12 |
| 52. | Geranyl acetate | 1383 | - | 0.1 | - | - | t | - |
| 53. | β-bourbonene | 1387 | | | | - | | |
| 54. | α-gurjunene | 1408 | - | 0.1 | - | - | - | - |
| 55. | **Methyl eugenol** | 1416 | - | 0.6 | **74.8** | **-** | **t** | **88.18** |
| 56. | E-β-caryophyllene | 1417 | 0.9 | 1.7 | - | 1.52 | 2.88 | - |
| 57. | Z-β-copaene | 1431 | - | - | 0.5 | - | - | - |
| 58. | α-guaiene | 1432 | | | | | 0.46 | |
| 59. | aromadendrene | 1438 | - | 0.4 | 0.2 | - | t | - |
| 60. | Selina-5,11-diene | 1441 | - | 0.1 | - | - | - | - |
| 61. | α-humulene | 1452 | 0.2 | 0.3 | 0.2 | 0.22 | 0.31 | - |
| 62. | E-Muurola-3,5-diene | 1453 | - | - | 0.1 | - | - | - |
| 63. | γ-gurjunene | 1470 | - | 0.1 | - | - | t | - |
| 64. | Cabreuva oxide D | 1475 | 0.1 | - | - | - | - | - |
| 65. | E-cadina-1(6),4-diene | 1475 | - | - | 0.1 | - | - | - |
| 66. | D-germacrene | 1480 | - | - | 1.1 | - | - | 0.60 |
| 67. | β-selinene | 1484 | - | 0.1 | - | - | - | - |
| 68. | α-amorphene | 1485 | | | | | 0.10 | |
| 69. | δ-selinene | 1489 | - | 0.1 | - | - | - | - |
| 70. | viridiflorene | 1493 | - | 0.5 | - | - | - | - |
| 71. | bicyclogermacrene | 1496 | - | - | 0.4 | - | - | - |
| 72. | E-methyl isoeugenol | 1497 | | | | | | 0.20 |
| 73. | α-muurolene | 1499 | - | 0.1 | 0.1 | - | 0.18 | - |
| 74. | β-cadinene | 1506 | - | 0.6 | - | - | - | - |
| 75. | E,E-α-farnesene | 1508 | 0.1 | - | - | - | - | - |
| 76. | γ-cadinene | 1513 | - | 0.1 | 0.1 | 0.10 | 0.12 | - |
| 77. | β-curcumene | 1515 | | | | | 0.43 | |
| 78. | E-calamenene | 1522 | - | 0.2 | - | - | 0.10 | - |
| 79. | δ-cadinene | 1522 | | | | - | | |
| 80. | Z-calamenene | 1524 | - | - | 0.7 | - | - | - |
| 81. | Z-nerolidol | 1531 | | | | 0.10 | | |
| 82. | Cadina-1,4-diene | 1532 | - | - | 0.2 | - | - | - |
| 83. | α-calacorene | 1542 | - | 1.1 | t | - | - | - |
| 84. | ledol | 1557 | - | 0.2 | - | - | - | - |
| 85. | elemicin | 1558 | - | - | 0.2 | - | - | 0.30 |
| 86. | germacrene-B | 1558 | | | | | 0.99 | |
| 87. | palustrol | 1565 | - | 0.2 | 0.1 | - | - | - |
| 88. | ***E*-nerolidol** | **1568** | **85.7** | **-** | **-** | **90.85** | 0.10 | - |
| 89. | spathulenol | 1575 | - | 0.2 | 0.3 | - | t | 0.22 |
| 90. | Caryophyllene oxide | 1581 | 3.7 | - | - | 0.99 | 0.10 | 0.14 |
| 91. | globulol | 1582 | - | 1.2 | 0.3 | 0.70 | - | - |
| 92. | viridiflorol | 1591 | 0.6 | 0.3 | 0.1 | 0.19 | - | - |
| 93. | Cubeban-11-ol | 1592 | - | 0.5 | 0.1 | - | 0.52 | - |
| 94. | fokienol | 1599 | 0.4 | - | - | - | - | - |
| 95. | juneperol | 1599 | | | | | t | |
| 96. | rosifoliol | 1600 | - | 0.4 | 0.1 | - | t | - |
| 97. | Humulene epoxide II | 1608 | 0.3 | - | t | - | | - |
| 98. | 1,10-di-epi-cubenol | 1612 | - | 1.3 | t | - | 0.10 | - |
| 99. | 10-epi-γ-eudesmol | 1622 | | | | 0.14 | | |
| 100 | epicubenol | 1626 | - | t | 0.1 | - | 0.25 | - |
| 101 | Allo-aromadendrene epoxide | 1632 | 0.1 | - | - | - | - | - |
| 102 | isospathulenol | 1636 | - | 0.2 | - | - | - | - |
| 103 | Caryophylla-4(12),8(13)-diene-5-α-ol | 1636 | 0.5 | - | - | - | - | - |
| 104 | τ-cadinol | 1638 | - | 0.2 | - | - | - | - |
| 105 | epi-α-cadinol | 1638 | | | | 0.20 | t | |
| 106 | τ-muurolol | 1640 | - | - | 0.5 | - | - | - |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 107 | α-muurolol | 1643 | | | | | | 0.20 |
| 108 | δ-cadinol | 1644 | - | 0.1 | 0.1 | - | - | - |
| 109 | β-eudesmol | 1649 | | | | t | | |
| 110 | α-cadinol | 1653 | - | 0.1 | 0.4 | 0.33 | - | 0.14 |
| 111 | E-14-hydroxy-9-epi caryophyllene | 1670 | 0.1 | - | t | - | - | - |
| 112 | cadalane | 1672 | - | 0.1 | - | | t | - |
| 113 | β-bisabolol | 1675 | | | | t | | |
| 114 | (Z)-nerolidyl acetate | 1679 | | | | t | | |
| 115 | α-bisabolol | 1685 | 0.2 | - | - | | - | - |
| 116 | 2Z,6Z-farnesol | 1698 | 0.1 | - | - | | - | - |
| 117 | 2Z,6E-farnesol | 1721 | 0.7 | 0.3 | - | | - | - |
| 118 | Iso-longifolol | 1732 | 0.1 | - | - | | - | - |
| 119 | methylconiferylaldehyde | 1777 | - | - | 0.3 | | - | - |
| 120 | phytol | 2112 | - | 0.3 | - | | - | - |
| **121** | **Total identified** | | **97.8** | **99.0** | **98.1** | **98.84** | **98.90** | **98.24** |
| **122** | **Monoterpene hydrocarbons** | | 0.9 | 11.2 | 4.0 | 1.34 | 5.53 | 1.30 |
| **123** | **Oxygenated monoterpenes** | | 1.6 | 75.6 | 12.4 | 2.16 | 86.63 | 2.42 |
| **124** | **Sesquiterpene hydrocarbons** | | 1.2 | 5.7 | 3.9 | 1.84 | 5.67 | 0.60 |
| **125** | **Oxygenated sesquiterpenes** | | 92.2 | 5.3 | 2.1 | 93.50 | 1.07 | 0.70 |
| **126** | **Others** | | 1.9 | 0.6 | 75.7 | - | - | 93.14 |

**Mode of identification: RI (retention index), MS, t = trace (<0.1%)**

The most abundant component of essential oil of *M.leucadendron* was *E*-nerolidol (85.7%). Other major components identified were caryophyllene oxide (3.7%), benzaldehyde (1.2%), 2*Z*,6*E*-farnesol (0.7%), viridifloral (0.6%) and caryophyllene (0.9%) etc. The major constituents identified in *M.linarifolia* essential oil were 1,8-cineole (61.1%) along with significant presence of α-terpineol (12.3%), α-pinene (4.0%), β-myrcene (3.8%), *E*-caryophyllene (1.7%), α-calacorene (1.1%), globulol (1.2%), 1,10-di-*epi*-cubenol (1.3%), β-pinene (1.6%) while eugenol methyl ether (74.8%) was identified as a principal component in *M.bracteata* essential oil along with methyl cinnamate (7.97%). The RI and percentage contribution are shown in Table 1. The essential oils from *M.bracteata* and *M. lineriifolia* showed quantitative variability in chemical composition when compared with the earlier reports [36,37]. The chemical composition of essential oil of *Melaleuca leucadendron* was mostly composed of oxygenated sesquiterpenes (92.2%), whereas *Melaleuca linariifolia* essential oil consisted of oxygenated monoterpenes (75.6%). The *Melaleuca bracteata* essential oil was characterized by dominance of phenylpropanoid methyl eugenol (74.8%).

The compounds α-phellandrene (1.0%), *p*-cymene (0.7%), methyl chavicol (0.4%), α-citral (0.2%), methylcinnamate (8.0%), β-copane (0.5%), D-germacrene (1.1%), bicyclogermacrene (0.4%), calamenene (0.7%), cadina-1,4-diene (0.2%), elimicin (0.2%), τ- muurolol (0.5%), methyl coniferylaldehyde (0.3%) were found in *M. bracteata* essential oil but were missing in *M. leucadendron* and *M. lineriifolia* essential oils, whereas caryophyllene oxide (3.7%), fokienol (0.4%), caryophylla-4(12),8(13)-dien-5-alphaol (0.5%), *epi*-α-bisabolol (0.1%), α-bisabolol (0.2%), 2*Z*,6*Z*-farnesol (0.1%), *iso*-longifolol (0.1%), the minor constituents of *M. leucadendron* were absent in the other two essential oils. Similarly, psuedolimonene (0.2%), δ-terpineol (0.6%), viridiflorene (0.5%), β-cadinene (0.6%), *E*-calamene (0.2%), ledol (0.2%), isospathulenol (0.2%), τ-cadinol (0.2%), phytol (0.3%) were identified in *M.lineriifolia* essential oil but were absent in *M.leucadendron* and *M.bracteata* essential oils. Previously 1,8-cineole has been reported as the major component in the essential oil of *M. leucadendron* grown in Cuba, Ivory Coast, India, Indonesia[7,38,39]. Another report from Thiland revealed terpinolene (29.21%), α-terpinene (22.55%), 2-carene (8.53%) and α-phellandrene (7.61%) as the major compounds of *M. leucadendron* essential oil. Recently Siddique and coauthors,reported eugenol methyl ether (95.4%) as the dominant component of *M. leucadendron* essential oil [40]. Our findings corroborate the earlier report , where *E*-nerolidol (90.85%) was the major component although the quantity of *E*-nerolidol (85.7%) was smaller than reported in previous findings[41]. Caryophyllene oxide (3.7%) in the present study is higher in amount than that of earliar report[41].

Our results of *M. linariifolia* essential oil characterized it as a rich source of monoterpenoides such as 1,8-cineole and α-terpineol, α-pinene, β-pinene, β-myrcene. As per earlier reports, the essential oil composition of *M. linariifolia* from Australia was characterized as 'terpinen-4-ol' and '1,8-cineole' type based upon the dominance of these monoterpenoids[16,42-44]. However, the volatile oil of *M.linariifolia* from Brazil was shown to be characterized with a high content of methyleugenol (86.8%) [13]. Our results show similarity with the results published by Padalia and coauthors, 2015a where 1,8-cineole (77.40%) and α-terpineol (7.72%) were the major components with some differences in quantitative makeup [36]. Aboutabl and coauthors investigated the essential oils from three *Melaleuca* species growing in Egypt and reported that eugenol is the major component of *M. bracteata* (97.7%)[8]. Pino and coauthors isolated the leaf essential oil of *M. leucadendron* from Cuba and identified it as a Virdifloral type chemotype with major presence of viridiflorol (28.2%) and 1,8-cineole (21.3%) [45].In another report, Pino and coauthors analyzed the phytochemical composition and antioxidant activity of leaf and fruit essential oil of *M. leucadendron* from Cuba[46]. A total of fourty one and sixty four compounds were identified and the major portion of leaf and fruit essential oil was composed of 1,8-cineole (43.0%) and virdifloral (47.6%). The other major compounds detected were viridiflorol (24.2%), α-terpineol (7.0%), α-pinene (5.3%), and limonene (4.8%) in the leaf oil, whereas the fruit essential oil consisted of globulol (5.8%), guaiol (5.3%), and α-pinene (4.5%)[46].

The composition report of the essential oil of *M.bracteata* was in agreement with the previous reports where methyl eugenol was the major component of the leaf essential oil [8,47,48].Based on the quantity of nerolidol, 1,8-cineole and methyl eugenol in present study it can be concluded that these shrubs can be good natural source of these neutaceutically important terpenoids by developing the agricultural practices to cultivate these species in wastelands which can be also a good source of revenue generation for local residents.

## Antioxidant activity

### DPPH Radical scavenging activity

All the essential oils showd scavenging activity in a dose dependent manner (Table 2). In this study, the antioxidant activity of essential oils were compared with BHT, a reference standard antioxidant compound. It was found that the essential oils of *Melaleuca* species exhibited good antioxidant activity. The essential oil from *M. bracteata* was found most effective antioxidant ($IC_{50}$ = 4.06 ± 0.02 µg/mL) followed by *M.linariifolia* ($IC_{50}$ = 10.96 ± 0.00 µg/mL) compared to BHT ($IC_{50}$ = 9.29 ± 0.09 µg/mL), whereas *M.leucadendron* essential oil revealed moderate potential ($IC_{50}$ = 16.24 ± 0.05 µg/mL) towards radical scavenging activity(Table2).

### Metal chelating activity

All the essential oils exhibited dose dependent metal chelating activity. *Melaleuca leucadendron* and *Melaleuca linariifolia* essential oils showed remarkable metal chelating activity with $IC_{50}$ of 9.56 ± 0.31 µg/mL and 10.51 ± 0.39 µg/mL compared to positive control citric acid ($IC_{50}$ = 4.04 ± 0.63 µg/mL) while *Melaleuca bracteata* essential oil with $IC_{50\ of}$ 8.80±0.44 µg/mL exhibited highest chelating activity among all the tested oils(Table2).

### Reducing power

The *Melaleuca bracteata* essential oil was found most effective in reduction of $Fe^{+3}$ ($IC_{50}$ = 2.11 ± 0.39 µg/mL) depicting comparable potential with standard reducing agent BHT ($IC_{50}$ = 2.46 ± 0.25 µg/mL).The *Melaleuca linariifolia* essential oil demonstrated significant reducing capability ($IC_{50}$ = 4.18 ± 0.33 µg/mL) whereas moderate activity was observed in *M.leucadendron* essential oil (Table2).

### Super oxide radical scavenging activity

*M.leucadendron* ($IC_{50}$ = 20.92 ± 0.31 µg/mL) and *M.bracteata* ($IC_{50}$ = 21.79 ± 0.85 µg/mL) essential oils demonstrated significant superoxide radical scavenging activity compared to standard ascorbic acid ($IC_{50}$ = 9.85 ± 0.16 µg/mL) as indicated by their $IC_{50}$ values. A moderate radical scavenging activity was observed for *M.linariifolia* essential oil ($IC_{50}$ = 25.83 ± 0.19 µg/mL).

## Nitric oxide(NO) radical scavenging activity

All the tested essential oils exhibited strong nitric oxide (NO) radical scavenging activity**.** Remarkably strong nitric oxide radical scavenging potential was observed in *Melaleuca bracteata* essential oil ($IC_{50}$ = 11.59 ± 0.09 µg/mL) compared to ascorbic acid ($IC_{50}$ = 10.36 ± 0.45 µg/mL). *M. linariifolia* ($IC_{50}$ = 12.68 ± 0.09 µg/mL) and *M. Leucadendron* were the least active among the three tested essential oils ($IC_{50}$ = 16.17 ± 0.06 µg/mL). All the tested oils demonstrated radical scavenging activity in a dose dependent manner with maximum scavenging at highest concentration (Table 2).

**Table 2.** Antioxidant activity ($IC_{50}$) of essential oils of Melaleuca Species.

| Essential oil/Standard | Antioxidant activity($IC_{50}$) | | | | |
|---|---|---|---|---|---|
| | DPPH radical Scavenging | Metal chelating | Reducing power | NO Radical scavenging | SO Radical Scavenging |
| ***M.leucadendron*** | 16.24±0.05 | 9.56±0.31 | 12.89±1.72 | 16.17±0.06 | 20.92±0.31 |
| ***M.linerifolia*** | 10.946±0.00 | 10.51±0.39 | 4.18±0.33 | 12.68±0.09 | 25.83±0.19 |
| ***M.bracteata*** | 4.06±0.02 | 8.80±0.44 | 2.11±0.39 | 11.60±0.09 | 21.79±0.85 |
| **Citric acid*** | - | 4.04±0.63 | - | - | - |
| **BHT*** | 9.29±0.09 | - | 2.46±0.25 | - | - |
| **Ascorbic Acid*** | - | - | - | 10.36±0.45 | 9.85±0.16 |

Values are mean ± S.D.,*Standard antioxidant

## Antifungal activity

The essential oils exhibited moderate to good antifungal potential by inhibiting the mycelial growth of pathogenic fungi. The inhibitory effect of the oil varied from 7.08% to 90% (Table 3). At the concentration of 500 ppm, the oils showed highest inhibitory effect on the radial growth of *S. sclerotiorum. S. sclerotiorum* and *E.turcicum* were found to be the most inhibited fungal pathogens by the oils. Essential oil of *M. bracteata* exhibited a strong antifungal effect against all the tested phytopathogens (up to 90%) as compared to other oils.

**Table 3.** Antifungal activity of essential oils of Melaleuca Species

| Essential oil | Conc. (ppm) | Mycelial growth inhibition (%) | | | |
|---|---|---|---|---|---|
| | | ***Fusarium oxysporum*** | ***Sclerotinia sclerotiorum*** | ***Curvularia lunata*** | ***Exserohilum* turcicum** |
| ***M.leucadendron*** | **500** | 18.95±1.301$^{a}$ | 80.83±1.30$^{a}$ | 39.66±3.53$^{a}$ | 56.66±1.11$^{a}$ |
| | **250** | 12.91±0.72$^{b}$ | 77.70±0.95$^{ab}$ | 38.75±2.5$^{a}$ | 55.18±0.64$^{ab}$ |
| | **100** | 12.08±1.90$^{bc}$ | 75.83±3.20$^{b}$ | 35.41±2.00$^{ab}$ | 54.81±0.64$^{b}$ |
| | **50** | 11.33± 1.01$^{bc}$ | 75.16±0.314$^{b}$ | 32.91±3.14$^{b}$ | 52.59±1.28$^{c}$ |
| | **25** | 10±1.25$^{c}$ | 71.66±1.44$^{c}$ | 32.91±1.57$^{b}$ | 51.11±0.96$^{c}$ |
| **$IC_{50}$(ppm)** | | **2332.65** | **1465.22** | **1136.35** | **318.57** |
| ***M.linariifolia*** | **500** | 28.95±2.00$^{a}$ | 58.58±0.88$^{a}$ | 38.16±0.57$^{a}$ | 59.07±1.15$^{a}$ |
| | **250** | 20.37±1.51$^{b}$ | 51.87±1.08$^{b}$ | 35.62±1.65$^{a}$ | 55.55±1.11$^{b}$ |
| | **100** | 9.79±1.57$^{c}$ | 50.87±3.68$^{b}$ | 31.25±1.25$^{b}$ | 54.62±3.61$^{bc}$ |
| | **50** | 9.16±1.57$^{c}$ | 25±1.25$^{c}$ | 27.91±3.14$^{b}$ | 54.44±2.22$^{bc}$ |
| | **25** | 7.70±0.368$^{c}$ | 7.08±1.44$^{d}$ | 23.54±3.55$^{c}$ | 52.40±2.50$^{c}$ |
| **$IC_{50}$(ppm)** | | **938.50** | **318.81** | **883.13** | **253.02** |
| ***M.bracteata*** | **500** | 46.87±1.65$^{a}$ | 82.91±0.36$^{a}$ | 75.20±0.36$^{a}$ | 90±1.11$^{a}$ |
| | **250** | 36.12±1.59$^{b}$ | 76.04±0.95$^{b}$ | 60.20±0.954$^{b}$ | 88.51±0.64$^{a}$ |
| | **100** | 31.87±2.86$^{c}$ | 63.87±0.45$^{c}$ | 32.29±2.81$^{c}$ | 65.18±0.64$^{b}$ |
| | **50** | 17.29±1.57$^{d}$ | 47.08±2.00$^{d}$ | 31.08±1.44$^{c}$ | 58.14±1.69$^{c}$ |
| | **25** | 7.29±0.95$^{e}$ | 27.70±0.36$^{e}$ | 20.12±0.66$^{d}$ | 54.62±3.61$^{c}$ |
| **$IC_{50}$(ppm)** | **25** | **498.11** | **85.88** | **239.97** | **89.70** |
| **Carbendazim** | | 100.00±0.00 | 100.00±0.00 | 100.00±0.00 | 100.00±0.00 |

Values are Mean ± S.D. , Within a column means followed by the same letter are not significantly different ($P > 0.05$). Means followed by a different letter are significantly different ($P < 0.005$).

## Antibacterial activity

The zone of inhibition around the well diameters and minimum inhibitory concentrations (MICs) of the essential oils against the microorganisms tested are shown in Table 4. The results obtained from the agar well diffusion method for the essential oils revealed *S. typhimurium* to be the most sensitive microorganism with the largest inhibition zones (20.67, 20.67 and 23.67 mm against *M. leucadendron*, *M. linariifolia* and *M. bracteata* essential oils respectively), while the minimum zone of inhibition were exhibited by *S.aureus* (16.33, 14.00, 15.67mm) against *M.leucadendron*, *M.linariifolia* and *M.bracteata* essential oils respectively.

**Table 4.** Antibacterial potential of essential oils of Melaleuca Species

| S.N. | Sample | Zone of inhibition(mm) | | | |
|---|---|---|---|---|---|
| | | ***B.megaterium*** | ***S.typhimurium*** | ***E.coli*** | ***S.aureus*** |
| **1.** | ***M.leucadendron*** | | | | |
| | **10** | 11.33 ± 0.58$^{c}$ | 12.67±0.58$^{d}$ | 16.33±1.15$^{b}$ | 10.33±1.52$^{c}$ |
| | **15** | 13.33 ±0.58$^{b}$ | 14.33±0.58$^{c}$ | 17±0.00$^{b}$ | 13.67±0.58$^{b}$ |
| | **20** | 14.33±0.58$^{b}$ | 16.33±0.58$^{b}$ | 18.33±0.58$^{a}$ | 15.67±0.58$^{a}$ |
| | **25** | 18.33±0.58$^{a}$ | 20.67±0.58$^{a}$ | 19.00±0.00$^{a}$ | 16.33±1.15$^{a}$ |
| | **MIC(µg/mL)** | 10 | 10 | 5 | 10 |
| **2.** | ***M.linariifolia*** | | | | |
| | **10** | 10.33±0.58$^{c}$ | 11.67±0.58$^{c}$ | 13.33±0.58$^{c}$ | 8.67±1.15$^{d}$ |
| | **15** | 12.67±0.58$^{b}$ | 15.33±0.58$^{b}$ | 14.67±0.58$^{b}$ | 10.33±0.58$^{c}$ |
| | **20** | 13.67±0.58$^{b}$ | 16.33±0.58$^{b}$ | 16±1.00$^{a}$ | 12.33±0.58$^{b}$ |
| | **25** | 19.67±0.58$^{a}$ | 20.67±1.15$^{a}$ | 17±0.00$^{a}$ | 14±0.00$^{a}$ |
| | **MIC(µg/mL)** | 10 | 10 | 5 | 10 |
| **3.** | ***M.bracteata*** | | | | |
| | **10** | 11±0.00$^{c}$ | 16.67±0.58$^{c}$ | 13.33±0.58$^{d}$ | 10.67±1.15$^{c}$ |
| | **15** | 11.67±0.58$^{c}$ | 17.67±0.58$^{c}$ | 15.67±0.58$^{c}$ | 12.67±1.15$^{b}$ |
| | **20** | 13.33±0.58$^{b}$ | 20.67±0.70$^{b}$ | 18.67±1.15$^{b}$ | 13.67±1.15$^{b}$ |
| | **25** | 14.67±0.58$^{a}$ | 23.67±0.58$^{a}$ | 20.67±0.58$^{a}$ | 15.67±0.58$^{a}$ |
| | **MIC(µg/mL)** | 10 | 5 | 5 | 10 |
| **4.** | **Refamycin** | | | | |
| | **25 µg/mL** | 31.33±1.22$^{a}$ | 35±1.00$^{a}$ | 32.67±1.15$^{a}$ | 27±1.00$^{a}$ |

Values are Mean ± S.D. Within a column means followed by the same letter are not significantly different ($P > 0.05$). Means followed by a different letter are significantly different ($P < 0.005$).

## *In-vitro* anti-inflammatory activity

As part of the investigation on the mechanism of the anti-inflammatory activity, ability of essential oils to inhibit protein denaturation was studied. All the tested oils were found effective in inhibiting heat induced albumin denaturation (Table 5). The $IC_{50}$ for *M. leucadendron* essential oil was 27.82 ± 0.24 µg/mL whereas that of *M. linariifolia* was 23.43 ± 0.25 µg/mL. *Melaleuca bracteata* essential oil was found most effective in inhibiting protein denaturation with $IC_{50}$ of 20.68 ± 0.004 µg/mL compared to the standard diclofenac sodium ($IC_{50}$ = 11.93 ± 0.03 µg/mL).

**Table 5.** Anti-inflammatory activity of essential oils of Melaleuca Species

| Anti inflammatory activity | Essential oil/Standard | | | |
|---|---|---|---|---|
| | ***M.leucadendron*** | ***M.linerifolia*** | ***M.bracteata*** | **Diclofenac sodium** |
| **$IC_{50}$ Value** | 27.82±0.242 | 23.43±0.247 | 20.68±0.004 | 11.93±0.033 |

## DISCUSSION

This study was undertaken to compare the essential oil composition, antioxidant and biological activity of the three *Melaleuca* species: *Melaleuca leucadendron, Melaleuca linariifolia* and *Melaleuca bracteata* from Northern plains of India. Differences and similarities in the groups of terpenes were established between the three *Melaleuca* species based on the present studies. The major findings from the three

species were (*E*)-nerolidol, 1,8-cineole and methyl eugenol. *M.bracteata* exhibited highest antioxidant activity followed by *M.linariifolia* and *M.leucadendron* essential oil. Oils exhibited significant antibacterial activity. The microorganisms selected in our studies are mostly human pathogens whose effects are noticeable in skin, intestinal and respiratory infections. The essential oils revealed remarkable antimicrobial potential against all the tested organisms. This observation is particularly noteworthy because essential oils are known to be more active against gram-positive than gram-negative bacteria but in our study essential oils exhibited noticeable activity against all the bacterial species tested [50]. All the oils showed moderate to strong anti-inflammatory potential within the tested range of concentrations. The results also revealed differential ability of oils in inhibiting the phytopathogenic fungal growth.

(*E*)-nerolidol a sesquiterpene alcohol with pleasant odour have several reported biological and pharmacological activities such as antimalarial, antileishmanial, antiulcer, antibacterial, antifungal, and as a topical skin penetration enhancer whereas 1,8-cineole is extensively used in food-flavor, pharmaceutical, and cosmetic industries [51-54]. Several pharmacological activities including anti-microbial, anticancer, anti-inflammatory, antioxidant, bactericidal, herbicidal, insecticidal have been reported for 1,8-cineole [13,55,56]. In addition 1, 8-cineole possess strong larvicidal, insecticidal, fumigant, repellent and antifeedent and anti-inflammatory activities [43, 56-58]. Methyl eugenol has been reported to possess antiepileptic and anti-inflammatory activity [59, 60]. Eugenol was found to inhibit the growth of various multi drug resistant pathogenic bacteria such as *E. coli*, *Staphylococcus*, *Proteus*, *Klebsiella*, *Enterobacter*, *H. pylori* and *Pseudomonas* isolated from human subjects [61, 62]. Thus various reports supports our claim that the antioxidant, antimicrobial and antinflammatory activity of essential oils could be due to their major components such as 1.8-cineole, methyl eugnol, *E*-nerolidol. Nevertheless, the presence of minor component could also play a role in the biological activity.

## CONCLUSION

The increasing population pressure and constraint of agriculture lands due to several developmental activities in India have raised alarm for food security and naturally the essential oil crops may limit their cultivation. Melaleuca species can grow in wild and are perennial species which have less agriculture cost inputs for their commercial production and can be grown in wastelands and used for filling of damp areas. The high content of (E)-nerolidol in the essential oil of M. leucadendron suggests this species as a new potential source of (E)-nerolidol which have a widespread use in the food-flavor, perfumery, cosmetics and pharmaceutical industries. The oil yield , 1,8-cineole content, and the biological activity of essential oil from *M. linariifolia* favours the cultivation and promising use of this species for isolation of 1,8-cineole and in pharmaceutical industry. Another species *Melaleuca bracteata* was found as a rich source of methyl eugenol, the commercially important molecule. All the oils showed significant *in-vitro* antifungal and antibacterial activity against tested fungal phytopathogens and bacterial strains responsible for plant diseases and several chronic conditions. The anti-inflammatory potential gives additional remarks to these species. Conclusively the outcomes of chemical composition and various activities suggested the cultivation and development of these species as commercial crops in agro forestry systems.

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