

Phytochemical Profiling and *in vitro* Assessment of *Annona muricata* L. Leaf Extracts for Antioxidant and Antibacterial Properties

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ABSTRACT

Background and Objective: *Annona muricata* L. is known for its rich diversity of secondary metabolites, yet limited data exist on the comparative bioactivity of its solvent-based leaf extracts. This study aimed to evaluate the phytochemical composition and assess the antioxidant and antibacterial properties of acetone and methanolic leaf extracts of *A. muricata*. **Materials and Methods:** Leaf phytocompounds were extracted using acetone and methanol. Functional groups were identified through FTIR-ATR, while phenolic and flavonoid contents were quantified using HPLC. Phytochemical profiling was further conducted using ESI-MS. Antioxidant activity was evaluated using DPPH and FRAP assays, and antibacterial activity was assessed against four human pathogenic bacteria using the agar well diffusion method. Statistical analyses were applied to compare extract performance across assays. **Results:** The FTIR confirmed the presence of phenolic and carboxylic functional groups in both extracts. HPLC analysis revealed phenolic and flavonoid compounds, with coumaric acid detected at 57.95 mg/g in the acetone extract and 52.85 mg/g in the methanol extract. The ESI-MS identified Octadecane, 1-(ethenyl)- as the major compound in the acetone extract and Cholesta-5,7,9(11)-trien-3-ol acetate in the methanol extract. Antioxidant assays showed higher activity in the acetone extract, with a DPPH IC₅₀ value of 105.43±5.94 µg/mL. The acetone extract at 75 µg/mL exhibited the strongest antibacterial effect, producing a 9±0.04 mm inhibition zone against *Salmonella typhi*. **Conclusion:** The study demonstrates that acetone leaf extracts of *A. muricata* possess superior antioxidant and antibacterial activity compared to methanolic extracts. These findings support the potential application of acetone-extracted phytocompounds from *A. muricata* in natural antioxidant and antimicrobial formulations. Future research should explore compound isolation and mechanism-based evaluations.

KEYWORDS

Annona muricata L., leaf extract, bioactive compounds, phenolics, antioxidants, antibacterial

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INTRODUCTION

The vast and varied ecological landscapes in India offers richest biodiversity with diverse medicinal and aromatic plants species. These diverse medicinal plant varieties contribute to the availability of natural bioactive compounds with great significance to nurture the human health¹. Natural compounds from



medicinal plants have long been used for drug discovery with diverse therapeutic potential². Globally, the traditional medicinal system has relied on plant-based remedies as therapeutic agents for both physical and mental health issues³. Recent studies have highlighted that plant secondary metabolites such as phenolics, alkaloids, saponins, and terpenoids have emerged as promising antibacterial agents⁴. The chemical diversity of the drugs obtained from medicinal plants possess synergistic activities such as antibacterial, antiviral, antiprotozoal and antioxidants. Since, the drugs obtained from medicinal plants are cost-effective; there exists create great demand for these drugs in the market with minimal side effects than synthetic drugs⁵. The use of herbal medicines in future therapeutic applications relies on complementing clinical data with real world evidence and outcome measures⁶.

Annona muricata is a tropical evergreen hat reaches a height of 5-6 m and belongs to the Annonaceae family. The leaves, bark, fruits, and roots of *A. muricata* are employed in traditional herbal remedies. Notably, the fruit and leaves are valued for their tranquilizing and sedative effects. Traditionally, decoction of *A. muricata* leaves is used as a pain reliever and for treating gall bladder ailments. Externally, the leaves can be applied to alleviate eczema, skin rashes, and swelling. In addition, topical application of *A. muricata* leaves is believed to accelerate wound healing and prevent infections. In herbal medicine, *A. muricata* fruits are utilized to alleviate joint pain, treat heart conditions, act as a sedative, and mitigate coughing or flu symptoms⁷. The roots and leaves of *A. muricata* have higher phenolic contents. The medicinal and pharmacological potential of *A. muricata* has led to its investigation as an alternative therapy for bacterial infections, diabetes, hypertension, and cancer⁸. Also, for pest control and insect repellent purposes, the parts of *A. muricata* plant, including its unripe fruit are used as natural alternatives to synthetic chemicals⁹.

Since various plant parts of *A. muricata* finds its use in ethnomedicinal practices, the current study deals with phytochemical profiling of acetone and methanol leaf extracts of *A. muricata* using Fourier transform infrared spectroscopy (FTIR), High Performance Liquid Chromatography (HPLC) and Gas Chromatography-Mass Spectrometry (GC-MS) analytical techniques. Furthermore, *in vitro* antioxidant and antimicrobial activities of *A. muricata* leaf extracts will be evaluated against four human pathogenic bacteria.

MATERIALS AND METHODS

Study area and duration: This research work was carried out in Vilar, located in the Thanjavur District, Tamil Nadu, India, during the time period of 2022 to 2024.

Chemicals and reagents: Chemicals and reagents used were analytical grade, purchased from Hi Media Laboratories Pvt Ltd., Mumbai, India. The solvents and synthetic compounds were obtained from S.D Fine Chem Ltd., and Sigma Aldrich, respectively.

Plant sample collection: *Annona muricata* L. leaves collected from Poondi, Thanjavur District, Tamil Nadu, India. Taxonomical identification, confirmation and certification were provided by Bharathi herbarium in the Department of Botany, Bharathiar University, Coimbatore, Tamil Nadu, India (Ref no: (BU/DB/25/23/2021/Tech./1054) by characteristic identification and compared with the type specimen depository in the herbarium.

Extraction of phytochemicals using the soxhlet method: The collected fresh leaves were washed with tap water to remove the dust particles and again washed with distilled water. The cleaned leaves were dried at 40°C using hot air oven and powdered using a mechanical blender. About 20 gm *A. muricata* leaf powder was extracted successively with organic solvents namely acetone and methanol using Soxhlet apparatus. The crude acetone and methanolic leaf extracts concentrated using a rotary evaporator and stored at 4°C after complete evaporation of the solvents¹⁰.

Analytical characterization of leaf extracts

Fourier transform infrared spectroscopy (FTIR-ATR) analysis: The phytochemicals were extracted from *A. muricata* leaves using acetone and methanol solvents were characterized using FTIR-ATR spectroscopy. About 5 mg of leaf extract was analysed in the spectrum range from 4000 cm to 600 cm. The functional groups were analysed using the peak values.

High performance liquid chromatographic analysis: Both acetone and methanol leaf extracts of *A. muricata* were subjected to HPLC analysis (Shimadzu, Japan) for the determination of phytochemicals. A C18 column coupled with a PDA detector and a mobile phase containing water and methanol with a flow rate of 0.8 mL/min for 60 min was used for phytochemical separation. The column oven temperature was 40°C. The synthetic compounds, namely coumaric acid, rutin, catechin, and quercetin, were used to identify the presence of bioactive compounds in leaf extract by comparing retention times of both synthetic compounds and leaf phytochemicals. The retention time of synthetic compounds were detected using our previous results¹¹.

Electrospray Ionization Mass Spectrometry Analysis (ESI-MS): The fingerprinting of acetone and methanol leaf extracts was performed using ESI-MS analysis. The temperature, capillary voltage and cone voltage were set to 100°C, 3.0 kV and 40 V, respectively. The ESI-MS was performed by direct infusion of the sample with a 10 µL/min flow rate using a syringe pump. Mass spectra were accumulated over 60 sec, and the spectral range was obtained between 10 and 500 m/z¹².

Antioxidant activity of leaf extracts

DPPH radical scavenging activity of extracts: DPPH activity of *A. muricata* acetone and methanol leaf extracts was determined according to Blois¹³. About 100, 200, 300, 400 and 500 µg/mL of leaf extracts were added to 5 mL of 0.1 mM methanolic DPPH solution and incubated at 27°C for 20 min. About 0.1 mM DPPH methanolic solution served as a negative control. Blank used was methanol. The DPPH, methanol, and ascorbic acid were the positive control. Sample absorbance measured at 517 nm using a UV-Vis spectrophotometer. Inhibition percentage of extracts was calculated by following the equation:

$$\text{Inhibition of (\%)} = \frac{\text{Control OD} - \text{Sample OD}}{\text{Control OD}} \times 100$$

Ferric reducing antioxidant power assay: The FRAP assay performed for both acetone and methanol leaf extracts of *A. muricata* according to the method of Pulido *et al.*¹⁴. The reagent containing 20 mM 2,4,6-tris (2-pyridyl)-s-triazine (TPTZ) and FeCl₃ (20 mM) was prepared in 0.2 M acetate buffer and added to the samples. Rutin served as a positive control. The samples were incubated for 30 min at 37°C, and blue color absorbance was measured at 593 nm using a UV-Vis spectrophotometer. The reducing power ability was expressed as antioxidant concentration with ferric-TPTZ reducing ability equivalent to 1 mM FeSO₄·7H₂O.

Antibacterial activity of *A. muricata* leaf extracts: The antibacterial effect of *A. muricata* leaf acetone and methanol extracts was analyzed against four bacteria belonging to the human pathogenic group, namely *Salmonella typhi*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Bacillus subtilis* by agar well diffusion method. The suspension culture at 10³ cfu/mL concentration was loaded and swabbed on a nutrient agar plate. Then, different concentration of each extract (25, 50 and 75 µg/mL) was added into 6 mm diameter wells in agar plate. The positive control was chloramphenicol, whereas acetone and methanol were negative controls. These plates were incubated at 37°C for 24 hrs. The inhibition zone around wells were observed and recorded¹⁵.

RESULTS

Analytical characterization of leaf extracts

FTIR analysis: The functional groups present in acetone and methanol leaf extracts of *A. muricata* were determined using FTIR-ATR in 4000/cm to 650/cm range (Fig. 1 and 2).

Almost similar functional groups were analysed in both acetone and methanol extracts and the results revealed intense peaks in spectrum that indicated existence of functional groups. The functional groups of lipids, proteins and cell wall components were observed in both the extracts. The acetone extract showed strong intense peak at 2979.48/cm, indicating the O-H stretching of carboxylic acid. The conjugated anhydride, conjugated alkene, nitro compound, alkane, aromatic ester and sulfonic acid stretching vibrations were observed in the peak ranges of 1736.58, 1609.31, 1514.81, 1461.78, 1376.93, 1251.58 and 1159.01/cm, respectively. Also, the sulfoxide, amine and alkene compounds corresponding to S = O, C-N and C = C bending were also determined in acetone leaf extract (Table 1).

In methanolic leaf extract, the presence of secondary amines and carboxylic acid corresponding to lipid compounds with N-H and O-H stretching vibrations were observed in the peak ranges of 3342.03, 2968.87 and 2922.59/cm, respectively. Also, the amine salt, esters, α , β -unsaturated ketone, nitro compound, alkane, amine alkyl aryl ether and alkenes were observed in the peak ranges from 2852.2 to 718.354/cm (Table 2).

HPLC analysis: The phytocompound analysis of leaf acetone and methanol extracts was performed using HPLC analysis. The peaks of synthetic compounds were compared with our previous results of phenolic and flavonoid synthetic compounds. The chromatogram of both acetone and methanolic leaf extracts of *A. muricata* showed various peaks in different retention times which indicated different secondary metabolites. Chromatogram of acetone extract showed a characteristic peak at the retention time of RT-4.963, representing the presence of coumaric acid, a phenolic compound. The compound catechin was detected in acetone leaf extract at the retention time of RT-6.475 when compared with synthetic compound. At the retention time of RT-8.385, the compound rutin was detected in the acetone leaf extract. Also, the compound quercetin was detected in acetone leaf extract also at the retention time of RT-13.25 (Fig. 3a). The amount of phytocompounds in crude acetone leaf extract was recorded to be 52.85 mg/g of coumaric acid followed by 24.25 mg/g catechin, 13.25 mg/g rutin and 4.2 mg/g quercetin respectively (Table 3).

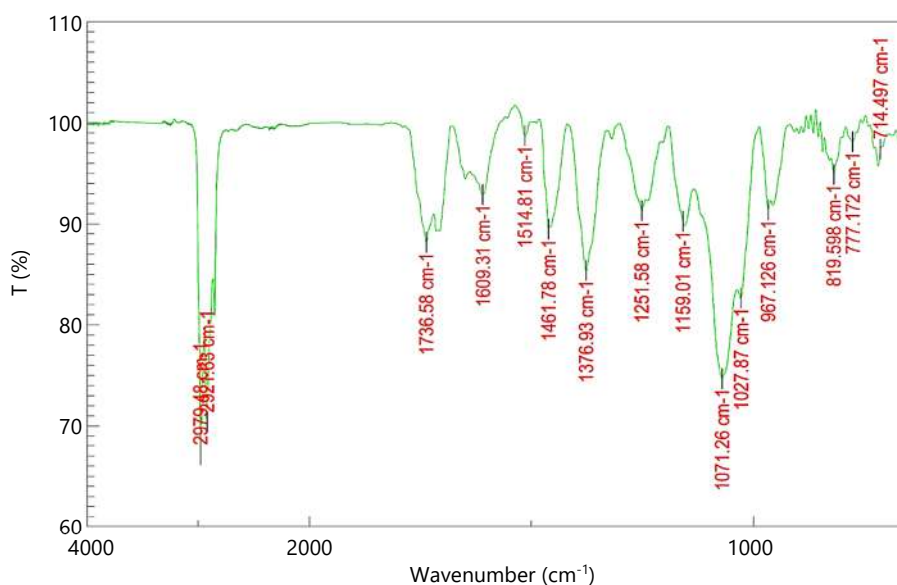


Fig. 1: FTIR-ATR spectrum of *Annona muricata* acetone leaf extract

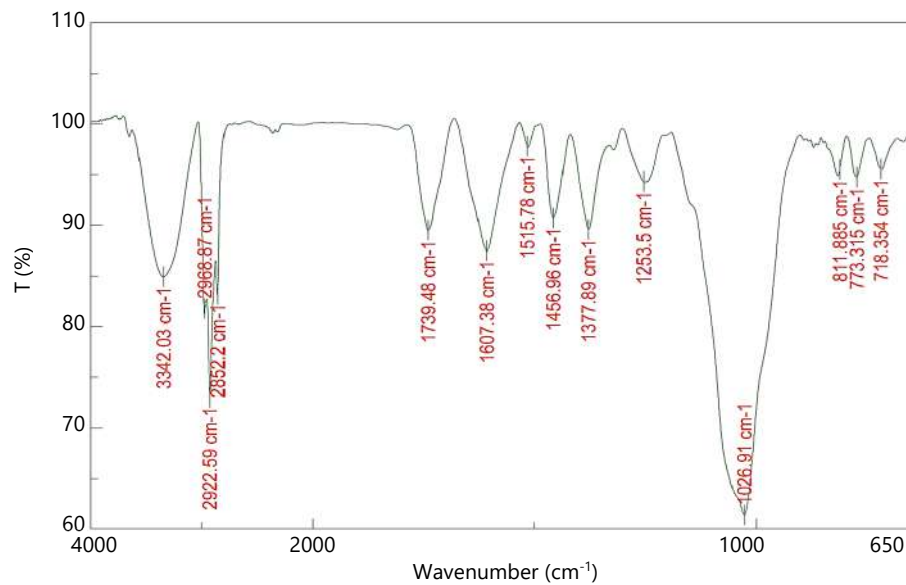


Fig. 2: FTIR-ATR spectrum of *Annona muricata* leaf methanolic extract

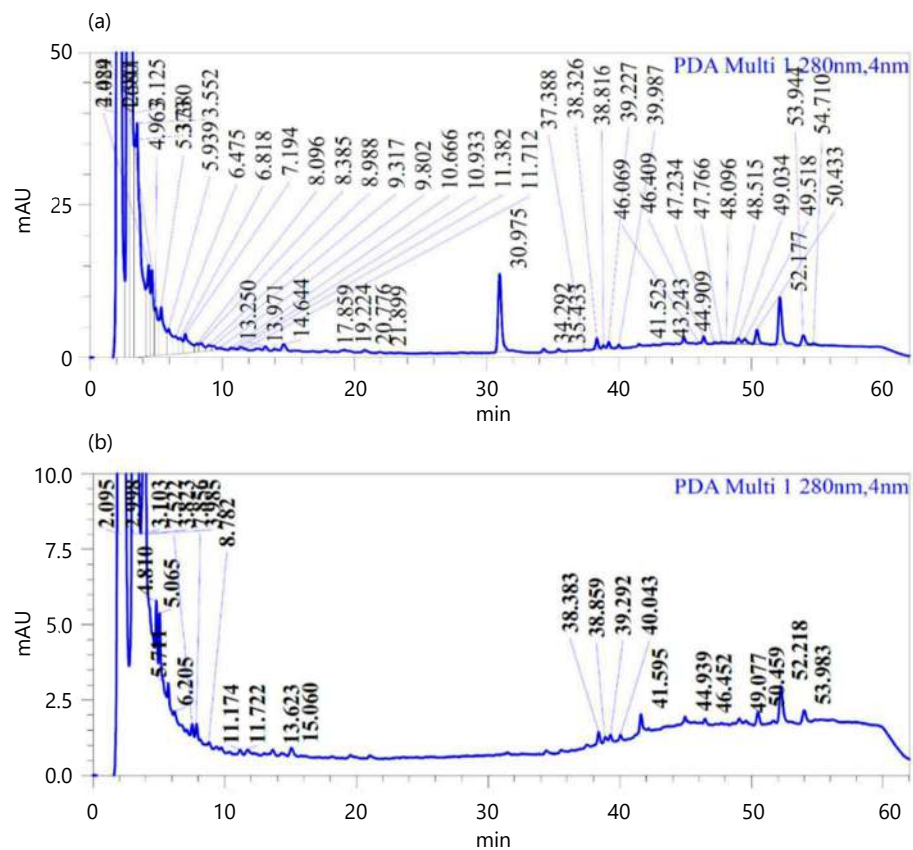


Fig. 3: HPLC chromatogram of leaf phytochemicals present in (a) Acetone extract and (b) Methanolic extract of *A. muricata*

The HPLC analysis of *A. muricata* methanolic leaf extract also exhibited phenolic and flavonoid compounds at the retention times of RT-4.810, RT-6.205, RT- 8.782 and RT-13.623 corresponding to coumaric acid, quercetin, catechin and rutin respectively (Fig. 3b).

The amount of phytochemicals in crude acetone leaf extract recorded to be 57.95 mg/g of coumaric acid followed by 10.05 mg/g catechin, 1.5 mg/g rutin and 2.2 mg/g quercetin respectively (Table 4).

Table 1: Functional groups of *A. muricata* acetone leaf extract analysed using FTIR

S. No.	Extract absorption (cm)	Functional group	Compound class
Lipids (3000-2000/cm)			
1	2979.48	O-H stretching	Carboxylic acid
Proteins (1800-1100/cm)			
2	1736.58	C=O stretching	Conjugated anhydride
3	1609.31	C=C stretching	Conjugated alkene
4	1514.81	N-O stretching	Nitro compound
5	1461.78	C-H bending	Alkane
6	1376.93	C-H bending	Alkane
7	1251.58	C-O stretching	Aromatic ester
8	1159.01	S=O stretching	Sulfonic acid
Cell wall components (1000-600/cm)			
9	1071.26	S=O stretching	Sulfoxide
10	1027.87	C-N stretching	Amine
11	967.126	C=C bending	Alkene
12	819.598	C=C bending	alkene
13	777.172	C=C bending	Alkene
14	714.497	C=C bending	Alkene

Table 2: Functional groups of *A. muricata* methanolic leaf extract analysed using FTIR

S. No.	Extract absorption (cm)	Functional group	Compound class
Lipids (3000-2000/cm)			
1	3342.03	N-H stretching	Secondary amine
2	2968.87	O-H stretching	Carboxylic acid
3	2922.59	O-H stretching	Carboxylic acid
Proteins (1800-1500/cm)			
4	2852.2	N-H stretching	Amine salt
5	1739.48	C=O stretching	Esters
6	1607.38	C=C stretching	α , β -unsaturated ketone
7	1515.78	N-O stretching	Nitro compound
8	1456.96	C-H bending	Alkane
9	1377.89	C-H bending	alkane
10	1253.5	C-N stretching	Amine
Cell wall components (1000-600/cm)			
11	1026.91	C-O stretching	Alkyl aryl ether
12	811.885	C=C bending	Alkene
13	773.315	C=C bending	Alkene
14	718.354	C=C bending	Alkene

Table 3: Secondary metabolites quantification in *A. muricata* acetone leaf extract using HPLC

Standard	Compound RT (min)	Concentration (mg/g extract)
Coumaric acid	4.963	52.85
Catechin	6.475	24.25
Rutin	8.385	13.25
Quercetin	13.25	4.2

Table 4: Secondary metabolites quantification in *A. muricata* methanolic leaf extract using HPLC

Standard	Compound RT (min)	Concentration (mg/g extract)
Coumaric acid	4.81	57.95
Catechin	6.205	10.05
Rutin	8.782	1.5
Quercetin	13.623	2.2

Hence, these HPLC results suggests that phenolic compounds are more in both acetone and methanolic leaf extract of *A. muricata*, when compared with the flavonoid compounds.

ESI-MS analysis: The electrospray ionization mass spectrum was obtained for the acetone and methanol leaf extracts of *A. muricata* (Fig. 4 and 5).

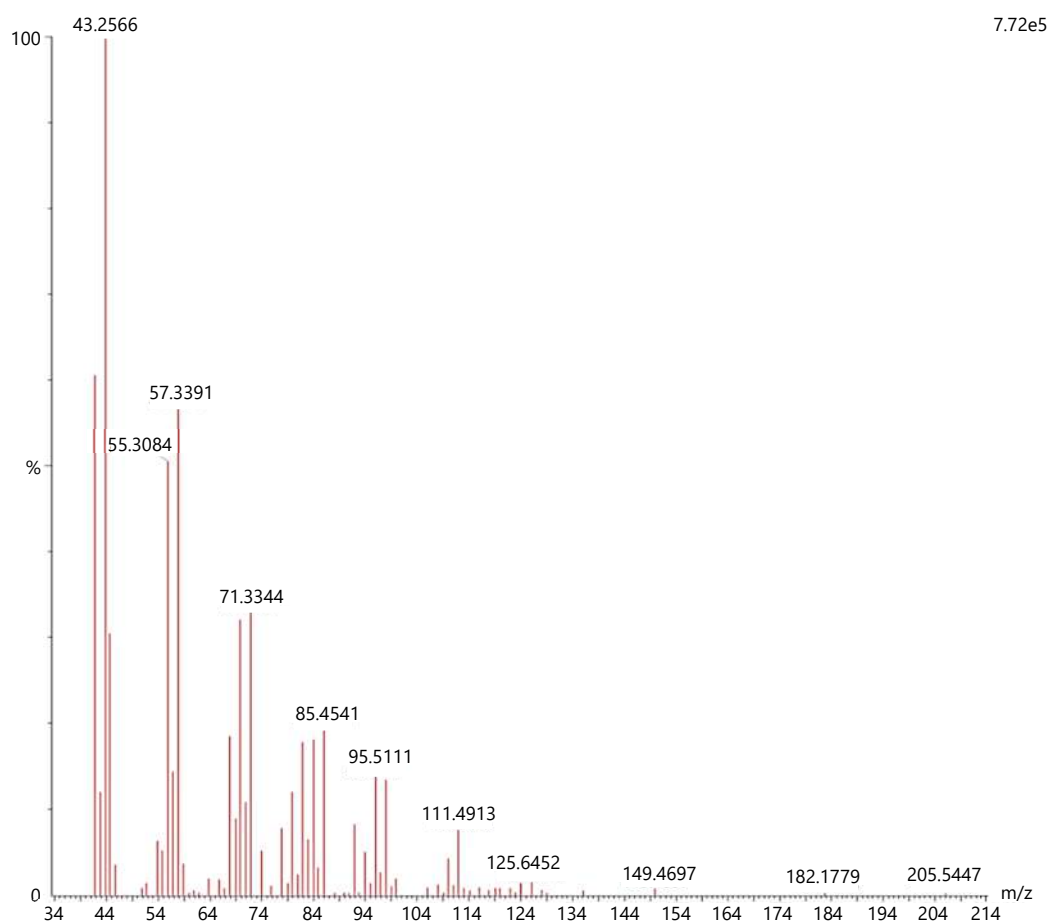


Fig. 4: ESI-MS spectrum of leaf phytocompounds present in acetone extract of *A. muricata*

Table 5: Phytocompounds detected in *A. muricata* acetone leaf extract using ESI-MS

S. No.	m/z	Compound name	Molecular weight	Molecular formula
1	41.9893	Oxalic acid, allyl pentadecyl ester	340	C ₂₀ H ₃₆ O ₄
2	43.2566	Octadecane, 1-(ethenyloxy)-	296	C ₂₀ H ₄₀ O
3	44.5675	Oxalic acid, allyl hexadecyl ester	354	C ₂₁ H ₃₈ O ₄
4	55.3084	Oxalic acid, allyl dodecyl ester	298	C ₁₇ H ₃₀ O ₄
5	57.3391	1-hexacosanol	382	C ₂₆ H ₅₄ O
6	69.9398	Oxalic acid, allyl undecyl ester	284	C ₁₆ H ₂₈ O ₄
7	71.3344	Oxalic acid, allyl tridecyl ester	312	C ₁₈ H ₃₂ O ₄
8	81.3342	Pentafluoropropionic acid, octadecyl ester	416	C ₂₁ H ₃₇ O ₂ F ₅
9	83.4541	Oxalic acid, allyl octadecyl ester	382	C ₂₃ H ₄₂ O ₄
10.	85.4541	Oxalic acid, allyl tetradecyl ester	326	C ₁₉ H ₃₄ O ₄
11	92.4959	Pentafluoropropionic acid, hexadecyl ester	388	C ₁₉ H ₃₃ O ₂ F ₅
12	95.5111	17-pentatriacontene	490	C ₃₅ H ₇₀
13	97.3642	2-piperidinone, n-[4-bromo-n-butyl]-	233	C ₉ H ₁₆ ONBr
14	110.9786	Silane, trichlorodocosyl-	442	C ₂₂ H ₄₅ C ₃ Si
15	111.4913	2-hexyl-1-octanol	214	C ₁₄ H ₃₀ O
16	124.1671	Heptafluorobutyric acid, n-pentadecyl ester	424	C ₁₉ H ₃₁ O ₂ F ₇
17	125.6452	Hydroxylamine, o-decyl-	173	C ₁₀ H ₂₃ ON
18	149.4697	Hexatriacontane	506	C ₃₆ H ₇₄
19	182.1779	1-decanol, 2-hexyl-	242	C ₁₆ H ₃₄ O
20	205.5447	1-octadecanesulphonyl chloride	352	C ₁₈ H ₃₇ O ₂ CS

Both the extracts possessed 20 different compound groups. The dominant compound observed in acetone extract was Octadecane, 1-(ethenyloxy)-(C₂₀H₄₀O) with the molecular weight of 296, followed by 1-hexacosanol (C₂₆H₅₄O) and Oxalic acid, allyl dodecyl ester (C₁₇H₃₀O₄) with a molecular weight of 382 and 298 respectively (Table 5).

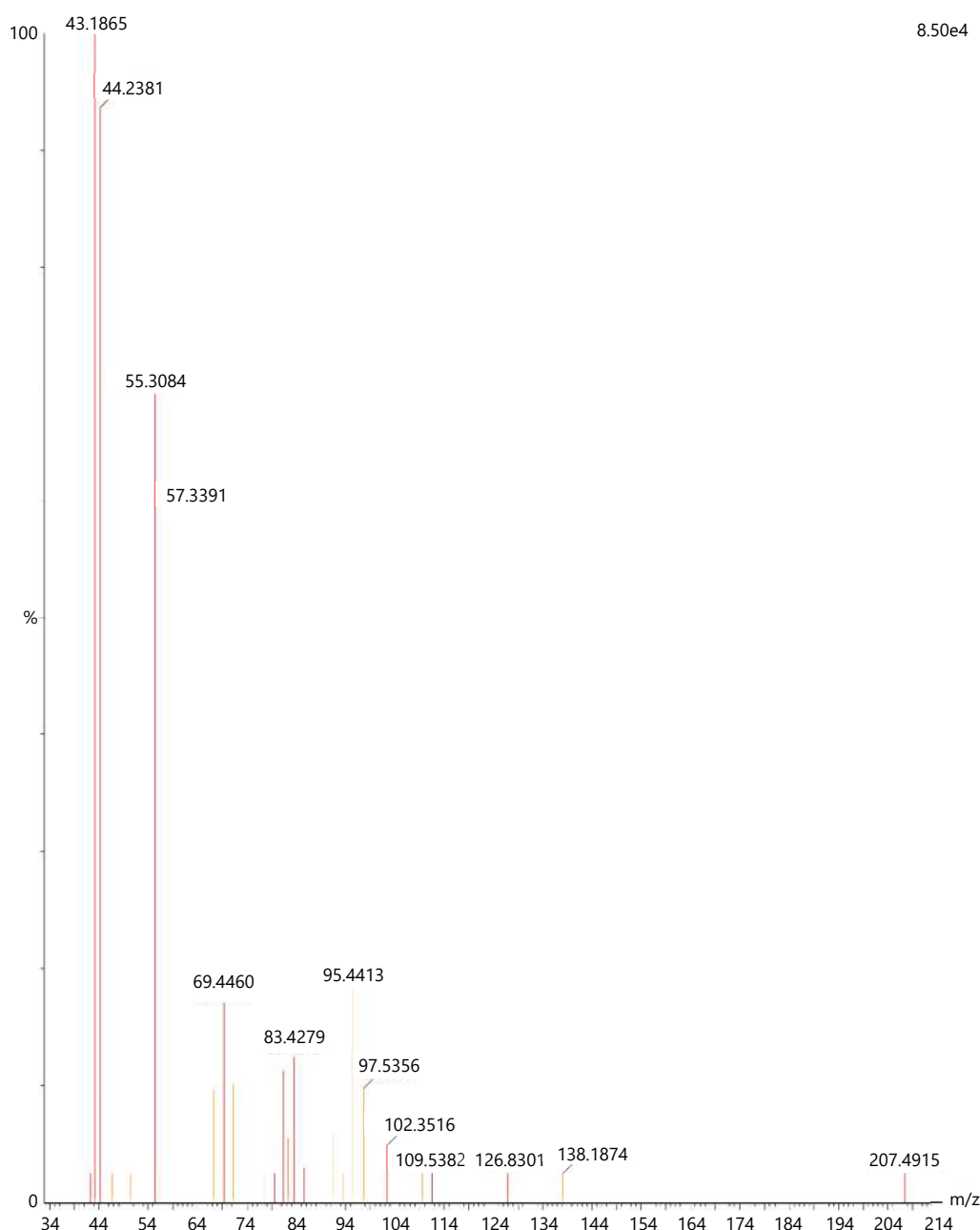


Fig. 5: ESI-MS spectrum of leaf phytocompounds present in methanol extract of *A. muricata*

In the methanolic leaf extract, the dominant compound was detected to be Cholesta-5,7,9(11)-trien-3-ol acetate ($C_{29}H_{44}O_2$) with the molecular weight of 424. The other dominant compounds observed in the methanolic leaf extract were 9-octadecenoic acid (z)-, 2-(acetyloxy)-1-[(acetylox ($C_{25}H_{44}O_6$), Octadecanoic acid, 2-oxo-, methyl ester ($C_{19}H_{36}O_3$) and 2,6,6-trimethyl-bicyclo[3.1.1]hept-3-ylamine ($C_{10}H_{19}N$) with the molecular weight of 440, 312 and 153 respectively (Table 6).

In vitro antioxidant activity of *A. muricata* leaf extracts: The antioxidant potential of both acetone and methanol leaf extracts of *A. muricata* were analysed by DPPH and FRAP assays. In DPPH assay, the acetone leaf extract showed higher percentage of inhibition of $62.40 \pm 2.98\%$ at $500 \mu\text{g/mL}$ concentration, with IC_{50} of $105.43 \pm 5.94 \mu\text{g/mL}$ compared with synthetic ascorbic acid ($71.71 \pm 2.44\%$ of inhibition and IC_{50} of $21.95 \pm 9.95 \mu\text{g/mL}$). Whereas at the same concentration, almost similar results were observed in methanolic leaf extract which showed $57.17 \pm 5.08\%$ percentage of inhibition with IC_{50} of $138.42 \pm 5.47 \mu\text{g/mL}$. The results of FRAP assay also exhibited that acetone and methanolic crude extracts of *A. muricata* possess increased antioxidant activities, compared to synthetic rutin. The extracts antioxidant activity increased

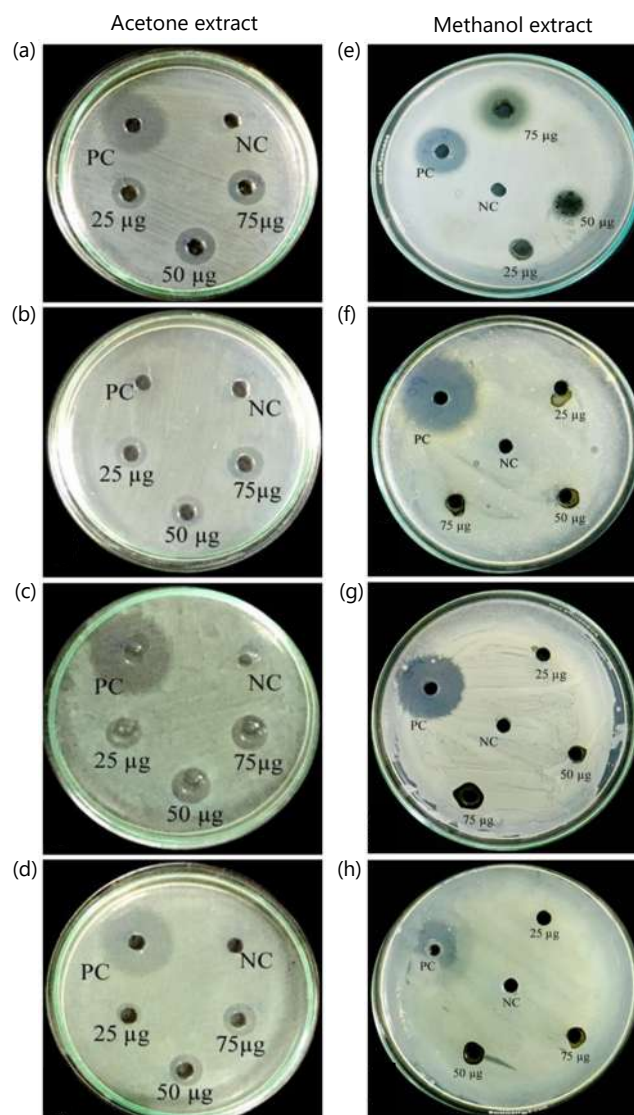


Fig. 6(a-h): Antibacterial activity of acetone and methanol leaf extract of *A. muricata* against (a-e) *Salmonella typhi* (b-f) *Pseudomonas aeruginosa* (c-g) *Escherichia coli* and (d-h) *Bacillus subtilis*
PC: Antibiotics chloramphenicol (positive control) and NC: Methanol and acetone solvent (negative control)

Table 6: Phytocompounds detected in *A. muricata* methanolic leaf extract using ESI-MS

S. No.	m/z	Compound name	Molecular weight	Molecular formula
1	41.9623	1,6;3,4-dianhydro-2-deoxy-.beta.-d-lyxo-hexopyrano	128	$C_6H_8O_3$
2	43.1865	Cholesta-5,7,9(11)-trien-3-ol acetate	424	$C_{29}H_{44}O_2$
3	44.2381	9-octadecenoic acid (z)-, 2-(acetyloxy)-1-[(acetylox	440	$C_{25}H_{44}O_6$
4	55.3084	Octadecanoic acid, 2-oxo-, methyl ester	312	$C_{19}H_{36}O_3$
5	57.3391	2,6,6-trimethyl-bicyclo[3.1.1]hept-3-ylamine	153	$C_{10}H_{19}N$
6	67.4788	9-methyl-z-10-pentadecen-1-ol	240	$C_{16}H_{32}O$
7	69.4460	9,12,15-octadecatrienoic acid, 2-(acetyloxy)-1-[(ace	436	$C_{25}H_{40}O_6$
8	71.2874	2,6-pyrazinediamine	110	$C_4H_6N_4$
9	81.5278	1-bromo-3-(2-bromoethyl)-nonane	312	$C_{11}H_{22}Br_2$
10	82.2651	2(3h)-furanone, 3-(15-hexadecynylidene)dihydro-4-h	334	$C_{21}H_{34}O_3$
11	83.4279	3-acetoxydodecane	228	$C_{14}H_{28}O_2$
12	91.5287	Octadecanal, 2-bromo-	346	$C_{18}H_{35}OBr$
13	95.4413	Nonanal	142	$C_9H_{18}O$
14	97.5356	Cyclohexanol, 2-methyl-5-(1-methylethyl)-, (1.alpha	156	$C_{10}H_{20}O$
15	102.3516	6-nitroundec-5-ene	199	$C_{11}H_{21}O_2N$
16	109.5382	Hexadecanal	240	$C_{16}H_{32}O$
17	111.529	7-methyl-z,z-8,10-hexadecadien-1-ol acetate	294	$C_{19}H_{34}O_2$
18	126.8301	1-hexyl-2-nitrocyclohexane	213	$C_{12}H_{23}O_2N$
19	138.1874	Myristic acid vinyl ester	254	$C_{16}H_{30}O_2$
20	207.4915	Cis-9,10-epoxyoctadecan-1-ol	284	$C_{18}H_{36}O_2$

Table 7: *In vitro* antioxidant activity of *A. muricata* leaf extracts

Concentration (µg/mL)	DPPH			FRAP		
	Acetone	Methanol	Ascorbic acid	Acetone	Methanol	Rutin
	Percentage of inhibition			mM Fe(II)E/mg		
100	31.40±4.28 ^e	25.58±2.03 ^e	49.61±4.37 ^e	379.47±2.54 ^e	154.27±1.22 ^e	538.01±3.07 ^e
200	44.67±2.18 ^d	28.78±1.77 ^d	58.72±1.05 ^d	518.90±5.32 ^d	260.77±4.62 ^d	672.15±3.92 ^d
300	50.68±2.06 ^c	44.67±1.10 ^c	63.18±0.44 ^c	581.91±3.92 ^c	382.32±3.66 ^c	820.12±3.66 ^c
400	54.75±1.21 ^b	47.38±3.03 ^b	69.19±0.58 ^b	633.54 ±4.40 ^b	396.95±2.44 ^b	858.74±3.73 ^b
500	62.40±2.98 ^a	57.17±5.08 ^a	71.71±2.44 ^a	698.17±2.11 ^a	559.96±1.41 ^a	955.49±2.44 ^a
IC ₅₀ (µg/mL)	105.43±5.94 ^b	138.42±5.47 ^c	21.95±9.95 ^a	-	-	-

Values represent Mean±Standard Deviation, were significant among the extracts p<0.05

Table 8: Antibacterial effect of *A. muricata* acetone and methanolic leaf extracts

Extracts	Concentration (µg/mL)	<i>Salmonella typhi</i>	<i>Pseudomonas aeruginosa</i>	<i>Escherichia coli</i>	<i>Bacillus subtilis</i>
Zone of inhibition (mm)					
Chloramphenicol (PC)	10	10±0.02 ^b	12±0.08 ^a	13±0.3 ^a	8±0.5 ^{cd}
Acetone (NC)	-	-	-	-	-
Acetone extract	25	5±0.03 ^d	4±0.01 ^b	3±0.01 ^b	2±0.01 ^c
	50	7±0.04 ^c	4±0.02 ^b	5±0.01 ^a	4±0.02 ^b
	75	9±0.04 ^a	6±0.07 ^a	5±0.06 ^a	5±0.03 ^a
Methanol (NC)	-	-	-	-	-
Methanolic extract	25	1±0.01 ^f	1±0.01 ^c	1±0.01 ^c	0±0.01 ^e
	50	3±0.02 ^e	1±0.02 ^c	1±0.03 ^c	1±0.02 ^d
	75	8±0.02 ^b	1±0.07 ^c	2±0.01 ^b	1±0.03 ^d

Values represent Mean±SE, means significantly different between extracts (p<0.05), PC: Positive control and NC: Negative control

in dose dependent manner in both acetone and methanol extracts. In FRAP assay, higher release of mM Fe(II) equivalents coincides with increased antioxidant activity of test sample. The results exhibited that acetone extract showed 698.17±2.11 mM Fe(II) equivalents per mg extract and methanolic leaf extract of *A. muricata* exhibited 559.96±1.41 mM Fe(II) equivalents per mg extract when compared with the standard rutin (955.49±2.44 mM Fe(II) equivalents per mg extract) (Table 7).

Antibacterial activity of leaf extracts: Antibacterial activity of acetone and methanol solvent extracts of *A. muricata* leaves were tested against four different bacterial pathogens. Inhibitory activity of the extracted crude leaf phytocompounds at different concentrations were tested against human bacterial pathogens such as *Salmonella typhi*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Bacillus subtilis*. Results compared with synthetic chloramphenicol drug (positive control), methanol and acetone was negative control. Acetone leaf extract at 75 µg/mL concentration exhibited stronger antibacterial activity against all human pathogenic bacteria tested (Fig. 6a-d). Acetone leaf extract showed maximum zone of inhibition against *Salmonella typhi* (9±0.04 mm), followed by *Pseudomonas aeruginosa* (6±0.07 mm), *Escherichia coli* (5±0.06 mm) and *Bacillus subtilis* (5±0.03 mm), respectively. Whereas, at 75 µg/mL concentration, the methanolic leaf extract exhibited only minimum inhibition activity in all the pathogens tested (Fig. 6e-h).

The methanolic leaf extract exhibited maximum zone of inhibition only against *Salmonella typhi* (8±0.02 mm). Also, only minimum inhibition zone was exhibited by methanolic crude extract of leaves against *Pseudomonas aeruginosa* (1±0.07 mm), *Escherichia coli* (2±0.01 mm) and *Bacillus subtilis* (1±0.03 mm) (Table 8).

DISCUSSION

Plants play an important role in providing products to help combat various diseases and ailments. The plants in its fresh, dry or in extract form are often utilized in traditional practices as remedy to cure various diseases. The plants belonging to the Annonaceae family serves as potential therapeutic agents because these plants contain acetogenins, alkaloids, flavonoids, terpenes and essential oils. Due to the medicinal value of *A. muricata*, this plant is being researched by the scientists in recent years⁹.

A study by Nga *et al.*¹⁶ reported that the phytochemical constituents namely, flavonoids, alkaloids, phenols, terpenoids, tannins, steroids, saponins, glycosides, carbohydrates and proteins were present in different solvent extracts of *A. muricata* leaves. In our study, the solvents acetone and methanol were used for phytocompound extraction from leaves, because these two solvents will be effective in extracting a wide range of compounds. Methanol is a highly polar solvent, whereas acetone can dissolve both polar and nonpolar substances. In addition, the acetone plant extracts will be more effective in extracting antioxidant and phenolic compounds with antibacterial properties^{17,18}.

The free radicals could be inhibited naturally by the compounds present in *A. muricata* leaves. Free radicals are compounds having unpaired electrons, which causes diseases such as cancer by searching reactive electron pairs. The harmful effects of the free radicals could be stopped by the plant antioxidant compounds usually containing phenolics and its derivatives¹⁹. In our study, initially the antioxidant activity of acetone and methanol leaf extract of *A. muricata* were analysed using DPPH and FRAP assays. Previous study by Nguyen *et al.*²⁰ reported that the ethanolic leaf extract of *A. muricata* possessed antioxidant activity with IC₅₀ value of 20.75±0.28 µg/mL in DPPH assay and 12.84±0.21 µg/mL in ABTS assay. In our study, the acetone leaf extract had shown highest IC₅₀ value of 105.43±5.94 µg/mL in DPPH assay. Also, the acetone extract of *A. muricata* leaves was found to exhibit highest antioxidant activity with 698.17±2.11 mM Fe(II) equivalents per mg extract. Sujatha *et al.*²¹ reported that the chloroform extract of *Cassia alata* possessed potent antioxidant activity.

In the present study, the extracted phytocompounds from acetone and methanol extracts of *A. muricata* was analytically characterized using FTIR analysis, and the results revealed different peaks corresponding to various functional groups of metabolites. The acetone extract of leaves showed various functional groups of conjugated anhydride, conjugated alkene, nitro compound, alkane, aromatic ester and sulfonic acid, and the methanolic extract showed functional groups of secondary amines, carboxylic acid, amine salt, esters, α, β-unsaturated ketone, nitro compound, alkane, amine alkyl aryl ether and alkenes. Similarly, Pharmawati and Wrasianti²² analyzed the functional groups present in chloroform: ethanol leaf extract of *Enhalus acoroides*, which contained alkanes, lipids, hydroxyl groups, fatty acids, secondary amines and benzenoid compounds. The FTIR analysis of the fruit extract of the medicinal plant *Ruta graveolens* showed the functional groups of alkenes, alkanes and alkyl halides²³.

Our study showed that the acetone and methanol leaf extract consists of phenolic and flavonoid compounds namely coumaric acid, catechin, rutin and quercetin at different retention times, which was quantified using HPLC analysis. The HPLC allows precise quantification of each single compound by associating with standard compounds' retention time. The phenolic compound coumaric acid (57.95 mg/g) were found to be more in the extracts when compared with the flavonoid compounds. Also, Salih *et al.*²⁴ used HPLC in separation and quantification of phenolic compounds namely gallic acid (9.2 µg/g), quercetin (18.2 µg/g) and tannic acid (29.3 µg/g) in the seeds of *Juniperus procera*.

In our study, the ESI-MS analysis showed that Octadecane, 1-(ethenyl)oxy and 1-hexacosanol were the dominant compounds present in *A. muricata* leaf acetone extract. Also, the ESI-MS analysis of methanolic leaf extract of *A. muricata* leaves showed that Cholesta-5,7,9(11)-trien-3-ol acetate was the major compound present in it. Similarly, a previous report by Affes *et al.*²⁵ studied the presence of phenolics in *Aeonium arboreum* leaf extracts using ESI-MS/MS analysis. Also, this phytocompound exhibited both antioxidant and antimicrobial activities.

The effect of the phytocompounds present in both acetone and methanolic leaf extract were analysed against disease causing bacterial pathogens. In our study, acetone extract showed maximum inhibitory activity at 75 µg/mL against the pathogens tested namely *Salmonella typhi*, *Escherichia coli*, *Bacillus subtilis* and *Pseudomonas aeruginosa*. Affes *et al.*²⁵ also tested the antimicrobial activity of *Aeonium arboreum* leaf

xtracts against different pathogens. Their results suggested that the leaf extracts from 12.5 to 50 µg/mL concentration was found to be effective against the bacteria namely, *Listeria ivanovii*, *Micrococcus luteus*, *Staphylococcus aureus*, *Escherichia coli*, *Salmonella enterica* and *Pseudomonas aeruginosa*. Settu and Arunachalam²⁶ also studied the phytochemical compound analysis and in vitro pharmacological activities of medicinal plants of Cucurbitaceae family. Similar to our results, the *Annona squamosa* leaf extracts showed antibacterial activity due to the existence of various pharmacologically important and active compounds²⁷.

CONCLUSION

Hence, current findings states that the acetone extract of *A. muricata* leaves possess better biological activities when compared with the methanol extracts. These results also support the development of antibacterial drugs with the available bioactive compounds from acetone extract of *A. muricata* leaves, which could find application in the pharmaceutical industries. In the present study *A. muricata* leaf extracts possessed various bioactive compounds belonging to the phenolics and flavonoid groups. The compound Octadecane, 1-(ethenylloxy)- was found to be dominant in acetone extract of *A. muricata* leaves. Hence, the presence of these phytochemicals in leaf crude extract are responsible for the antioxidant activity. In addition, these compounds also contributed to the effective control of disease-causing human pathogens. Therefore, our study suggests the effective utilization of *A. muricata* leaf extract for antibacterial drug development.

SIGNIFICANCE STATEMENT

Despite traditional medicinal use, the phytochemical profile and combined antioxidant–antibacterial potential of *Annona muricata* leaves remain insufficiently characterised under standardised conditions. In response to rising antibiotic resistance and demand for plant-based antioxidants, this study quantitatively profiles key phytochemicals and evaluates free-radical scavenging and antibacterial activities of leaf extracts. The findings provide scientific validation of traditional claims and support the development of safe, natural therapeutic agents against chronic and drug-resistant infections.

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