

Original Research Article

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Antimicrobial and Phytochemical Analysis of Methanolic and Aqueous Extract of *Annona muricata* (Leaf and Fruit)

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ABSTRACT

Annona muricata is a species of the genus *Annona* of the custard apple tree family, Annonaceae, which has edible fruit. The aim of this study was to carry out for check the Antibacterial, Antifungal and Phytochemical activity from methanolic and aqueous extract of leaf and fruit of *Annona muricata*. An antibacterial activity of *Annona muricata* was evaluated on Pathogenic bacteria (*Staphylococcus aureus*, *Streptococcus pyogenes*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Klebsiella pneumoniae*, *Escherichia coli* and *Proteus mirabilis*) by Well diffusion method. Antifungal activity of *Annona muricata* was evaluated on *Cryptococcus neoformans* and *Candida albicans*. Phytochemical analysis was done by using standard methods. Phytochemical screening was used to determine the presence of Alkaloids, Flavonoids, Carbohydrates, Glycosides, Proteins, Amino acids, Saponins, Tannins, Terpenoids in Methanolic and Aqueous extract of *Annona muricata*. These findings support the traditional use of *Annona muricata* in various disorders.

Keywords

Annona muricata,
Anti fungal,
Anti bacterial,
Phytochemical.

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Introduction

Plants are one of the most important sources of medicines for treating illnesses since the beginning of human civilization (Jyothi *et al.*, 2011). In recent times, and due to historical, cultural, and other reasons, folk medicine has taken an important place, especially in developing countries, where health services are limited. The study on the medicinal plants is essential to promote the proper use of herbal medicine in order to determine their potential as a source for the new drugs (Gajalakshmi *et al.*, 2012).

Ayurvedic medicine uses all of these either in diet or as a medicine. Some of these medicinal plants like Tulsi (*Ocimum sanctum*), Haridra (*Curcuma longa*), sarpagandha (*Rauvolfia serpentina*) and Ghritkumari (*Aleo barbadensis*) have been featured on Indian postage stamps. Among this one of the important medicinal plants which show many medicinal properties is *Annona muricata* (In Tamil, Mullu seetha). *Annona muricata* is a member of the family of custard apple tree called Annonaceae and

species of the genus *Annona* known mostly for its edible fruit Annona. It is a small upright evergreen tree 5-6 meter height with large, glossy, dark green leaves. It produce a large heart shaped, edible fruit, 5-20 cm in diameter, yellow green in colour and has white flesh inside. *Annona muricata* produce fruit that are usually called soursop due to its slightly acidic taste when ripe (Hutchinson *et al.*, 2011). The fruit is juicy, acidic, whitish and aromatic with abundant seeds. The average weight of 1000 fresh seeds is 479g and has an average oil content of 24% (Hutchings, 2006). The creamy and delectable flesh of fresh fruit consists of 80% of water, 1% protein, 18% carbohydrate and fair amount of Vitamin B, B2 and C and Potassium and dietary fiber. Annonaceous acetogenins are powerful phytochemicals found in the Graviola plant (*Annona muricata*), which are found only in Annonaceae family. These chemicals in general have been documented with Antitumor, Antiparasitic, Insecticidal and Anti-microbial activities. These acetogenins are superb inhibitors of enzyme processes that are found only in the membranes of cancerous tumour cells.

The leaves and stems of soursop show active cytotoxicity against cancer cells, due to bioactive compounds called acetogenins. These acetogenins are non-toxic to normal cells, but are highly toxic to cancer cells (Oberlies *et al.*, 1995). Acetogenins from the soursop plant are usually extracted using ethanol as the organic solvent (Zeng *et al.*, 1996). These compounds, collectively, have shown antitumor, parasiticidal, pesticidal, and antimicrobial activities (McLaughlin, 2008). The cytotoxicity of annonaceous acetogenins is due to the depletion of ATP levels *via* the inhibition of the NADP-ubiquinone oxidoreductase (Complex I) of the mitochondrial electron transport system (Zafra-Polo *et al.*, 1996). The lactone ring

present in acetogenin molecules plays an important role in the anticancer mechanism, present (Kreuger *et al.*, 2012). Annonacin is known as the most prevalent acetogenin present in soursop leaves (Champy *et al.*, 2004; Yuan *et al.*, 2003), however, the method for separation of annonacin from soursop leaves is not widely reported. More reports are available on various methods to obtain new acetogenins from soursop leaves (Kim *et al.*, 1998; Zeng *et al.*, 1996).

The objective of this research work is to check the antimicrobial activity of *Annona muricata* against pathogenic organisms like *Staphylococcus aureus*, *Streptococcus pyogenes*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Klebsiella pneumonia* and *Salmonella typhi*. Antifungal activity was checked against *Candida albicans* and *Cryptococcus neoformans*. And to screen the aqueous and methanolic extract of *Annona muricata* for their biologically active chemicals, with a view to provide a scientific basis for use of the leaves for prevention and treatment of diseases.

Materials and Methods

Sample collection and authentication

A.muricata L. Leaves and fresh ripe fruits (5 kg) were collected locally at Gudalur, Ooty in the month of July 2016. Leaves and fruits were authenticated (BSI/SRC/5/23/2016/Tech/1223) by the authority of the botanical survey of India (BSI), Tamil Nadu Agricultural University, Coimbatore.

Sample preparation

A.muricata leaves were washed thoroughly under running tap water, air dried and homogenized to fine powder and stored in sterile air tight container for the

experimental work. *A.muricata* fruits were washed in tap water. Seeds and pericarps were carefully removed and pulp was lyophilized for further use.

Preparation of Methanolic and Aqueous extracts

Air dried powder of leaves and lyophilized fruit pulp was extracted by using Soxhlet apparatus. 10 g of *A.muricata* leaf and fruit powder was taken in a paper cone and placed into soxhlet apparatus. 100 ml of solvent (methanol and water) was taken in the round bottom flask attached to this setup. Then the whole setup was placed on the heating mantle. The temperature was setup to 65-80°C solvents get vaporized and rises up to the condenses back in to the liquid and falls in to the plant sample in the cone and extract certain compounds falls in to the round bottom flask.

Methanol extract of leaf (dark green color) and fruit (yellowish brown) was evaporated to dryness at low temperature, by using rotary evaporator. And aqueous extract of leaf (brown color) and fruit (light brown) was evaporated by hot plate method (Satyanarayana, 2006). Concentrated extracts were dissolved in DMSO for antimicrobial studies.

Microbial cultures

Microorganisms *Staphylococcus aureus*, *Streptococcus pyogenes*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Klebsiella pneumoniae*, *Escherichia coli*, *Proteus mirabilis*, *Cryptococcus neoformans* and *Candida albicans* were obtained from KMCH Hospital, Coimbatore, Tamilnadu. And the cultures were further conformed by staining and biochemical methods. Antimicrobial activity was examined for aqueous and methanol extract from the fruit and leaves of *Annona muricata*.

Antimicrobial activity by well diffusion method

Molten Mueller Hinton agar was prepared and poured into petri dishes and the bacterial culture was swabbed on the surface of agar to check the antibacterial activity. And a well was made by using 6mm well cutter. Aqueous and methanolic extracts of fruit and leaves of *Annona muricata* was added into well in the concentration of 50µl, 100µl and 200µl respectively. For each bacterial strain positive and negative controls were maintained. Antibacterial activity of the extract was determined by measuring the diameter of zone of inhibition.

To check the antifungal activity Sabouraud dextrose agar was prepared and poured into petri dishes and the fungal culture was swabbed on the surface of agar. A well was made by using 6mm well cutter.

Aqueous and methanolic extracts of fruit and leaves of *Annona muricata* was added into well in the concentration of 50µl, 100µl and 200µl respectively. For each fungal culture positive and negative controls were maintained. Antifungal activity of the extract was determined by measuring the diameter of zone of inhibition.

Phytochemical screening

Phytochemical examinations were carried out for all the extracts as per the standard methods. (Roopashree *et al.*, 2008; Obasi *et al.*, 2010).

Results and Discussion

Antibacterial activity

Antibacterial activity for ethanolic and aqueous extract of *Annona muricata* was seen against to the several organisms namely

Staphylococcus aureus, *Streptococcus pyogenes*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Klebsiella pneumoniae*, *Escherichia coli* and *Proteus mirabilis*.

The methanol and aqueous leaf extract showed maximum activity against *Streptococcus pyogenes*. And the methanol and aqueous fruit extract showed maximum activity against *Staphylococcus aureus* and *Salmonella typhi* as shown in table 1 and 2.

Antifungal activity

Antifungal activity for ethanolic and aqueous extract of *Annona muricata* was seen against *Candida albicans* and *Cryptococcus neoformans*. The methanol and aqueous leaf extract showed maximum activity against *Cryptococcus neoformans*. And the methanol and aqueous fruit extract showed maximum activity against *Candida albicans* as shown in Table 3 and 4.

Table.1 Antibacterial activity for methanolic and aqueous leaf extract of *Annona muricata*

Test organisms	Concentration of methanol extract(µl)			Concentration of aqueous extract(µl)			Positive control (Streptomycin 0.1mg/ml)	Negative control (DMSO)
	50	100	200	50	100	200		
	Diameter of inhibition (mm)							
<i>Staphylococcus aureus</i>	14	20	22	10	12	15	18	-
<i>Streptococcus pyogenes</i>	18	20	24	11	14	18	20	-
<i>Pseudomonas aeruginosa</i>	15	21	22	10	13	16	17	-
<i>Klebsiella pneumonia</i>	14	22	23	13	15	18	20	-
<i>Proteus mirabilis</i>	13	19	21	-	-	13	15	-
<i>Escherichia coli</i>	20	22	24	10	13	15	20	-
<i>Salmonella typhi</i>	18	20	22	15	18	20	20	-

Table.2 Antibacterial activity for methanolic and aqueous fruit extract of *Annona muricata*

Test organisms	Concentration of methanol extract(µl)			Concentration of aqueous extract(µl)			Positive control (Streptomycin 0.1mg/ml)	Negative control (DMSO)
	50	100	200	50	100	200		
	Diameter of inhibition (mm)							
<i>Staphylococcus aureus</i>	13	18	24	18	20	22	18	-
<i>Streptococcus pyogenes</i>	-	15	20	-	11	17	20	-
<i>Pseudomonas aeruginosa</i>	11	15	18	-	13	18	17	-
<i>Klebsiella pneumonia</i>	-	18	22	-	-	14	20	-
<i>Proteus mirabilis</i>	-	-	18	-	-	15	15	-
<i>Escherichia coli</i>	-	18	22	-	-	14	20	-
<i>Salmonella typhi</i>	18	22	28	15	18	26	20	-

Table.3 Antifungal activity for methanolic and aqueous leaf extract of *Annona muricata*

Test organisms	Concentration of methanol extract(µl)			Concentration of aqueous extract(µl)			Positive control (Amphotericin B 0.1mg/ml)	Negative control (DMSO)
	50	100	200	50	100	200		
	Diameter of inhibition (mm)							
<i>Candida albicans</i>	-	14	16	14	16	18	20	-
<i>Cryptococcus neoformans</i>	18	20	24	17	19	21	18	-

Table.4 Antifungal activity for methanolic and aqueous fruit extract of *Annona muricata*

Test organisms	Concentration of methanol extract(µl)			Concentration of aqueous extract(µl)			Positive control (Amphotericin B 0.1mg/ml)	Negative control (DMSO)
	50	100	200	50	100	200		
	Diameter of inhibition (mm)							
<i>Candida albicans</i>	18	20	23	18	20	22	18	-
<i>Cryptococcus neoformans</i>	18	20	22	15	17	18	20	-

Fig.1 Antibacterial activity for Aqueous extract of *Annona muricata*

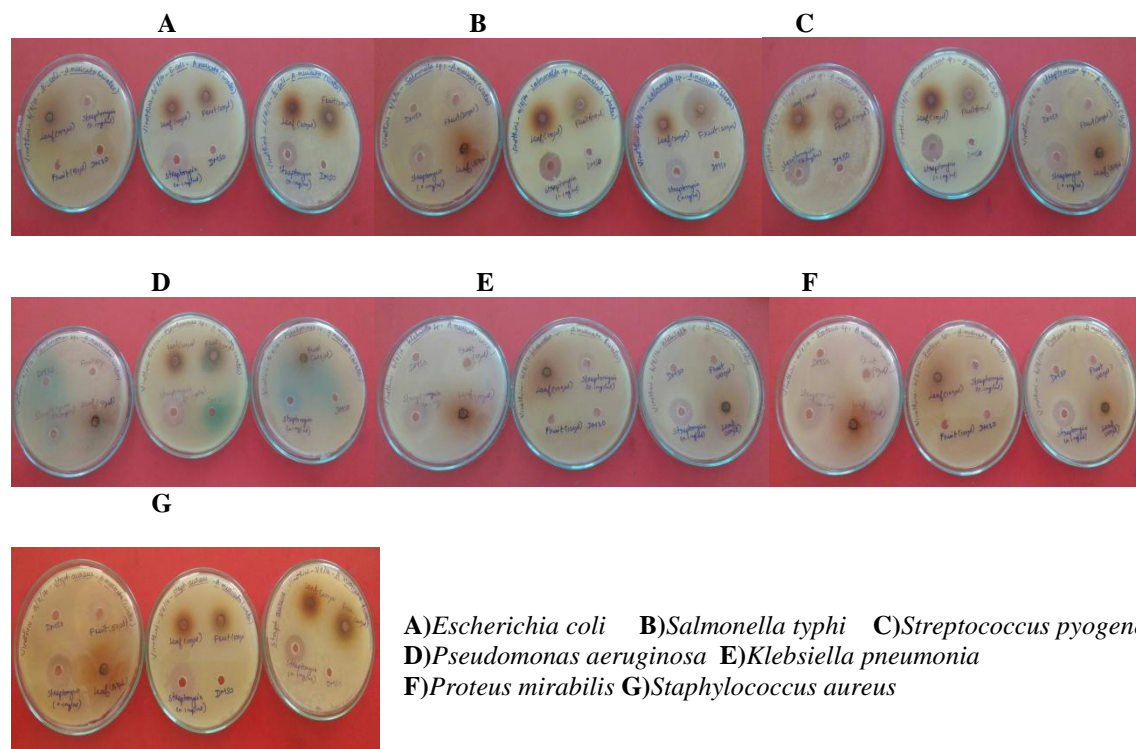


Table.5 Qualitative phytochemical screening of aqueous and methanol extract of *Annona muricata*

S.No	Test	Aqueous extract		Methanol extract	
		Leaf	Fruit	Leaf	Fruit
1.	Test for alkaloids				
	a)Mayer's test	+	+	+	+
	b)Wagner's test	+	-	+	-
	c)Hager's test	+	+	+	+
2.	Test for flavonoids				
	a)Alkaline reagent test	+	+	+	+
	b)Lead acetate test	+	-	+	+
3.	Test for carbohydrates				
	a)Benedict's test	+	+	+	+
	b)Molisch's test	+	+	+	+
	c)Fehling's Test	+	+	+	+
4.	Test for glycosides				
	a)Borntrager's test	+	+	+	+
	b)Legal's test	+	+	+	+
5.	Test for saponin				
	a)Froth test	+	+	+	+
	b)Foam test	+	+	+	+
6.	Test for phenols				
	a)Ferric chloride test	-	-	+	+
7.	Test for tannins				
	a)Gelatin test	+	-	+	-
8.	Test for protein and amino acids				
	a)Xanthoproteic test	+	+	+	+
	b)Ninhydrin test	+	+	+	+
9.	Test for terpenoids				
	a)Salkowski test	+	-	+	-
	b)Copper acetate test	+	+	+	+
10.	Test for anthraquinone				
	a)Ammonia test	-	-	-	-

Fig.2 Antibacterial activity for methanol extract of *Annona muricata*

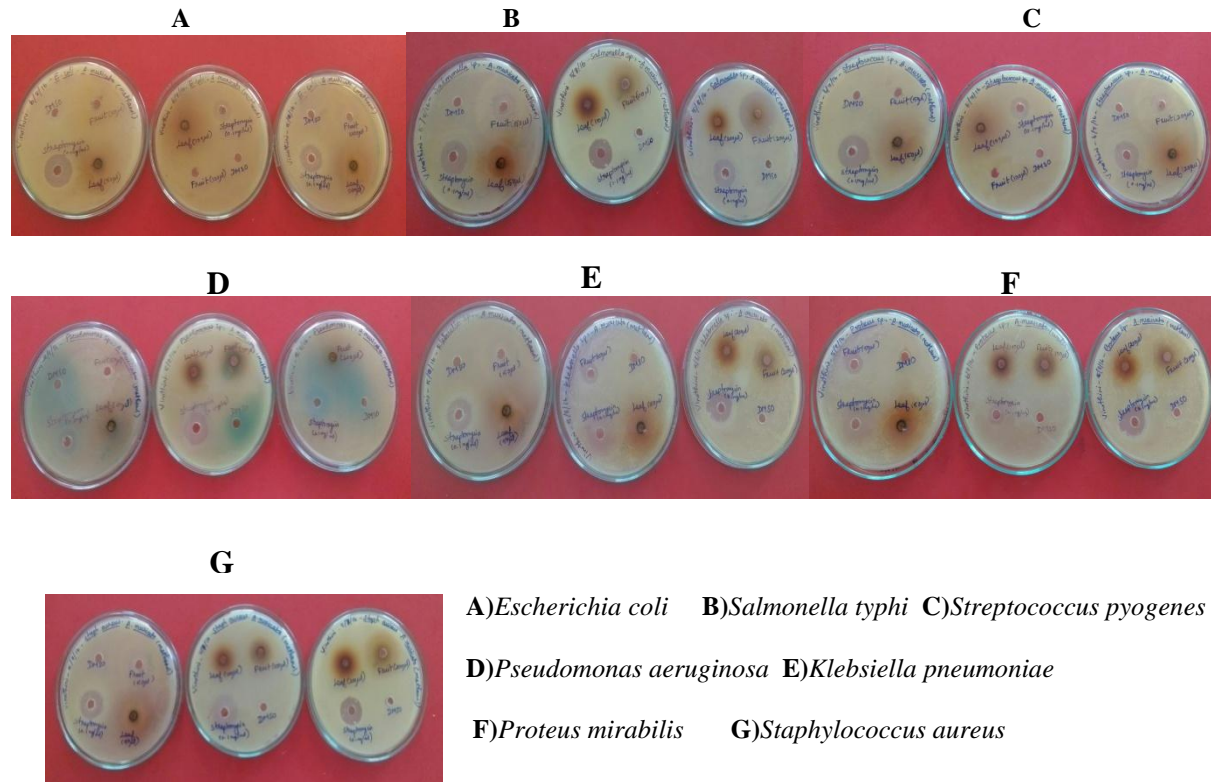
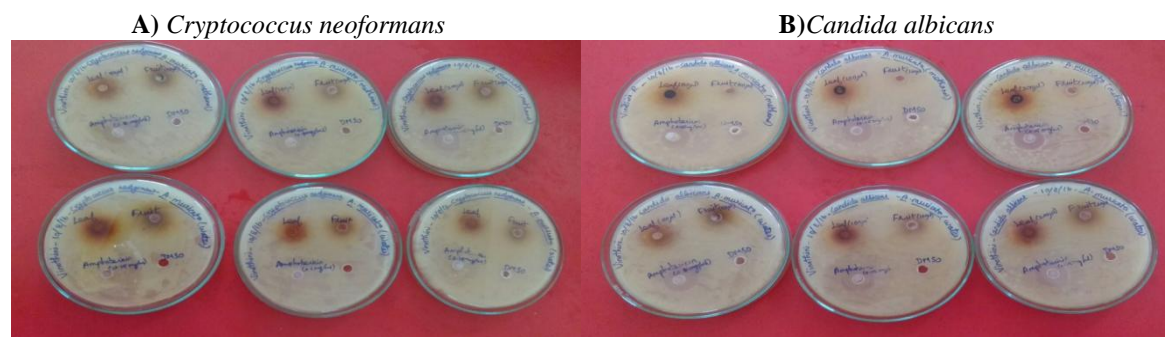


Fig.3 Antifungal activity for methanol and aqueous extract of *Annona muricata*



Phytochemical screening

The present study reveals that *Annona muricata* plant shows the presence of phytochemical constituents like alkaloids, carbohydrates, glycosides, proteins, aminoacids, saponins, tannins, terpenoids and anthraquinones in methanol and aqueous leaf and fruit extract of *Annona muricata* as shown in Table 5.

Antimicrobial activity of methanol and aqueous extract of *Annona muricata* (fruit and leaf) was determined by well diffusion method (Abubacker *et al.*, 2012). Methanol extract of *Annona muricata* showed maximum antimicrobial activity than aqueous extract. Results of the phytochemical screening of *Annona muricata* methanolic leaf and fruit extract showed the absence of anthraquinone while

flavonoids, saponins, tannins, phenols, alkaloids, terpenoids, protein, amino acids and glycosides were present. And *Annona muricata* aqueous leaf and fruit extract showed the absence of phenol and anthraquinone. (Roopashree *et al.*, 2008; Obasi *et al.*, 2010). These phytochemicals exhibit various pharmacological and biochemical actions when ingested by animals. The comparative antimicrobial activity between methanolic and aqueous extracts of *Annona muricata* and the standard antibiotic streptomycin and amphotericin B revealed that the methanolic extract showed significant antimicrobial efficacy and could compete with the standard antibiotic, streptomycin and amphotericin B. This study discovered that *Annona muricata* extract possess a broad spectrum of activity against a panel of microbial responsible for the most common bacterial and fungal diseases.

In conclusion, the present study demonstrates that *Annona muricata* has antibacterial and antifungal activity against various test organisms like *Staphylococcus aureus*, *Streptococcus pyogenes*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Klebsiella pneumoniae*, *Escherichia coli*, *Proteus sp.*, *Lactobacillus sp.*, *Cryptococcus neoformans* and *Candida albicans*. And this study shows that *Annona muricata* is a good source for various phytochemicals like alkaloids, flavonoids, carbohydrates, glycosides, saponins, tannins, terpenoids, proteins and anthraquinone. All these preliminary reports form a primary platform for further phytochemical and pharmacological studies.

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