IARJSET ISSN (O) 2393-8021, ISSN (P) 2394-1588

International Advanced Research Journal in Science, Engineering and Technology

Impact Factor 8.311 ⁜ Peer-reviewed & Refereed journal ⁜ Vol. 13, Issue 2, February 2026

DOI: 10.17148/IARJSET.2026.13204

Phytochemical Analysis, Antioxidant and Antimicrobial Activities of an Endemic and Threatened Nutmeg Species of Andaman and Nicobar Islands, India, with a Conservation Appraisal

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Abstract: *Myristica andamanica* Hook.f., an endemic and vulnerable nutmeg species of the Andaman and Nicobar Islands, was investigated for its phytochemical composition, antioxidant potential, antimicrobial activity, and conservation status. Methanolic leaf extracts were subjected to qualitative and quantitative phytochemical analyses, in-vitro antioxidant assays (DPPH and ABTS), antimicrobial screening, and HPLC profiling. Preliminary screening revealed the presence of alkaloids, saponins, flavonoids, steroids, terpenoids, phenols, and carbohydrates. The extract exhibited moderate total phenolic and high flavonoid contents, with IC_{50} values of 52.36 µg/ml and 74.99 µg/ml in DPPH and ABTS assays, respectively. HPLC analysis identified protocatechuic acid and kaempferol as major constituents. Moderate antimicrobial activity was observed against selected bacterial and fungal strains. *Ex-situ* and *in-situ* conservation strategies were successfully implemented. The present study revealed such phytochemical profiling and antioxidant property of *M. andamanica* for the first time and highlights the medicinal potential and conservation importance of.

Keywords: *Myristica andamanica*, Phytochemicals, Antioxidant activity, Antimicrobial activity, Endemic species, Conservation

I. INTRODUCTION

Medicinal plants constitute one of the most important sources of bioactive compounds used in traditional and modern healthcare systems [1]. Secondary metabolites such as phenolics, flavonoids, alkaloids, terpenoids, and glycosides play crucial roles in plant defence mechanisms and exhibit diverse pharmacological activities, including antioxidant, antimicrobial, anti-inflammatory, and anticancer effects [2,3]. These compounds are widely exploited in the pharmaceutical, nutraceutical, and cosmetic industries [4].

The genus *Myristica* (family Myristicaceae) comprises several economically and medicinally important species, among which *Myristica fragrans* is extensively studied for its essential oils, phenolic compounds, and therapeutic properties [5].

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IARJSET ISSN (O) 2393-8021, ISSN (P) 2394-1588

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Studies have demonstrated that nutmeg-derived compounds possess strong antioxidant, antimicrobial, and neuroprotective activities [6].

However, comparatively little scientific attention has been directed towards endemic species of this genus [7]. *Myristica andamanica* Hook.f. is an endemic nutmeg species confined to the Andaman and Nicobar Islands and is traditionally used by aboriginal tribal communities (Jarawas and Nicobarese) of this Archipelago for treating fever, malaria, stomach trouble, and wounds [8,9,10]. Preliminary investigations have reported wound-healing and limited bioactivity of this species; however, comprehensive studies integrating phytochemical profiling, antioxidant assessment, antimicrobial evaluation, and conservation appraisal remain scarce [11].

The Andaman and Nicobar Islands represent a biodiversity hotspot characterized by high degree of endemism (approximately 10% of the total island flora) and ecological sensitivity [12]. Rapid urbanization, deforestation, habitat fragmentation, and unsustainable exploitation of forest resources have severely threatened many endemic plant species, including *M. andamanica* [13]. This lead to categorise this species as vulnerable in the IUCN Red List with reduction in population size (14).

Waman and Bohra [15] reported the importance of assisted regeneration and nursery-based propagation for the conservation of *M. andamanica*. These findings underscore the need for integrating propagation-based conservation approaches with phytochemical and pharmacological validation, as scientific validation of medicinal potential is essential to promote sustainable utilization and strengthen conservation strategies [16].

In this context, the present study aimed to systematically evaluate the phytochemical composition, in-vitro antioxidant activity, antimicrobial potential, and conservation status of *M. andamanica*. The investigation integrates biochemical analysis with biodiversity conservation to provide a comprehensive scientific basis for the sustainable management of this endemic and vulnerable medicinal plant.

II. MATERIALS AND METHODS

2.1. Collection and identification of plant material

Fresh and healthy leaf samples of *Myristica andamanica* Hook.f (Figure 1) were collected from various locations of Andaman and Nicobar Islands during August 2019 to January 2023. Plant specimens were authenticated with reference to herbarium collections maintained at PBL, Andaman and Nicobar Regional Centre (ANRC), Botanical Survey of India (BSI), Port Blair. Voucher specimen (No. 32395) was prepared and deposited at PBL.

2.2. Preparation of crude plant powder

Leaf samples were thoroughly washed with tap water followed by distilled water to remove dust and contaminants. The samples were shade-dried at room temperature and further dried in a hot air oven at 50 °C for 4–5 h to remove residual moisture. Dried leaves were pulverized into fine powder using an electric grinder and stored in airtight containers at room temperature for further analysis.

2.3. Sequential solvent extraction

Sequential Soxhlet extraction was performed using solvents of increasing polarity: n-hexane, diethyl ether (DEE), dichloromethane (DCM), ethyl acetate (EA), and methanol (MeOH) according to established methodology [17]. Approximately 100 g of powdered leaf material was extracted with 250 ml of each solvent for 24–48 h under continuous reflux. After extraction, the solvent extracts were filtered through Whatman No. 1 filter paper and concentrated under reduced pressure using a rotary evaporator at 38–40 °C. The dried crude extracts were weighed and stored at 4°C in airtight containers until further use. Finally, the methanolic extract was used for subsequent analysis.

2.4. Qualitative phytochemical screening

Methanolic extract was subjected to qualitative phytochemical screening to detect major secondary metabolites including alkaloids, tannins, saponins, flavonoids, terpenoids, phenols, glycosides, steroids, cardiac glycosides, carbohydrates, proteins, and coumarins using standard protocols such as Ferric chloride test (tannins), Foam test (saponins), Mayer's test (alkaloids), Salkowski test (terpenoids), Folin–Ciocalteu test (phenols), Molisch's test (carbohydrates), and Biuret test (proteins) described by Harborne, Kokate, and Trease and Evans [18-20].

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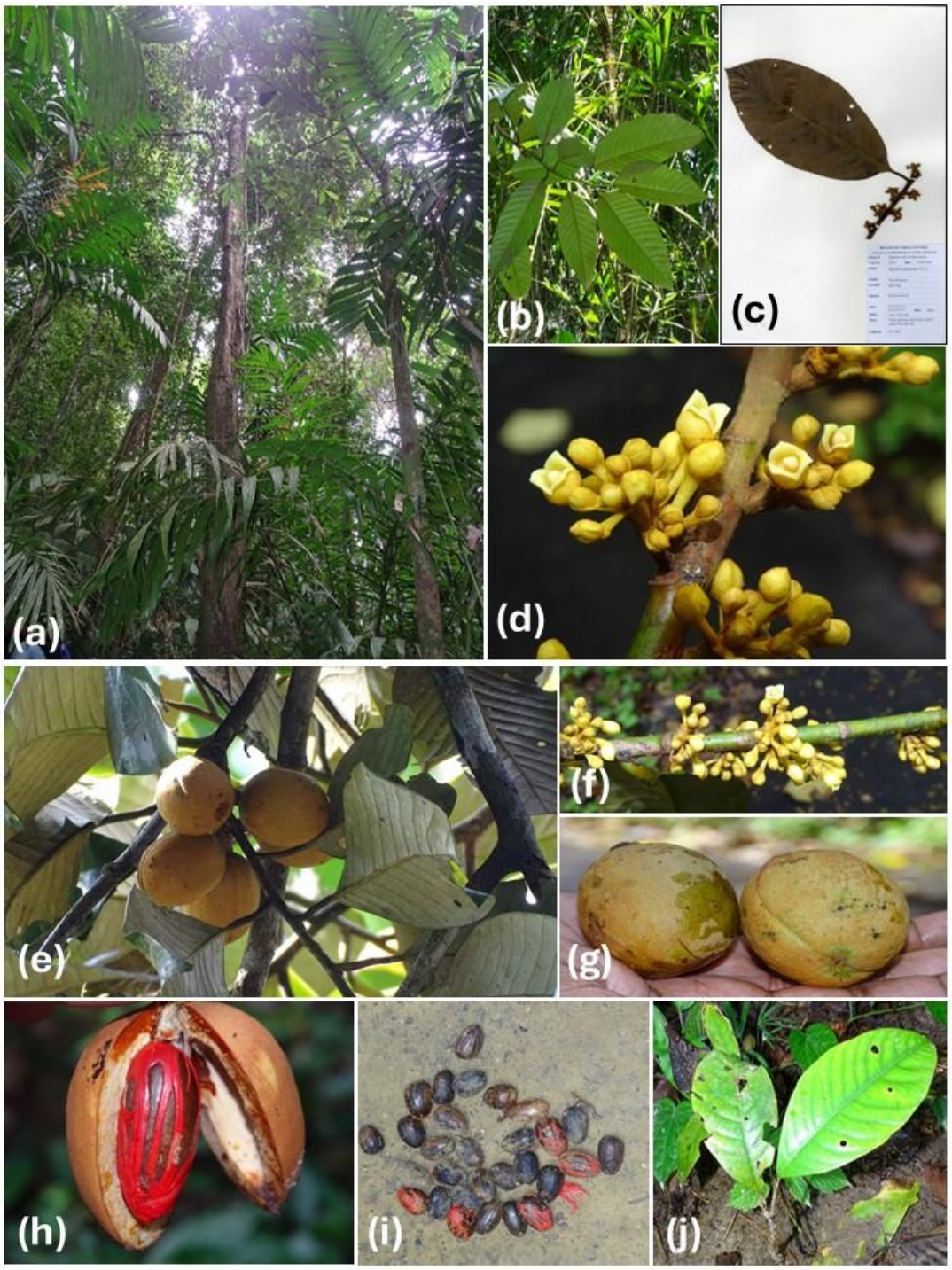

Figure 1. *Myristica andamanica* Hook.f. (Myristicaceae): (a) Habit, (b) A branch, (c) Voucher specimen (No. 32395), (d) Flowers, (e) Fruiting branch, (f) Flowering branch, (g) Ripen fruits, (h) Dehisced fruit with seed aril, (i) Seeds, (j) Seedling in wild habitat.

2.5. Quantitative phytochemical analysis

2.5.1. Estimation of Total Phenolic Content (TPC)

Total phenolic content was estimated using the Folin–Ciocalteu method [21]. Briefly, diluted Folin–Ciocalteu reagent was mixed with extract solution followed by sodium carbonate addition. After incubation, absorbance was measured at 765 nm using a UV–Visible spectrophotometer. Gallic acid was used as standard, and results were expressed as mg GAE/g dry extract.

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2.5.2. Estimation of Total Flavonoid Content (TFC)

Total flavonoid content was determined using the aluminium chloride colorimetric method [22]. Extract was treated with 5% sodium nitrite (w/v) and 10% aluminium chloride, and 1M NaOH. Absorbance was measured at 415 nm. Quercetin was used as standard, and results were expressed as mg QE/g dry extract.

2.6. In-vitro antioxidant assays

2.6.1. DPPH radical scavenging assay

DPPH radical scavenging assay was performed as described by Dzoyem and Eloff [23]. Different concentrations (10–100 µg/ml) of methanolic extract were mixed with DPPH solution and incubated in the dark for 30 min. Absorbance was measured at 517 nm using a UV–Visible spectrophotometer. Ascorbic acid was used as a standard. Percentage inhibition was calculated using the following equation:

$$\text{DPPH scavenging activity (\%)} = (A_0 - As)/A0 \times 100$$

Where, Ao is the absorbance of the blank, and As is the absorbance of the leaf extract.

The antioxidant activity was expressed as percentage inhibition (%), and IC_{50} value (µg/ml) was calculated by plotting percentage inhibition against extract concentrations.

2.6.2. ABTS radical scavenging assay

ABTS assay was carried out according to Khanam et al. [24]. $ABTS^{\cdot+}$ radical solution was prepared using ABTS and potassium persulfate. The reaction mixture was incubated for 2 hours in the dark and the absorbance was measured at 734 nm using a UV–Visible spectrophotometer. Ascorbic acid was used as the positive control. The percentage inhibition of $ABTS^{\cdot+}$ radicals was calculated using, following equation.

$$\text{ABTS scavenging activity (\%)} = (A_0 - As)/A0 \times 100$$

Where, Ao is the absorbance of the blank, and As is the absorbance of the leaf extract.

Antioxidant activity was expressed as percentage inhibition (%) and IC_{50} value (µg/ml) were calculated by plotting percentage inhibition against extract concentrations.

2.7 Quantification of phenolic acids and flavonoids by High-Performance Liquid Chromatography (HPLC)

High Performance Liquid Chromatography (HPLC) was employed for qualitative and quantitative analysis of phenolic acids and flavonoids present in methanolic extracts following the method proposed by Seal et al. [25]. HPLC analysis was performed using a Thermo Scientific Dionex Ultimate 3000 system equipped with a diode array detector (DAD) and an Acclaim C18 column (250 × 4.6 mm, 5 µm). Separation and quantification of phenolic acids and flavonoids were carried out using a gradient mobile phase consisting of methanol and 0.5% aqueous acetic acid at 25 °C. Standard solutions of selected phenolic acids and flavonoids were prepared in HPLC-grade methanol and used for calibration. Working standards (20–100 µg/ml) were employed to generate calibration curves based on peak area versus concentration. Detection was performed at 272, 280, and 310 nm. Compounds were identified by comparing retention times with reference standards and confirmed by spiking. Quantification was achieved using external standard calibration, and results were expressed as mean values from triplicate analyses.

2.8. *In-vitro* antimicrobial assay

The antimicrobial activity of the methanolic leaf extract of *M. andamanica* was assessed using the agar spot-on-lawn method as described by Mandal et al. [26]. Selected pathogenic bacterial strains, namely *Escherichia coli*, *Klebsiella pneumoniae*, and *Enterococcus faecalis*, as well as fungal strains including *Candida glabrata*, *C. parapsilosis*, and *C. tropicalis*, were employed in the assay. Sterile nutrient agar plates for bacteria and Sabouraud dextrose agar plates for fungi were uniformly inoculated with the respective microbial suspensions. An aliquot of 10 µL of the methanolic extract was aseptically spotted onto the inoculated agar surface. The plates were incubated at 37 °C for 24 h for bacterial strains and at 28-30 °C for 48 h for fungal strains. Following incubation, the diameter of the zones of growth inhibition was measured in millimetres. All experiments were performed in triplicate, and the results were expressed as mean values.

2.9. Conservation assessment

Ex-situ conservation was carried out through nursery raising at the Dhanikhari Experimental Garden-cum-Arboretum (DEGCA), Botanical Survey of India (BSI), Andaman and Nicobar Regional Centre (ANRC), South Andaman. Freshly

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collected seeds were soaked in water overnight, sown in polybags, and maintained under controlled nursery conditions. After successful germination and establishment, healthy seedlings were reintroduced into their natural habitats, and wild populations were regularly monitored. In addition, seedlings collected from various locations across the Andaman Islands were nurtured in the nursery for acclimatization prior to transplantation.

III. RESULTS

3.1. Preliminary phytochemical screening

Qualitative phytochemical analysis of methanolic leaf extracts of *M. andamanica* revealed the presence of several important secondary metabolites, including alkaloids, saponins, flavonoids, steroids, terpenoids, phenols, and carbohydrates. Proteins, glycosides, and coumarins were not detected (Table 1).

The abundance of flavonoids and saponins suggests potential antioxidant and antimicrobial activities. Alkaloids and terpenoids are known for their pharmacological relevance, including antimicrobial and anti-inflammatory properties. The presence of multiple bioactive classes indicates the therapeutic versatility of this endemic species.

Table 1: Preliminary phytochemical screening of *Myristica andamanica* methanolic leaf extract.

Phytochemical Test	Phytochemical Constituent	Result
Mayer's test	Alkaloids	+
Ferric chloride test	Tannins	–
Foam test	Saponins	++
Alkaline reagent test	Flavonoids	++
Chloroform test	Glycosides	–
Test for steroids	Steroids	++
Salkowski test	Terpenoids	+
Folin–Ciocalteu reagent test	Phenols	+
Keller–Killiani test	Cardiac glycosides	–
Molisch's test	Carbohydrates	++
Biuret test	Proteins	–
Test for coumarins	Coumarins	–

'–' Absent; '+' Present; '++' Highly present

3.2. Quantitative phytochemical analysis

3.2.1. Total phenolic content

The total phenolic content of *M. andamanica* methanolic extract was found to be 6.345 mg gallic acid equivalent/g (GAE/g) dry extract. Phenolic compounds are important contributors to antioxidant activity due to their hydrogen-donating ability and free radical scavenging properties. The moderate phenolic content suggests that phenolics contribute partially to the observed antioxidant effects.

3.2.2. Total flavonoid content

The total flavonoid content was estimated as 126.033 mg quercetin equivalent/g (QE/g) dry extract, indicating that *M. andamanica* is a rich source of flavonoids. Flavonoids possess strong antioxidant, antimicrobial, and anti-inflammatory activities. The high flavonoid concentration observed in this study suggests that flavonoids may play a dominant role in the biological activities of the extract

3.3. *In-vitro* antioxidant activity

3.3.1. DPPH radical scavenging activity

The methanolic extract of *M. andamanica* exhibited dose-dependent DPPH radical scavenging activity with an IC_{50} value of 52.36 µg/ml. Although this activity was lower than that of ascorbic acid, the extract demonstrated substantial hydrogen-donating ability. The moderate antioxidant activity may be attributed to the combined effect of flavonoids, phenols, and terpenoids.

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3.3.2. ABTS radical scavenging activity

In the ABTS assay, the extract showed an IC_{50} value of 74.99 µg/ml, indicating moderate scavenging capacity. The consistency between DPPH and ABTS results confirms the reliability of the antioxidant potential of *M. andamanica*. The lower activity in ABTS compared to DPPH may reflect differences in radical reactivity and solubility of phytoconstituents.

3.4. Quantification of phenolic acids and flavonoids by High-Performance Liquid Chromatography (HPLC)

High-performance liquid chromatography (HPLC) analysis of the methanolic leaf extract of *M. andamanica* revealed the presence of several phenolic acids and flavonoids, as shown in the chromatographic profile recorded at 280 nm (Table 2, Figure 2).

Among the phenolic acids, protocatechuic acid was detected as the predominant compound with a concentration of 3.603 µg/g dry extract, followed by gentisic acid (0.660 µg/g), chlorogenic acid (0.502 µg/g), *p*-coumaric acid (0.292 µg/g), ferulic acid (0.264 µg/g), and *p*-hydroxybenzoic acid (0.057 µg/g). Minor quantities of caffeic acid (0.029 µg/g) and sinapic acid (0.120 µg/g) were also detected. In contrast, gallic acid, vanillic acid, syringic acid, salicylic acid, and ellagic acid were not detected in the extract.

With respect to flavonoids, kaempferol was identified as the major flavonoid constituent with a concentration of 1.042 µg/g dry extract, followed by myricetin (0.282 µg/g) and naringin (0.091 µg/g). Other flavonoids, including rutin, quercetin, naringenin, apigenin, and catechin, were not detected under the experimental conditions.

Table 2. Quantitative estimation of phenolic acids of the methanolic extract of *M. andamanica* by HPLC (µg/mg dry plant extract.

Phytochemicals	**Content (µg/g dry extract)**
Phenolic acids	
Gallic acid	–
Protocatechuic acid	3.603
Gentisic acid	0.660
p-Hydroxybenzoic acid	0.057
Chlorogenic acid	0.502
Vanillic acid	–
Caffeic acid	0.029
Syringic acid	–
p-Coumaric acid	0.292
Ferulic acid	0.264
Sinapic acid	0.120
Salicylic acid	–
Ellagic acid	–
Flavonoids	
Naringin	0.091
Rutin	–
Myricetin	0.282
Quercetin	–
Naringenin	–
Apigenin	–
Kaempferol	1.042
Catechin	–

'–' Not detected

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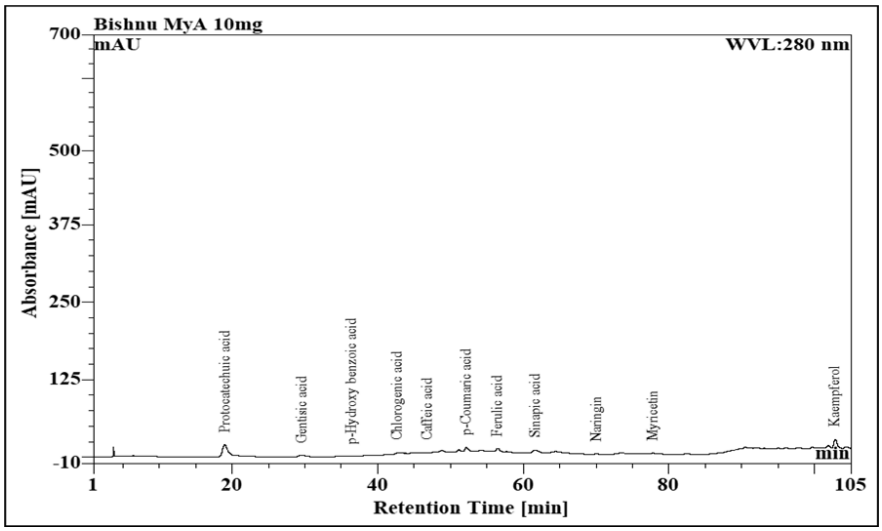

Figure 2. HPLC chromatogram showing separation of phenolics and flavonoids from methanolic leaf extracts of *M. andamanica.*

3.5. *In-vitro* antimicrobial activity

The methanolic leaf extract of *M. andamanica* exhibited moderate antimicrobial activity against both bacterial and fungal strains (Table 3, Figure 23).

Among the bacterial pathogens, the highest zone of growth inhibition was observed against *E. coli* (8.0 mm), followed by *E. faecalis* (6.5 mm), while *K. pneumoniae* showed the lowest inhibitory response (6.0 mm).

With respect to fungal pathogens, moderate inhibitory activity was recorded against *C. glabrata* and *C. parapsilosis*, each exhibiting inhibition zones of 7.0 mm. *C. tropicalis* showed a slightly lower inhibition zone of 6.5 mm.

Table 3 Antimicrobial activity study against pathogenic bacterial and fungal strains.

Microorganism	**Zone of growth inhibition (mm)**
Bacterial strains	
Escherichia coli MTCC 730 (Gram negative)	8.0
Klebsiella pneumoniae MTCC 7407 (Gram negative)	6.0
Enterococcus faecalis (Gram positive)	6.5
Fungal strains	
Candida glabrata	7.0
Candida parapsilosis	7.0
Candida tropicalis	6.5

Values are mean of triplicate data.

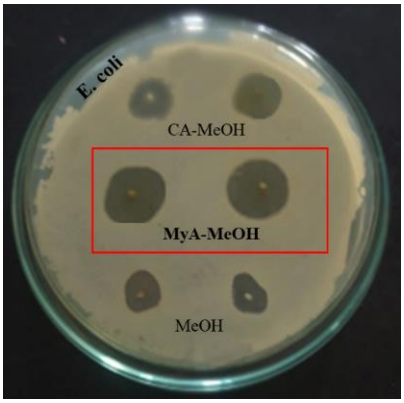

Figure 3. *In-vitro* antimicrobial activity of methanolic leaf extract of *M. andamanica* showing diameter of zones of growth inhibition (in mm) against selected human pathogenic bacterial and fungal strains.

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3.6. Conservation assessment

Ex-situ conservation through nursery raising and in-situ conservation through habitat restoration were successfully implemented. Seeds and saplings of *M. andamanica* were established in Botanical Survey of India nurseries under controlled environmental conditions (Figure 4). High survival rates were achieved through regular monitoring and maintenance. *In-situ* conservation efforts involved reintroduction of propagated plants into natural habitats and protection of existing populations. These measures enhance genetic diversity, population stability, and long-term sustainability of the species.

IV. DISCUSSION

The present investigation provides comprehensive insights into the phytochemical composition, antioxidant potential, antimicrobial activity, and conservation status of *M. andamanica*, an endemic and underexplored medicinal plant.

The presence of flavonoids, phenols, alkaloids, and terpenoids observed in this study is consistent with earlier reports on *Myristica* species [5,7,27]. These compounds are widely recognized for their therapeutic relevance and synergistic biological effects [2,28].

Moderate phenolic content and high flavonoid concentration strongly correlate with antioxidant activity, as reported in several medicinal plants [20-22,29]. The predominance of flavonoids may therefore account for the observed radical scavenging capacity.

The DPPH and ABTS assays demonstrated moderate antioxidant activity comparable to related *Myristica* species [6,23,30]. Difference in scavenging capacity may be attributed to solvent polarity and compound solubility [30].

The HPLC analysis of *M. andamanica* methanolic extract revealed protocatechuic acid and kaempferol as the major polyphenolic compounds. Protocatechuic acid is known for its strong antioxidant and antimicrobial properties, while kaempferol exhibits effective free radical scavenging and cytoprotective activities [28,29,31]. The predominance of these compounds in this study suggests that *M. andamanica* possesses a distinct chemical profile compared to other nutmeg species and are contributing to the observed biological activities of this plant.

Figure 4. Seedlings raised in the nursery under controlled conditions. (a): Established seedlings collected from fields, Seedlings germinated from seeds collected from (b): South Andaman Island and (c): Great Nicobar Island.

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Moderate levels of gentisic acid, chlorogenic acid, and ferulic acid further support the antioxidant potential of the extract, as these phenolic acids are reported to inhibit oxidative stress and lipid peroxidation [29,30]. The absence or low concentration of some compounds may be attributed to species-specific metabolism and environmental factors influencing secondary metabolite biosynthesis [27]. Previous studies on *Myristica* species have reported considerable variation in phytochemical composition depending on plant part and extraction method [5,7,27]. The HPLC results were consistent with the total phenolic and flavonoid contents and the moderate antioxidant and antimicrobial activities observed in DPPH, ABTS, and microbial assays. The combined action of detected phenolic acids and flavonoids may enhance the overall biological efficacy of the extract through synergistic interactions [31-33].

The moderate antimicrobial activity of the methanolic extract against both bacterial and fungal strains may be attributed to the synergistic effects of flavonoids, phenolic compounds, and terpenoids detected during phytochemical screening, which are known to disrupt microbial cell membranes and inhibit essential metabolic pathways [31-33]. Similar antimicrobial patterns have been reported in other members of the genus *Myristica*, indicating the presence of conserved bioactive mechanisms within the family [5,7].

The present conservation efforts are in agreement with earlier findings of Waman and Bohra [15], who standardized assisted regeneration techniques for *M. andamanica* through nursery raising. Their study demonstrated that appropriate seed selection and pre-sowing treatments significantly enhance germination and seedling vigour. The successful establishment of propagated plants in the present study further supports the effectiveness of integrated *ex-situ* and *in-situ* conservation approaches for long-term preservation of this vulnerable species. The integration of phytochemical research with conservation assessment enhances awareness regarding the medicinal and ecological value of endemic plants [12]. *Ex-situ* conservation success supports long-term preservation strategies.

V. CONCLUSION

The present study demonstrates that *Myristica andamanica* is a valuable source of bioactive phytochemicals with moderate antioxidant activity and notable antimicrobial potential. The high flavonoid content and the presence of major compounds such as protocatechuic acid and kaempferol support its therapeutic potential. HPLC profiling established a reliable phytochemical fingerprint for quality assessment and standardization. Furthermore, successful nursery-based propagation and habitat restoration confirm the effectiveness of integrated conservation strategies. The combined phytochemical, biological, and conservation findings emphasize the importance of sustainable utilization and long-term protection of this vulnerable endemic species. Future studies on isolation, characterization, and pharmacological evaluation of bioactive pure compounds are needed to facilitate drug development.

ACKNOWLEDGEMENTS

The authors are grateful to the Director of the Botanical Survey of India, Kolkata, for providing all facilities and support, and to the officials and staff of the Botanical Survey of India, Andaman and Nicobar Regional Centre, Port Blair, for their assistance during the plant sample collection. Acknowledgements are due to the Forest Department, Andaman and Nicobar Administration, for their necessary permission and support during the field survey.

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