Original Article

Pleiotropic Chemodiversity in Extracts and Essential Oil of *Melaleuca viminalis* and *Melaleuca armillaris* of Myrtaceae Family

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Abstract

Background and objective: *Melaleuca viminalis* (syn. *Callistemon viminalis,* red bottle brush) and *Melaleuca armillaris* (white Bottle brush) belong to the family Myrtaceae and are reported for their traditional medicinal properties. The objective of this study was to explore and compare the chemical compositions and biological properties of these two species.

Methods: Sequential extraction and hydro-distillation methods were employed to extract essential oils for further analysis of chemical composition by gas chromatography-mass spectrometry (GC-MS). The biological potential as antioxidants was investigated for both species by assessing 1,1-diphenyl-2-picrylhydrazyl (commonly known as DPPH) scavenging activity and by use of ferric iron reducing assay. The biological potential as antibacterials was investigated by agar well diffusion assay. The *in vitro* cytotoxicity analysis was carried out by MTT assay.

Results: GC-MS analysis of the essential oil of *Melaleuca viminalis* indicated the presence of eucalyptol as the principal chemical constituent, while that of *Melaleuca armillaris* indicated the presence of methyl eugenol. Comparative studies indicated that *Melaleuca viminalis* had higher potential for antioxidant and antibacterial activities than *Melaleuca armillaris.* Also, the essential oil of *Melaleuca viminalis* exhibited *in vitro* cytotoxicity against the cancer cell lines of A549 (lung; IC_{50} 24.12 µg/mL), HCT-116 (colon; IC_{50} 21.5 µg/mL) and T47D (breast; IC_{50} 21.78 µg/mL), in comparison to *Melaleuca armillaris* for which cytotoxicity was only observed against theA549 (IC_{50} 10.2 µg/mL) lung cancer cell line.

Conclusions: The present findings suggest that essential oil of *Melaleuca viminalis* (leaves) hold potential for future application in various medical procedures. However, the presence of methyl eugenol in *Melaleuca armillaris* raises concern of its being acarcinogenic compound, so further detailed toxicological studies are required to validate its therapeutic potential.

Introduction

Myrtaceae (121 genera, 3,800–5,800 spp.) is one of the largest families of tropical forest plants, comprises of aromatic trees or shrubs. Economically important essential oils have been reported from several species of the *Melaleuca* genus and are known to possess diverse bioactivity (*viz*., antidiarrheal, antimicrobial, antioxidant, antirheumatic, anti-inflammatory anticough, antibronchitis, insecticidal, antiphylococcal, nematicidal, larvicidal, pupicidal and antithrombotic, *etc*.).[1,2]

Traditionally, *M. viminalis* leaves have been used as tea substitute, having refreshing flavor and fragrance; they have also been used as treatment against various diseases, including gastroenteritis, diarrhea and skin infections.[3,4] Apart from medical applications, it has also been reported to act as environmental bioindicators and to have antiquorum sensing activities.[5–7] Another species, *M. armillaris*, is most widely cultivated around the world as an ornamental plant, but there is scarce literature regarding investigations into its biological activities.[8–11] However, studies of the chemical composition of its oil have shown predominance of ses-

Keywords: Antibacterial; Antioxidant; Cytotoxicity; Essential oil; Eucalyptol; *Melaleuca armillaris*; *Melaleuca viminalis*; Methyl eugenol; Myrtaceae.

Abbreviations: DPPH, 1,1-diphenyl-2-picrylhydrazyl; ELISA, enzyme-linked immunosorbent assay; IC_{50}, concentration of drug inhibiting cell growth by 50%; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide.

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quiterpenes; unfortunately, variation has been frequently observed during the quantitative analysis, which depends majorly upon the taxonomic identity and population being studied.[12–14]

As continuation to our earlier studies on essential oils isolated from plants of the Northwestern Himalayan region, herein we describe our most recent studies on the chemical composition and *in vitro* biological properties of the essential oils from *Melaleuca viminalis* (red bottle brush) and *Melaleuca armillaris* (white bottle brush).

Methods

Collection of plants and extraction

The aerial parts (leaves) of both the plant species were collected from the botanical garden of the University of Jammu, Jammu and Kashmir (India) and were identified by the taxonomist of the University. Different extracts were prepared with different solvents based on polarity. For chloroform extract, powdered plant material (100 g) was soaked in chloroform for 24 hand then filtered. The resultant filtrate was further evaporated using a rotary evaporator, and the extract obtained was lyophilized and stored at 4 °C. Similarly, the residue was then sequentially processed with ethyl acetate, acetone, methanol and water.

Extraction of essential oil

Two hundred grams of fresh leaves of each *Melaleuca* species were hydro-distillated with a Clevenger-type apparatus for 3 h.[15] The yield of essential oil from both the species was calculated based on the fresh weight. Samples were stored at 4 °C.

Chemical screening

Qualitative screening of extracts of leaves of *M. viminalis* and *M. armillaris*

To test the presence or absence of phytochemical constituents (*viz*., tannins, saponins, terpenoids, coumarins, quinines, anthraquinones, cardiac glycosides, *etc*.), extracts were qualitatively screened.[16]

Saponins

A 1 mg/mL stock solution of the extracts in methanol and distilled water were vortexed, after which a few drops of olive oil/refined oil was added. Persistence of foam indicated the presence of saponins.

Tannins

A 1 mg/mL stock solution of the extract was mixed with methanol and stirred with 5% ferric chloride solution. Appearance of a black precipitate indicated the presence of tannins.

Terpenoids

A 1 mg/mL stock solution of the extract was mixed with methanol, chloroform (1 mL) and concentrated H_2SO_4 (1.5 mL). Appearance of reddish-brown coloration at the interface indicated the presence of terpenoids.

Coumarins

A 1 mg/mL stock solution of the extract was mixed with methanol, 10% NaOH (1 mL) and chloroform (1 mL). Presence of yellowish color indicated the presence of coumarins.

Quinones

A 1 mg/mL stock solution of the extract was mixed with methanol and NaOH (1 mL). The disappearance of bluish green or red color indicates the presence of quinines.

Anthraquinones

A 1 mg/mL stock solution of the extract was mixed with methanol and chloroform (2.5 mL), further vortexed (5 m), filtered and an equal volume of 10% ammonia (3.5 mL) was added. The appearance of pink, violet or red coloration indicated the presence of anthraquinones.

Cardiac glycosides

A 1 mg/mL stock solution of extract was mixed with methanol, glacial acetic acid (2 mL), ferric chloride solution (one drop) and 1mL of concentrated H_2SO_4. Presence of a brown colored ring at the interface indicated the presence of deoxysugar, characteristic of carotenoids.

Total phenolic content

Total phenolic content was determined according to the Folin–Ciocalteau method.[17] A 0.5 mL aliquot of the 1 mg/mL extractstock solution was mixed with 0.5 mL of 1N Folin–Ciocalteau reagent. After 5 m, 1 mL of 20% Na_2CO_3 was added. After 10 m of incubation at room temperature, the absorbance was measured at 750 nm using a spectrophotometer. The concentration of phenolic compounds was calculated according to the following equation obtained from the standard gallic acid: Absorbance = 0.0364 gallic acid (µg) + 0.009.

Total flavonoid content

Total flavonoid content was determined by a colorimetric method.[17,18] A 0.5 mL aliquot of the 1mg/mL extract stock solution was mixed with 150 µL of a 5% $NaNO_2$ solution. After 5 m, 300 µL of 10% $AlCl_3 \cdot H_2O$ solution, 300 µL of 1 M NaOH and 550 µL of distilled water were added. The solution was mixed well and the absorbance was observed at 510 nm using a UV-VIS spectrophotometer. The concentration of flavonoid compounds was calculated according to the equation obtained from the standard quercetin graph: Absorbance = 0.001 quercetin (µg) + 0.032.

Chemical analysis by gas chromatography-mass spectrometry (GC-MS)

Analysis of the oil was carried out at the CSIR-Indian Institute of

Integrative Medicine(India) using the Varian GC-MS 4000 (USA) system equipped with a Varian CP-SIL 8CB column (30 m × 0.32 mm i.d., 1 µm film thickness). The injector temperature was 230 °C. The oven temperature program used consisted ofholding at 60 °C for 5 m, heating to 250 °C at 3 °C/mand keeping the temperature constant at 250 °C for 10 m. Helium was used as a carrier gas at a constant flow of 1.0 mL/m, and an injection volume of 0.20 µL was employed. The MS scan parameters included electron impact ionization voltage of 70 eV, and a mass range of 40–500 m/z. The identification of components of the essential oil was based on comparison of their mass spectra with those of the NIST05 (version 2.0) library.

Biological activities

1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay

The free radical scavenging activity was determined according to the method of Blois (1958) with some modifications.[19] Aliquots of 50 µg/mL, 100 µg/mL, 150 µg/mL, 200 µg/mL and 250 µg/mL of the test sample were mixed with 1 mL of 0.5 mM solution of DPPH in methanol. To these, 2 mL of 0.1 M sodium acetate buffer (pH 5.5) was added respectively. The mixtures were then allowed to stand at room temperature in the dark for 30 m. The absorbance was measured at 517 nm using a double beam UV-VIS spectrophotometer. The radical scavenging activity was calculated as a percentage of DPPH radical discoloration from dark purple to yellow, using the equation: % radical scavenging activity = $[(A_0 - = _s)/A_0] \times 100$, where $A_0$was the absorbance of the control and A_s was the absorbance of the test compound.

Ferric ion reducing antioxidant power (FRAP) assay

FRAP activity was measured according to the method of Benzie and Strain (1996).[20] Acetate buffer (300 mM, pH 3.6), 10 mM TPTZ (2,4,6-tripyridyl-s-triazine in HCl) and $FeCl_3 \cdot 6H_2O$ (20 mM) were prepared and mixed in the ratio of 10:1:1. The test sample (100 µL) was mixed with 3 mL of working FRAP reagent, and absorbance was measured at 593 nm after vortexing. $FeSO_4 \cdot 7H_2O$ (100–2,000 µM) were prepared in methanol and used as the standard for the calibration curve of known Fe^{2+} concentration. The parameter equivalent concentration was defined as the concentration of antioxidant having a ferric-TPTZ reducing ability equivalent to that of 1 mM $FeSO_4 \cdot 7H_2O$.

Antibacterial screening

The antibacterial activities of the crude extracts of *M. viminalis*and *M. armillaris*were determined by the agar well diffusion method.[21] The bacterial isolates were first grown by streaking on a nutrient agar plate and incubating for 24 h at 37 °C before use. Cell suspension was prepared by dissolving a loop full of culture from the culture plate in 200 µL of autoclaved nutrient broth. Then, a 100 µL aliquot of the respective cell suspensions was spread on nutrient agar plates using a sterile glass spreader and allowed to absorb in the agar for 30 m. Furthermore, four wells (6 mm) were bored in each agar plate and a 20 µL solution of 1 µg/µL extracts/essential oil was added. Plates were incubated at 37 °C for 18 h and then observed for zones of inhibition. The effect was compared with that of the positive reference ampicillin to determine the sensitivity of bacterial growth. Each sample was applied in duplicate for the determination of antibacterial activity.

In vitro cytotoxicity assay against human cancer cell lines

In vitro cytotoxicity against human cancer cell lines was measured by the tetrazolium-based colorimetric assay which measures the reduction of the tetrazolium salt 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) into a blue formazan product, mainly by the activity of the mitochondrial enzymes, cytochrome oxidase and succinate dehydrogenase.[22] Typically, 100 µL of a cell suspension was plated at a density of approximately 2×10^4 cells per well in a 96-well plate and were subsequently incubated at 37 °C in a 5% CO_2 humidified incubator for 24 h. Different concentrations of the essential oil and extracts were added to each group (in triplicate wells) and were incubated for 24 h, followed by the addition of l0 µL (5 mg/mL) MTT dye solution to each well and incubation for 4 h at 37 °C. After removal of the MTT dye solution, the cells were treated with 100 µL DMSO and absorbance at 490 nm was quantified using an enzyme-linked immunoassay (ELISA) plate reader. The cytotoxicity was calculated after comparing with the control (treated with 0.1% DMSO). Cytotoxicity was expressed as the concentration of drug inhibiting cell growth by 50% (IC_{50}). All tests and analysis were run in 100 µL DMSO and the absorbance at 490 nm was quantified by using ELISA reader. The cytotoxicity was calculated after comparing with the control (treated with 0.1% DMSO). Cytotoxicity was expressed as the concentration of drug inhibiting cell growth by 50% (IC_{50}). All tests and analyses were run in triplicate.

Results

Percentage yield

M. viminalis showed the highest yield in water extract (3.12%), followed by methanol extract (2.50%), chloroform extract (2.15%), ethyl acetate extract (1.90%) and acetone extract (1.95%); whereas, *M. viminalis* essential oil yield was 0.39%. In the case of *M. armillaris,* the highest yield was found in acetone extract (2.42%), followed by water extract (2.07%), methanol extract (1.33%) and ethyl acetate extract (1.11%); whereas the essential oil yield was 1.14%.

Qualitative phytochemical assays of extracts

The qualitative phytochemical analysis of *M. viminalis* and *M. armillaris* (leaves) are depicted in Table 1. The presence of terpenoids was found to be highest in *M. viminalis* methanolic extract; whereas, the presence of tannins was observed in the ethyl acetate and methanol extracts. Anthraquinones, coumarins, saponins and cardiac glycosides were moderately present in the chloroform, methanol and acetone extracts, while quinones were absent in *M. viminalis*. *M. armillaris* showed moderate presence of coumarins in acetone and methanol extracts; quinones, cardiac glycosides, tannins and anthraquinones were absent in all the extracts of *M. armillaris*.

Total phenolic and flavonoid content

A high content of phenolics was observed in *M. viminalis* methanol

Table 1. Phytochemical profiles of extracts of *M. viminalis* (*M.v*) and *M. armillaris* (*M.a*)

No.	Phytochemical	Extracts									
		Chloroform		Ethyl acetate		Acetone		Methanol		Water	
		M.v	*M.a*	*M.v*	*M.a*	*M.v*	*M.a*	*M.v*	*M.a*	*M.v*	*M.a*
1	Tannins	–	–	+++	–	–	–	+++	–	–	–
2	Coumerins	++	+	–	–	++	++	+	++	–	–
3	Terpenoids	+	+	–	++	++	++	+	+++	–	–
4	Cardiac glycosides	–	–	–	–	++	–	+++	–	–	–
5	Saponins	–	–	–	–	–	+	–	+	–	–
6	Quinones	–	–	–	–	–	–	–	–	–	–
7	Anthraquinones	–	–	+	–	–	–	+	–	–	–

+++, strongly present; ++, moderately present; +, present; –, absent.

extract (16 ± 0.11 mg/g GAE), followed by acetone extract (14 ± 0.010 mg/g GAE), ethyl acetate extract (12 ± 0.009 mg/g GAE) and chloroform extract (10 ± 0.076 mg/g GAE); whereas, *M. armillaris* showed the highest phenolic content in methanolic extract (14 ± 0.010 mg/g GAE), followed by acetone extract (8 ± 0.005 mg/g GAE), ethyl acetate extract (6 ± 0.005 mg/g GAE), chloroform extract (4 ± 0.003 mg/g GAE) and water extract (2 ± 0.001 mg/g GAE).

Similarly, high-content flavonoids in *M. viminalis*was observed in methanolic extract (10 ± 0.072 mg/g QE), followed by acetone extract (6 ± 0.067 mg/g QE), ethyl acetate extract (5 ± 0.064 mg/g QE), water extract (4 ± 0.066 mg/g QE) and chloroform extract (3 ± 0.051mg/g QE); whereas, the *M. armillaris* highest flavonoid content was present in methanolic extract (11 ± 0.072 mg/g QE), followed by acetone extract (10 ± 0.007 mg/g QE), ethyl acetate extract (7 ± 0.060 mg/g QE), chloroform extract (5 ± 0.062 mg/g QE) and water extract (2 ± 0.056 mg/g QE).

GC-MS findingsof the essential oils

The chemical composition of the essential oil of *M. viminalis* revealed the presence of six major compounds (Fig. 1A). Among all the other compounds, eucalyptol (74.613%) was present in the highest amount, followed by alpha-pinene (8.889%), alpha-terpineo (5.573%), para cymene (5.272%), limonene (4.290%) and 4-terpineno (1.363%). The chemical composition of *M. armillaris* revealed the presence of two major compounds, with the highest content being for methyl eugenol (94.283%) followed by methyl cinnamate (5.717%) (Fig. 1B).

Biological activities

DPPH radical scavenging assay findings

The radical scavenging capacities of extracts and oils of *M. viminalis* and *M. armillaris* were tested by using the free radical DPPH assay. Both essential oils showed antioxidant properties, with the methanolic extract of *M. viminalis* showing high antioxidant activity followed by the acetone and chloroform extracts; whereas, the methanolic extract of *M. armillaris* was found to be a good antioxidant. Among the essential oils, that of *M. viminalis* possessed high antioxidant activity compared with that of *M. armillaris*. The IC_{50} µg/mL scavenging activity of extracts and essential oils are depicted in Figure 2.

FRAP assay findings

High FRAP was observed in both the plants (*i.e. M. viminalis* and *M. armillaris*). Among all the extracts, the chloroform extracts of both plants showed good ferric reducing power potential (Fig. 3).

Antibacterial activities

The essential oils and extracts of both the plants showed varied activities against the tested bacteria. Both the extracts and oils of *M. viminalis* and *M. armillaris* were found to be effective against

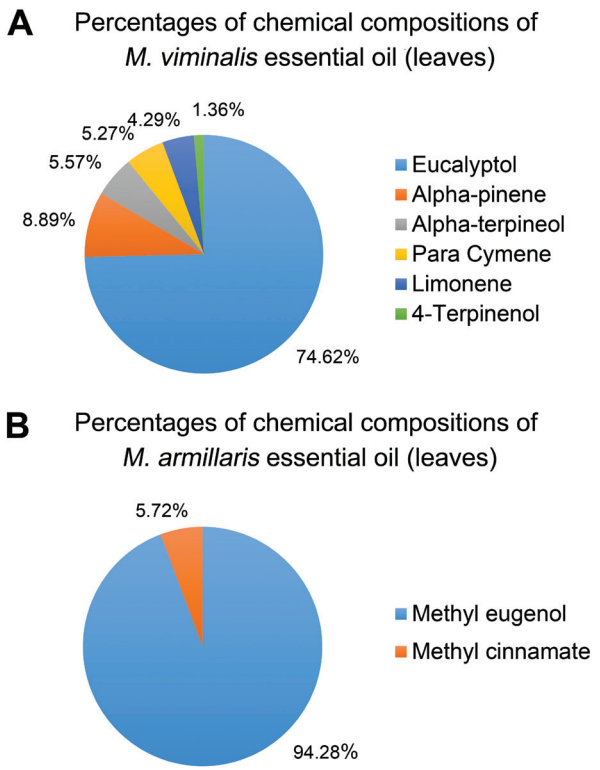

Fig. 1. Percentages of chemical compositions of M. viminalis and M. armillaris essential oil (leaves).

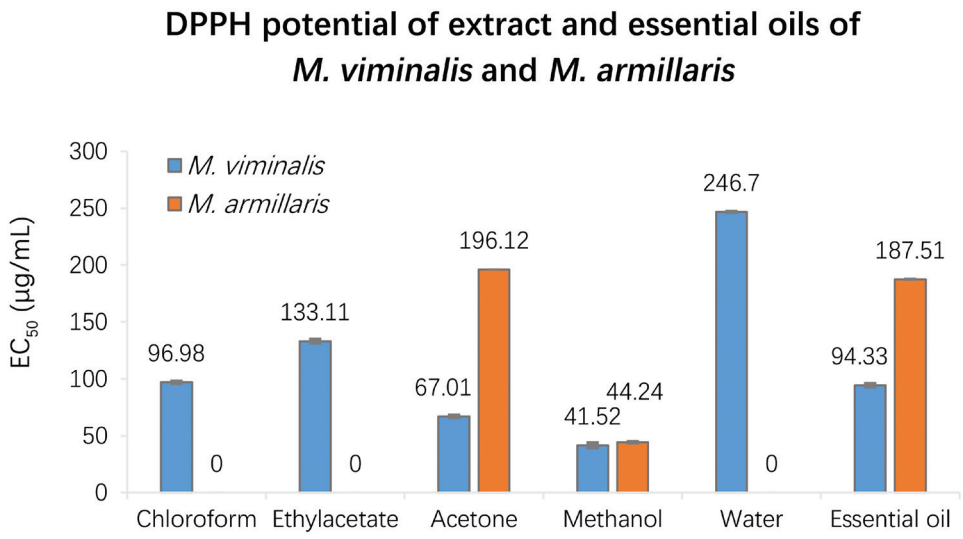

Fig. 2. Free radical scavenging (DPPH) potential of extracts and essential oils of *M. viminalisand M. armillaris.*

all the microbes tested. The highest antimicrobial activity was observed against Gram-positive bacteria, in comparison to Gram-negative bacteria. In the case of *M. viminalis*, the essential oil showed the largest zone of inhibition (23 mm) against *B. subtilis*, followed by the chloroform extract (23 mm) against *S. aureus*. Whereas for *M. armillaris*, the essential oil showed the largest zone of inhibition against *S. aureus* (19 ± 1.22 mm), followed by the chloroform extract against *K. pneumoniae* (11 ± 1.22 mm), and the methanol extract showed activity against *B. subtilis* (10 ± 1.5 mm) (Tables 2 and 3).

In vitro cytotoxicity against human cancer cell lines

M. viminalis and *M. armillaris* essential oils demonstrated significant *in vitro* cytotoxic activity against the cancer cell lines for lung (A549), colon (HCT-116) and breast (T47 d). *M. viminalis* essential oil was found to be more effective against the colon HCT-116 (IC_{50} 21.5 µg/mL) and breast T47D, (IC_{50} 21.78 µg/mL) cancer cell lines, whereas *M. armillaris* essential oil was found to be effective against lung A549 (IC_{50} 10.2 µg/mL) only (Fig. 4).

Discussion

Nature is a rich source of biological and chemical diversity. The unique and complex structures of natural products cannot be obtained easily by chemical synthesis.[23] Plant essential oils and extracts have been used for many thousands of years in food preservation and as pharmaceuticals, alternative medicines and natural therapies.[24–26] Some of them constitute effective alternatives or complements to synthetic compounds of the chemical industry, without exerting the secondary effects of the latter.[27]

As reported earlier, essential oils of the Myrtaceae family are

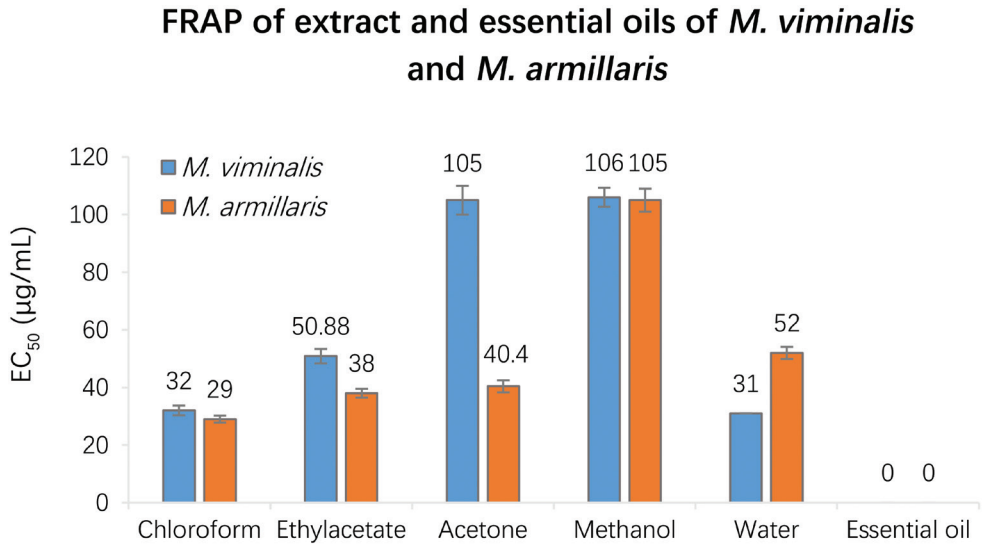

Fig. 3. FRAP of extracts and essential oils of *M. viminalis and M. armillaris.*

Table 2. Antibacterial activity of extracts and essential oil of *M. viminalis*

No.	Sample, 20 µg/µL	*B.subtilis*	*A.denitrificans*	*K.pneumonie*	*S.aureus*
1	Ampicillin	22 ± 4.7 mm	19.5 ± 3.7 mm	24.5 ± 4.3 mm	27.5 ± 2.57 mm
2	Essential oil	22 ± 2.73 mm	18 ± 4.7 mm	15 ± 0.97 mm	23 ± 2.56 mm
3	Chloroform extract	14 ± 0.75 mm	10 ± 1.75 mm	12 ± 0.97 mm	15 ± 1.27 mm
4	Ethylacetate extract	16 ± 0.67 mm	13 ± 1.17 mm	10 ± 2.47 mm	–
5	Acetone extract	20 ± 1.27 mm	7 ± 0.26 mm	–	12 ± 1.87 mm
6	Methanol extract	17 ± 0.75 mm	11 ± 0.57 mm	–	6 ± 1.17 mm
7	Aqueous extract	–	–	–	–

Table 3. Antibacterial activity of extracts and essential oil of *M. armillaris*

No.	Sample, µg/µL	*B. subtilis*	*A. denitrificans*	*K. pneumoniae*	*S. aureus*
1	Ampicillin	15 ± 2.37 mm	18 ± 3.3 mm	20 ± 1.27 mm	22 ± 0.37 mm
2	Essential oil	12 ± 2.07 mm	16 ± 0.14 mm	13 ± 2.17 mm	19 ± 1.34 mm
3	Chloroform extract	9 ± 0.87 mm	8 ± 07 mm	11 ± 1.12 mm	7 ± 1.15 mm
4	Ethylacetate extract	6 ± 0.65 mm	12 ± 2.7 mm	–	5 ± 2.11 mm
5	Acetone extract	–	5 ± 3.01 mm	8 ± 0.32 mm	10 ± 1.7 mm
6	Methanol extract	10 ± 1.5 mm	6 ± 3.01 mm	7 ± 0.11 mm	–
7	Aqueous extract	–	–	–	–

well known for their pharmaceutical properties[28]; therefore, in this study, we compared the chemical constituents and biological properties of *M. viminalis* and *M. armillaris.* The chemical constituents varied between the species. Ethyl acetate, acetone and methanol extracts of *M. viminalis* and *M. armillaris* demonstrated the strong presence of terpenoids and various other secondary metabolites, such as phenolics, glycosides, flavanoids, saponins, tannins and cardiac glycosides. The major difference in essential oil composition between two species revealed thatthe major compound of *M. viminalis*is eucalyptol (74.61%), while methyl eugenol (94.24%) was the major compound of *M. armillaris*.

Also as mentioned earlier, eucalyptol is known to possess antibacterial properties.[29,30] Although methyl eugenol (methyl ether of eugenol) is present as the major constituent in *M. armillaris* essential oil, this compound has been listed by the 'National Toxicology Program's Report on Carcinogens' as a human carcinogen.[31,32] It is well known that the variations in essential oil content and chemical composition are influenced by many factors, including location, age of the plant, climate, cultivar, method of distillation, type of distillation apparatus employed, *etc*.[33,34] These parameters may represent factors influencing the chemical variation in both the species.

Evaluation of activities against various *in vitro* biological parameters indicated that the methanol extract, acetone extract and essential oil of *M. viminalis* possess the highest scavenging activities, followed by the chloroform and ethyl acetate extracts;

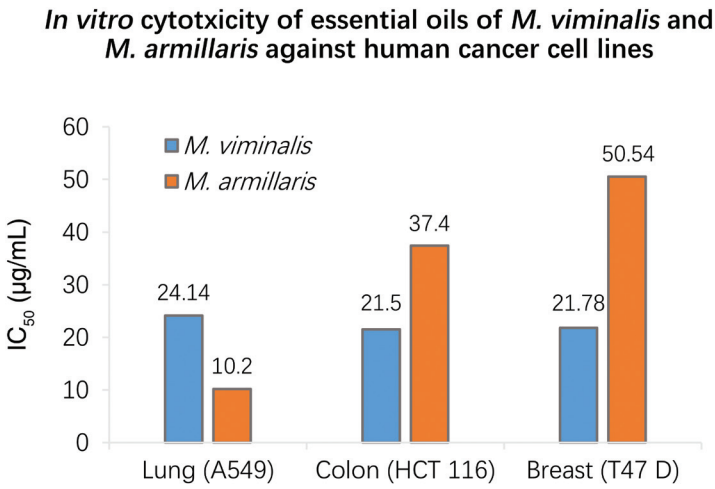

Fig. 4. *In vitro* cytotoxic activity of essential oils of *M. viminalis* and *M. armillaris*.

whereas, the aqueous extract had the least activity. The ferrous chelating assay indicated that extracts of *M. armillaris* have good ferrous chelation activity, as compared to extracts of *M. viminalis*; meanwhile, the essential oil of *M. viminalis* showed more chelation power than the *M. armillaris* essential oil. Hence, the antioxidant activity in both *M. viminalis* and *M. armillaris* was found in accordance with Zubair *et al*,[3] Salem *et al*[35] and Chabir *et al*.[9]

The antibacterial activity of essential oils and extracts of both *M. viminalis* and *M. armillaris* plants were evaluated and found to be effective against the tested bacteria. The highest antimicrobial activity was observed on Gram-positive bacteria. An important characteristic of essential oils and their components is their hydrophobicity, which enables them to partition the lipids of the bacterial cell wall and disturb the cell structures thereby, rendering them more permeable.[36,37] Extensive leakage from bacterial cells or the exit of critical molecules and ions will lead to death.[38] Gram-positive bacteria were more resistant to the essential oils than Gram-negative bacteria.[39] Hence, in the present study, *M. viminalis* and *M. armillaris* were found to be equally effective against both Gram-positive and Gram-negative organisms.

Cytotoxicity of *M. viminalis* and *M. armillaris* was evaluated by MTT assay, a simple and reliable experimental method which measures cell viability and cytotoxicity and is used for screening cytotoxic agents.[40] Findings of this experiment demonstrated that *M. viminalis* and *M. armillaris* essential oils were strongly effective against all the cell lines tested in a dose-dependent manner and were more effective against human lung (A549) cancer cells, with an IC_{50} value of 10.2 µg/mL. A previous study reported the cytotoxic activity of the essential oils from the flowers and leaves of *Callistemon citrinus* and the presence of highly active phytochemicals, like 1,8-cineole, and monoterpenoids.[41] Perhaps the cytotoxicity of *M. viminalis* and *M. armillaris* essential oils may also be attributed to the presence of phyto-constituents as well as their synergistic activities.

Future research direction

Despite promising results demonstrated by both *M. viminalis* and *M. armillaris* species against various *in vitro* biological parameters, there are problems that still need to be addressed, such as stability, selectivity and bioavailability of these essential oils in the human body, and for any possible adverse herb-drug interaction. Additionally, the optimal ratio and dosing regimens should be explored for higher efficacy and decreased toxicity. Thus, the future research direction would be focused on establishing these critical parameters in order to provide sufficient evidence to support going through the phases of clinical trials.

Conclusions

The present study concludes that due to the presence of phytochemicals, like eucalyptol and monoterpenoids, in the essential oils of *M. viminalis* leaves, this plant (particularly its leaves) may serve as potent source of natural anti-cancer compound(s) and should be considered for clinical trials. However, the high content of methyl eugenol, a carcinogenic compound, from *M. armillaris* (the aerial part) may raise concern, which needs to be addressed by detailed analysis, including *in vivo* studies and mechanistic analyses for further consideration of its potential as a human therapeutic.

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Conflict of interest

The authors have no conflicts of interest regarding this paper.

Author contributions

Study design (MB), critical revision and critical funding (MB), performance of experiments (SP, V, SG), manuscript writing (MS), analysis of data and statistical analysis (MS), administrative support (KB).

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