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Research Article

CHEMICAL CHARACTERIZATION AND RADICAL SCAVENGING ACTIVITY OF *AMOMUM AGASTYAMALAYANUM* V. P. THOMAS AND M. SABU (ZINGIBERACEAE)

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ABSTRACT

Objectives: The objective of the study is to analyze the chemical composition of the methanolic extract of *Amomum agastyamalayanum*, an endemic species to the Western Ghats of South India, and to evaluate its antioxidant potential.

Methods: The chemical constituents were analyzed by gas chromatography/mass spectrometry (GC/MS) and high-resolution liquid chromatography (HR-LC)/MS. Quantitative estimation of major phytocomponents, including phenolics, terpenoids, and flavonoids, was done. Antioxidant activity was assessed using 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging, superoxide radical scavenging, lipid peroxidation inhibition, and ferric reducing antioxidant power (FRAP) assays.

Results: GC/MS and HR-LC/MS analyses revealed the presence of several bioactive components. The extract was found to be rich in terpenes such as 5β-cholestan-3-one ethylene acetal, 1-hexacosene, β-guaiene, caryophyllene, aromadendrene, α-cedrene oxide, and rosifoliol. The methanolic extract showed high phytochemical content, with phenolics (88.41±3.06 mg gallic acid equivalents [GAE]/g dry weight [DW]), terpenoids (106.67±3.83 mg linalool/g DW), and flavonoids (94.05±6.37 mg QE/g DW) as the major constituents. The extract exhibited strong antioxidant activity, with IC_{50} values of 47.26±1.00 µg/mL for DPPH, 40.66±3.53 µg/mL for superoxide radical scavenging, and 27.72±2.37 µg/mL for lipid peroxidation inhibition. FRAP was significant, showing a reducing capacity equivalent to 7.12±0.48 µmoL/mL $FeSO_4 \cdot 7H_2O$ at 10 µg/mL.

Conclusion: The methanolic extract of *A. agastyamalayanum* is rich in bioactive phytochemicals and exhibits potent antioxidant activity, highlighting its potential as a natural source of antioxidant compounds for pharmaceutical and nutraceutical applications.

Keywords: *Amomum agastyamalayanum*, Antioxidant, Chemical composition, Quantitative estimation.

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INTRODUCTION

Medicinal and aromatic plants are of considerable importance in traditional or indigenous healthcare systems, where they are used to treat a wide range of illnesses and they account for a sizable amount of the global pharma industry [1]. The family Zingiberaceae which is well known for its medicinal values, has a wide distribution throughout the tropics, particularly in Southeast Asia [2]. The ginger family consists of 53 genera and over 1200 species [3]. India is one of the richest and most diverse regions for Zingiberaceae, having 20 genera and around more than 200 species [4]. *Amomum* is the second largest genus of Zingiberaceae after *Alpinia.* It includes about 150–180 species, widely distributed in Southeast Asia [5]. In India, the genus is represented by 22 species, mostly restricted to North-Eastern India and Southern India [6]. Many species are medicinally used. The chemical composition and bioactivities of many species of *Amomum* have been reported by many researchers [7-15]. *Amomum agastyamalayanum* Thomas and Sabu is a herbaceous species, endemic to the Western Ghats, and is known only in the Agasthyamalai of South India. It is found growing as an undergrowth in the evergreen forests at an altitude of 900–1300 m. *A. agastyamalayanum* is characterized by the presence of smaller, diffuse habit, glabrous leaves with flat veins, slender inflorescence, small yellow flowers, and red, echinate fruits. Rhizome is stoloniferous, slender, creamy-white inside, covered with scales [16]. The chemical composition of the rhizome essential oil of this species has been reported [17]. Further chemical estimations and bioactivities of this species have not been reported yet. This study was conducted to identify the chemical constituents, quantify the major phytoconstituents, and evaluate the antioxidant potential of the methanolic rhizome extract of *A. agastyamalayanum*. Methanolic extracts are known to effectively solubilize polar compounds such as phenols and flavonoids, which play an important role in antioxidant activity.

METHODS

Preparation of methanolic extract

The plant was collected (CALI No. 123768) and authenticated by Dr. Thomas VP., Assistant Professor, Department of Botany, Catholicate College, Pathanamthitta, Kerala, India. The rhizomes of the plant materials were washed thoroughly, chopped into small pieces, and shade dried. The dried samples were powdered in a blender and sieved (100 mesh). The powdered plant material (10 g) was extracted with 100 mL of 100% methanol using a Soxhlet apparatus for 6 h under continuous reflux, as a single extraction cycle. The extract thus obtained was cooled, filtered, and concentrated by removing the solvent in a vacuum evaporator. The final dried extract was stored at 4°C in amber colored glass bottles. Further experiments were done using this dried extract.

Phytochemical characterization

The phytochemical constituents of plants can be detected employing various techniques. In the present study, qualitative tests, gas chromatography/mass spectrometry (GC/MS), and high-resolution liquid chromatography (HR-LC/MS) studies were used for the phytochemical characterization of methanolic extracts of *A. agastyamalayanum*. Estimation of major groups of compounds was also done.

GC/MS analysis

Bioactive volatile constituents were detected using gas chromatography coupled with mass spectrometry technique (GC/MS). The identification and quantification of the volatile fraction present in the species studied were done by GC/MS analysis of the plant methanol extract. Varian model CP-3800 GC interfaced with Varian Saturn 2200 ion trap spectrometer (Varian Inc., USA) operating at 70eV and 250°C, equipped with a CP-1177 Split/Splitless capillary injector and Combi PAL auto sampler, was used for GC/MS analysis. A cross-linked factor for capillary column, VF 5 ms with 30 m×0.25 mm ID and 0.25 µm film thickness was utilized. Helium was used as the carrier gas at a flow rate of 1 mL/min. Injection volume was 1 µL and the split ratio was 1:20. The temperature program for the chromatographic analysis was set at 60°C for 1 min (initial) and then heated up at a rate of 3°C/min to 280°C. The final temperature of 280°C was maintained for a total analysis time of 60 min. The mass spectra and retention indices of individual compounds were identified by comparing them with those in GC-MS libraries (National Institute of Standards and Technology) and with published data. Final quantification was carried out based on their percentage peak area.

HR-LC/MS analysis

Non-volatile components in the extract were detected by employing HR-LC/MS techniques. The analysis was performed using an Agilent 1290 Infinity Ultra-high performance liquid chromatography System, with 6550 iFunnel Q-TOFs (Agilent Technologies, USA). The chromatographic separation was achieved on a reverse-phase Agilent Zorbax-C18 110 Å analytical column (250×4.6 mm ID., 5 µm) operated at 40°C. Elution was achieved with a gradient mobile phase consisting of methanol (A) and 0.5 µM ammonium formate (B) in water at a flow rate of 1 mL/min. The gradient system used was as follows: Initially, 9% A and 91% B, which was increased to 100% A over 30 min. The mode of injection was Automatic liquid sampling. Electrospray ionization (ESI) was used as the interface and was operated in positive selected ion monitoring (SIM) mode. The analytes were monitored by tandem-mass spectrometry with positive ESI. The injection volume was 20 µL. The probe temperature was set at 500°C and needle voltage was set at 20 V. The cone voltage was set at 50 V for all SIM scans.

Quantitative estimation of major phytocomponents

Quantification of major classes of compounds such as phenols, flavonoids, terpenoids, alkaloids, and tannins was done as per the standard methods. Total phenolic content of the extract was determined using Folin–Ciocalteu (FC) reagent using standard procedures [18-20]. Gallic acid was used as the standard and calibration curve was plotted. The absorbance of the standard and the sample was measured at 760 nm. The phenolic content of the extract was expressed in mg of gallic acid equivalents per gram of dry weight (mg [GAE]/g DW) using a regression equation. Total terpenoid content was determined using colorimetric assay described previously [19,21]. Linalool was used as the standard to obtain the calibration curve, and the total terpenoid content in the extract was measured using the regression equation generated. The absorbance was measured at 538 nm. It was expressed as mg of linalool equivalent per gram of dry weight (mg linalool/g DW). Total flavonoid content of the extract was measured using the aluminum chloride colorimetric method [22]. Quercetin was used as the standard. The absorbance was measured at 510 nm. Calibration curve of quercetin was plotted and the total flavonoid content, expressed as mg quercetin equivalent per gram of dry weight (mg QE/g DW), was determined using regression equation. Estimation of total alkaloid content was done using standard procedures [23]. Caffeine was used as the standard, and absorbance was measured at 470 nm. Tannin content in the plant extract was quantified by previously established methods [24,25]. The absorbance was measured at 500 nm. Tannic acid was used as the standard for plotting the calibration curve and the total tannin content was expressed as mg of tannic acid equivalent per gram of dry weight (mg tannic acid/g DW) using the regression equation. Samples were analyzed in triplicate.

Antioxidant activity

The antioxidant potential of a plant is a multifaceted phenomenon. It cannot be analyzed by a single assay. Hence, the activity of the taxa was investigated using 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay, superoxide radical scavenging assay, lipid peroxidation assay, hydroxyl radical scavenging assay, and ferric reducing antioxidant power (FRAP) assay [26-30]. These assays were calibrated using standard antioxidants such as ascorbic acid and α-tocopherol. The percentage inhibition was calculated by comparing with that of the control.

$$\text{Percentage inhibition} = \frac{\text{Ac} - \text{As}}{\text{Ac}} \times 100$$

Where Ac is the absorbance of the control and as is the absorbance of the sample.

Antioxidants act as free radical scavengers. This property of antioxidants can be effectively used to test the scavenging potential of compounds. Five different concentrations (10, 20, 50, 100, and 200 µg/mL) of the extracts were used. All the experiments were performed in triplicate.

RESULTS

GC/MS analysis

The phytoconstituents of *A. agastyamalayanum* were assessed by GC/MS analysis. The gas chromatogram is represented in Fig. 1. The extract was found to have 29 compounds (Table 1). Compounds which belonged to various classes like terpenoids, alkaloids, phenolics, fatty acids, esters, etc., were identified. The major compounds were octadecyl palmitate (16.60%) and 3-methylbenzaldehyde (13.85%). Several other compounds occurred in considerable amounts, including 1,2-dimethyl indole and 5-methyl-2-furaldehyde, indicating the presence of nitrogenous and aromatic bioactivities. Terpenoids constituted a major fraction of the extract and included sesquiterpenes and triterpenes such as β-guaiene, caryophyllene, aromadendrene, α-cedrene oxide, rosifoliol, and 5β-cholestan-3-one ethylene acetal, many of which are known for their biological activities. Phenolic constituents were represented mainly by 2-methoxy-4-vinyl phenol (3.10%) and 2,6-dimethoxy phenol (5.09%). In addition, phytosterols such as campesterol and γ-sitosterol were also detected.

The GC/MS analysis revealed an array of bioactive compounds. Phenolic compounds and terpenes were the predominant constituents. Phytosterols were found in varying amounts in the extract.

HR-LC/MS analysis

HR-LC/MS analysis of the species was done to detect the presence of phytoconstituents that were not revealed through GC/MS. The components are listed in Table 2 and the chromatogram in Fig. 2. A total of 20 compounds were identified from the methanolic extract of

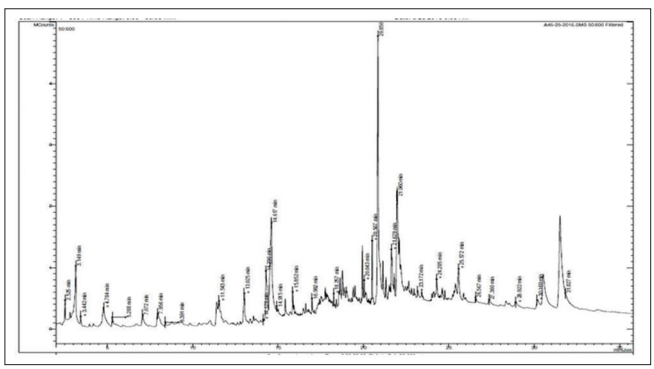

Fig. 1: Total ion chromatogram of methanolic extract of *Amomum agastyamalayanum* from gas chromatography-mass spectrometry analysis

Table 1: Phytochemical constituents of *Amomum agastyamalayanum* as revealed by GC/MS analysis

Sl. No.	RT (min)	Compound	Peak area (%)
1	3.442	Cyclobutanethiol	3.97
2	5.716 3-	Furanmethanol	2.41
3	7.956	5-Methyl-2-furaldehyde	9.10
4	8.391 2,4-	Dihydroxy-2,5-dimethyl-3 (2H)-furanone	1.19
5	11.543	2,5-Furandicarbaldehyde	6.27
6	13.094	1,2-Dimethyl indole	10.55
7	13.565	Caprylic acid	1.09
8	14.290	3-Methyl benzaldehyde	13.85
9	15.415	2-Methoxy-4-vinyl phenol	3.10
10	15.852	2,6-Dimethoxy phenol	5.09
11	16.621	Caryophyllene	0.36
12	16.982	Aromadendrene	0.70
13	17.126	Tricyclo [20.8.0.0 (7,16)] triacontane, 1 (22),7 (16)-diepoxy	0.66
14	18.257	β-Asarone	2.02
15	18.630	Boronal	0.62
16	18.772	β-Guaiene	1.10
17	19.356	2-[4-methyl-6-(2,6,6-trimethylcyclohex-1-enyl) hexa-1,3,5-trienyl] cyclohex-1-en-1	0.48
18	19.504	α-Cedrene oxide	0.60
19	19.936	4a, 5,6,7,8,8a-Hexahydro-6-[1(hydroxymethyl) ethenyl]-4,8a-dimethyl-2-naphthalenone	1.92
20	20.043	Rosifoliol	1.40
21	20.507	Methyl 13-methylpentadecanoate	2.15
22	20.858	Octadecyl palmitate	16.60
23	21.960	6,9,12,15-Docosatetraenoic acid methyl ester	6.70
24	22.102	2-Monoolein	1.20
25	22.819	5β-Cholestan-3-one ethylene acetal	0.43
26	23.408	1-Hexacosene	0.81
27	24.611	2-Nonadecanone 2,4-dinitrophenylhydrazine	0.62
28	30.169	Campesterol	0.20
29	31.500	γ-Sitosterol	4.81

RT: Retention time, Compounds were tentatively identified based on mass spectral matching with the NIST library (match factor>85%) and comparison of calculated retention indices with literature values, allowing a tolerance of±10 RI units. The relative percentages were calculated from GC peak areas without correction factors. NIST: National Institute of Standards and Technology, GC/MS: Gas chromatography/mass spectrometry

Table 2: Phytochemical constituents of *Amomum agastyamalayanum* identified through HR- LC/MS analysis

Sl. No.	RT (min)	Compound	Class
1	1.308	2-Methyl valeric acid	Carboxylic acid
2	1.444	Norcotinine	Alkaloid
3	1.585	Choline	Vitamin
4	7.375	Phe GluGln	Biopeptide
5	7.438	Epothilone D	Polyketide
6	8.226	Rutin	Flavonoid
7	9.528	Val Asn Asp	Biopeptide
8	10.584	Asp Lys Glu	Biopeptide
9	11.062	Ser Asn Leu	Biopeptide
10	11.152	Carbetapentane	Ester
11	11.314	Selinidin	Coumarin
12	12.874	Swietenine	Triterpenoid
13	13.637	Resveratrol	Phenol
14	15.431	Atractyloside	Heteroglucoside
15	15.751	Clovanediol diacetate	Sesquiterpene
16	17.577	Dehydroepiandrosterone	Sterol
17	18.07	Koparin 2'-methyl ether	Ether
18	18.84	Ala Pro Arg	Biopeptide
19	18.853	Punctaporin B	Terpene
20	22.176	11-Hydroxy palmitic acid	Fatty acid

HR-LC/MS: High-resolution liquid chromatography/mass spectrometry, RT: Retention time

Table 3: Quantitative estimation of major phytocomponents in the extract of *Amomum agastyamalayanum*

Sl. No.	Phytocomponent	Quantity
1	Phenolic content (mg GAE/g DW)	88.41±3.06
2	Terpenoid content (mg linalool/g DW)	106.67±3.83
3	Flavonoid content (mg QE/g DW)	94.05±6.37
4	Tannin content (mg tannic acid/g DW)	17.20±1.39
5	Alkaloid (mg caffeine/g DW)	1.75±0.36

GAE: Gallic acid equivalents, QE: Quercetin equivalents, DW: Dry weight. Values expressed as mean±standard deviation

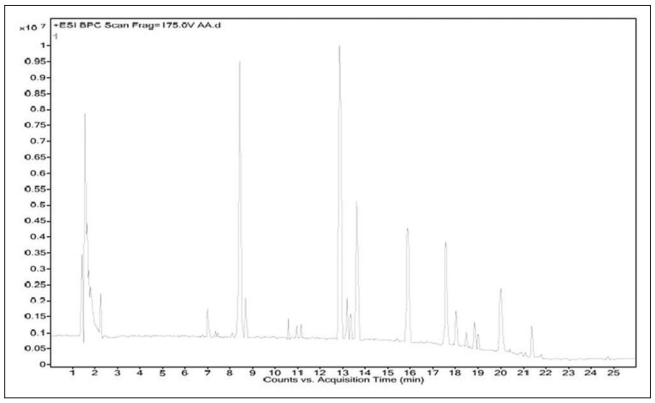

Fig. 2: Liquid chromatogram of the methanolic extract of *Amomum agastyamalayanum*

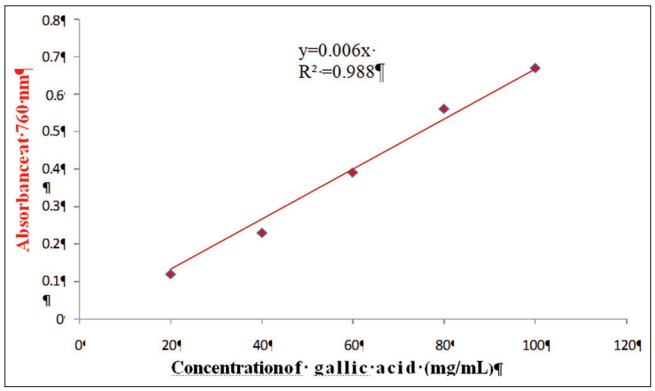

Fig. 3: Standard calibration curve of gallic acid for the estimation of total phenol content

A. agastyamalayanum which belonged to major classes of compounds such as alkaloids, flavonoids, terpenoids, and phenols. A coumarin compound, selinidin, was also identified. Interestingly, several biopeptides were also identified from the extract, many of which are known to be biologically very active.

Compounds were tentatively identified based on accurate mass measurements obtained from HR-LC/MS analysis and by comparison with available HR-MS databases and published literature; Tripeptides are represented using standard three-letter amino acid codes.

Quantitative estimation of major phytocomponents

Estimation of phytocomponents was performed in the extract and the results are summarized in Table 3.

The total phenolic content in the extract was estimated using the FC reagent and was expressed as GAE using the standard equation from the linear calibration curve (Fig. 3). The phenolic content was observed to be 88.41±3.06 mg GAE/g DW (mean±standard deviation [SD]). The total terpenoid content in the extract was expressed in terms of mg linalool/g DW. The content was calculated from the standard curve

(Fig. 4). The terpenoid content was estimated to be 106.67±3.83 mg linalool/g DW (mean±SD). The total flavonoid content was established using aluminum chloride and was expressed in terms of mg QE/g DW from the standard curve (Fig. 5). The content in the extract was estimated to be 94.05±6.37 mg QE/g DW (mean±SD). The tannin content in the extract was estimated using vanillin reagent and was expressed as mg tannic acid/g DW from the standard curve (Fig. 6). The tannin content in the extract was 17.20±1.39 mg tannic acid/g DW (mean±SD). The alkaloid content in the extract was estimated as caffeine equivalent and was expressed as mg caffeine/g DW of the extract from the standard curve (Fig. 7). The alkaloid content was found to be 1.75±0.36 mg caffeine/g DW (mean±SD).

The methanolic extract of the taxa was thus found to contain a considerable amount of phytocomponents such as phenols, terpenoids, and flavonoids. Tannins and alkaloids were also found to be present, even though in lesser amounts.

Antioxidant activity

In DPPH radical scavenging activity, *A. agastyamalayanum* showed a range of percentage inhibitions, 18.24±6.67–90.74±0.78 for 10 µg/mL–200 µg/mL of the extracts. The IC_{50} value was 47.26±1.00. The maximum value for superoxide radical scavenging activity was found to be 75.32±4.65, and the IC_{50} values were seen to be 40.66±3.53, for the species. In the lipid peroxidation assay, the inhibition percentage was significant, with an IC_{50} value of 27.72±2.37. The inhibition percentage was in the range of 5.27±3.70–73.00±1.49 for the various concentrations. The methanolic extracts at 10 µg/mL were analyzed for the ferric ion-reducing property. The concentration of antioxidants was measured as the ferric-TpTz reducing ability equivalent to that of 1 µmoL/mL $FeSO_4.7H_2O$ employing the corresponding regression equation (Fig. 8). The ferric reducing activity of 10 µg/mL of the methanolic extract was equivalent to the reducing power of 7.12±0.48 of $FeSO_4.7H_2O$ which indicates significant FRAP activity of the species (Table 4).

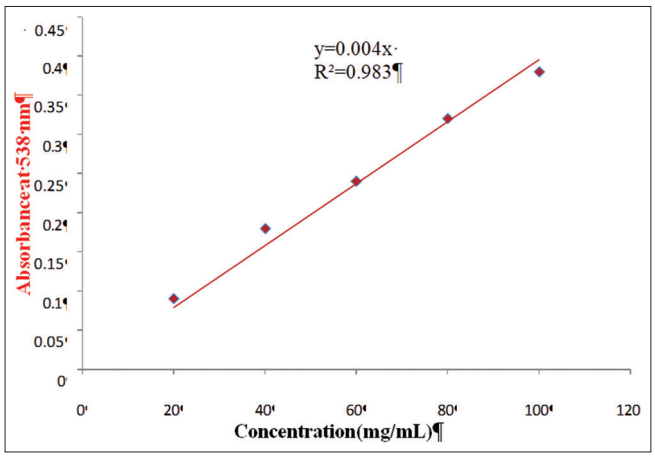

Fig. 4: Standard calibration curve of linalool for the estimation of total terpenoid content

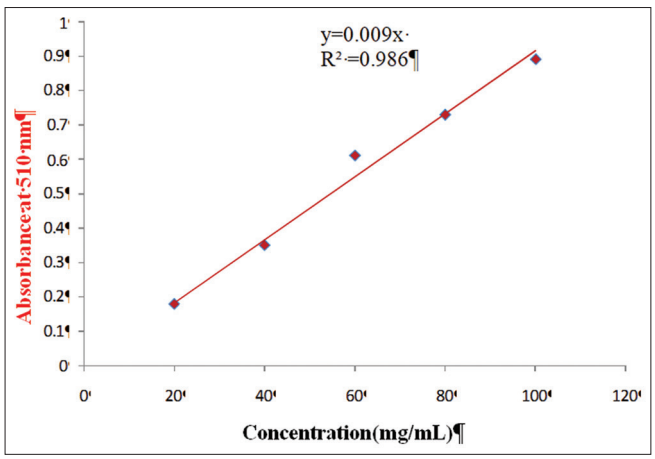

Fig. 5: Standard calibration curve of quercetin for the estimation of total flavonoid content

DISCUSSION

Secondary metabolites found in plants are mainly of three primary categories: Alkaloids, phenols, and terpenoids among others [31]. Natural antioxidants, such as flavonoids, coumarins, tocopherols, carotenoids, and phenolic acids, are widely distributed in medicinal plants [32]. The methanolic extract of *A. agastyamalayanum* was found to have 29 compounds. The extract exhibited high total phenolic and flavonoid contents, which can be directly correlated with its potent antioxidant activity observed in the present study. Phenolic compounds are known to act as effective electron donors, and the strong FRAP of the extract is therefore likely a direct reflection of its phenolic compounds. A significant relationship between total phenolic content and ferric reducing capacity has been widely reported, reinforcing the role of polyphenols as major contributors to antioxidant potential [33-37]. The flavonoid-rich nature of the extract further strengthens this association, as flavonoids such as rutin and quercetin derivatives possess multiple hydroxyl groups capable of scavenging free radicals and chelating pro-oxidant metal ions [38-40]. The comparatively low IC_{50} values obtained in free radical scavenging assays indicate a high antioxidant efficiency of the extract.

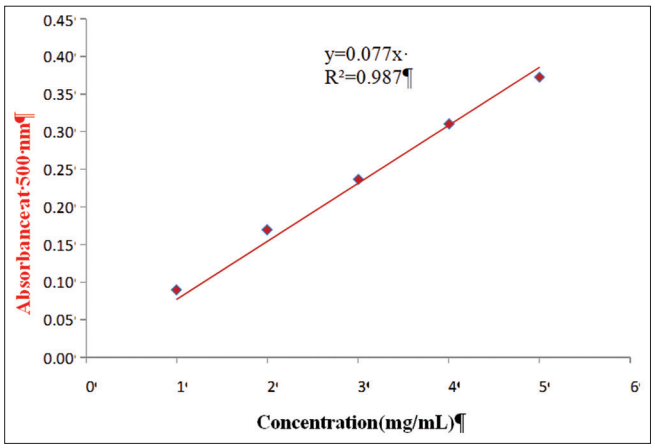

Fig. 6: Standard calibration curve of tannic acid for the estimation of total tannin content

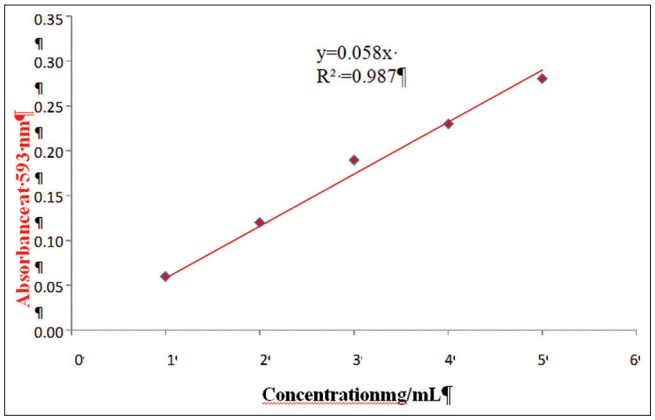

Fig. 7: Standard calibration curve of caffeine for the estimation of alkaloid content

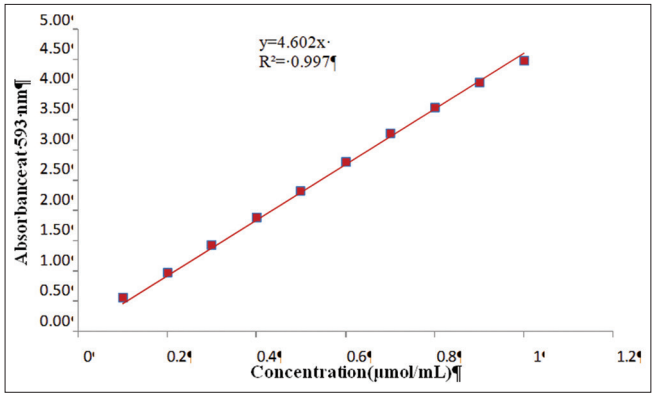

Fig. 8: Standard calibration curve of $FeSO_4 7H_2O$ for the ferric reducing antioxidant power assay

Table 4: IC_{50} of methanolic extract of *Amomum agastyamalayanum* in different antioxidant assays.

Sl. No.	Assays	IC_{50} value
1	DPPH radical scavenging	47.26±1.00
2	Superoxide radical scavenging	40.66±3.53
3	Lipid peroxidation	27.72±2.37
4	Hydroxyl radical scavenging	15.87±2.23
5	Ferric ion reducing activity	7.12 ± 0.48

IC_{50}-Inhibitory concentration at 50% activity. Ferric iron reducing activity expressed as EC±SD (µmoL/mL). EC: Effective concentration with ferric-TpTz reducing ability. Values expressed as mean±standard deviation. SD: Standard deviation, DPPH: 2,2-Diphenyl-1-picrylhydrazyl

The extract was rich in a variety of terpenes. Many of the terpenes present in this extract are proven to be of therapeutic value by earlier workers [41-46]. Another compound, selinidin from *A. agastyamalayanum,* is a coumarin. Its topical application inhibited croton oil-induced ear dermatitis in mice. It induced a significant edema reduction as comparable to the reference drug indomethacin [47]. Resveratrol, a phytoalexin in *A. agastyamalayanum*, has been found to be antimutagenic and anticarcinogenic in diverse animal and *in vitro* system studies conducted earlier [48].

An interesting observation in the HR-LC/MS analysis was the detection of bioactive peptides. Bioactive peptides have been defined as specific protein fragments that have a positive impact on body functions or conditions and may ultimately influence health [49,50]. They are increasingly recognized for their antioxidant, antimicrobial, anti-inflammatory, and anticancerous activities [51,52].

In recent years, the search for plant products possessing antioxidant properties has been on the rise due to their potential use in the therapy of various chronic and infectious diseases. In the present study, the results of the various antioxidant assays vary considerably, which emphasize that a single assay cannot be relied upon for establishing the antioxidant property of a compound. However, the results suggest that the extracts of *A. agastyamalayanum* are highly effective and are a promising antioxidant.

The phytochemical analysis of the species under study revealed the presence of potent phenolics and flavonoids, which account for the scavenging property of these plants. The results are in corroboration with earlier reports on herbs and spices of Zingiberaceae which have been used for long in folk medicines that function as excellent antioxidants [53-55]. Although the use of multiple *in vitro* antioxidant assays provides a broad assessment of radical scavenging and reducing capacities, such assays do not fully replicate the complexity of biological systems. Factors such as bioavailability, metabolism, and cellular interactions cannot be inferred from chemical assays alone. Therefore, while the present findings strongly support the antioxidant potential of *A. agastyamalayanum*, further validation through cell-based assays and *in vivo* studies is necessary to establish its physiological relevance and therapeutic applicability.

Overall, this study provides the first detailed chemical profiling and antioxidant evaluation of *A. agastyamalayanum*. The strong correlation between its high phenolic and flavonoid contents and potent antioxidant activity highlights its promise as a natural source of bioactive compounds and lays a solid foundation for future pharmacological investigations.

CONCLUSION

The methanolic rhizome extract of *A. agastyamalayanum* contains bioactive phytochemicals and shows significant antioxidant activity in various assays. The observed reducing capacity is likely due to its phenolic and flavonoid constituents, indicating its potential as a natural antioxidant source.

ACKNOWLEDGMENT

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AUTHORS' CONTRIBUTIONS

The first author designed the study, collected the data, and performed the experiments. The second author analyzed the data and prepared the initial draft of the manuscript. The third author contributed to data analysis and interpretation and edited the draft. All authors read, revised, and approved the final version of the manuscript.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial, or otherwise.

FUNDING

Nil.

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