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Bioactive Potential of Melaleuca Alternifolia: In Vitro Assessment of Antioxidant, Antimicrobial, and Anti-Parkinsonian Activities

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ABSTRACT: Melaleuca alternifolia (tea tree) essential oil is a rich source of bioactive terpenoids with promising antioxidant, antimicrobial, and neuroprotective properties. The present study investigated the phytochemical composition, antioxidant, antimicrobial, and in vitro neuroprotective potential of M. alternifolia essential oil. Essential oil was extracted from shade-dried leaves by hydrodistillation using a Clevenger-type apparatus, yielding 0.53% (w/w) oil. Preliminary phytochemical screening and GC–MS analysis were performed to identify major constituents. Antioxidant activity was evaluated using the Ferric Reducing Antioxidant Power (FRAP) assay. Neuroprotective potential was assessed using the MTT assay on SH-SY5Y human neuroblastoma cells and the monoamine oxidase (MAO-A/B) inhibition assay. Antimicrobial activity was determined against Staphylococcus aureus, Escherichia coli, Aspergillus niger, and Candida albicans using the agar well diffusion method. Phytochemical screening confirmed the presence of steroids, flavonoids, phenols, tannins, saponins, glycosides, and proteins. GC–MS analysis identified 42 volatile compounds, with eugenol (43.52%), β-caryophyllene (19.51%), γ-terpinene dimer (17.72%), α-selinene (2.78%), and γ-muurolene (2.35%) as the predominant constituents. The essential oil exhibited potent antioxidant activity with a FRAP IC_{50} of 0.01316 µL/mL. In the MTT assay, the essential oil demonstrated moderate cytotoxicity toward SH-SY5Y cells (IC_{50} = 68.69 ± 0.05 µg/mL) compared with doxorubicin (48.62 ± 0.04 µg/mL). However, it showed negligible MAO inhibitory activity, with an IC_{50} above the maximum tested concentration. The essential oil displayed concentration-dependent antimicrobial activity, with stronger antifungal effects against A. niger and C. albicans than antibacterial effects against S. aureus and E. coli.Melaleuca

alternifolia essential oil possesses significant antioxidant and broad-spectrum antimicrobial activities together with moderate neuroprotective potential, supporting its potential as a natural therapeutic candidate for managing oxidative stress-associated disorders and microbial infections.

1. INTRODUCTION

Essential oils are complex mixtures of volatile bioactive compounds, predominantly terpenes, terpenoids, and phenylpropanoids, obtained from aromatic plants. Owing to their diverse phytochemical composition, essential oils have attracted considerable attention as multifunctional therapeutic agents with antioxidant, antimicrobial, and neuroprotective properties. Oxidative stress is a major contributor to the pathogenesis of several chronic disorders, particularly neurodegenerative diseases such as Parkinson's disease (PD). Excessive generation of reactive oxygen species (ROS) results in lipid peroxidation, mitochondrial dysfunction, and dopaminergic neuronal degeneration. The antioxidant constituents of essential oils can neutralize free radicals, enhance endogenous antioxidant defence systems, and reduce oxidative damage, thereby providing neuroprotection. In addition to their antioxidant effects, essential oils exhibit broad-spectrum antimicrobial activity against pathogenic bacteria and fungi through disruption of microbial cell membranes, inhibition of enzyme activity, and interference with biofilm formation. These properties make them promising natural alternatives to synthetic antimicrobial agents, particularly in the face of increasing antimicrobial resistance. Recent studies also suggest that several essential oils possess anti-Parkinson potential by attenuating neuroinflammation, preventing α-synuclein aggregation, improving mitochondrial function, and protecting dopaminergic neurons from oxidative injury. The close relationship between oxidative stress, microbial infections, inflammation, and neurodegeneration highlights the therapeutic relevance of essential oils. Therefore, investigating the antioxidant, antimicrobial, and neuroprotective activities of essential oils provides valuable insights into their potential as natural, multi-target therapeutic agents for the prevention and management of Parkinson's disease and other oxidative stress-related disorders [1-5]. *Melaleuca alternifolia* (Maiden & Betche) Cheel, commonly known as tea tree, is an aromatic medicinal plant belonging to the family Myrtaceae and is native to Australia. The plant is widely recognized for its essential oil, which is obtained by steam distillation of the leaves and terminal branches. Tea tree essential oil (TTO) contains a complex mixture of bioactive terpenes, with terpinen-4-ol, γ-terpinene, α-terpinene, 1,8-cineole, α-terpineol, and p-cymene being the principal constituents responsible for its pharmacological activities. These phytoconstituents exhibit potent antioxidant properties by scavenging reactive oxygen species and reducing oxidative stress, a major contributor to neuronal degeneration. Terpinen-4-ol has also demonstrated neuroprotective potential through the suppression of neuroinflammation, attenuation of microglial activation, and preservation of neuronal viability. Moreover, tea tree essential oil possesses broad-spectrum antimicrobial activity against Gram-positive and Gram-negative bacteria as well as pathogenic fungi by disrupting microbial cell membranes and inhibiting biofilm formation. The synergistic actions of its phytochemicals suggest that *M. alternifolia* essential oil is a promising natural therapeutic candidate for combating oxidative stress-related disorders, microbial infections, and neurodegenerative diseases, including Parkinson's disease [6-7].

2. MATERIALS AND METHODS

2.1 Plant material collection and identification: *Melaleuca alternifolia* leaves were employed in this investigation. The authors gathered the samples at Maharana Pratap College of Pharmacy's and approved the plant by the pharmacognosy department.

2.2 Extraction of Essential Oil from *Melaleuca alternifolia* Leaves (Hydrodistillation Method): Fresh, healthy leaves of *Melaleuca alternifolia* were collected, washed thoroughly with distilled water to remove dust and foreign matter, and shade-dried at room temperature (25–30 °C) for 5–7 days until a constant weight was achieved. The dried leaves were coarsely ground using a laboratory grinder. Approximately 500 g of the powdered leaves were transferred into a 5 L round-bottom flask containing 3 L of distilled water. The essential oil was extracted by hydrodistillation using a Clevenger-type apparatus for 3–4 hours, following the procedure described in the European Pharmacopoeia. During distillation, the volatile oil vapors condensed and separated from the aqueous phase in the graduated arm of the Clevenger apparatus. The collected essential oil was separated carefully using a micropipette and dried over anhydrous sodium sulfate to remove residual moisture. The dried oil was filtered through Whatman No. 1 filter paper and stored in amber-colored glass vials at 4 °C until further phytochemical and biological analyses [8]. The percentage yield of essential oil was calculated based on the dry weight of the plant material using the following equation:

$$\text{Essential Oil Yield (\%, w/w)} = \frac{\text{Weight of Essential Oil Obtained (g)}}{\text{Weight of Dried Plant Material (g)}} \times 100$$

2.3 Qualitative Phytochemical Screening: To confirm the existence of different bioactive components, a standard qualitative investigation of the Essential Oil from *Melaleuca alternifolia* Leaves was performed.

2.3.1 Liebermann-Burchard Test: Two millilitres of essential oil was mixed with 2 mL of acetic acid and concentrated sulfuric acid. The development of initial red coloration followed by blue and finally green colour indicated steroid presence.

2.3.2 Salkowski-Reaction: The presence of steroids in the plant extract was determined using the Salkowski reaction. Approximately 2 mL of the essential oil was mixed with 2 mL of chloroform, followed by the careful addition of concentrated sulfuric acid along the side of the test tube to form a separate layer. The appearance of a red or reddish-brown coloration in the chloroform layer, accompanied by a greenish-yellow fluorescence in the sulfuric acid layer, indicated the presence of steroidal compounds. This colour change occurs due to the reaction of sterol molecules with concentrated sulfuric acid, making the Salkowski test a reliable preliminary method for steroid detection.

2.3.3 Lead Acetate Test: To detect tannins, 2 mL of the essential oil was treated with a few drops of 10% lead acetate solution. The formation of a white or cream-colored precipitate indicated the presence of tannins. This reaction occurs because tannins form insoluble complexes with lead ions, resulting in visible precipitation.

2.3.4 Ferric Chloride Test: For the detection of phenolic compounds, 2 mL of the essential oil was treated with a few drops of 5% ferric chloride solution. The development of a bluish-black, dark green, or violet coloration confirmed the presence of phenolic constituents. The ferric ions react with the hydroxyl groups of phenolic compounds to produce colored complexes, making this test a widely accepted qualitative method for identifying phenolics.

2.3.5 Alkaline Reagent Test: The presence of flavonoids was evaluated by adding a few drops of 10% sodium hydroxide solution to 2 mL of the essential oil. The immediate appearance of an intense yellow coloration indicated the possible presence of flavonoids. Upon the addition of dilute hydrochloric acid, the yellow colour disappeared, confirming the presence of flavonoid compounds. This reversible colour change is attributed to the formation and subsequent neutralization of flavonoid sodium salts under alkaline and acidic conditions, respectively.

2.3.6 Foam Test: To identify saponins, 2 mL of the essential oil was diluted with approximately 5 mL of distilled water and shaken vigorously for several minutes. The persistence of a stable froth or foam for at least 10 minutes indicated the presence of saponins. The foam-forming property results from the surfactant nature of saponins, which reduce the surface tension of water.

2.3.7 Froth Test: In a separate assay, essential oil was diluted with distilled water in a graduated cylinder and shaken vigorously for about 2–3 minutes. The formation of a stable froth layer measuring approximately 1 cm in height that persisted for at least 10 minutes confirmed the presence of saponins. The stability and persistence of the froth provide qualitative evidence of saponin-containing phytoconstituents in the extract [9].

2.4 In Vitro Antioxidant Activity

2.4.1 Antioxidant Assay FRAP: 10µl of different stock of the test compound and standard (Ascorbic Acid – (SRL, Cat no-23006) was added to the wells of the 96-well plate in treatment and treatment blank while 10 µl of demineralized water was added to the wells of control and control blank. Then, 50 µl of Solution A- potassium ferricyanide [K3Fe (CN) 6] (SRL, Cat no- 15766) was added to the wells in treated and control, and 50 µl of demineralized water was added to the blank wells of control and treated. The wells without treatment were considered as control. The plate was then incubated at 50 °C for 20 minutes. After incubation, 50 µl of Solution B- trichloroacetic acid (SRL, Cat no-92390) was added to all wells of the 96-well plate. Then 40µl of demineralized water and 50µl of Solution C-ferric chloride (Fischer scientific – Cat no. 23585) were added. The colored solution was read at 700 nm against the blank using microplate reader (iMark, BioRad). IC50 was calculated by using software Graph Pad prism 6. Graph was prepared between X axis (Sample Concentration) Vs. Y axis (% inhibition wrt control) [10].

Calculations

% FRAP = (A Sample - A Control /A Control) ×100

A Control = Absorbance of control

A Sample = Absorbance of sample

2.5 GC-MS Analysis: GC-MS analysis of Essential Oil from *Melaleuca alternifolia* was performed using a PerkinElmer Clarus 600 system equipped with an Rtx-5MS capillary column. Helium (99.99%) was used as the carrier gas at a constant flow rate of 1.0 mL/min. Injection volume: 1 µL, split ratio: 10:1, ionization energy: 70 eV, scan range: 40–650 m/z, scan time: 0.2 sec. Injector temperature: 260°C. Oven temp held at 50°C for 3 min, then ramped to 300°C at 10°C/min. Compounds were identified by comparing retention times and mass spectra with those in the NIST and Wiley-8 libraries [11].

2.6 In Vitro Models Of Anti Parkinson Activity

2.6.1 MTT assay: Cytotoxicity of the provided samples on SHSY5Y (Passage No. 23, Procured from NCCS Pune, India, RRID- CVCL_0019) cell line was determined by MTT Assay. The cells (10000 cells/well) were cultured in 96 well plate for 24 h in DMEM medium (Dulbecco's Modified Eagle Medium-AT149-1L) supplemented with 10% FBS (Fetal Bovine Serum - HIMEDIA-RM 10432) and 1% antibiotic solution (Penicillin-Streptomycin-Sigma-Aldrich P0781) at 37°C with 5% CO2. Next day cells were treated from different concentrations of the samples (concentration as per mentioned in excel sheet). Stock solution of samples was prepared in DMSO and further diluted to get different concentrations in incomplete Cell culture Medium (Without FBS). After incubation for 24 hours, MTT Solution (5 mg/ml) was added to cell culture and further incubated (Air- jacketed CO2 incubator-Heal force-HF90) for 2 h. Cells without treatment were considered as Control and cells without MTT were considered as Blank. At the end of the experiment, culture supernatant was removed, and cell layer matrix was dissolved in 100 µl Dimethyl Sulfoxide (DMSO –SRL- Cat no.- 67685) and read in an Elisa plate reader (iMark, Biorad, USA) at 540 nm. IC50 was calculated by using Graph Pad Prism -6. Images were captured under inverted microscope (Olympus ek2) using Camera (AmScope digital camera 10 MP Aptima CMOS). 50% inhibitory concentration (IC50) was presented as Mean ± SEM (Standard Error of Mean) [12].

Calculation

% Viable cells = (Atest / AControl) *100

(Atest = Absorbance of test sample)

2.7 Enzyme Inhibition Assay -Monoamine Oxidase A/B: 5 µl of different stock dilutions of the test sample (0 to 1000 µg/ml) or inhibitor (0 to 50 µM) were added to the defined wells of the 96-well plate. 5 µl of enzyme were added to the wells of all the sets. After that, the plate was incubated (Basil Scientific Corp. India- Incubator) for 10 minutes at 37°C. 5 µl of tyramine (Cat No: T90344) 40 mM were added to the defined wells. 15 µl of the reaction mixture (containing 5 µl of hydrogen peroxidase (Cat No: HP6520) (10 U/ml), 5 µl of amino antipyrine (1 mM), and 5 µl of 5-dichloro-2-hydroxybenzenesulfonic acid (DCHBS)) were added to all wells of the plate. The plate was incubated for 30 minutes at 37°C. The absorbance was measured at 515 nm using ELISA micro plate reader. (iMark, BioRad). Inhibitor, Acarbose (SRL- Cat no-65457) (50 µg/ml final Concentration) was used as a positive control. *IC-50* was calculated using Software Graph Pad Prism 6[13].

2.8 Antimicrobial Assay

2.8.1 Anti-Microbial-Zone Inhibition Test (Well diffusion Method) –*Staphylococcus aureus:* The Antibacterial activity was checked by following Zone Inhibition Method (Kirby-Bauer method). The MHA plates were inoculated by spreading with 100 µl of Bacterial culture, *Staphylococcus aureus* (Inoculum was prepared by adjusting 0.5 McFarland Unit - Approx. cell density (1.5 X 10^8 CFU/mL from Mueller- Hinton Broth) and followed by making the wells containing 10 µl of different concentration (0 to 100 %). 10 % of the sample was taken and serially diluted to achieve the required amount to be loaded in the well. One well in each plate was loaded with solvent alone which served as vehicle control and Ciprofloxacin well (3 µg) was taken as positive control. The plates of *Staphylococcus aureus* were incubated in incubator (Basil Scientific Corp. India) at 37 °C for 24 hrs. The clear zones created around the well were measured and recorded.

2.8.2 Anti-Microbial-Zone Inhibition Test (Well diffusion Method) – *Escherichia coli:* The Antibacterial activity was checked by following Zone Inhibition Method (Kirby-Bauer method). The MHA plates were inoculated by spreading with 100 µl of Bacterial culture, *Escherichia coli* (Inoculum was prepared by adjusting 0.5 McFarland Unit - Approx. cell density (1.5 X 10^8 CFU/mL from Mueller- Hinton Broth) and followed by making the wells containing 10 µl of different concentration (0 to 100 %). 10 % of the sample was taken and serially diluted to achieve the required amount to be loaded in the well. One well in each plate was loaded with solvent alone which served as vehicle control and Ciprofloxacin well (3 µg) was taken as positive control. The plates of *Escherichia coli* were incubated in incubator (Basil Scientific Corp. India) at 37 °C for 24 hrs. The clear zones created around the well were measured and recorded.

2.8.3 Anti-fungal -Zone Inhibition Test (Well diffusion Method) -*Aspergillus Niger:* The Antifungal activity was checked by following Zone Inhibition Method (Kirby-Bauer method). The SDA plates were inoculated by spreading with 100 µl of fungal culture, *Aspergillus niger* (Inoculum was prepared by adjusting 0.5 McFarland Unit - Approx cell density (1.5 X 10^8 CFU/mL from Sabouraud dextrose broth) and followed by placing the wells containing 10 µl of different concentration (0 to 100 %). 10 % of the sample was taken and serially diluted to achieve the required amount to be loaded in the well. One well in each plate was loaded with solvent alone which served as vehicle control and Fluconazole (40µg) was taken as positive control. The plates of *Aspergillus Niger* were incubated in incubator at 28 °C for 24 hrs. The clear zones created around the well were measured and recorded.

2.8.4 Anti-Fungal-Zone Inhibition Test – (Well diffusion Method) *Candida albicans:* The Antifungal activity was checked by following Zone Inhibition Method (Kirby-Bauer method). The PDA plates were inoculated by spreading with 100 µl of Fungal culture, *Candida albicans* (Inoculum was prepared by adjusting 0.5 McFarland Unit - Approx cell density (1.5 X 10^8 CFU/mL from Sabouraud dextrose broth) and followed by the wells containing 10 µl of different concentration (0 to 100 %). 10 % of the sample was taken and serially diluted to achieve the required amount to be loaded in the well. One well in each plate was loaded with solvent alone which served as vehicle control and Fluconazole well (40 µg) was taken as positive control. The plates of *Candida albicans* were incubated in incubator at 37 °C for 24 hrs. The clear zones created around the well were measured and recorded [14-16].

2.8.9 Statistical investigation: All data were investigated by means of the analysis of variance (ANOVA) followed by the Post Hoc Tukey's test (Graph Pad Prism Software-6). All data represent three independent values and results are presented as mean ± S.E. Statistical significance was considered as P value ≤ 0.05.

3. RESULTS AND DISCUSSION

The hydrodistillation process successfully extracted essential oil from *Melaleuca alternifolia* leaves with a yield of **0.53% (w/w)**. The obtained yield falls within the range commonly reported for *M. alternifolia* leaves extracted by conventional hydrodistillation, indicating efficient recovery of volatile constituents suitable for subsequent phytochemical characterization and biological evaluation.

3.1 Phytochemical Results:

Table 1: Results of Phytochemical Screening of Essential Oil from Melaleuca alternifolia Leaves

Test for	Name of the Test	Result
Steroids	Liebermann–Burchard test	+
	Salkowski reaction	+
Tannins	Lead acetate test	+
Flavonoids	Alkaline reagent test	+
	Shinoda test	+
Carbohydrates	Molisch test	−
Phenols	Ferric chloride test	+

Saponins	Foam test	+
	Froth test	+
Glycosides	Borntrager's test	+
	Legal's test	+
Proteins and Amino Acids	Millon's test	+
	Xanthoproteic test	+

Note: (+) Presence of phytochemical constituent; (−) Absence of phytochemical constituent

3.2 Identification of Components: Identification was done using the calculated fragments, molecular mass, and molecular structure. The NIST database and the Willy8-library, which has more than 62,000 pattern entries, were used to interpret the GC-MS spectra. Together with their molecular weights and chemical structures, the components of the test materials were determined. The percentage amounts discovered in the sample were contrasted with component spectra from the Willy8 collection and the NIST library. This is done in order to determine whether this plant species has any chemicals or a combination of them that could support its traditional and commercial therapeutic purposes. It also helps determine the most effective methods for removing harmful compounds. The test materials' constituent parts were determined. Table-2 and figure-1 represent the compounds details.

Table 2: Compounds of Essential Oil from Melaleuca alternifolia

Peak	R.Time	Area	Area%	Name	Common Name
1.	7.264	8774889	0.13	Bicyclo[3.1.1] Hept-2-Ene 2,6,6-Trimethyl-	A-Pinene
2.	7.842	8013360	0.12	Bicyclo[2.2.1] Heptane, 2,2-Dimethyl-3-Methylene	B-Pinene
3.	8.725	1183393	0.02	Sabinene	Sabinene
4.	8.890	6858437	0.10	Pinene Oxide <Beta->	B-Pinene Oxide
5.	9.425	1715734	0.03	Myrcene	Myrcene (B-Myrcene)
6.	10.859	1860141	0.03	Cymene <Para->	P-Cymene
7.	11.054	7655928	0.11	Limonene	Limonene
8.	11.157	21012463	0.31	Eucalyptol	1,8-Cineole (Eucalyptol)
9.	11.863	16841186	0.25	.Beta.-Ocimene	B-Ocimene
10.	13.070	5817058	0.09	1-Octanol	Octyl Alcohol (1-Octanol)
11.	14.328	23778505	0.35	Linalool	Linalool
12.	15.994	3311913	0.05	Geijeren	Geijerene
13.	16.248	12552874	0.19	Camphor	Camphor
14.	17.572	47253198	0.70	Bicyclo[2.2.1] Heptan-2-Ol, 1,7,7-Trimethyl-, (1s-Endo)-	Borneol (Endo-Borneol)
15.	17.887	1768367	0.03	Terpinen-4-Ol	Terpinen-4-Ol
16.	18.621	2783311	0.04	(+)-Alpha-Terpineol (P-Menth-1-En-8-Ol)	A-Terpineol
17.	19.368	3961603	0.06	(3e,5e)-2,6-Dimethylocta-3,5,7-Trien-2-Ol	Hotrienol
18.	25.268	12691722	0.19	4-Isopropyl-3,7-Dimethyl-3a,3b,4,5,6,7-Hexahydro 1h	Aromadendrene

				Cyclopenta[2,3]Cyclopropa[1,2a]Benzene	
19.	27.123	2946108909	43.52	Phenol, 2-Methoxy-3-(2-Propenyl)-	Eugenol
20.	27.841	1199733989	17.72	2,4-Diisopropenyl-1-Methyl-1-Vinylcyclohexane	Γ-Terpinene Dimer / No Common Trivial Name
21.	28.378	54440484	0.80	Benzene, 1,2-Dimethoxy-4-(2-Propenyl)-	Methyl Eugenol
22.	29.017	1320554051	19.51	Caryophyllene <(E)->	B-Caryophyllene (Trans-Caryophyllene)
23.	29.304	111507644	1.65	Bergamotene <Alpha-, Cis->	Cis-A-Bergamotene
24.	30.110	102395620	1.51	Humulene <Alpha->	A-Humulene
25.	30.774	44253804	0.65	Selina-4,11-Diene	Selina-4,11-Diene
26.	31.152	141603900	2.09	1,6-Cyclodecadiene, 1-Methyl-5-Methylene-8-(1-Methylethyl)-, [S-(E,E)]-	Germacrene D
27.	31.490	159309277	2.35	Naphthalene, Decahydro-4a-Methyl-1-Methylene-7-(1-Methylethenyl)-, [4ar-(4a.Alpha.,7.Alpha.,8a.Beta.)]-	Γ-Muurolene
28.	31.805	187859907	2.78	.Alpha.-Selinene	A-Selinene
29.	32.095	9033461	0.13	Lanceol Acetate <(Z)->	(Z)-Lanceol Acetate
30.	32.574	46519176	0.69	Cadinene <Delta->	Δ-Cadinene
31.	32.899	13255551	0.20	Kessane	Kessane
32.	33.791	17052579	0.25	Hedycaryol	Hedycaryol
33.	34.032	5906282	0.09	Coniferyl Alcohol	Coniferyl Alcohol
34.	35.025	110272506	1.63	Caryophyllene Oxide	Caryophyllene Oxide
35.	35.981	7026507	0.10	(1r,3e,7e,11r)-1,5,5,8-Tetramethyl-12-Oxabicyclo[9.1.0]Dodeca-3,7-Diene	Humulene Epoxide II
36.	36.332	18904859	0.28	Selin-11-En-4-Alpha-Ol	A-Selinol (Selin-11-En-4α-Ol)
37.	37.258	6811222	0.10	Naphth-1-Ol <1,2,3,4,4a,7,8,8a-Octahydro-, 4-Isopropyl-, 1,6-Dimethyl->	Eudesmol Isomer (Commonly Reported As A-Eudesmol)
38.	37.911	39518520	0.58	Intermedeol<Neo->	Neo-Intermedeol
39.	39.700	10472696	0.15	Valencene<13-Hydroxy->	13-Hydroxyvalencene
40.	40.297	27353393	0.40	Larixol	Larixol
		6769557001	100.00		

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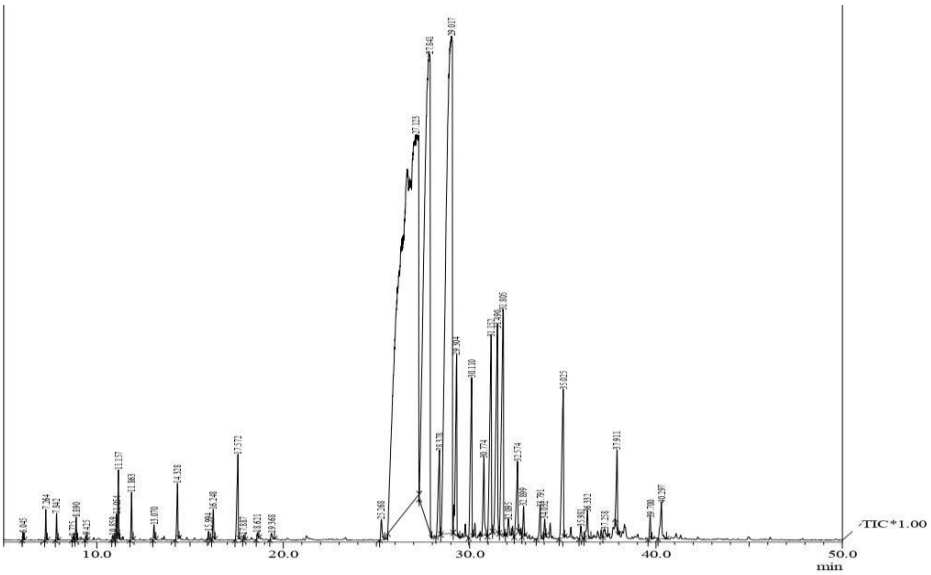

Figure 1: Chromatogram of Essential Oil from Melaleuca alternifolia

The GCMS analysis of *Melaleuca alternifolia* revealed the presence of many compounds. In essential oil total of 42 plant compounds were identified, which are already mentioned in above table.

3.3 Antioxidant results: Based on the results obtained from the study, Antioxidant activity (FRAP) was estimated in sample, and 50% inhibitory concentration was mentioned in Table-3. *Melaleuca alternifolia* was found to be highly active. 0.01316 µl of sample Essential Oil from *Melaleuca alternifolia* was found equivalent to 3.235µg of the standard ascorbic acid. The standard compound, being a purified compound and highly active substance, was assessed using a lower concentration range (0 to 50µg/ml). In contrast, the sample's potency remains unbound; therefore, a broader concentration range (0 to 0.1 µl/ml) was employed for the sample analysis.

Table 3: Results of antioxidants effects of Essential Oil from Melaleuca alternifolia

Sample	***IC*$_{50}$ Value** (Mean ± SEM)
Ascorbic Acid	3.235±0.01 (µg/ml)
Essential Oil from *Melaleuca alternifolia*	0.01316±0.12 (µl/ml)

3.4 Enzyme Inhibition and Neuroprotective: The enzyme inhibition and MTT cytotoxicity assays were performed to evaluate the neuroprotective potential of the essential oil from *Melaleuca alternifolia*. In the monoamine oxidase (MAO) inhibition assay, the essential oil exhibited negligible inhibitory activity, with an IC_{50} value above the maximum tested concentration, indicating that it does not effectively inhibit MAO-A or MAO-B enzymes under the experimental conditions. In contrast, the standard inhibitors Chlorgyline and Pargyline demonstrated potent inhibition with IC_{50} values of 30.04 ± 0.004 and 41.69 ± 0.02, respectively. The cytotoxicity of the essential oil was evaluated using the MTT assay on SH-SY5Y human neuroblastoma cells. The essential oil exhibited an IC_{50} value of 68.69 ± 0.05 µg/mL, whereas the positive control, doxorubicin, showed a lower IC_{50} value of 48.62 ± 0.04 µg/mL, indicating greater cytotoxicity. The relatively higher IC_{50} value of the essential oil suggests moderate cytotoxicity and comparatively lower toxicity toward neuronal cells than doxorubicin. Since IC_{50} represents the concentration required to reduce cell viability by 50%, these findings indicate that although *M. alternifolia* essential oil does not possess significant MAO inhibitory activity, it exhibits acceptable cellular compatibility at moderate concentrations. Therefore, its potential neuroprotective effects, if any, are likely mediated through mechanisms other than monoamine oxidase inhibition, such as antioxidant or anti-inflammatory pathways, which warrant further investigation.

Table 4: Enzyme Inhibition and Neuroprotective (MTT Cytotoxicity) Activity of Melaleuca alternifolia Essential Oil

Assay	Sample	IC_{50} Value (Mean ± SEM)
Monoamine Oxidase (MAO) Inhibition	Essential Oil from *Melaleuca alternifolia*	Above maximum dose limit
	Chlorgyline (MAO-A inhibitor, Positive control)	30.04 ± 0.004
	Pargyline (MAO-B inhibitor, Positive control)	41.69 ± 0.02
Neuroprotective Activity (MTT Assay on SH-SY5Y Cells)	Doxorubicin (Positive control)	48.62 ± 0.04 µg/mL
	Essential Oil from *Melaleuca alternifolia*	68.69 ± 0.05 µg/mL

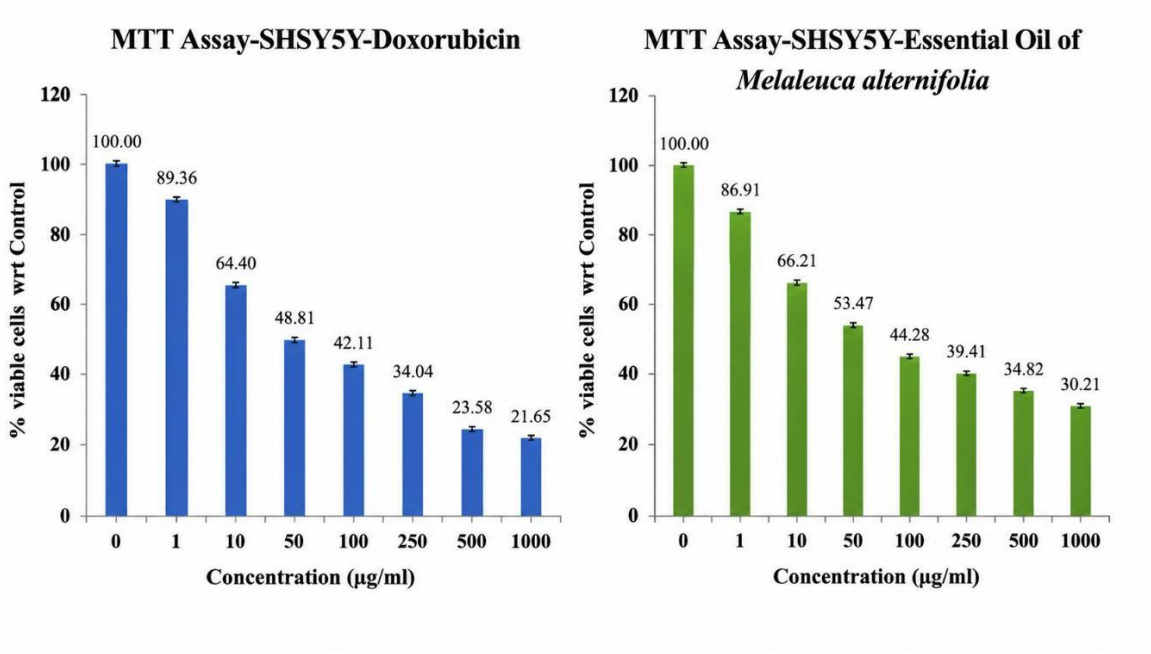

Figure 2: Represents the Neuroprotective activity of Essential Oil from *Melaleuca alternifolia* and Percentage Viability of cells by MTT assay

3.5 Antimicrobial Analysis: The antimicrobial activity of of Essential Oil from *Melaleuca* ***alternifolia*** was evaluated against *Staphylococcus aureus*, *Escherichia coli*, *Aspergillus niger*, and *Candida albicans* using a loaded volume of 10 µL. The results demonstrated a concentration-dependent increase in the zone of inhibition for all tested microorganisms. Against *S. aureus*, the inhibition zone increased from 6.67 mm at 6.25% concentration to 12.67 mm at 100%. Similarly, *E. coli* exhibited inhibition zones ranging from 9.00 mm to 15.00 mm. Among the fungal strains, *A. niger* showed pronounced susceptibility, with inhibition zones increasing from 16.00 mm to 29.00 mm, while *C. albicans* exhibited inhibition zones ranging from 19.00 mm to 28.00 mm. Overall, of Essential Oil from *Melaleuca* demonstrated broad-spectrum antimicrobial activity, with comparatively greater antifungal activity than antibacterial activity, indicating its potential as a natural antimicrobial agent.

Table 5: Antimicrobial Activity of Essential Oil from Melaleuca alternifolia Against Different Microorganisms (Zone of Inhibition, mm)

Microorganism	Loaded Volume (µL)	0%	6.25%	12.5%	25%	50%	100%
Staphylococcus aureus	10	0.00	6.67	9.67	10.33	11.67	12.67
Escherichia coli	10	0.00	9.00	10.00	11.00	14.00	15.00
Aspergillus niger	10	0.00	16.00	16.00	27.00	28.00	29.00
Candida albicans	10	0.00	19.00	21.00	26.00	28.00	28.00

4. DISCUSSIONS

The present study demonstrated that the essential oil of *Melaleuca alternifolia* possesses significant antioxidant and antimicrobial activities, together with moderate neuroprotective potential. Hydrodistillation yielded 0.53% (w/w) essential oil, which is within the range commonly reported for *M. alternifolia* leaves, indicating efficient extraction of volatile constituents. Preliminary phytochemical screening confirmed the presence of steroids, flavonoids, phenols, tannins, saponins, glycosides, and proteins, while GC–MS analysis identified 42 volatile compounds, with eugenol, β-caryophyllene, γ-terpinene dimer, α-selinene, and γ-muurolene as the predominant constituents. These phytochemicals are well recognized for their antioxidant, antimicrobial, and anti-inflammatory properties. The essential oil exhibited strong antioxidant activity in the FRAP assay, with an IC_{50} value of 0.01316 µL/mL, suggesting a high ferric reducing capacity that may contribute to protection against oxidative stress. Since oxidative stress plays a central role in the progression of neurodegenerative disorders, this antioxidant potential may be beneficial in preventing neuronal damage. In the MTT assay using SH-SY5Y cells, the essential oil exhibited moderate cytotoxicity (IC_{50} = 68.69 ± 0.05 µg/mL) compared with doxorubicin, indicating relatively lower toxicity toward neuronal cells. Conversely, the essential oil showed negligible monoamine oxidase inhibitory activity, suggesting that its neuroprotective effects are unlikely to be mediated through MAO inhibition but rather through antioxidant or anti-inflammatory mechanisms. Furthermore, the essential oil exhibited concentration-dependent antimicrobial activity against both bacterial and fungal pathogens, with greater efficacy against *Aspergillus niger* and *Candida albicans* than against *Staphylococcus aureus* and *Escherichia coli*. Overall, these findings support the potential of *M. alternifolia* essential oil as a promising natural source of antioxidant and antimicrobial agents with complementary neuroprotective properties, warranting further mechanistic and in vivo investigations.

5. CONCLUSION

The present study demonstrates that the essential oil of *Melaleuca alternifolia* is a valuable source of bioactive phytochemicals with significant antioxidant and broad-spectrum antimicrobial activities. Hydrodistillation yielded 0.53% (w/w) essential oil containing 42 volatile compounds, with eugenol and β-caryophyllene identified as the major constituents by GC–MS analysis. The essential oil exhibited potent ferric reducing antioxidant activity and concentration-dependent antimicrobial effects, particularly against *Aspergillus niger* and *Candida albicans*. Although it showed negligible monoamine oxidase inhibitory activity, the MTT assay indicated moderate cytotoxicity toward SH-SY5Y neuronal cells, suggesting acceptable cellular compatibility and the possibility of neuroprotective effects through antioxidant and anti-inflammatory mechanisms rather than MAO inhibition. Overall, these findings highlight the therapeutic potential of *M. alternifolia* essential oil as a natural antioxidant and antimicrobial agent with promising applications in the management of oxidative stress-related disorders. Further in vivo studies and molecular investigations are required to validate its efficacy and elucidate its underlying mechanisms of action.

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AUTHOR CONTRIBUTIONS

Dr. Anubhav Dubey: Conceptualization, Investigation.

Saumya Gupta and, Dr. Sanyogita Shahi, Dr. Deepika Shukla: Methodology, Laboratory Analysis.

Dr. Chandan Mukherjee and Priyanka Yadav: Data Curation, Formal Analysis.

Dr. Garima Ojha and Dr. Anubhav Dubey: Supervision, Writing—Review & Editing.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ETHICS STATEMENT

Ethical approval was not required for this study.

DATA AVAILABILITY STATEMENT

Data supporting the findings of this study are available from the corresponding author upon reasonable request.

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