PLANT SCIENCE TODAY
Vol 12(4): 01–08
https://doi.org/10.14719/pst.8914

eISSN 2348-1900

RESEARCH ARTICLE

Antioxidant, antimicrobial and phytochemical analysis of *Microtoena patchoulii* leaves: A rare plant species from North East India

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Received: 16 April 2025; Accepted: 15 July 2025; Available online: Version 1.0: 24 September 2025; Version 2.0: 16 October 2025

Cite this article: Aleena K, Mrinal Kumar D, Sanjoy Singh N, Kamal G, Rajiv CG. Antioxidant, antimicrobial and phytochemical analysis of *Microtoena patchoulii* leaves: A rare plant species from North East India. Plant Science Today. 2025;12(4):01–08. https:/doi.org/10.14719/pst.8914

Abstract

Microtoena patchoulii [(C.B. Clarke ex J.D. Hooker) C.Y. Wu et Hsuan] is a lesser-known plant of family Lamiaceae found in Asia traditionally used for medicinal and cosmetic purposes. The present study focuses on the phytochemical constituents, antioxidant properties and antimicrobial activity of the hexane and methanolic extract of *M. patchoulii* leaves collected from Manipur, Northeast India. Phytochemical screening was conducted on both hexane and methanolic extracts to detect the presence of phenols and flavonoids. Radical scavenging and reducing power were estimated by quantitative antioxidant assays, such as 2,2-diphenyl-1-picrylhydrazyl (DPPH), [2,2′-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid)] (ABTS) and ferric reducing antioxidant power (FRAP). GC-MS analysis was performed on the methanolic extract to identify bioactive constituents. Antimicrobial activity was assessed against selected bacterial and fungal strains using standard methods. Phytochemical analysis of the methanolic extracts of the leaves confirmed the presence of phenols and flavonoids. GC-MS analysis of the methanolic and hexane extracts identified key bioactive compounds, including n-Hexadecanoic acid, 1,3 -Benzodioxole and various terpenoids and phenolics. Some of these compounds are reported to exhibit antioxidant, antimicrobial and anti-inflammatory properties. The methanolic extract of leaves exhibited high antioxidant activity with an FRAP value of 2.89 ± 0.03 mM $FeSO_4$ equivalent/mL. ABTS IC_{50} of 15.12 µg/mL and DPPH IC_{50} of 433.78 µg/mL. Antibacterial studies using methanolic extract revealed selective activity against *Escherichia coli*. There were significant antifungal effects against *Saccharomyces cerevisiae* and *Candida tropicalis*. However, no inhibition effect was observed against the bacteria *Staphylococcus aureus*. The study supports the therapeutic potential of *M. patchoulii* for its antioxidant and antifungal efficacy. The GC-MS analysis identified several compounds with reported antimicrobial and antioxidant properties, supporting the observed bioactivities. These findings support the plant's traditional use and highlight its potential for drug development.

Keywords: antimicrobial; antioxidant; GCMS; medicinal plants; *Microtoena patchoulii*; phytochemical screening

Introduction

Since time immemorial, plants have been utilized for various purposes, including medicines, dyes, essential oils, cosmetics and other applications. Even today, they remain a major source of natural products and play a major role in pharmaceutical research (1). The search for potent therapeutics from natural products becomes a core focus in the present era, as medicinal plants and their secondary metabolites have potential as sources of new bioactive compounds to combat various diseases. Synthetic drugs often have drawbacks such as limited access, side effects and high costs. As a result, there is a growing preference for natural product-based therapies, leading to increased reliance on herbal medicines (2). Furthermore, the growing problem of antibiotic resistance has intensified the search for antimicrobial agents derived from natural sources. From these various aspects, natural products remain at the forefront of drug discovery and therapeutic advancements.

Microtoena patchoulii [(C.B. Clarke ex J.D. Hooker) C.Y. Wu et Hsuan] is an herbaceous plant classified under the family Lamiaceae. It is commonly known as *Assam Patchouli* or Chinese Patchouli and is locally called *Sangbrei* in Manipuri (3). The plant is native to northeastern India, Myanmar, Southeast Asia, the Malay region and Yunnan Province in China (4). Fresh leaves of *M. patchoulii* have strong fragrance and are commonly used in various traditional medicines (5). In the traditional medicine, this plant has been used to treat menstrual disorders, coughs, asthma, abdominal pain and enteritis (5, 6). Previous studies on this plant reported a high content of patchouli alcohol in the essential oil (5) and significant antioxidant activity in by the phenolic compounds (7). Despite these findings, comprehensive studies on antioxidant and antimicrobial properties of this

plant are limited. The current study attempts to fill this gap in scientific data of *M. patchoulii*.

This study aims to analyze the phytochemical constituents of the hexane and methanolic extract of *M. patchoulii* and evaluate their antioxidant potential of these extracts and assess their antimicrobial efficacy against selected bacterial and fungal pathogens. By providing comprehensive phytochemical and biological analysis, this study can contribute to the growing field of natural product research and highlights the potential pharmacological applications of *M. patchoulii*.

Materials and Methods

Fresh leaves of *M. patchoulii* were collected from the Manipur, India. The plant was identified in the Department of Life Sciences (Botany), Manipur University and a voucher specimen was deposited at the herbarium of Department of Botany, Assam Down University, Guwahati, Assam. The leaves were washed, shade-dried and ground into a fine powder. A 10 g powder was put in a thimble and loaded into the Soxhlet apparatus. About 250 mL of methanol was used as the solvent for the extraction process. The extraction was carried out for 8 cycles over 8 hr. After the extraction process, the excess solvent was removed using a rotary evaporator. Then the extracted sample was concentrated by volume reduction. The concentrated methanolic extract was reconstituted in methanol to obtain a 1 mg/mL stock solution (8). The procedure was also repeated for hexane as the solvent. Methanolic extraction was used for phytochemical screening of polar and moderately polar compounds of phenols and flavonoids. Both methanolic and hexane extracts were used in GC-MS analysis to detect a wide spectrum of volatile and semi-volatile compounds across different polarity ranges.

Phytochemical screening

Phytochemical analysis was conducted to estimate the total phenolic and flavonoid content using spectrophotometric methods. GC-MS analysis was also conducted to identify and characterize bioactive compounds in the extracts. The detailed methodologies are described in the following sections.

Total phenolic content (TPC)

The total phenolic content of the methanolic extract of the sample was determined by the Folin-Ciocalteu method spectrophotometrically following the modified protocol (9). 100 µL of the methanolic extract was mixed with 1.5 mL of Folin-Ciocalteu's reagent then added 1 mL 7.5 % Sodium Carbonate and allowed solution to incubate for 30 min at 40 °C. The solution absorbance value was measured using UV-Vis spectrophotometry at 765 nm. Gallic acid (GA) is used as standard for these. The total phenolic content of the extract was mg GAE/g (milligram of gallic acid equivalent per gram of dry weight of each extract). The total phenolic content was determined by the following formula

$$C = \frac{cV}{M}$$

Where

C = Total phenolic content in mg GAE/g.

c = concentration of gallic acid obtained from calibration curve in mg/mL.

V = Volume of extract in mL.

M = Mass of the extract in gram.

Total flavonoid content (TFC)

The total content of the methanolic extract of the sample was determined by aluminium chloride colorimetric method spectrophotometrically following the modified protocol (9-11). 750 µL of the sodium nitrate added to plant extract and allowed to incubate in dark place for 6 min. Then 150 µL of 10 % aluminium chloride was added and incubated in dark for 5 min. Then 500 µL of sodium hydroxide and 1 mL of methanol were added to the rest to make 3 mL final volume. The flavonoid content of the sample was calculated using the quercetin standard curve. The absorbance of the sample was measured at 510 nm using UV-Vis spectrophotometer. Total flavonoid content was calculated by the formula

$$C = \frac{cV}{M}$$

Where

C = Total flavonoid content in mg QE/g.

c = concentration of gallic acid obtained from calibration curve (mg/mL).

V = Volume of extract in mL.

M = Mass of the extract in gram.

GC-MS analysis of hexane and methanol extracts of the sample

The crude extracts of sample were done Soxhlet extraction with the help of Hexane solvent (HPLC grade) and methanolic extracts. After the extraction, GCMS analysis performed was performed using a Clarus 680 GC and Clarus 600C MS, both from PerkinElmer, USA; Liquid auto sampler with a capillary column 'Elite- 5MS' having dimensions of length 60 m, ID 0.25 mm and film thickness 0.25 µm and the stationary phase being 5 % diphenyl 95 % dimethyl polysiloxane, at GBP, Assam. The operating conditions of the GC-MS set for the analysis were as follows: Oven temperature at 60 °C for 3 min, then 200 °C at 5°C/ min for 3 min and finally 300 °C at 60 °C /min for 10 min. With the help of Perkin Elmer Programmable Split/Split less injector (PSSI) the initial set point of temperature is 280 °C and initially holds for 999.00 min. The sample injection was 1 µL with helium as the carrier gas at 1 mL/min. The ionization of the sample components was conducted at 70 eV. The total running time of the GC was 40 min. The chromatogram ranges from 50 to 600 Da in a column length of 60.0 m with 250 µm diameter. The interpretation of mass spectral data for GC-MS was conducted using the database of the National Institute of Standards and Technology (Library Software: Turbo Mass NIST 2014). Potential co-elution at identical or similar retention times was resolved through spectral deconvolution using the instrument's library search algorithm (12).

Antioxidant properties

The 2,2-diphenyl-1-picrylhydrazyl (DPPH), [2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid)] (ABTS) and ferric reducing antioxidant power (FRAP) assays were employed to evaluate antioxidant activity through different mechanisms, including hydrogen atom transfer (HAT) and single electron transfer (SET), to ensure a more comprehensive and reliable assessment (13).

FRAP assay

FRAP assay was performed following the protocol (14). The FRAP reagent was prepared by mixing 300 mM acetate buffer (pH 3.6), 10 mM TPTZ (2,4,6-tripyridyl-s-triazine) solution in 40 mM HCl (hydrochloric acid) and 20 mM $FeCl_3$ (ferric chloride) in water at volume ratio of 10:1:1. The mixture was warmed in a water bath maintained at 37 °C for 15 min. A 100 µL aliquot of the sample extract was added to 1 mL of methanol and mixed with 3 mL of the working FRAP reagent. The solution was then incubated at 37 °C for 5 min. Then, the absorbance was measured at 593 nm. A standard curve of $FeSO_4$ was prepared using varying concentrations and FRAP values were determined by comparing the absorbance change in the test mixture with the standard curve of ferrous sulfate. The resulting FRAP value was expressed as mM equivalent per mL of the sample.

ABTS assay

The ABTS radical scavenging assay was performed as described in the protocol (15, 16). A stable stock solution was prepared by mixing 7 mM aqueous solution of ABTS with a 2.45 mM potassium persulfate solution. The mixture was incubated for 16 hr at room temperature in the dark. The ABTS working solution was prepared by diluting the stock solution with methanol until an absorbance value of 0.70 ± 0.02 at 745 nm is achieved. Sample solutions were prepared at varying concentrations of 2, 5, 7 and 10 µg/mL by diluting with methanol. A 3 mL aliquot of the ABTS working solution was added to each sample. Absorbance was taken at 745 nm after an incubation period of 10 min. A control sample was prepared using 1 mL of methanol and 3 mL of ABTS solution and its optical density was measured under the same conditions. The optical density was recorded and the percentage scavenging of ABTS radicals was calculated using the following formula:

$$\% \text{ ABTS radical scavenging} = \frac{\text{Abs Control - Abs Sample}}{\text{Abs Control}} \times 100$$

IC_{50} was calculated from % scavenging activity calculated at different concentration of sample. Calculation was performed by replacing y with 50 in line equation and calculated the × value (10).

DPPH assay

The free radical scavenging activity was measured by DPPH assay according to the method (9, 11). First, a DPPH stable stock solution was prepared by dissolving 4 mg of DPPH in 100 mL of methanol, resulting in a final concentration of 40 µg/mL. The DPPH working solution was prepared by diluting the stock solution with methanol until its absorbance reached 0.98 ± 0.02 at 517 nm. For the assay, 1.5 mL of the diluted DPPH solution was added to sample solutions of varying concentrations (10, 150 and 350 µg/mL). The mixture was incubated for 5 min in the dark at room temperature. Following incubation, the absorbance was measured at 517 nm using a spectrophotometer. The DPPH radical scavenging percentage was calculated using the following formula:

$$\% \text{ DPPH radical scavenging} = \frac{\text{Abs Control - Abs Sample}}{\text{Abs Control}} \times 100$$

IC_{50} was calculated from % scavenging activity calculated at different concentration of sample. Calculation was performed by replacing 'y' with 50 in line equation and calculated the 'x' value (10).

Antimicrobial studies

Antimicrobial studies on the methanolic leaf extract were conducted by determining the agar disk diffusion method against selected microbial strains. The test organisms were procured from the Microbial Type Culture Collection and Gene Bank, Chandigarh, India. These included *E. coli* (MTCC 13120), *Staphylococcus aureus* (MTCC 10787), *C. tropicalis* (MTCC 184) and *S. cerevisiae* (MTCC 180).

Antibacterial test

Antibacterial activity of the methanolic extract was determined by agar disk diffusion method, following the standard protocol (17). A sample stock solution (1 mg/mL) was diluted by following the serial dilution method. Mueller-Hinton Agar (MHA) medium was prepared and sterilized by autoclaving at 15 lbs pressure for 15 min. It was then allowed to solidify for a few minutes. A uniform layer of bacterial culture was spread onto the medium, followed by drying for 10 min. Sterile disks were placed in four different zones: one disk was dipped in the concentrated extract, while the others were dipped in the 10^{-1}, 10^{-2} and 10^{-3} dilutions. A positive control-Kanamycin (30 µg) for *E. coli* and Gentamycin (30 µg) for *Staphylococcus aureus*-was placed at the centre of the plate. All plates were incubated at 37 °C for 24 hr.

Antifungal test

Antifungal activity was determined by using the agar disk diffusion method. A 1 mg/mL stock solution of the sample was diluted using the serial dilution method to obtain 10^{-1}, 10^{-2} and 10^{-3} dilutions. Potato Dextrose Agar (PDA) was poured into two plates. One plate was inoculated with 150 µL of *S. cerevisiae*, while the other was inoculated with 150 µL of *C. tropicalis* (18). Sterile disks were placed in four separate zones on the agar plate.: One disk was dipped in the concentrated extract, while the others were dipped in the 10^{-1}, 10^{-2} and 10^{-3} dilutions. A positive control, voriconazole for *S. cerevisiae* and ketoconazole for *C. tropicalis* was placed at the center of each plate. All plates were incubated at 28 °C (19) for 24 hr.

Results and Discussion

Total phenolic content and flavonoid content

TPC and TFC of the methanolic extract of were determined to be 20.434 ± 0.045 mg GAE/g and 18.89 ± 0.01 mg QE/g, respectively (Table 1). These findings indicate the presence of substantial amount of both phenolic compounds and flavonoid concentration. The finding is comparable to reports of previous studies (7). The results demonstrated the presence of bioactive compounds with known antioxidative potential (9). The presence of phenolic and flavonoid compounds highlighted the potential of *M. patchoulii* in pharmaceutical and nutraceutical applications (20).

Table 1. Phenolic and flavonoid content of the methanolic extract

Parameter	Contents
Total phenolic content	24.34 ± 0.045 mg GAE/g
Total flavonoid content	18.89 ± 0.01 mg QE/g

GC-MS analysis of hexane and methanolic extracts

The GC-MS analysis of the hexane extract identified several bioactive compounds, including terpenoids, phenolics and alkanes (Table 2 and Supplementary Fig. 1). Compounds detected are Phensuximide, Indane, Trans-2,4-Dimethylthiane, (+)-2-Carene, 3-N-Hexylthiolane, Eicosyl Octyl Ether, Carbonic Acid, Eicosyl Vinyl Ester, Caryophyllene, (S,1z,6z)-8-Isopropyl-1-Methyl-5-Methylenecyclodeca-1,6-Diene,Trans-Alpha Bergamotene, Alpha-Bulnesene, Phytol, Patchouli Alcohol and Azulene. The detection of alpha-bergamotene, beta-caryophyllene and patchouli alcohol is consistent with previous studies (5).

Comparatively, less compounds are detected in the GC-MS analysis of the methanolic extract (Table 3 and Supplementary Fig. 2). Compound detected in the methanolic extract are Dihydrotachysterol, Panaxjapyne A, Thymol, Androstan-17-One and Arsenous Acid.

Many compounds identified in the study have reported pharmaceutical and therapeutic properties potential applications in neurology, cancer therapy, metabolic regulation, immune modulation, anti-inflammatory treatments and antimicrobial therapy (Fig. 1 and 2). Phensuximide (Milontin), identified in the study, is used in neurological treatments, particularly in managing seizure disorders (21). Arsenous Acid (Arsenic Trioxide), another compound identified in the study, has application in chemotherapy (22). particularly in acute promyelocytic leukemia (APL). Dihydrotachysterol, identified in the study, is another derivative of vitamin D. This compound plays an important role in bone mineralization and skeletal health by enhancing calcium and phosphate absorption (23), thus making it useful in rickets and osteomalacia treatment. Another compound, Panaxjapyne A was also identified in the study. This compound has been associated with immune regulation, digestive health and chronic fatigue management (24).

Trans-2,4-Dimethylthiane identified in the study possess anti-inflammatory activity by modulating cyclooxygenase-2 (COX-2), making it a potential candidate for managing chronic inflammatory diseases such as arthritis (25). Caryophyllene (β-Caryophyllene), a dietary cannabinoidm, is also identified in the study. This compound can play a role in immune modulation, pain relief and neuroprotection by selectively binding to CB2 receptors (26). Alpha-Bulnesene, a common constituent in patchouli oil, is also detected in the study. This compound is known for its anti-inflammatory and anti-allergic effects, making it beneficial in dermatological treatments and respiratory conditions (27).

(+)-2-Carenepossesses broad-spectrum antimicrobial properties, including antibacterial, antifungal and anti-inflammatory effects (28). Thymol has been recognized for its antibacterial and antifungal properties, with applications in respiratory conditions, metabolic disorders and skin infections (29). Azulene, a non-benzenoid aromatic compound, has been utilized in skincare formulations due to its antioxidant and anti-inflammatory effects. It can help in neutralizing reactive oxygen species (ROS) and reduce oxidative stress (30). (S,1Z,6Z)-8-Isopropyl-1-Methyl-5-Methylenecyclodeca-1,6-Diene, identified in the study, has been found to possess antimicrobial properties against bacterial and fungal pathogens, making it useful in dermatological applications (31). Indane has been explored for its medicinal application, particularly in the treatment of infectious diseases and biosensing technologies (32). 3-Hexylthiophene was identified as a potential antimicrobial compound, though further research is necessary to fully establish its mechanism of action (33).

Table 2. Phytochemicals detected by GCMS analysis of hexane extract

Sl No	Compound name	Retention time (Rt, min)	Area (%)	Molecular weight	Molecular formula
1	Phensuximide	12.890	1.921	189	$C_{11}H_{11}NO_2$
2	Indane	16.862	1.797	118	C_9H_{10}
3	Trans-2,4-Dimethylthiane	17.737	1.797	130	$C_7H_{14}S$
4	(+)-2-Carene	17.862	1.671	136	$C_{10}H_{16}$
5	3-N-Hexylthiolane	18.598	4.784	168	$C_{10}H_{16}S$
6	Eicosyl Octyl Ether	22.894	0.560	410	$C_{28}H_{58}O$
7	Carbonic Acid, Eicosyl Vinyl Ester	22.894	0.560	368	$C_{23}H_{44}O_3$
8	Caryophyllene	26.416	0.612	204	$C_{15}H_{24}$
9	(S,1z,6z)-8-Isopropyl-1-Methyl-5-Methylenecyclodeca-1,6-Diene	26.416	0.612	204	$C_{15}H$
10	Trans-Alpha-Bergamotene	26.661	0.556	204.3511	$C_{15}H_{24}$
11	Alpha-Bulnesene	27.716	1.091	204.35	$C_{15}H_{24}$
12	Azulene	28.076	0.757	128.217	$C_{10}H_8$
13	Patchouli Alcohol	31.768	1.899	222.37	$C_{15}H_{26}O$
14	Phytol	36.510	18.625	296	$C_{20}H_{40}O$

Note: Peaks at 22.894 min and 26.416 min indicate co-elution of compounds. Identities were determined through spectral deconvolution and confirmed by high library match scores.

Table 3. Phytochemicals detected by GCMS analysis of methanolic extract

Sl No	Compound name	Retention time (Rt, min)	Area (%)	Molecular weight	Molecular formula
1	Dihydrotachysterol	27.152	0.263	398	$C_{28}H_{26}O$
2	Panaxjapyne A	27.152	0.263	246.4	$C_{17}H_{26}O$
3	Androstan-17-One	28.107	0.118	274.4	$C_{19}H_{30}O_2$
4	Arsenous Acid	30.103	0.934	125.944	AsH_3O_3
5	Thymol	32.204	0.645	150.22	$C_{10}H_{14}O$

Note: Co-elution is indicated at 27.152 min for Dihydrotachysterol and Panaxjapyne A.

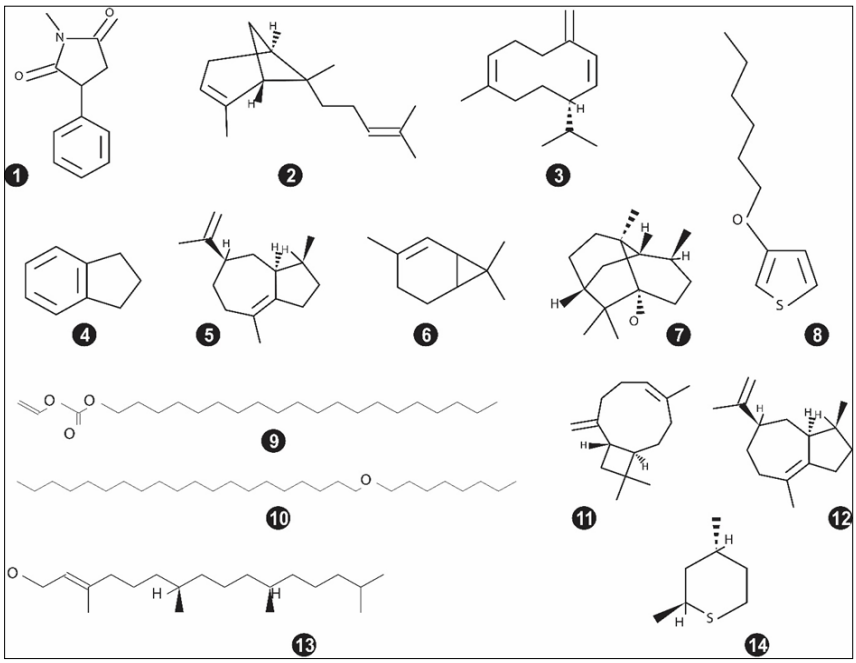

Fig. 1. Phytochemicals observed from GCMS analysis of hexane extracts.

In addition to pharmaceutical applications, several compounds identified in the study have significant industrial, cosmetic and fragrance-related properties. as fragrance enhancers, flavoring agents and surfactants. Patchouli Alcohol is a sesquiterpene alcohol, extensively used in the perfume and cosmetic industries because of its long-lasting fragrance and skin-conditioning properties. It also possesses antimicrobial and anti-inflammatory effects, making it useful in topical formulations (34). Trans-Alpha-Bergamotene, a naturally occurring terpene, serves as a flavoring agent in the food and beverage industry. It is also studied for its potential role in drug bioavailability enhancement (35). Phytol detected in the analysis plays a crucial role as biosynthetic precursors for vitamins E and K1. It is also being studied for drug delivery applications because of its lipophilic nature (36). The presence of these bioactive compounds aligns with previously reported medicinal benefits of plants from the Lamiaceae family, further reinforcing the potential pharmacological applications of *M. patchoulii*.

Antioxidant properties

The antioxidant potential of *M. patchoulii* was determined by using FRAP, ABTS and DPPH assays (Table 4). The FRAP assay determined a (FRAP) value of 2.89 ± 0.03 mM $FeSO_4$ equivalent/mL, indicating high electron-donating capacity. The ABTS assay reported an IC_{50} value of 15.12 µg/mL, while the DPPH assay resulted in an IC_{50} value of 433.78 ± 0.05 µg/mL. These values demonstrated the methanolic extract's potential to neutralize free radicals. The antioxidant activity may be attributed to the presence of phenolic and flavonoid compounds identified in phytochemical screening. These values of FRAP, ABTS and DPPH are comparable to those reported for antioxidant-rich plant extracts. In a previous study, ABTS IC_{50} values ranges from 10 to 30µg/mL in antioxidant rich plants of *Rosmarinus officinalis*, *Origanum vulgare* and *Thymus vulgaris* (10). The ABTS assay is effective in evaluating both hydrophilic and lipophilic antioxidants such as *Trolox* and *green tea extracts* (15). While DPPH responses are more variable, IC_{50} values above 400µg/mL have been reported in *Mentha spicata*, *Marrubium vulgare* and *Inula viscosa* (11) comparable to the activity of present study. extract's activity. FRAP assay results also reinforce its antioxidant capacity, aligning with applications across phenolic-rich plants (14).

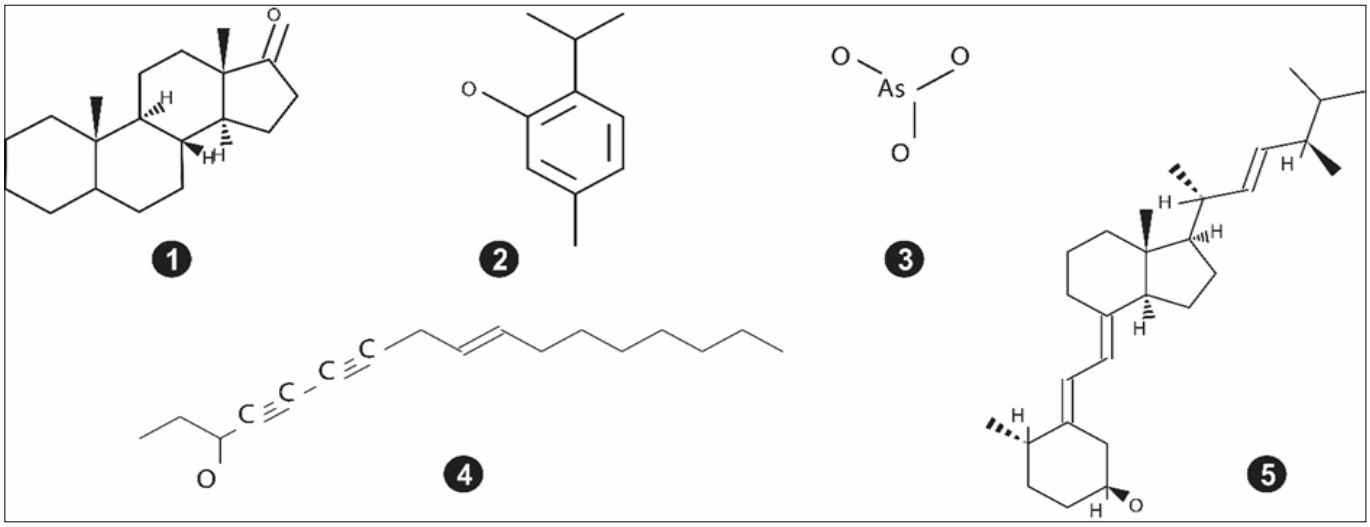

Fig. 2. Phytochemicals observed from GCMS analysis of methanolic extracts.

Table 4. Antioxidant properties of methanolic extract of *M. patchoulii* leaves

Assay	Measured parameter	Value (Mean ± SD)
FRAP	FRAP Value (mM $FeSO_4$ eq./mL)	2.89 ± 0.03
ABTS	IC_{50} (µg/mL)	15.12
DPPH	IC_{50} (µg/mL)	433.78 ± 0.05

Antimicrobial studies

The methanolic extract of *M. patchoulii* demonstrated potent antibacterial activity against *E. coli*, with inhibition zones decreasing as the extract was diluted (Table 5). However, no inhibition was observed against *Staphylococcus aureus*.

Table 5. Zone of inhibition (in mm) at serial dilutions of crude methanolic extract against selected microorganisms. Positive controls used were Ciprofloxacin (bacteria) and Amphotericin B (fungi)

Microorganism	Methanolic extract (1 mg/mL)	10^{-1} Dilution	10^{-2} Dilution	10^{-3} Dilution	Positive control
E. coli	5.0	4.0	0.6	0.2	26.0
S. aureus	Nil	Nil	Nil	Nil	25.0
S. cerevisiae	5.0	3.0	1.0	0.5	10.0
C. tropicalis	4.0	2.0	1.5	0.4	12.0

The antifungal activity remained consistent, with inhibition observed in both *S. cerevisiae* and *C. tropicalis*, with larger inhibition zones at higher concentrations (Fig. 3). These findings highlight the significant antimicrobial and antioxidant potential of *M. patchoulii*, making it a promising candidate for the development of natural therapeutic agents, particularly for bacterial and fungal infections resistant to conventional treatments (18, 19).

Conclusion

Phytochemical screening of *M. patchoulii* leaf extracts demonstrated the presence of phenols and flavonoids, which are commonly associated with antioxidant and antimicrobial properties. GC-MS analysis identified several bioactive compounds such as thymol, patchouli alcohol, caryophyllene and azulene. The antioxidant property of the methanolic extract was determined through DPPH, ABTS and FRAP assays, with particularly strong activity observed in the ABTS and FRAP tests and moderate activity in the DPPH assay. The extract showed selective antimicrobial activity, effectively inhibiting *E. coli*, *S. cerevisiae* and *C. tropicalis*, but not *S. aureus*, suggesting its potential application in certain fungal pathogens and gram-negative bacteria. The finding from the study highlights the therapeutic relevance of *M. patchoulii* and its potential for further studies in plant-based drug development and functional formulations.

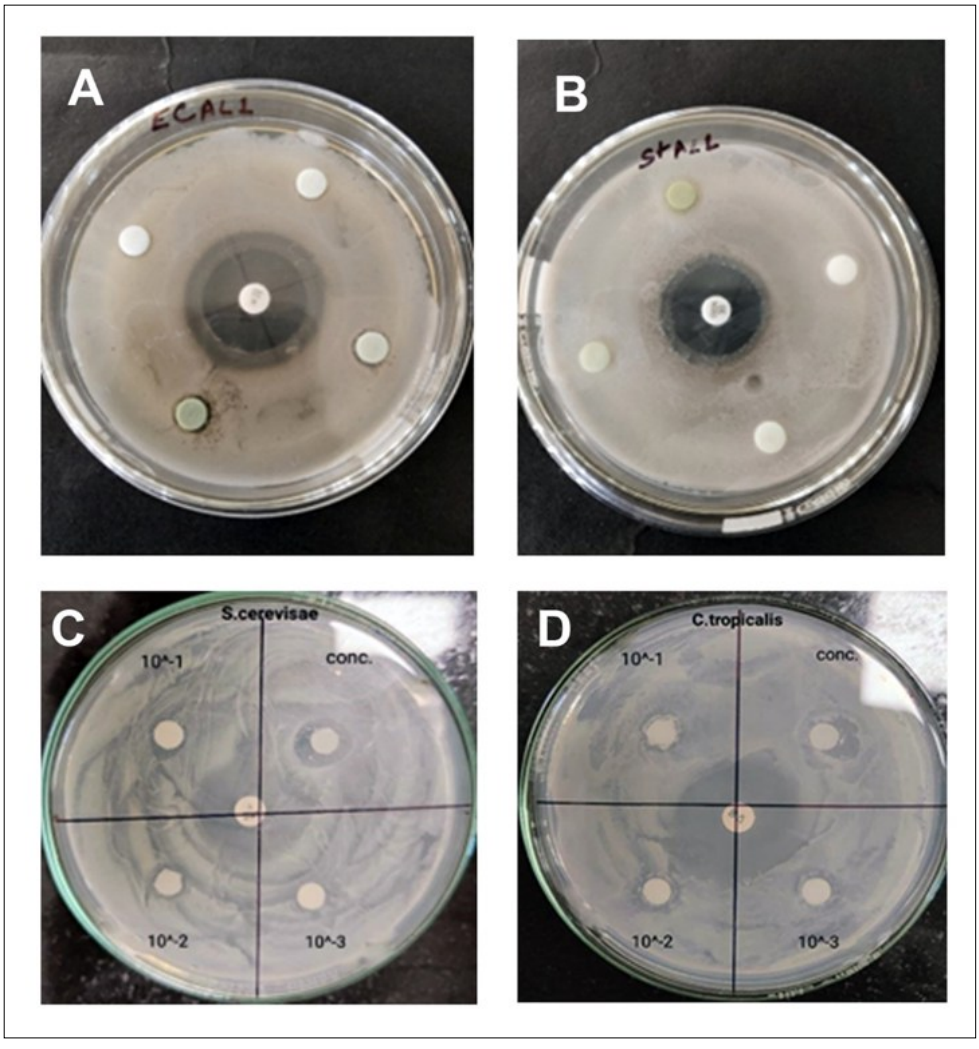

Fig. 3. Zone of inhibition patterns for different antimicrobials (A) *E. coli*, (B) *S. aureus*, (C) *S. cerevisiae* and (D) *C. tropicalis*.

Acknowledgements

The authors express their sincere gratitude to Guwahati Biotech Park for permitting the use of their laboratory facilities for phytochemical studies and GC-MS analysis.

Authors' contributions

AK carried out the experiments and drafted the manuscript. MKD conceived the study design and supervised the work. KG and RCG participated in the GC-MS analysis. SSN contributed to the study design and coordination. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: The authors declare that they have no competing interests.

Ethical issues: None

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Peer review: Publisher thanks Sectional Editor and the other anonymous reviewers for their contribution to the peer review of this work.

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