RESEARCH PAPER

Metabolite Profiling Of Amomum Muricatum Using Gc/Ms and Hr-Lc/Ms: Insights into Antioxidant and Anti-Inflammatory Potential

Sinitha K. [1*,2], Archana E. R.[1,3], Renjana P. K. [1,2] and John Ernest Thoppil[1]

[1]*Cell & Molecular Biology Division, Department of Botany, University of Calicut, Kerala, India, 673635*
[2]*Department of Botany, Govt. Arts & Science College Calicut, Kozhikode, Kerala, India, 673018*
[3]*Department of Botany, Providence Women's College (Autonomous), Kozhikode, Kerala, India, 673010*
[*]***Corresponding Author:*** *sinithak@gmail.com*

Received: 24th April, 2026; Revised: 20th May 2026; Accepted: 30th June, 2026; Available Online: 10th July, 2026

ABSTRACT

The present study aimed to investigate the phytochemical composition, antioxidant potential, and anti-inflammatory activity of the methanolic extract of Amomum muricatum. Phytochemical profiling using Gas Chromatography/Mass Spectrometry and High resolution- Liquid chromatography/ Mass Spectrometry was done. The in vitro anti-inflammatory potential was screened using three biochemical assays viz., proteinase inhibitory assay, albumin denaturation, membrane stabilization and Cyclooxygenase-2 assay was done on RAW 264.7 macrophage cell lines. GC/MS identified 17 compounds and HR- LC/MS revealed 29 compounds. Quantitative estimation showed high levels of phenolics (95.01 ± 1.01 mg GAE/g DW), terpenoids (87.24 ± 3.50 mg/g DW), and flavonoids (65.85 ± 1.75mg QE/g DW), with lower amounts of tannins and alkaloids. The extract exhibited significant antioxidant activity in multiple in vitro assays. DPPH radical scavenging showed 79.31 ± 2.98% inhibition (IC_{50}: 56.82 ± 3.09µg/mL), while lipid peroxidation and hydroxyl radical scavenging assays demonstrated strong activity with IC_{50} values of 12.89 ± 1.02µg/mL and 9.16 ± 2.38µg/mL, respectively. The extract also showed appreciable ferric reducing antioxidant power. Anti-inflammatory activity was confirmed through inhibition of protein denaturation, proteinase activity, and membrane stabilization, with IC_{50} values ranging from 43.07 to 72.67µg/mL. Furthermore, significant inhibition of COX-2 activity (65.20 ± 1.19% at 200µg/mL; IC_{50} - 58.11 ± 1.76 µg/mL) was observed in RAW 264.7 macrophage cell lines. The findings indicate that Amomum muricatum possess potent antioxidant and anti-inflammatory properties, likely mediated by its diverse phytochemical constituents.

Keywords: Antioxidant, Anti-inflammation, Cyclooxygenase-2, Macrophage cell line, Phytochemistry, Zingiberaceae

How to cite this article: Sinitha K, Archana ER, Renjana PK, Thoppil JE, Metabolite Profiling Of Amomum Muricatum Using Gc/Ms and Hr-Lc/Ms: Insights into Antioxidant and Anti-Inflammatory Potential. Int J Drug Deliv Technol. 2026;16(7): 800-807; DOI: 10.25258/ijddt.16.7.84
Source of support: Nil.
Conflict of interest: None

INTRODUCTION

The family Zingiberaceae is well known for its medicinal importance and diverse phytochemical composition. The genus Amomum, the second largest genus in the family after Alpinia, comprises about 150–180 species distributed widely across Southeast Asia [1]. Species of Amomum are traditionally used for the treatment of heart-related ailments and as stomachic, antiemetic, antibilious, astringent, and alexipharmic agents, and also used in the management of cough, indigestion, biliousness, abdominal pain, and liver congestion [2]. Many species of Amomum have been reported to be medicinally important [3-9].

Amomum muricatum is a herbaceous plant with slender, stoloniferous rhizome and densely packed yellow flowers with red streaks. Volatile constituents from the leaf oil of *A. muricatum* has been reported[10]. Further phytochemical characterization and bioactivity evaluation of this species remain limited. Therefore, the present study was conducted to identify the chemical constituents using GC/MS and HR-LC/MS analysis, quantify the major phytoconstituents and evaluate the antioxidant and anti-inflammatory potential of methanolic rhizome extract of *Amomum muricatum*.

MATERIALS AND METHODS

Collection and authentication

The plant was collected from Wayanad and Idukki districts of Kerala and was authenticated by the Angiosperm Taxonomy Division, Department of Botany, University of Calicut and Department of Botany, Catholicate College, Pathanamthitta, Kerala. Voucher specimens (CALI No. 123763**)** were deposited at the Herbarium (CALI) of Department of Botany, University of Calicut, Kerala, India.

Preparation of methanolic extract

The rhizome of the plant materials were collected, washed thoroughly, chopped into small pieces and shade dried. The dried samples were powdered in a blender. The

**Author for Correspondence: sinithak@gmail.com*

powdered plant materials, 10 g of each were extracted with 100 ml of 100% methanol for 6 h using Soxhlet apparatus. The extract thus obtained was cooled, filtered and concentrated by removing the solvent in a vacuum evaporator. The final dried extracts were stored at 4 °C in amber coloured glass bottles. Further experiments were done using this dried extract.

Phytochemical profiling

Detailed phytochemical analysis for the detection of specific compounds in the extract was done by the analytical technique GC/MS. Bioactive volatile constituents were detected using gas chromatography coupled with mass spectrometry technique (GC/MS).

The model Varian CP-3800 GC with Varian Saturn 2200 Ion Trap Spectrometer (ITMS) operating at 70eV and 250 °C equipped with a CP-1177 Split/Splitless capillary injector and Combi PAL auto sampler was used for the analysis. The carrier gas was Helium. The linear retention indices of the constituents were compared for identification. The MS fragmentation patterns were matched with National Institute of Standards and Technology and mass spectra libraries for identification of individual components. Final quantification was carried out based on their percentage peak area.

Non-volatile components in the extract was detected by employing High resolution liquid chromatography coupled with mass spectrometry (HR-LC/MS) techniques. This technique is a widely used powerful tool to establish the constituents of extracts. HR-LC/MS analysis of the plant extract was performed using an Agilent 1290 Infinity UHPLC System, 1260 infinity Nano HPLC with Chipcube, 6550 iFunnel Q-TOFs (Palo Alto, CA, USA).

Estimation of major classes of compounds such as phenols, flavonoids, terpenoids, alkaloids and tannins were done as per the standard methods. Total phenolic content of the extract was determined using Folin-Ciocalteu reagent (FC) [11]. Gallic acid was used as the standard and calibration curve was plotted. The phenolic content of the extract was expressed in mg of gallic acid equivalents per gram of dry weight (mg GAE/g DW) using regression equation. Total flavonoid content of the extract was measured according to the method of Chang et al. [12]. Quercetin was used as the standard. 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. The total terpenoid content of the plant extract was estimated by the method described by Ghorai et al. [13]. 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. It was expressed as mg of linalool equivalent per grams of dry weight (mg linalool/g DW). Estimation of total alkaloid content was done by standard protocol [14]. Caffeine was used as the standard. Tannin content in the plant extract was quantified by the method of Bainbridge et al. [15]. 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 grams of dry weight (mg tannic acid/g DW) using the regression equation. Samples were analysed in triplicate.

Antioxidant activity

Screening for the antioxidant property has been done by various assays using the methanolic extract. Antioxidants act as free radical scavengers. This property of antioxidants can be effectively used to test scavenging potential of compounds. Five different assays were used on various concentrations (10, 20, 50, 100 and 200µg/mL) of the extract. DPPH radical scavenging potential of the extracts was measured by the method proposed by Coruh et al. [16]. DPPH has an absorption peak at 515 nm and its purple colour rapidly fades when it encounters any proton radical scavenger. Ascorbic acid was taken as the standard. The percentage inhibition was calculated by comparing with that of the control. Reduction of nitroblue tetrazolium (NBT) by the superoxide generated from riboflavin is the basis of estimation of superoxide (O_2^-) radical scavenging potential of the extract. The method described by Mc Cord and Fridovich [17] was followed. Hydroxyl radical scavenging activity was measured by studying the competition between deoxyribose and test compounds for hydroxyl radicals generated from the Fe^{3+}/Ascorbate/EDTA/H_2O_2 system (Fenton reaction), as proposed by Kunchandy and Rao [18]. Lipid peroxidation was induced in rat liver homogenate by the method described by Bishayee and Balasubramanian [19]. Percentage inhibition was calculated by the given formula.

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

Where, Ac is the absorbance of the control and As is the absorbance of the sample.

FRAP assay is based on the reduction capacity of the extract wherein the Fe^{3+}-TpTz complex is reduced to ferrous (Fe^{2+}) form, visualised by an intense blue coloured complex. The assay proposed by Benzie and Strain [20], followed with modifications by Pulido et al. [21] was used. All the experiments were performed in triplicate.

Anti-inflammatory activity

The *in vitro* anti-inflammatory potential of the species of *Amomum* was screened using three biochemical assays *viz*., proteinase inhibitory assay, albumin denaturation, and membrane stabilization assays. Three concentrations of the extract (50, 100 and 200 µg/mL) was tested. The percentage inhibition and IC_{50} value of each experiment were also calculated. Aspirin was taken as the standard drug. Proteinase inhibitory test was performed according

to the modified method of Oyedepo and Femurewa [22]. The percentage of proteinase inhibitory activity was calculated by the formula:

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

Where, Ac is the absorbance of the control and As is the absorbance of the sample.

Method of Mizushima and Kobayashi was followed for the assay of inhibition of albumin denaturation. [23] Percentage inhibition of protein denaturation was calculated as mentioned earlier. For Membrane stabilization test fresh whole human blood (10 mL) was collected and transferred to the heparinized centrifuged tubes. The tubes were centrifuged at 3000 rpm for 10 min and were washed three times with equal volume of normal saline. The volume of the blood was measured and reconstituted as 10% v/v suspension with normal saline [24]. Heat induced haemolysis and percentage inhibition of haemolysis was calculated by the formula mentioned earlier [25].

Anti-inflammatory activity on RAW 264.7 macrophage cell lines

Anti-inflammatory activity was tested on RAW 264.7 macrophage cell lines. The effect on inhibition of COX-2, was analysed.

Cell culture and LPS stimulation

The RAW 264.7 macrophage cell lines were procured from National Centre for Cell Sciences (NCCS), Pune, India and maintained in Dulbecco's modified eagle's medium (DMEM) supplemented with 10% fetal bovine serum and 100 U/mL of streptomycin and maintained at 37 °C in a humidified CO_2 incubator. The cells were grown to 60% confluency in culture flasks. The cells were then seeded in 96 well microtitre plate followed by activation with 1µL lipopolysaccharide (LPS).

Preparation of plant extract

A stock of the plant solution at 1mg/mL was prepared in 0.1% DMSO. Various concentrations of the extract (50, 100 and 200µg/mL) were prepared from this stock solution using appropriate volume of cell culture medium DMEM. LPS stimulated RAW 264.7 cells were exposed to different concentrations of the extract. Diclofenac sodium, a standard anti-inflammatory drug and DMSO (0.1%) in varying concentrations, corresponding to the sample were used as positive control and negative control respectively. The treated cells were incubated for 24 h. After incubation the COX activity was analysed using the cell lysate.

Cyclooxygenase (COX) activity

COX assay was performed by the method of Walker and Gierse [26]. The cell lysate was incubated in Tris-HCl buffer (pH 8), glutathione 5 mM/L, and haemoglobin 5 mM/L for 1 min at 25 °C. The reaction was initiated by the addition of arachidonic acid (200 mM/L) and terminated after 20 min incubation at 37 °C by the addition of 10% trichloroacetic acid in 1 N hydrochloric acid. COX activity was determined by reading absorbance at 632 nm. The percentage inhibition of COX was calculated by the following formula:

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

Where, Ac is the absorbance of the control and As is the absorbance of the sample.

Statistical Analysis

All analysis were done in triplicate, and data were depicted as the Mean ± Standard Error (SE). The statistical significance was calculated using a one-way Analysis of Variance (ANOVA). The statistical differences were taken as significant at $p<0.05$.

RESULTS

Phytochemical profiling

In GC/MS analysis (Table 1) the extract of *A. muricatum* showed the presence of potent compounds with **2,3-dihydro-1-benzofuran (30.82%) being the major one.** The amount of hexanoic acid and the fatty acid **octadecyl palmitate** were also found to be noteworthy with 7.32 and 9.70% respectively. The extract contained terpenes like 2,3-oxidosqualene and D:A-friedoolean-7-ene. Alcohols like Z,E-2,13-octadecadien-1-ol and ergost-8(14)-en-3-ol were seen in minor amounts. A total of 17 compounds were detected from the extract.

HR-LC/MS analysis of the species of *Amomum* was done to detect the presence of phytoconstituents which were not revealed through GC/MS. The components are enlisted in Table 2. The extract was found to have 29 compounds, which belonged to various classes *viz*., flavonoids, phenols, alkaloids, terpenoids etc. Several biopeptides were also identified from the extract, many of which are known to be biologically very active. The analysis revealed that the extract contained bioactive compounds.

Estimation of phytocomponents was performed in the extract and the results are given in Table 3. The Folin-Ciocalteu reagent was used for the estimation of total phenolic content in the extract, and was expressed as gallic acid equivalents. The phenolic content was observed to be 95.01 ± 1.01 mg GAE/g DW (mean ± SE). The terpenoid content was found to be 87.24 ± 3.50 mg linalool/g DW (mean ± SE). The total flavonoid content was established using aluminium chloride and was expressed in terms of quercetin equivalent (mg QE/g DW). The content in the extract was estimated to be 65.85 ± 1.75 mg QE/g DW (mean ± SE). The tannin content in the extract was estimated using vanillin reagent and was expressed as mg tannic acid/g DW. The tannin content in the extract was 3.99 ± 0.89 mg tannic acid/g DW (mean ±

SE). The alkaloid content in the extract was estimated as caffeine equivalent and was expressed as mg caffeine/g DW of the extract. The alkaloid content was found to be 2.01 ± 0.47 mg caffeine/g DW(mean ± SE). The methanolic extract of the taxa was thus found to contain considerable amount of phytocomponents like phenols, terpenoids and flavonoids. Tannins and alkaloids were also found to be present, even though in lesser amounts.

Table 1: Phytochemical constituents of *A. muricatum* as revealed by GC/MS analysis

Sl No.	RT (min)	Compound	Class	Peak area %
1	5.552	Hexanoic acid	Carboxylic acid	7.32
2	9.998	Cyclotene	Organic compound	3.43
3	12.411	3-Ethyl-2-hydroxy-2-cyclopenten-1-one	Alkene	1.56
4	14.280	2,3-Dihydro-1-benzofuran	Phenolic compound	30.82
5	15.071	5-Heptyl resorcinol	Phenol	2.21
6	16.306	1-(3,6,6-Trimethyl-1,6,7,7a-tetrahydrocyclopenta [c]pyran-1-yl) ethanone	Ketone	1.83
7	20.314	Z,E-2,13-Octadecadien-1-ol	Alcohol	3.1
8	20.374	Z-7-Hexadecenoic acid	Fatty acid	0.62
9	20.510	Ergost-8(14)-en-3-ol	Alcohol	0.35
10	20.858	Octadecyl palmitate	Ester	9.70
11	21.323	2,3-Oxidosqualene	Triterpene	0.14
12	21.707	Arachidyl oleate	Ester	0.77
13	21.965	Monolinolein	Triglyceride	3.80
14	22.660	D:A-Friedoolean-7-ene	Terpenoid	0.52
15	25.416	4,4-Dimethylandrost-5-en-17-ol	Steroid	0.32
16	30.574	4,4-Dimethylcholesta-22,24-dien-6-ol	Sterol	0.16
17	31.500	γ-Sitosterol	Sterol	1.21

Table 2. Phytochemical constituents detected in *A. muricatum* through HR-LC/MS

Sl No.	RT (min)	Compound	Class of compound
1	0.908	Betaine	Amino acid derivative
2	1.901	Carnitine	Amino acid
3	2.407	N-Acetyl-L-glutamic acid	Amino acid
4	2.747	(S)-2-Hydroxyglutarate	Hydroxy acid
5	2.80	Lactaldehyde	Aldehyde
6	2.906	3,7-Epoxycaryophyllan-6-one	Terpene
7	2.958	2-Hydroxyadipic acid	Amino acid
8	4.909	Gln Cys Cys	Biopeptide
9	5.341	Pantothenic Acid	Vitamin B5
10	5.881	Butyryl-L-carnitine	Carnitine derivative
11	5.92	Dehydrorotenone	Flavonoid
12	6.059	Gentisic acid	Phenolic acid
13	6.11	Pyrocatechol	Phenolic compound
14	6.292	Gly Met Phe	Biopeptide
15	6.723	Tyr Thr Asn	Biopeptide
16	6.761	Leucine	Amino acid
17	6.77	Lys Cys His	Biopeptide
18	6.949	His Thr Lys	Biopeptide
19	7.325	m-Salicylic acid	Phenolic acid
20	7.903	Glu Tyr Lys	Biopeptide
21	8.12	His Asn Ile	Biopeptide

22	8.246	Asn Phe Thr	Biopeptide
23	8.405	Asn Tyr Phe	Biopeptide
24	8.524	α-Mangostin	Xanthone
25	8.541	Tyr Asn Pro	Biopeptide
26	8.573	Benzoic acid	Carboxylic acid
27	8.852	p-Coumaric acid	Phenolic compound
28	8.857	Pro Tyr Gln	Biopeptide
29	10.742	Lys Phe Glu	Biopeptide

Table 3. Quantitative estimation of major phytocomponents in the extract of *A. muricatum*

Sl No.	Phytocomponent	Quantity
1	Phenolic content (mg GAE/g DW)	95.01 ± 1.01
2	Terpenoid content (mg linalool/g DW)	87.24 ± 3.50
3	Flavonoid content (mg QE/g DW)	65.85 ± 1.75
4	Tannin content (mg tannic acid/g DW)	3.99 ± 0.89
5	Alkaloid (mg caffeine/g DW)	2.01 ± 0.47

GAE – Gallic acid equivalents; QE – Quercetin equivalents; DW – Dry Weight; values expressed as mean ± Standard Error

Antioxidant activity

The antioxidant potential of the selected taxa of *Amomum* was investigated using DPPH radical scavenging assay, superoxide radical scavenging assay, lipid peroxidation assay, hydroxyl radical scavenging assay and FRAP assay. These assays were calibrated using standard antioxidants like ascorbic acid and α-tocopherol. DPPH radical scavenging activity of methanolic extract was performed with five different concentrations (10µg/mL to 200µg/mL). The extract showed a dose dependent effect. Ascorbic acid was taken as the standard. The extract showed 79.31 ± 2.98% inhibition at 200µg/mL. The IC_{50} was 56.82 ± 3.09 µg/mL. In super oxide radical scavenging assay the maximum value was found to be 70.75 ± 5.42%. The IC_{50} value was found to be 40.59 ± 3.25µg/mL. In lipid peroxidation assay, significant activity was observed with IC_{50} of 12.89 ± 1.02µg/mL and a range of inhibition of 15.09 ± 1.59 to 93.31 ± 1.60% for the concentrations 10µg/mL to 200µg/mL. The standard used was α-tocopherol. In scavenging hydroxyl radical, the IC_{50} was 9.16 ± 2.38µg/mL and inhibition percentage of 21.33 ± 1.59 to 93.34 ± 1.60. The extract at 10µg/mL was analysed for the ferric ion reducing property. The concentration of antioxidant was measured as the ferric-TpTz reducing ability equivalent to that of 1µmol/mL $FeSO_4.7H_2O$. The ferric reducing activity of 10µg/mL of the methanolic extract was equivalent to the reducing power of 9.01 ± 0.82µmol/mL of $FeSO_4.7H_2O$ for *A. muricatum* (Table 4).

Table 4. IC_{50} of methanolic extract of *A. muricatum* in different antioxidant assays.

Sl No.	Assays	IC_{50} value
1	DPPH radical scavenging	56.82 ± 3.09
2	Superoxide radical scavenging	40.59 ± 3.25
3	Lipid peroxidation	12.89 ± 1.02
4	Hydroxyl radical scavenging	9.16 ± 2.38
5	Ferric ion reducing activity	9.01 ± 0.76

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 error

Anti-Inflammatory Activity

The *in vitro* anti-inflammatory potential of the selected species of *Amomum* was screened using three biochemical assays *viz*., inhibition of protein denaturation, proteinase inhibition and membrane stabilization assays. The percentage of inhibition of albumin denaturation was analysed for the extract. The standard, diclofenac was used at 50 µg/mL. The extract of *A. muricatum* showed an inhibition rate of 25.23 ± 1.53 and 72.91 ± 1.58% for the 50 µg/mL to 200 µg/mL concentrations and IC_{50} was 43.07 ± 0.46 µg/mL. The potential for inhibiting proteinase enzyme by the extract was evaluated using three concentrations of the extract (50, 100 and 200 µg/mL). The inhibition rate was seen as 73.44 ± 1.20 for 200 µg/mL and IC_{50} 61.58 ± 0.97 µg/mL. Inhibition of heat induced haemolysis and thus the membrane stabilisation potential of the extract was analysed for three concentrations (50, 100, and 200 µg/mL). The extract showed a dose dependent activity. The percentage of inhibition was 65.01 ± 1.55 and IC_{50} was 72.67 ± 0.53 (Table 5).

Table 5. In vitro anti-inflammatory activity of *A. muricatum*

Sl No.	Assays	IC_{50} value

1	Protein denaturation	43.07 ± 0.46
2	Proteinase inhibition	61.58 ± 0.97
3	Membrane stabilisation	72.67 ± 0.53
4	COX inhibition	58.11 ± 1.76

IC_{50} - inhibitory concentration at 50% activity. Values expressed as mean ± Standard error

Anti-inflammatory activity on RAW 264.7 macrophage cell lines

The anti-inflammatory activity of the extract was tested on RAW 264.7 macrophage cell lines using cyclooxygenase activity assay. Evaluation of COX-2 enzyme inhibition is a direct measurement of anti-inflammatory activity of the extract. COX assay was taken to analyse the activity on inflammatory mediators in RAW 264.7 cells. The extract was evaluated for its cyclooxygenase activity for three concentrations (50, 100 and 200 µg/mL) and percentage inhibition was calculated. The extract showed an activity of 65.20 ± 1.19% at 200 µg/mL and IC_{50} 58.11 ± 1.76 µg/mL (Figure 1).

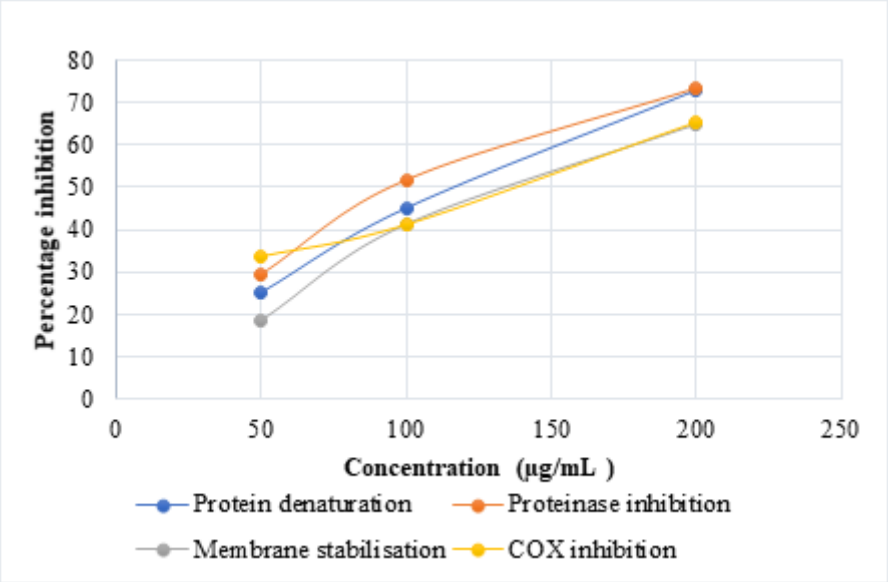

Fig. 1. Anti-inflammatory activity of *A. muricatum* in various assays

DISCUSSION

Phytomedicinal components have always attracted the attention of the pharmaceutical and scientific communities [27]. Quantitative techniques like GC/MS and HR-LC/MS can be used to find out the specific components of plant extracts**.** Gas chromatography (GC) and mass spectrometry (MS) analytical technique is very suitable for trace analysis of plant ingredients [28]. GC/MS analysis revealed 17 compounds, with 2,3-dihydro-1-benzofuran (30.82%) as the predominant constituent. Benzofuran derivatives are widely reported for their antioxidant, anti-inflammatory, antimicrobial and anticancer properties, which may significantly contribute to the observed bioactivities of the extract [29]. Compounds like 5-heptyl resorcinol and arachidyl oleate in the extract, are also reported to be biologically active [30-33].

The HR-LC/MS analysis of the methanolic extracts revealed the presence of a total of 29 compounds belonging to flavonoids, phenols, alkaloids, terpenoids and biopeptides. Salicylic acid, detected in the extract is an established anti-inflammatory agent. It can inhibit the transcription of COX-2 gene. According to Paterson & Lawrence [34], the compound also has anticancer property, especially colorectal cancer. Compounds like Epigallocatechin, α-mangostin, are reported to have significant biological activities [35-37]. Biopeptides detected in the extract may further contribute to its biological potential due to their known antioxidant and anti-inflammatory properties [38,39]. The diversity of phytoconstituents suggests that the therapeutic effects of *A. muricatum* may arise from synergistic interactions among multiple compounds rather than a single active principle.

The antioxidant assays demonstrated that the methanolic extract possess substantial free radical scavenging activity. In the DPPH assay, the extract exhibited dose-dependent inhibition with an IC_{50} of 56.82 ± 3.09µg/mL, indicating effective hydrogen-donating ability. Significant superoxide radical scavenging activity (IC_{50} 40.59 ± 3.25µg/mL) suggests its capability to neutralize reactive oxygen species involved in oxidative stress–mediated disorders. The extract showed particularly strong activity in lipid peroxidation (IC_{50} 12.89 ± 1.02µg/mL) and hydroxyl radical scavenging assays (IC_{50} 9.16 ± 2.38 µg/mL), indicating potent protection against membrane damage and highly reactive hydroxyl radicals. The ferric reducing

antioxidant power (FRAP) assay further confirmed its reducing capacity, demonstrating electron-donating ability comparable to $FeSO_4 \cdot 7H_2O$ equivalents. Collectively, these results suggest that the antioxidant activity of *A. muricatum* may be attributed to its rich phenolic and terpenoid content.

The anti-inflammatory potential of the extract was evaluated through both in vitro biochemical assays and cell-based analysis. The extract significantly inhibited protein denaturation, proteinase activity and heat-induced haemolysis in a dose-dependent manner. The inhibition of albumin denaturation (IC_{50} 43.07 ± 0.46µg/mL) and proteinase activity (IC_{50} 61.58 ± 0.97µg/mL) suggests stabilization of proteins and suppression of inflammatory enzyme activity. Membrane stabilization results indicate its potential to prevent lysosomal enzyme release during inflammation [40]. Furthermore, the extract demonstrated considerable cyclooxygenase (COX-2) inhibitory activity in RAW 264.7 macrophage cells, with 65.20% inhibition at 200 µg/mL (IC_{50} 58.11µg/mL). Since COX-2 plays a central role in the synthesis of pro-inflammatory mediators, its inhibition confirms the extract's anti-inflammatory efficacy at the cellular level [41].

CONCLUSION

Overall, the findings indicate that *Amomum muricatum* possess potent antioxidant and anti-inflammatory properties, likely mediated by its diverse phytochemical constituents. The results support its potential as a natural source of therapeutic agents for managing oxidative stress and inflammatory disorders. Further studies focusing on isolation, characterization and mechanistic evaluation of individual bioactive compounds are needed.

Financial assistance: NIL

Conflict of Interest : The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

The first author acknowledges research support from the University Grants Commission (UGC), new Delhi, India, under the Faculty Improvement Programme (FIP).

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