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Chemical characterization, antioxidant and antimicrobial activities of essential oil from *Melaleuca quinquenervia* leaves

Saima Siddique*[1], Sania Mazhar[2], Firdaus-e-Bareen[1] & Zahida Parveen[3]

[1]College of Earth & Environmental Sciences, University of the Punjab, Ferozpur Road, Lahore, Pakistan

[2]Food & Biotechnology Research Centre; [3]Applied Chemistry Research Centre, PCSIR Laboratories Complex, Lahore, Pakistan

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Niaouli oil is an essential oil known for its applications in aromatherapy and pharmaceutical preparations for coughs, colds, rheumatism and neuralgia. It also serves as a sedative, possesses antifungal activity, and used in perfume industry. *Melaleuca quinquenervia* (Cav.) S.T. Blake, commonly called, paper bark tea tree or punk tree, is a potential source of niaouli oil. Here, we analyzed the chemical composition of essential oil from *M. quinquenervia* leaves and evaluated its antioxidant and antimicrobial potential. Chemical analysis of the oil by GC-FID and GC-MS revealed 1,8-cineole (31.0%) as a major component followed by *p*-cymen-8-ol (19.7%), *p*-cymene (16.5%), α-terpineol (9.9%), limonene (6.8%), α-pinene (4.2%) and terpinolene (4.2%). *M. quinquenervia* essential oil demonstrated good antioxidant activity by inhibiting 84.3 % of 2,2′-diphenyl-1-picrylhydrazyl radical and ferric reducing power (1.94±0.007) at 100 µg/mL. Further, it was highly effective against tested food borne bacterial as well as fungal pathogens inducing 11.0-46.0 mm and 11.8-46.0 mm zones of inhibition, respectively at concentration of 8-250 µg/mL. The high degrees of antibacterial and antifungal activities were further confirmed at 8 µg/mL minimum bactericidal concentrations and minimum fungicidal concentrations, respectively. Time kill assay showed significant bactericidal and fungicidal effects of essential oil for four weeks. The high antimicrobial and antioxidant activities of *M. quinquenervia* essential oil substantiate its potential use as alternative to chemical preservatives in food industry.

Keywords: 1,8-Cineole, 2′-Diphenyl-1-picrylhydrazyl radical, Food borne pathogens, Limonene, Minimum fungicidal concentration, Niaouli oil, 2Paper bark tea tree, Preservatives, Punk tree, Time kill assay

Microorganisms and oxidation are the major causes of food deterioration. Microbial growth is a major concern as some microorganisms can potentially cause food-borne illnesses. Auto-oxidation of lipids in food can have a deteriorating effect on food colour, flavour, texture, quality, wholesomeness and safety[1]. Synthetic preservatives are usually preferred in food industry to retard discoloration, spoilage and microbial contamination due to their effectiveness and low price[2]. However, concerns for the safety of some chemical preservatives and negative customers feedbacks over their use have encouraged growing interest in natural green alternatives for the extension of product shelf-life[3]. Among emerging natural preservatives, essential oils have been widely investigated and have gained momentum in recent years due to their antimicrobial (antifungal, antibacterial and antiviral), antioxidants, antimutagenic and anticarcinogenic properties[4].

Myrtaceae family comprising of at least 133 genera and approximately 3800 is the rich source of essential oils. It is found abundantly in Australia, Southeast Asia and tropical to southern temperate America while few are domesticated in Africa[5]. Essential oils belonging to Myrtaceae have been reported to have diverse bioactivity, such as insecticidal, antimicrobial and nematicidal activity[6-8].

Melaleuca quinquenervia (Cave) S. T. Blake (Fam. Myrtaceae) is a source of 1,8-cineole-rich essential oil called Niaouli oil, which is used in aromatherapy and pharmaceutical preparations for the relief of coughs, colds, rheumatism and neuralgia[9]. The compounds *E*-nerolidol and linalool present in niaouli oil have widespread use in the perfume industry[10]. Besides, linalool has demonstrated acaricidal[11], bactericidal and fungicidal properties[12].

Pioneer reports have elucidated chemical composition[13-16] and antifungal activity[17,18] of essential oil of *M. quinquenervia* from different parts of the world. To the best of our knowledge, the chemical composition of Pakistani variety of *M. quinquenervia* has not been described previously. The present work, thus, aims to study the chemical composition of locally growing *M. quinquenervia* species and to evaluate its antioxidant potential and antimicrobial activity against food borne pathogens and spoilage microorganisms, for a potential use as a natural preservative in food industry.

* Correspondence:
Phone: +92 992 30688
E-mail: saimesiddique@gmail.com

Material and Methods

Collection of Materials

Plant materials

Fresh leaves (1.16 Kg) of *M. quinquenervia* were collected during 2013 from the botanical garden, Hattar, Pakistan and authenticated at the Herbarium, Department of Botany, University of Punjab, Lahore, Pakistan. The voucher specimen (code 4057) was also deposited in the same herbarium.

Chemicals

Homologous series of C_8–C_{25} n-alkanes, 2,2′-diphenyl-1-picrylhydrazyl (90.0%), butylated hydroxytoluene (BHT, 99.0%), and various reference chemicals used in this study were obtained from Sigma Chemical Co. (St. Louis, MO, USA). All other chemicals (analytical grade), i.e., anhydrous sodium sulphate, ferrous chloride, potassium ferricyanide, trichloroacetic acid, ethanol and methanol used in this study were purchased from Merck (Darmstadt, Germany). All culture media (Nutrient broth, Nutrient agar, Potato dextrose agar, Plate count agar) were purchased from OXOID Ltd. Hampshire, UK and HiMEDIA, Mumbai. India.

Isolation and Chemical analysis of essential oil

The air-dried and finely ground plant material (1.16 kg) was subjected to hydrodistillation for 3 h using Clevenger-type apparatus according to the method recommended in the European Pharmacopoeia[19]. Distillate of essential oil was dried over anhydrous sodium sulfate, filtered and stored at –4°C until analyzed.

GC analysis of the essential oil was carried out on Shimadzu GC 2010 using DB-5 MS (30 m×0.25 mm id, 0.25 µm film thickness) capillary column. The column oven temperature was programmed initially at 40-90°C at the rate of 2°C/min and then 90-240°C at the rate of 3°C/min. The final temperature was held constant for 5 min. Injector and detector temperatures were maintained at 240 and 280°C, respectively. Essential oil (0.5 µL) was injected in a split mode ratio of 1:5. Helium was used as a carrier gas at the flow rate of 1 mL/min. GC-MS analysis was carried out on GCMS-QP 2010 Plus, Shimadzu, Japan operating in electron ionization mode at 70 eV. Column conditions were same as in GC analysis. The mass spectrometer was capable of scanning from 35 to 500 AMU every second or less. The data acquisition system continuously acquires and stores all data analyses.

The components were identified by comparing their mass spectra with those of NIST mass spectral library (Mass spectral library 2001) and Adam (2001) as well as by comparing their retention indices either with those of authentic compounds or with literature values[20,21].

Evaluation of antimicrobial activities of essential oil

Test microorganisms

Seven bacterial strains from American Type Culture Collection (ATCC, Rockville) and six local fungal strains (fungal bank, Institute of Agricultural Sciences, University of the Punjab, Lahore, Pakistan) were selected for *in vitro* antimicrobial activity of essential oil. Of 7 bacterial strains, *Bacillus spizizenii* (ATCC 6633) and *Staphylococcus aureus* (ATCC 25923) were Gram positive while *Enterobacter aerogenes* (ATCC 13048), *Escherichia coli* (ATCC 8739), *Salmonella enterica* (ATCC 14028), *Klebsiella pneumonie* (ATCC 13882) and *Pseudomonas aeruginosa* (ATCC 27853) were Gram negative strains. *Aspergillus niger* (AC 1109), *A. flavus* (AC 1110), *Fusarium oxysporum* (AC 1175), *F. solani* (AC 1199), *Alternaria alternata* (AC 1200) and *Penicillium digitatum* (AC 1160) were selected for antifungal activity. All the bacterial strains were subcultured at 35°C for 24 h on nutrient agar slants prior to being grown in nutrient broth overnight whereas the fungal strain was sub-cultured at 25°C for 120 h on potato dextrose agar (PDA) slants to prepare spore suspension before testing.

Antimicrobial activity

Antimicrobial activity of *M. quinquenervia* essential oil was evaluated by agar well diffusion method[22]. Twenty millilitres of molten agar medium were inoculated with microbial suspension containing indicator strain at 10^6 cfu/mL. The inoculated medium was poured into a Petri plate and allowed to solidify. Wells were made on solidified agar and 90 µg of *M. quinquenervia* essential oil was added to each. The plates with bacterial strains were incubated at 35ºC for 24 h and at 25°C for 48 h for the fungal strain. The diameters of inhibition zones were measured in millimeters and results were recorded in triplicate.

Minimum inhibitory concentration (*MIC*) *assay*

Four different concentrations of oil (8, 25, 65 and 250 µg/mL) were used in triplicate to determine MIC levels by agar well method[22]. The lowest concentration of oil inhibiting visible growth of each microbe was taken as the MIC.

Minimal bactericidal concentration (*MBC*) and *fungicidal concentration* (*MFC*)

Minimum bactericidal concentration (MBC) and minimum fungicidal concentration. (MFC) were determined by broth microdilution method[23]. Bacterial/fungal spore load (10^6 cfu/mL) was poured in tubes containing respective culture broth and oil with concentration of MIC. Broth tubes with and without bacterial/fungal spore load were used as controls. The tubes were incubated for 24 h at 35°C for bacterial and at 25°C for a period of 48 h for fungal growth. After incubation, 100 µL from tubes having no visible growth was removed and poured in plates along with agar to enumerate total viable counts and molds counts. The lowest concentration killing 99.9% of the original inoculum with no visible growth after 24 h of incubation at 35°C was defined as the MBC while the minimum oil concentration that entirely seized the fungal growth and didn't allow slightest growth revival even after 48 h of incubation was considered as MFC (Minimum fungicidal concentration).

Time kill assays

Time kill study was carried out with the MIC values found previously in agar well method to evaluate the microbiostatic or microbiocidal effect of tested oil for four weeks[24]. The purpose of this assay was to evaluate the efficacy of tested essential oil as a food preservative. Microorganisms (bacterial/fungal spore suspension) with 10^6 cfu/mL and oil having concentration equal to MIC were added respectively in the tube of corresponding culture medium. Broth tubes with and without microbial suspension were used as controls. The cultures were incubated for one month at 35°C for bacterial and at 25°C for fungal growth. An inoculant of 100 µL, removed after 2, 5, 8, 11, 14 and 30 d was poured in agar plates in triplicate to determine the total reduction in viable counts. The mean number of the colonies ($cfu.mL^{-1}$) was counted and compared with that found in the control culture at the end of the incubation period. The test tubes with turbidity after a certain time period of incubation depict microstatic effect of the tested essential oil at the applied concentration. To determine the bactericidal and fungicidal concentration of *M. quinquenervia* essential oil against that particular strain, the higher concentrations (15, 65 and 250 µg/mL) were applied and lethal effect of essential oil was observed as mentioned above.

DPPH assay

The antioxidant activity of the essential oil from *M. quinquenervia* was assessed by measuring its ability to scavenge 2,2′-diphenyl-1-picrylhydrazyl (DPPH) stable radical. The assay was carried out spectrophotometrically as described by Shimada *et al.*[25] with some modifications.

Various concentrations (20-100 µL/mL) of oil sample were prepared in methanol. To the 0.1 mL of each test concentration, 3 mL of methanolic solution of DPPH (0.004%) was added. The resulting mixtures were incubated in dark for 30min at room temperature. The decrease in absorbance was measured at 517 nm using a spectrophotometer (Cecil CE7200). Scavenging (%) of DPPH free radical by essential oil was calculated as follows:

$$\text{Scavenging (\%)} = (A_{blank} - A_{sample}/A_{blank}) \times 100$$

where A_{blank} is the absorbance of the control reaction (containing all reagents except the test compound) and A_{sample} is the absorbance of the test oil. All determinations were performed in triplicate. The DPPH scavenging activity of butylated hydroxytoluene (BHT) was evaluated for comparison.

Total reduction ability by Fe^{3+}- Fe^{2+} transformation

The total reduction ability of essential oil was determined by the method of Oyaizu *et al*., 1986[26]. The capacity of essential oil to reduce the ferric ion (Fe^{3+}) to the ferrous ion (Fe^{2+}) was evaluated by measuring the absorbance at 700 nm. Different concentrations of the essential oil (20-100 µL/mL) were added to 2.5 mL of phosphate buffer (0.2M, pH 6.6) and 2.5 mL of potassium ferricyanide (1%). The mixture was incubated at 50°C for 20 min. After which 2.5 mL of trichloroacetic acid (10 %) was added. The mixtures were revolved at 3000 rpm for 10 min. The supernatant (2.5 mL) was mixed with 2.5 mL of distilled water and 0.5 mL of ferric chloride. The absorbance at 700 nm was measured on UV spectrophotometer after allowing the solution to stand for 30 min. High absorbance of the reaction mixture indicated greater reducing power. The ferrous reducing antioxidant power was compared with butylated hydroxytoluene (BHT) used as standard.

Statistical analysis

The mean values ± standard deviations were calculated using MS Excel 2007. The One-way ANOVA test was used to assess mean differences between sample mean sizes of the inhibitory zones

at different volumes of the same oil followed by Post hoc Tukey HSD test. Statistical software SPSS version 22 was used for data analysis. A *P*-value <0.05 was considered statistically significant.

Results and Discussion

The hydrodistillation of fresh leaves of *M. quinquenervia* yielded 1.34% of oil. The yield of essential oil was lower than that reported previously from *M. quinquenervia* dried leaves (2.97%) from Cuba[27]. The variability in essential oil yield can be attributed to the age of plant, the state of plant material used (dried or fresh), the part of the plant used, time of collection, season of harvest, condition of the soil, ecological and climatic conditions, etc.[28].

Nineteen components amounting to 98.7% of the total oil were identified in *M. quinquenervia* leaves essential oil by GC-MS analysis. The oil mainly consisted of monoterpenoids (42.4%) followed by aromatic compounds (37.3%) and monoterpene hydrocarbons (17.4%). Oxygenated sesquiterpenes were also present in noticeable amount (1.3%) and represented by carbophyllene oxide (0.41%) and epiglobulol (0.93%) while caryophyllene (0.09%) was the only sesquiterpene hydrocarbon present in *M. quinqueneveria* essential oil. The major components detected were 1, 8-cineole (31.0%) followed by α-terpineol (9.91%) in oxygenated monoterpene fraction while monoterpene hydrocarbon fraction contained limonene (6.8%), α-pinene (4.2%) and terpinolene (4.2 %). Aromatic fraction of essential oil contained *p*-cymen-8-ol (19.7%) followed by *p*-cymene (16.5%) and eugenol methyl ether (1.1%) (Table 1).

Previous studies on the composition of *M. quinquenervia* essential oil reported the 1,8-cineole as a principal component in oil in conjunction with viridiflorol, terpinolene and E-nerolidol[13-15]. On the contrary, Pino *et al*.[27] reported longifolene (95.0%), 1,8-cineole (43.0%), viridiflorol (76.0%) and allo-aromadendrene (50.0%) in the essential oil of *M. quinquenervia* variety growing in Cuba. Wheeler *et al.*[15] reported two chemotypes of *M. quinquenervia* leaf essential oil existing in Florida, each dominated by sesquiterpenes, either E-nerolidol or viridiflorol. Our findings on *M. quinquenervia* essential oil showed similarity in the previous results, 1,8-cineole being the major component. However, the *p*-cymen-8-ol (19.7%), β-linalool (1.5%), eugenol methyl ether (1.1%) and α-phellandrene (1.9%) among the monoterpenes and caryophyllene (0.1%), caryophyllene oxide (0.4%) and epiglobulol (0.9%) as sesquiterpenes, present in essential oil of locally grown of *M. quinquenervia* have not been reported before. These differences in the essential oil compositions might arise from difference in environmental (climatic, seasonal, geographical) factors[29].

The antimicrobial activity of *M. quinquenervia* essential oil was evaluated by measuring the inhibition zone against the common food borne pathogens. The oil manifested good antimicrobial properties against all the tested microbes but the level of microbial growth inhibition induced was proved to be dependent on the oil concentrations and the microbial strain. *Melaleuca quinquenervia* exhibited excellent activity against *B. spizizenii* with inhibition zone (IZ) of 13-46 mm at 8-250 µg/mL concentration while moderate activity was shown towards *S. aureus* with IZ=11.2-12 mm. Unlike the reports on resistance of essential oils towards gram negative bacteria[30],

Table 1 — Chemical composition of *Melaleuca quinquenervia* essential oil

Compounds	RI_{exp}	RI_{lit}	Area (%)	Mode of identification
α-Pinene	930	932	4.2	RT, RI, MS
Camphene	944	946	0.1	RT, RI, MS
α-Phellandrene	1002	1002	2.0	RT, RI, MS
δ-3-Carene	1004	1008	tr	RT, RI, MS
Limonene	1029	1024	6.8	RT, RI, MS
p-Cymene	1026	1020	16.5	RT, RI, MS
1,8-Cineole	1032	1026	31.0	RT, RI, MS
cis-β-Ocimene	1043		tr	RT, RI, MS
δ-Terpinene	1055	1054	0.1	RT, RI, MS
Terpinolene	1083	1086	4.2	RT, RI, MS
Linalool	1095	1095	1.5	RT, RI, MS
p-Cymen-8-ol	1183	1179	19.7	RT, RI, MS
2-Isopropenyl-5-methylhex-4-enal	1198	–	0.1	RT, MS
α-Terpineol	1186	1186	9.9	RT, RI, MS
Eugenol methyl ether	1402	1403	1.1	RT, RI, MS
β–caryophyllene	1417	1417	tr	RT, RI, MS
Germacrene D	1484	1484	0.1	RT, RI, MS
Caryophyllene oxide	1582	1582	0.4	RT, RI, MS
Epiglobulol	1585	–	0.9	RT, MS
Total identified			98.7	
Monoterpene hydrocarbons			17.4	
Oxygenated monoterpenes			42.4	
Sesquiterpene hydrocarbons			0.2	
Oxygenated sesquiterpenes			1.3	
Aromatic compounds			37.3	

Table 2 — Antimicrobial activity of *Melaleuca quinquenervia* essential oil

Tested microbial strains	Zone of inhibition (mm) concentration in µg/mL				
	250	100	65	15	8
Bacillus spizizenii (ATCC 6633)	46.0±0.0	26.0±0.5	14.3±0.3	13.7±1.05	12.5±1.6
Enterobacter aerogenes (ATCC 13048)	15.7±1.8	13.8±0.8	13.5±0.0	12.0±0.0	11.7±0.3
Escherichia coli (ATCC 8739)	17.2±1.04	15.0±0.9	14.2±0.6	12.5±0.0	11.8±0.3
Klebsiella pneumonia (ATCC 13882)	46.0±0.0	27.7±0.8	15.7±0.6	12.8±0.3	12.5±0.5
Pseudomonas aeruginosa (ATCC 27853)	11.8±0.3	11.7±0.3	11.3±0.3	11.2±0.3	11.2±0.3
Salmonella enteric (ATCC 14028)	16.0±0.0	14.0±0.5	13±0.0	12±0.0	11.5±0.5
Staphylococcus aureus (ATCC 25923)	12.0±0.0	12±0.0	12±0.0	11.3±0.0	11.2±0.3
Aspergillus niger (AC 1109)	42.7±2.9	27.0±0.9	17.8±0.3	13.7±1.5	11.8±0.3
A. flavus (AC 1110)	42.5±0.8	16.8±0.5	14.2±0.2	13.3±0.2	12.2±0.2
Fusarium oxysporum (AC 1175)	42.8±0.2	18.3±0.2	15.8±0.2	13.8±0.2	12.2±0.2
F. solani (AC 1199)	46.0±0.0	24.8±0.5	20.5±0.3	15.7±0.2	13.0±0.0
Alternaria alternate (AC 1200)	45.3±0.5	22.5±0.3	19.5±0.3	16.3±0.2	15.0±0.2
Penicillium digitatum (AC 1160)	46.0±0.0	25.2±0.5	20.5±0.3	17.2±0.2	15.2±0.2

[*The diameter of the inhibition zones (mm), including the disc diameter (6 mm), are given as mean ± SD of triplicate experiments]

M. quinquenervia showed good activity against them with IZ ranging from 11-46 mm. *M. quinquenervia* showed large zone of inhibition against *K. pneumoniae* (13-46 mm) followed by *E. coli* (11.8-16.2 mm). It showed moderate activity against *E. aerogenes* and *S. enterica* i.e.11.5-16 mm while minimum activity was exhibited against *P. aeruginosa* (11.2-11.8 mm). *M. quinquenervia* oil displayed excellent antifungal activity against all tested fungal strains with zones of inhibition ranging from 11.8-46.0 mm. (Table 2). The lower microbial activity shown by *P. aeruginosa* could be related to its ability to metabolise a wide range of organic compounds in the essential oils that are inhibitory to many of the other bacteria[31].

Results of one-way ANOVA showed that there was a statistically significant effect of concentration on ZI at the $P < 0.05$ level for all concentrations [$F(4, 205) = 47.806$, $p = 0.000$]. Post hoc comparisons using the Tukey HSD test indicated that the mean score for the highest concentration of 250 µg/mL was significantly different than 100, 65, 15 and 8 µg/mL. However, there was no statistical significant difference between 100, 65 and 8 µg/mL.

The MIC value was found to be 8 µg/mL against all microbial strains. The oil showed bactericidal and fungicidal effects at the same inhibitory concentration i.e. 8 µg/mL (Table 3).

The kill time assay was carried out to check the potency of *M. quinquenervia* essential oil to be used as a food preservative. The study was carried out to evaluate the microbiostatic or microbiocidal effect of tested oil for four weeks. The oil showed fungicidal effect against all tested fungal strains at 8 µg/mL for a month. Among bacterial strains *S. aureus* and *K. pneumoniae* demonstrated bactericidal effect for four weeks at 8 µg/mL. *B. spezizenii*, *E. coli* and *E. aerogenes* showed only bacteriostatic activity at 8 µg/mL. The bactericidal activity *E. coli* and *E. aerogenes* was observed 65 µg/mL. *P. aeruginosa* showed bactericidal activity at 250 µg/mL (Table 4). A probable explanation of highest susceptibility of fungal strains to essential oil as large zones of inhibition is that antimicrobial activity of volatile compounds results from the combined effect of direct vapour absorption on microorganism and indirect effect through the medium that absorbed the vapour[32]. Fungi grow mainly on the surface of the agar medium

Table 3 — Minimum inhibitory concentration (MIC) and Minimum bactericidal/fungicidal concentration (MBC/MFC) *Melaleuca quinquenervia* essential oil

Tested microbial strains	MIC (µg/mL)	MBC (µg/mL)	MFC (µg/mL)
Bacillus spizizenii	8	8	-
Staphylococcus aureus	8	8	-
Enterobacter aerogenes	8	8	-
Escherichia coli	8	8	-
Klebsiella pneumonia	8	8	-
Pseudomonas aeruginosa	8	8	-
Salmonella enteric	8	8	-
Aspergillus niger	8	-	8
A. flavus	8	-	8
Fusarium oxysporum	8	-	8
F. solani	8	-	8
Alternaria alternate	8	-	8
Penicillium digitatum	8	-	8

and might be more susceptible to direct vapour contact while the antimicrobial effect against bacteria might be more dependent on the vapour accumulation into the agar.

The antimicrobial activity of *M. quinquenervia* essential oil could be associated with the presence of 1,8-cineole; the major component found in oil which is previously known for its antimicrobial activity[33]. However, it is difficult to correlate the antimicrobial activities of essential oils to a specific compound due to their complexity and variability. Linalool and α-terpineol; other oxygenated monoterpenes present in the oil are also known to have very efficient antimicrobial properties[34,35]. Among the monoterpenes, limonene has been demonstrated to have bacteriostatic activity against several microorganisms[35,36]. Alpha pinene has been previously reported to be active against many organisms (*B. subtilis*, *S. aureus*, *S. epidermidis*, *E. coli*, *P. aeruginosa*, *S. pyogenes*, *S. pneumoniae*, *Saccharomyces cerevisiae*, *Candida albicans*, methicillin-resistant *S. aureus* (MRSA)[37-39]. Many researchers reported antibacterial effects of terpinolene and α-phellandrene present in the oil[40].

In addition, the components with lower concentrations, such as camphene, terpinolene, β-*cis*-ocimene, 4-carene may also have been contributing to the antimicrobial activity of the oil. Therefore, the high antimicrobial activity of *M. quinquenervia* essential oil could be due to synergistic effects of many diverse major and minor components of the essential oil.

DPPH assay and reducing power assay were used to assess antioxidant potential of *M. quinquenervia* essential oil. Synthetic antioxidant BHT was used as an equivalence parameter for the antioxidant activity. The essential oil showed 84.3% of DPPH scavenging effect by reducing stable, purple coloured radical DPPH into yellow coloured DPPH-H. *M. quinquenervia* oil showed comparable IC_{50} (44.5 ± 0.44) mg/mL value with butylated hydroxytoluene (BHT) i.e. 41.5 ± 0.50 mg/mL. In the reducing power assay, *M. quinquenervia* essential oil showed higher absorbance at tested concentrations (20-100 µg/mL) and consequently good antioxidant activity (Table 5).

No data is available for comparison of antioxidant activity of *M. quinquenervia* essential oil. However, the literature showed that essential oils rich in monoterpenoids (1,8-cineole) showed good antioxidant properties[41-43]. Many researchers have also reported antioxidant activities of monoterpene present in studied oil. Wei & Shibamoto[44] related antioxidant activity to the α-pinene in *Citharexylum caudatum* L.

Table 4 — Time kill assay; bactericidal/fungicidal effect of *Melaleuca quinquenervia* essential oil for 30 days

Tested microbial strains	MBC (µg/mL)	MFC(µg/mL)
Bacillus spizizenii	65	-
Staphylococcus aureus	8	-
Enterobacter aerogenes	65	-
Escherichia coli	65	-
Klebsiella pneumonia	8	-
Pseudomonas aeruginosa	250	-
Salmonella enterica	8	8
Aspergillus niger	-	8
A. flavus	-	8
Aspergillus oxysporum	-	8
F. solani	-	8
Alternaria alternate	-	8
Penicillium digitatum	-	8

Table 5 — Antioxidant activity of *Melaleuca quinquenervia* essential oil measured in term of DPPH radical scavenging capacity and Ferric reducing ability

Test System	Concentrations (µg/mL)	*M. quinquenervia* oil	BHT	IC_{50} value/mg/mL
DPPH radical scavenging capacity (%)	20	28.3±01.1	30.8±0.6	44.5±0.44 (Oil) 41.5±0.50 (BHT)
	40	49.8±1.1	52.4±1.0	
	60	65.5±0.5	66.5±0.7	
	80	75.5±0.5	76.8±0.6	
	100	84.9±0.9	85.8±0.8	
Reducing power (Absorbance at 700 nm)	20	0.87±0.006	1.16±0.18	-
	40	1.21±0.06	1.36±0.03	
	60	1.43±0.04	1.57±0.05	
	80	1.58±0.01	1.82±0.07	
	100	1.94±0.007	1.95±0.04	

Limonene, terpinolene, and γ-terpinene have also been reported to show considerable activity[45]. de Oliveira *et al.*[46] reported antioxidant activity of *p*-cymene i*n vivo*. Thus, antioxidant activity of essential oils can be attributed to the combination of their various major and minor oils constituents.

Conclusion

The results of the study show that the essential oil from *Melaleuca quinquenervia* has good antimicrobial and antioxidant activities. Therefore, the study concludes the promising possibility of using the essential oil from *M. quinquenervia* as an alternative to chemical preservatives in food. However, *in vivo* studies are recommended to evaluate the toxicity profile of essential oil.

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