Trends in Phytochemical Research (TPR)

Journal Homepage: https://tpr.shahrood.iau.ir/

Original Research Article

HPLC analysis, phytochemical screening, *in-vitro* antioxidant and antibacterial activity of *Annona muricata* L. fruit extracts

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ABSTRACT

Annona muricata L. belongs to the family Annonaceae with a huge number of secondary metabolites representing a wide variety of pharmacological actions. This study evaluated the secondary metabolites, antioxidant and antimicrobial properties of *A. muricata* fruits extracts. The methanolic fruit extracts showed higher amounts of total phenols, tannin and flavonoids (289.63 ± 2.3 GAE mg/g, 121.02 ± 3.4 GAE mg/g, and 86.24 ± 3.1 RE mg/g), whereas HPLC analysis of methanol fruit extracts revealed the presence of major phenolic isoforms, especially coumaric acid (2 mg/g), catechin (0.22 mg/g), rutin (0.54 mg/g), and quercetin (0.64 mg/g). The FTIR spectrum analysis showed the presence of phenolic and carbolic groups. In addition, 100 µg/mL methanolic fruit extract of *A. muricata* displayed the maximum zone of inhibition against *Staphylococcus aureus* (12.3 ± 0.07 mm) and *Klebsiella pneumoniae* (11 ± 0.09 mm) altogether accounting for the potential *in vitro* antibacterial activity of the methanol extract of *A. muricata* L.

ARTICLE HISTORY

Received: 23 April 2023
Revised: 24 June 2023
Accepted: 26 June 2023
ePublished: 29 June 2023

KEYWORDS

Annona muricata fruit extract
Annonaceae
Antibacterial
Antioxidant
Bioactive compounds
Phytochemicals
Tannins

dor: 20.1001.1.25883623.2023.7.2.5.0

1. Introduction

India is known for its varied plant biodiversity due to the variation in geography and environment with vast unexplored regions. In recent years, research on medicinal plants has increased worldwide due to their broad pharmaceutical applications (Raja et al., 2023). Traditionally, several generations have used various medicinal plants for the treatment of different human ailments for a long time. In the modern era, people are using several important medicinal plants for curing chronic diseases worldwide especially for cancer treatment (Vignesh et al., 2022a). The traditional anticancer research and drug discovery approaches are preferred because of their less time-consuming feature and also lower price (Rayan et al., 2017). Furthermore, the search for anticancer drugs from plant-derived bioactive compounds is highly recommended due to their minimal side effects. However, the development of biochemical and pharmacological mechanisms is a key factor to utilize the contribution of plants as a proper remedy (Khan et al., 2019). Plant-based compounds are more potent and their synergistic effects lead to the further development of novel drugs that are more beneficial with lower risk of adverse effects against cancer treatment (Shrihastini et al., 2021). The therapeutics of cancer diseases by plant metabolites can destruct the target cancer cells *vs.* normal cells and helps to rejuvenate wounded cells (Patel et al., 2011).
Indian plant medicine system provides a new way for anticancer treatment due to involving of a variety of alkaloids, flavonoids, terpenoids, polyphenols, steroids, etc. which are frequently used as anticancer drugs (Vignesh et al., 2022b). These compounds are also used in combination with other chemotherapeutic drugs for controlling the relevant side effects (Cragg and Newman, 2009). The major phytocompounds, *e.g.*, limonoids, triterpenoids and coumarins are present in the genus

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Ekebergia that show various biological activities including antiplasmodial, antimicrobial, antiproliferative or uterotonic activities (Mouthe kemayou et al., 2021). The most potential polyphenolic compounds such as *p*-arbutin, quinic acid, ferulic acid, gallic acid, vanillic acid, and *p*-coumaric acid are reported in *Vitis vinifera* leaves and *Ocimum* species which exhibit remarkable pharmacological effects (Sobhani et al., 2023; Sarkar et al., 2023). Medicinal plants are rich sources of the essential oils which are extracted by different methods, *e.g.*, the genus *Perovskia* contains oxygenated monoterpenes extracted by ethanol and displays potential antioxidant properties (Mohammadhosseini et al., 2019). Recent reports also state that the phytoconstituents of some herbal genera imply ethnopharmacological and therapeutic properties. Essential oils from some plants play an important role in the preservation of human and animal health from various diseases including digestive, gastrointestinal and urinary tract infections (Mohammadhosseini et al., 2021; Olaoluwa et al., 2022). The herbal species *A. muricata* L. is an evergreen plant belonging to Annonaceae family which is widely distributed throughout the tropical and subtropical regions of the world like India and many other Caribbean and American countries (Rady et al., 2018). Its fruit is known as soursop that contains high levels of vitamin C along with some valuable bioactive compounds which are used to cure several diseases like arthritic pain, neuralgia, arthritis, diarrhea, and some scientific reports show that *Annona* fruits are capable of curing type 2-diabetes (Adefegha et al., 2015; Karthik et al., 2021). The plant contains highly potent bioactive compounds that are promoting inhibition of free radicals and oxidative cell damages (Vignesh et al., 2021). The whole plant has been utilized for various pharmaceutical purposes. The nutritional composition and secondary metabolites of this plant have been attributed to possess beneficial effects such as antimicrobial, antidiabetic, antioxidant and anticancer activities (Rocha et al., 2018; Kumar et al., 2019). The previous reports have also shown that leaf extracts of *A. muricata* L. could be used to treat various bacterial and fungal diseases (Gajalakshmi et al., 2012).

Based on the ethnobotanical knowledge of *A. muricata* L., the present study aims to preliminary analyze, screen and quantify the phytocompounds of the fruits of *A. muricata* L. by various extracting solvents. In addition, the antioxidant, antibacterial efficacy of extraction and the corresponding phytochemicals are characterized by high performance liquid chromatography (HPLC) and Fourier transform infrared (FTIR) analysis.

2. Experimental

2.1. Reagents and Chemicals

In this study, analytical grade solvents and chemicals were used. The solvents acetone, ethyl acetate and methanol were purchased from S.D Fine Chem Ltd, India. The standards ascorbic acid and rutin were supplied from Sigma Aldrich (Mumbai, India). All the other fine chemicals used in this study were provided by Hi Media Laboratories Pvt Ltd (Mumbai, India).

2.2. Plant sample collection

A. muricata L. fruits were collected from Poondi, Thanjavur District, Tamil Nadu, India. The taxonomic identity of the plant was confirmed and certified by the Bharathi herbarium, Department of Botany, Bharathiar University, Coimbatore, Tamil Nadu, India (Ref no: (BU/DB/25/23/2021/Tech./1054) through comparing with the type specimen depository at the herbarium and also by characteristic identification. The ripened fruits were thoroughly washed with tap water followed by distilled water and shade dried in room temperature.

2.3. Plant extraction by Soxhlet method

The collected fruit samples of *A. muricata* L. were first sliced and dried at room temperature (36 °C). In the next step, the dried materials were ground into a fine powder using a mechanical grinder (Sujatha Dynamx, India). About 20 g of the obtained powder was then packed in small thimbles and separately extracted with organic solvents such as acetone, ethyl acetate, methanol in the increasing order of their polarity using soxhlet apparatus. Finally, the extracted fruit samples were macerated using hot water with constant stirring for 24 hrs and the water extract was also filtered using Whatman No. 1 filter paper. The crude extracts were concentrated by rotary evaporator (SUPERFIT ROTAVAP Model: PBU-6D) and the air dried extract was stored at 4 °C for further analysis (Agu and Okolie, 2017).

2.4. Quantitative phytochemical analysis

2.4.1. Total phenol content

The different solvents were subjected to analyze the total phenolic content *A. muricata* L. according to Folin-Ciocalteu colorimetric method described by Vignesh et al. (2021). Briefly, about 50 µL of each solvent was taken in the test tubes, then 1 mL of distilled water was added followed by the addition of Folin-Ciocalteu reagent (1:1 v/v). Then, 250 µL of a solution of sodium carbonate was added to each tube. The gallic acid was used as standard (positive control) and all test tubes were incubated in dark for 30 min. The absorption of each sample was recorded at 725 nm using a UV-spectrophotometer.

2.4.2. Total tannin content

The total tannin content in the extracts of *A. muricata* L. was estimated by polyvinyl polypyrrolidone (PVPP) method according to Vignesh et al. (2021). Accordingly, about 75 mg of PVPP was weighed and 900 µL of distilled water and 750 µL of the fruit extract were subsequently added to it. The resulting mixture was vortexed well and the tube was kept in refrigerator for 4 hrs at 4 °C. The sample was then centrifuged at 4000 × g for 10 min at room temperature and the supernatant was collected and its optical density (absorbance) was measured at 725 nm using a spectrophotometer. The non-phenolic content in the sample was calculated

using the following formula (Eqn. 1).
Tannin (%) = Total phenolics (%) - Non tannin phenolics (%) (Eqn. 1)

2.4.3. Total flavonoid content

Flavonoid content of the fruit extracts was determined by an aluminium chloride colorimetric assay (Zhishen et al., 1999). In this sense, rutin as a standard flavonoid standard compound was used to compare the quantity of flavonoids present in the extracts. An adequate of the sample extracts (0.5 mL) were taken and added to 0.5 mL of aluminium chloride (2%) and kept at room temperature for 1 h. The absorbance was finally recorded at 415 nm using UV-Vis spectrophotometer.

2.5. Assessment of antioxidant activity

2.5.1. DPPH radical scavenging test

The DPPH free radical scavenging activity of the fruit extracts of *A. muricata* L. was determined by the method reported by Blois (1958). In this relation, the extracts at various concentrations (100, 200, 300, 400 and 500 µg/mL) were added to 5 mL of methanolic solution of DPPH (0.1 mM) and allowed to stand for 20 min at 27 °C. The negative control was prepared by adding 100 µL of methanol with 0.5 mL of DPPH (0.1 mM). The methanol served as blank and mixture of methanol, DPPH and ascorbic acid treated as positive control. The absorbance of the all samples measured at 517 nm using a UV spectrophotometer. The percentage of inhibition and IC_{50} value of the extracts were also calculated. The scavenging effect was expressed as follows (Eqn. 2).
Scavenging activity (%)= [(A blank – A control) – (A sample – A control)]/(A blank – A control) × 100 (Eqn. 2)

2.5.2. Hydrogen peroxide scavenging activity

The hydrogen peroxide scavenging ability of *A. muricata* L. fruit extracts was determined according to the method of Ruch et al. (1989). Accordingly, a solution of hydrogen peroxide (2 mM) was prepared by using a phosphate buffer (0.2 mM; pH-7.4). About 100 µL of sample extracts were added into the mixture containing 300 µL phosphate buffer with 600 µL of H_2O_2. The phosphate buffer without H_2O_2 served as blank, as well. The positive control prepared by a mixture of phosphate buffer with H_2O_2 and standard rutin was used to construct the relevant calibration curve. The absorbance of the all samples was measured at 230 nm under UV spectrophotometer against blank. The scavenging activity (%) was finally calculated as follows (Eqn. 3).

Scavenging activity (%) = [(Control OD - Sample OD) / Control OD] × 100 (Eqn. 3)

2.6. Analytical characterization of *A. muricata* L. extracts

2.6.1. Fourier transforms infrared spectroscopy analysis (FTIR)

The extracted compounds were characterized by FTIR spectroscopy (Jasco N-4700, Japan). To record the corresponding spectra, about 5 mg of fruit extracted samples were first ground to make KBr pellet for FTIR measurement over the spectral range of 4000 to 600 cm^{-1}. The peak values of the extracts were noted to assign their functional groups.

2.6.2. High performance liquid chromatography (HPLC)

HPLC analytical technique was carried out for separation and determination of phytcompounds from selected methanol extracts of *A. muricata* L.. The HPLC analysis of the phytocompounds was performed by an HPLC Shimadzu, (Japan) and Shimadzu Shim-Pack Solar C18 column (5u,4.6 X 250 mm), part no: 227-30600-02, coupled with PDA detector at 254 nm. The mobile phase used in an isocratic elution mode consisting of polar solvents like mixture of water (70%) and methanol (30%) (HPLC gradient grade, ≥99.9%) with a flow rate of 0.8 mL/min for 45 min. The column oven was set at 40 °C. The standard compounds such as quercetin, catechin, rutin and coumaric acid were used to identify bioactive compound in the extracts through comparing the retention times.

2.7. Assessment of inhibitory effect of *A. muricata* L. extracts against bacteria

The antimicrobial effect of *A. muricata* L. fruit methanolic extracts were investigated against six human pathogenic bacteria, namely *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Streptomyces* sp. by agar well diffusion method. The suspension culture 10^{-3} cfu/mL concentration was loaded on agar plate containing nutrient agar (NA). Then, 6 mm in diameter size well were made in agar plate and different concentrations (25, 50, 75 and 100 µg/mL) of extracts were poured. The chloramphenicol and methanol were used as positive and negative control, respectively. All the plates were incubated at 35-37 °C for 24 hrs and the zone of inhibition around the wells was observed accordingly (VasanthKumar et al., 2022).

2.8. Statistical analysis

All the experiments were performed in triplicates and the results were expressed as Mean ± Standard deviation. The data were statistically analyzed using one-way ANOVA followed by Duncan's test. Mean values were considered statistically significant at $p < 0.05$ using SPSS package version 17.0 (SPSS Inc., Chicago, Illinois, USA).

3. Results and Discussion

3.1. Quantitative analysis

The quantitative analysis of *A. muricata* L. extracts showed the presence of various secondary metabolites using different extracting solvents. *A. muricata* L. fruit methanolic extracts showed highest phenolic (289.63

± 2.3 mg GAE/g dry wt) and tannin (121.02 ± 3.4 mg GAE/g dry wt) contents than the other prepared extracts. The flavonoid content was found to be more in water extracts (92.13 ± 2.8 RE mg/g extract) (Table 1). In contrast, least amount of phenol, tannins and flavonoids were recorded in acetone extracts. It has been well documented that the natural compounds from plant origin have high potential to reduce the communicable and non-communicable disease risk (Fang and Bhandari, 2010). The methanolic fruits extracts of *A. muricata* L. contain high amount of phenols, flavonoids and tannin when compared with other solvent extraction. However, Muthu and Durairaj (2015) reported the highest amount of total phenol, tannin and flavonoid contents in the ethanol extract from the leaves of *A. muricata* L. (0.44 ± 0.0013 mg/g, 1.92 ± 0.02 mg/g, 104.43 ± 0.013 mg/g). However, according to the findings of our study on the fruit methanolic extract of *A. muricata* L. higher quantities of secondary metabolites were characterized. The yield and quantity of the extracted compounds depend on the solvent polarity as well as the optimum conditions that conserve various secondary metabolites (Kumar et al., 2021). Previous studies reported that *Annona squamosa* leaves extracts obtained by various solvents exhibited a large number of potential antioxidant and antimicrobial compounds (El-Chaghaby et al., 2014). The extracted phyto-composition from plants can be used as ingredients in foods and pharmacological products (Punia and Kumar, 2021).

Table 1
Total phenol, tannin and flavonoid content of *A. muricata* L. fruit extracts.

Solvent extraction	Total phenol (GAE mg/g extract)	Tannin (GAE mg/g extract)	Flavonoid (RE mg/g extract)
Acetone	40.16 ± 0.8^{d}	15.64 ± 1.1^{d}	55.22 ± 1.5^{a}
Ethyl acetate	132.21 ± 1.7^{b}	86.41 ± 2.8^{b}	42.21 ± 1.9^{b}
Methanol	289.63 ± 2.3^{a}	121.02 ± 3.4^{a}	86.24 ± 3.1^{d}
Water	112.12 ± 2.1^{c}	46.21 ± 2.2^{c}	92.13 ± 2.8^{c}

Values are mean of triplicate analysis (n = 3) ± standard deviation a>b>c>d statistical different at $p < 0.05$ among the extracts. **GAE**: Gallic acid equivalent, **RE**: Rutin equivalent.

3.2. Evaluation of *in vitro* antioxidant activity

The antioxidant potential of different extracts of *A. muricata* L. was also assessed. In this context, the free radical assays of 2,2-diphenyl-1-picrylhydrazyl (DPPH$^{\bullet}$) and H_2O_2 are commonly used to analyze the antioxidant property of different plant samples. The DPPH free radical scavenging activity was measured using standard ascorbic acid and the numerical IC_{50} values were calculated. Taking into account the obtained results, the methanolic fruit extract of *A. muricata* L. showed high percentage of inhibition 69.63 ± 0.34% at 400 µg/mL concentrations, with IC_{50} of 192.14 ± 5.2 µg/mL. Similarly, ethyl acetate extract showed 62.64 ± 0.43 percentage of inhibition with IC_{50} of 203.43 ± 4.3 µg/mL (Table 2). The other extracts (acetone and water) showed minimal inhibitory effects when compared with ascorbic acid. Similarly, Reddy et al. (2021) also reported the *Gardenia jasminoides* fruit methanolic extracts showed the IC_{50} value of 65.82 µg/mL. Justino et al. (2020) also found that ethyl acetate extracts of *Eugenia dysenterica* fruit pulp extracts showed an IC_{50} value of 10 µg/mL when using the DPPH assay.

H_2O_2 radical is a weak oxidizing agent and reacts with Fe^{2+} or Cu^{2+} ion and gets converted into hydroxyl radical. The hydroxyl radical was strongly encountered by bioactive molecules. In this study, the efficacy of crude extracts was tested against hydroxyl free radicals. The results exhibited that crude extract of *A. muricata* L. possesses higher antioxidant activities when compared with the standard rutin. All the studied extracts caused a strong dose-dependent inhibition of hydrogen peroxide. The achieved results represent that methanloic fruit extract of *A. muricata* L. exhibits a percentage of inhibition of 74.02 ± 3.84 followed by ethyl acetate (67.27 ± 1.96%) and acetone (62.53 ± 2.24%) with the IC_{50} values of 401.08 ± 5.4 µg/mL, 457.24 ± 5.6 µg/mL and 852.75 ± 4.8 µg/mL for methanol, ethyl acetate and acetone, respectively. However, the standard rutin shows a remarkable IC_{50} value (85.81 ± 2.04 µg/mL) (Table 3). The lowest value of inhibition was observed for the water extract that showed 58.15 ± 2.81% with a high IC_{50} value (651.23 ± 4.2 µg/mL). The H_2O_2 radical scavenging activity was also studied by Hegazy et al. (2019) in the methanolic fruit extract of *Coccinia grandis* and exhibited higher activity with 71.53%.

3.3. FTIR analysis

The Fourier transform infrared spectroscopy (FTIR) techniques are used to determine the various functional groups of the extracted compounds. Based on the quantification of metabolites, methanol extract of *A. muricata* L. fruit was subjected to FTIR analysis over

Table 2
Effect of *A. muricata* L. fruit extracts on DPPH free radical scavenging activity.

Concentration (μg/mL)	Acetone	Ethyl acetate	Methanol	Water	Ascorbic acid
Percentage of inhibition					
100	17.98 ± 0.43^{d}	36.24 ± 0.16^{d}	45.81 ± 0.09^{d}	28.24 ± 0.21^{a}	57.47 ± 0.19^{c}
200	29.74 ± 0.26^{c}	43.16 ± 0.12^{c}	53.28 ± 0.15^{c}	32.12 ± 0.55^{c}	63.23 ± 0.73^{d}
300	37.21 ± 0.78^{b}	50.52 ± 0.41^{b}	60.91 ± 0.63^{a}	38.11 ± 0.61^{d}	78.15 ± 0.92^{b}
400	49.25 ± 0.90^{a}	62.64 ± 0.43^{a}	69.63 ± 0.34^{b}	51.73 ± 0.17^{a}	88.31 ± 0.88^{a}
IC_{50}	488.25 ± 4.1^{a}	203.43 ± 4.3^{c}	192.14 ± 5.2^{e}	350.52 ± 3.2^{b}	42.32 ± 2.4^{d}

Values are mean of triplicate determination (n = 3) ± standard deviation where significance among the extracts $p < 0.05$.

Table 3
H_2O_2 scavenging activity of *A. muricata* L. fruit extracts.

Concentration (μg/mL)	Acetone	Ethyl acetate	Methanol	Water	Rutin
Percentage of inhibition					
100	43.15 ± 2.31^{c}	35.30 ± 1.51^{d}	58.13 ± 2.15^{d}	29.53 ± 1.03^{b}	29.13 ± 0.77^{d}
200	49.31 ± 2.12^{d}	52.21 ± 2.32^{a}	62.32 ± 1.93^{b}	19.22 ± 0.95^{d}	48.07 ± 2.01^{b}
300	58.72 ± 1.43^{b}	53.18 ± 2.61^{b}	61.45 ± 3.23^{c}	32.50 ± 1.32^{c}	69.12 ± 3.49^{c}
400	62.53 ± 2.24^{a}	67.27 ± 1.96^{c}	74.02 ± 3.84^{a}	58.15 ± 2.81^{a}	90.07 ± 4.51^{a}
IC_{50}	852.75 ± 4.8^{a}	457.24 ± 5.6^{b}	401.08 ± 5.4^{d}	651.23 ± 4.2^{c}	85.81 ± 2.04^{e}

Values are mean of triplicate determination (n = 3) ± standard deviation where significance among the extracts $p < 0.05$.

the range 4000-650 cm^{-1}. Various functional groups were observed in the methanol extract and the results revealed the presence of different intense peak each indicating a specific functional group. The FTIR spectrum of methanol fruit extracts of *A. muricata* L. showed a strong intense peak at 3361.32 cm^{-1} indicating the aliphatic primary amine at N-H stretching bond.
The strong, week and broad bands with C-H and C=O stretching vibration at 2922.59 and 1702.84 cm^{-1} were attributed to the C-H stretching and C=O stretching group (Fig. 1). The alkene, nitro compound, carboxylic acid, phenol or tertiary alcohol assigned with medium intensity peaks occurring at 1665.23, 1521.56 and 1363.41 cm^{-1}. The peak signals at 1231.33 cm^{-1} with strong S=O stretching indicated the aromatic ester. The strong and medium signal of 1021.12 and 817.67 cm^{-1} indicated the presence of carboxylic acids and the peak at 775.24 cm^{-1} with C-O stretching assigned the presence of alkane (Table 4). Similarly, Ragavendran et al. (2011) screened the functional groups of carboxylic acids, amines, amides, sulphur derivatives, polysaccharides, organic hydrocarbons and halogens that are responsible for various medicinal properties of *Aerva lanata*. The FTIR analysis of methanolic and aqueous leaf extracts of *Bauhinia racemosa* revealed the presence of protein, oil, fats, phenolic compounds, flavonoids, saponins, tannins and carbohydrate as major functional groups (Kumar et al., 2010). Starlin et al. (2012), while analyzing the ethanolic extracts of *Ichnocarpus frutescens* by FTIR, revealed functional group components of amino acids, amides, amines, carboxylic acid, carbonyl compounds, organic hydrocarbons and halogens.

3.4. HPLC analysis

The HPLC analysis was used to characterize the various phytocompounds of *A. muricata* L.. The chromatogram of extracts showed different peaks at various retention times that indicated the presence of secondary metabolites. In this study, the methanolic extract of *A. muricata* L. fruit was analyzed by HPLC and results revealed that extract showed more than 30 peaks at various retention times (Fig. 2e). The phytocompounds were quantified by standard bioactive compounds such as coumaric acid, catechin, rutin and quercetin (Fig. 2a-d). The major peaks are detected at various retention times (min) at 280 nm such as RT-6.8, RT-7.1, RT-8.533, and RT-13.274 and were compared with standard phytocompounds. The values of phytocompounds recorded in crude extracts are coumeric aicd (2 mg/g) followed by catechin (0.22 mg/g), rutin (0.54 mg/g) and quercetin (0.64 mg/g) (Table 5). Similar to our results, the HPLC analysis of methanolic fruit extracts of *Ziziphus spina-christi* showed different types of polyphenolic compounds within the retention times of 3.058 to 16.075 (El-Shahir et al., 2022). The methanolic extract of *Luffa echinata* was also found to have alkaloids, flavonoids and tannins (Patel and Ghane, 2021).

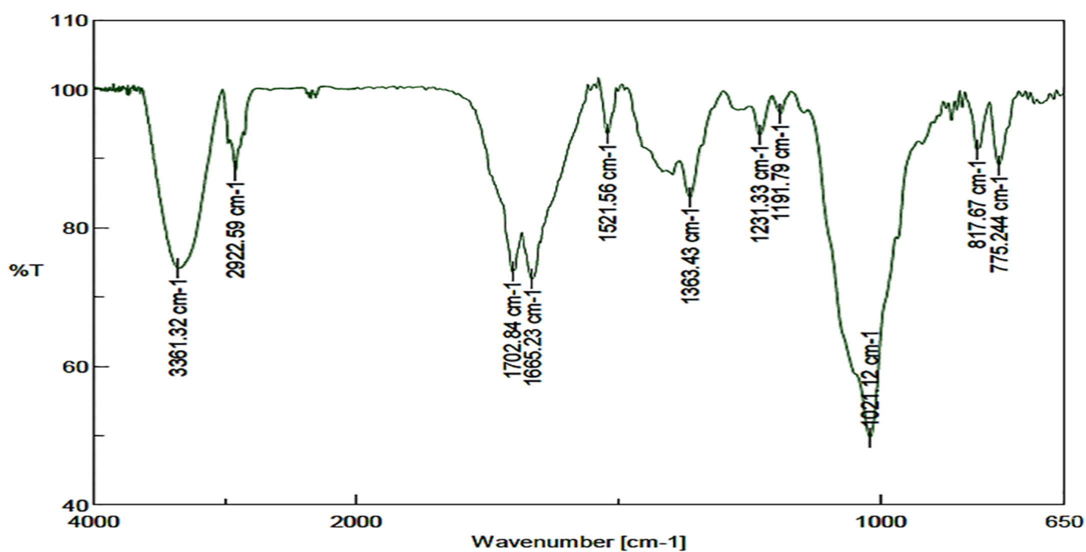

Fig.1. FTIR-ATR spectrum analysis of *Annona muricata* L. methanolic fruit extract.

Table 4
Functional groups of phytocompounds from *A. muricata* L. extract by FTIR analysis.

Extract Absorption (cm^{-1})	Group	Compound Class
Lipids (3000-2000 cm^{-1})		
3361.32	N-H stretching	Aliphatic primary amine
2922.59	C-H stretching	Alkane
Proteins (1800-1500 cm^{-1})		
1702.84	C=O stretching	Conjugated aldehyde
1665.23	C=C stretching	Alkene
1521.56	N-O stretching	Nitro compound
1363.43	S=O stretching	Sulfonate
1231.33	C-O stretching	Alkyl aryl ether
1191.79	C-O stretching	Ester
1021.12	S=O stretching	Sulfoxide
Cell wall components (1000-600 cm^{-1})		
817.67	C=C bending	Alkene
775.24	C=C bending	Alkene

Table 5
Quantification of secondary metabolites in methanolic fruit extract of *A. muricata* L. using HPLC method.

Reference standard	Compound RT (min)	mg/g (Sample)	Peakpurity index
Coumaric acid	4.68	2	0.964
Catechin	7.1	0.22	0.657
Rutin	8.533	0.54	0.584
Quercetin	13.274	0.64	0.049

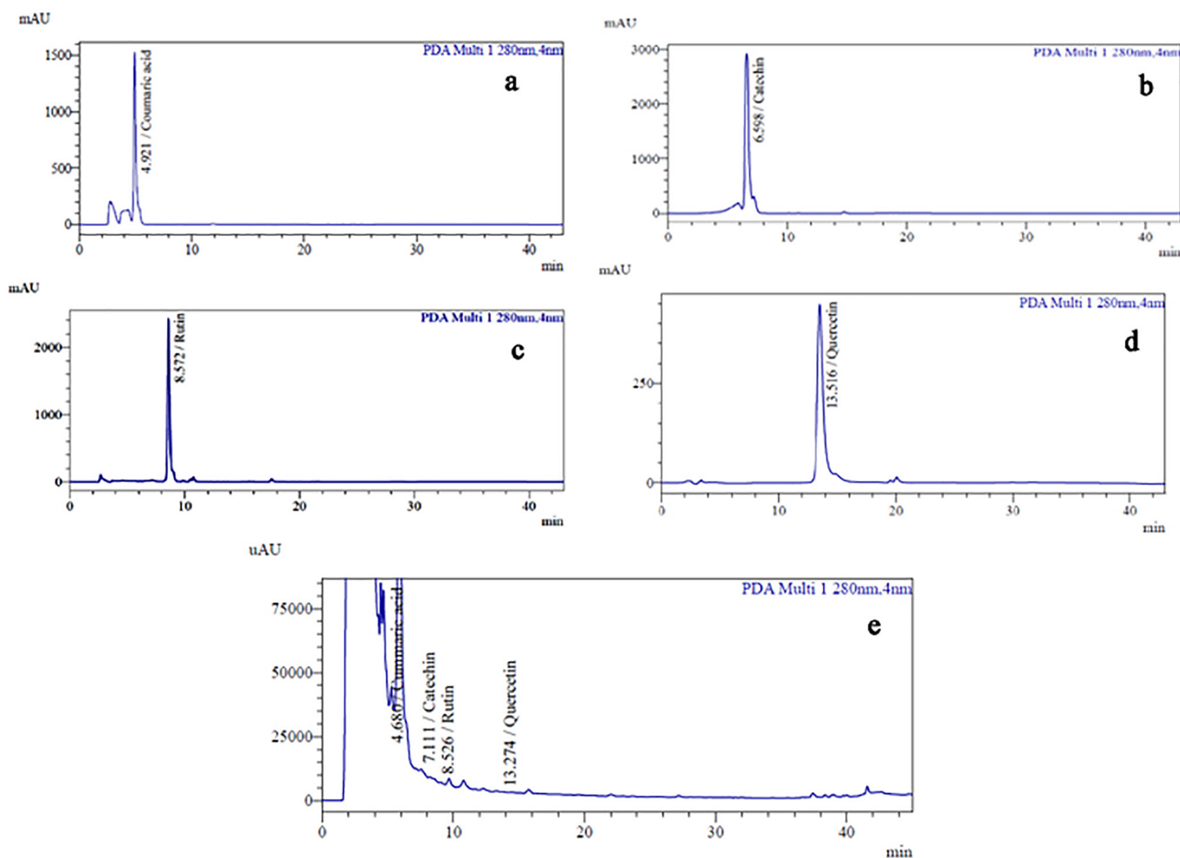

Fig. 2. HPLC chromatogram of standard and phytocompounds on methanolic fruit extract of *A. muricata*. [(**a**) Coumaric standard, (**b**) Catechin standard, (**c**) Rutin standard, (**d**) Quercetin standard, and (**e**). *A. muricata* methanol fruit extract.]

3.5. Antibacterial activity

The antibacterial activity of the different extracts of *A. muricata* L. was determined against bacterial pathogens. The minimal inhibitory activities of extracted plant samples were tested at various concentrations against some disease causing bacterial pathogens such as *Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus, Bacillus subtilis*, and *Pseudomonas aeruginosa* and *Streptomyces sp.* The results were compared with standard drug chloramphenicol (positive control) and methanol used as negative control. Methanol fruits extract at 100 µg/mL strongly exhibited effective antibacterial activity against all pathogens (Fig. 3). The maximum zone of inhibition recorded against *Staphylococcus aureus* (12.3 ± 0.07 mm), *Klebsiella pneumoniae* (11 ± 0.09 mm) and *Streptomyces sp.* (9 ± 0.03 mm), respectively. The minimum effect of methanolic crude extract was recorded against *Escherichia coli* (6.2 ± 0.06 mm) followed by *Bacillus subtilis* (7 ± 0.04 mm) (Table 6). Reddy et al. (2021) also found that the methanolic fruit extracts of *Gardenia latifolia* possessed inhibitory effect on bacterial strains at the concentrations of 15.62, 62.5 and 62.5 µg/µL respectively for *Staphylococcus aureus*, *Escherichia coli* and *Klebsiella pneumoniae*. *Annona squamosa* leaves extracts contain enormous pharmacologically important compounds that showed antibacterial activity against Gram-positive bacteria which were similar to the obtained results of our group (Kumar et al., 2021). Effect of oleoresin from *A. muricata* L. leaf extract showed potent antibacterial activity against *Enterobacter cloacae* and *Pseudomonas aeruginosa* (Cagnini et al., 2021). The results supported the development of antimicrobial drugs and the bioactive compounds in *A. muricata* L. fruit could be used against micro-organisms and further developed into medicinal products against various diseases by pharmaceutical industries.

4. Concluding remarks

The present study revealed that the fruit extracts of *A. muricata* L. possessed various active compounds such as poly phenols, flavonoids, tannins and alkaloids. The phytocompound extraction was characterized by analytical methods which confirmed the presence

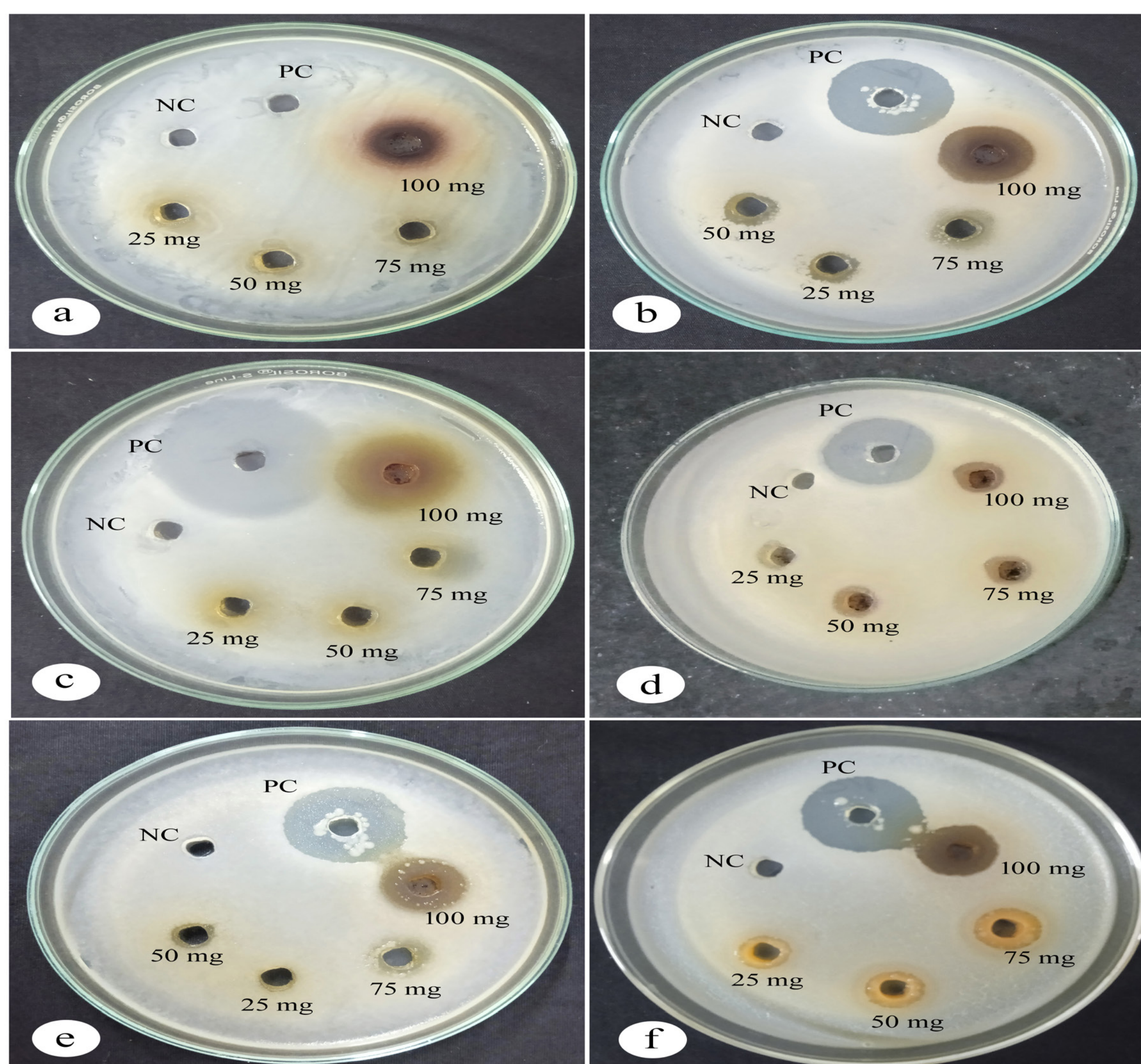

Fig. 3. Antibacterial activity of methanol fruit extract of *A. muricata* against (a) *Escherichia coli* (b) *Klebsiella pneumonia* (c) *Staphylococcus aureus* (d) *Bacillus subtilis* (e), *Pseudomonas aeruginosa* (f), *Streptomycetes sp.*, (PC). Antibiotics chloramphenicol was used as positive control and (NC), while methanol solvent as negative control.

of various functional metabolites. These different compound groups were found to be responsible for higher antioxidant activity. Also, crude extracts of *A. muricata* L. were so effective in controlling the growth of human disease-causing pathogenic bacteria. Thus, plant extracts have substantial antimicrobial properties that are considered to be cost-effective and safe alternatives for commercial synthetic antibiotics.

Conflict of interest

The authors declare that there is no conflict of interest.

Acknowledgments

The authors are thankful to the Department of Botany, A.V.V.M Sri Pushpam College (Autonomous), Poondi 613 503, Thanjavur (Dt) Tamil Nadu. The authors also thank DST-FIST II and UGC -SAP programme, Department of Botany, Bharathiar University for providing the infrastructure facilities to carry out the research work.

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Table 6
Antibacterial effect of methanolic fruit extract of *A. muricata* L.

Extracts	Concentration (µg/mL)	*Escherichia coli*	*Klebsiella pneumonia*	*Staphylococcus aureus*	*Bacillus subtilis*	*Pseudomonas aeruginosa*	*Streptomycetes sp*
		Zone of inhibition (mm)					
Chloramphenicol (PC)	10	2 ± 0.02^{cd}	12 ± 0.08^{a}	18 ± 0.3^{a}	13 ± 0.5^{a}	11 ± 0.3^{b}	10 ± 0.5^{b}
Methanol (NC)	--	--	--	--	--	--	--
Methanolic extract	25	4 ± 0.01^{b}	4 ± 0.01^{d}	5 ± 0.01^{a}	2 ± 0.01^{e}	2 ± 0.02^{e}	4 ± 0.03^{e}
	50	5 ± 0.02^{c}	3 ± 0.02^{e}	4 ± 0.03^{a}	5 ± 0.02^{c}	4 ± 0.01^{d}	6 ± 0.02^{d}
	75	4 ± 0.02^{b}	6 ± 0.07^{c}	5 ± 0.01^{a}	4 ± 0.03^{d}	6 ± 0.02^{c}	9 ± 0.04^{c}
	100	6 ± 0.06^{a}	11 ± 0.09^{b}	12.3 ± 0.07^{b}	6 ± 0.02^{b}	7 ± 0.04^{a}	9 ± 0.03^{a}

Values are presented as means ± SE of triplicate determinations and expressed per g of plant extracts. Means significantly different between the extract ($p < 0.05$). **Note**: **PC**: Positive control. **NC**: Negative control.

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