

## Original Research Article

# Phytochemical screening and assessment of antibacterial activity for the bioactive compounds in *Annona muricata*

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### ABSTRACT

#### Keywords

Anti-oxidant components;  
Phosphorous;  
Iron;  
Antibacterial activity;  
*Annona muricata*.

*Annona muricata* is a fruit native of Central America, which has become an important crop because of its tasty flavor, high pulp content, and nutritional value and antioxidant properties. This study was aimed to determine the phytochemicals as well as antioxidants present in the plant and antibacterial activities of various parts of *Annona muricata*. The aqueous leaf extract contains a high protein and phenol content of 36.66 mg% and 134.28 mg% respectively. The non enzymatic Anti-Oxidant components like Vitamin-C and Vitamin-E of aqueous leaf and seed extract contains 66.6 mg% and 26.68 mg% respectively. The enzymatic anti-oxidant components like Super Oxide Dismutase (SOD) and Catalase of aqueous leaf and seed extract contains 255 U/mg and 83.4  $\mu$ mol of /min/mg respectively. The ethanolic leaf extract shows high phosphorous and iron content of 128 mg% and 1.075 mg% respectively. The ethanolic root extract shows highest calcium content of 22 mg% and the aqueous extract of seed contains high carbohydrate content of 11.025 mg% respectively. The ethanolic extract of leaf shows highest antibacterial activity towards *Pseudomonas aeruginosa* and *Staphylococcus aureus*.

### Introduction

In India, many forms of alternative medicines are available for those who do not want conventional medicine (or) who cannot be helped by conventional medicine. Ayurvedha and Kabiraji (Herbal medicine) are two important forms of alternative medicine that is widely available in India. Many herbs and species are used in Indian cooking such as onion, Garlic, Ginger, Turmeric, Cardamom, Coriander etc. 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 (*Rauwolfia 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). 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 is 5-20 cm in diameter, yellow green in colour and has white flesh inside. 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, Anti-parasitic, 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 antioxidant activity is also related to their ability to quench reactive oxygen species such as singlet molecular oxygen and peroxy radicals thus acting as deactivators of excited molecules or as chain breaking agents respectively (Scoppola et al., 2001). The leaf, stem, root and seed extract of *Annona muricata* have the antibacterial activity against numerous pathogens (Sundarrao et al., 1993).

The leaves, roots and seeds of graviola demonstrated insecticidal properties, with these seeds demonstrating strong insecticidal activity (Tattersfield et al., 1940). The phytochemicals present in *Annona muricata* are alkaloids, Flavonoids, carbohydrates, cardiac glycosides, saponins, tannins, phytosterol, terpenoids and proteins (Edeoga et al., 2005). *Annona muricata* acts as ideal antidiabetic drug since it possesses both hypoglycemic and antioxidant properties without any adverse effects (Lenk et al., 1992). The leaves of *Annona muricata* act as molluscicidal and Anti-parasitical agents (Bieber et al., 2005). *Annona muricata* used to cure inflammatory conditions like Flu and cough. People in the older days used to mix *Annona muricata* tree extract from the roots, leaves and stem to make tea and other solution which was given to people suffering from various illnesses. *Annona muricata* extract solution was applied on the body to treat rheumatism, arthritis and any other pain in joints (Padma et al., 2001).

*Annona muricata* oils can be extracted from its leaves. The leaves can be crushed along with raw fruit from the plant and mixed with olive oil. The oil can be used to treat various skin disorders like rashes, boils and sores (Padma et al., 2001). The present study determined the phytochemical constituents, antioxidant property (Enzymatic and Non-Enzymatic methods), antibacterial activity, Mineral contents and protein contents present in various parts of *Annona muricata*.

## **Materials and Methods**

### **Collection and processing of plant samples**

Plant sample (*Annona muricata*) (Figure.1a,b) was collected from Agricultural college and Research institute, Agricultural University, Madurai, Tamil Nadu. The plant was washed thoroughly in tap water and the stem was removed from the plant. The leaf, root, bark and seed parts of the plant were air dried in the shade for 4 weeks. The plant samples were ground in to uniform powder using milling machine. The powder was used for the further studies.

### **Preparation of various solvent extracts**

Aqueous extract of the sample was prepared by soaking 5gm of dried powder in 100ml of distilled water for 12hrs. The extract was filtered using the Whatmann filter paper and it was used for further studies.

5gm of *Annona muricata* leaf bark and root powder was taken in a paper cone and placed in to Soxhlet apparatus. 100ml of methanol and ethanol 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 methanol gets vaporized and rises up to the condenser where it 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 (Satyanarayana, 2006).

### Phytochemical screening procedure

Qualitative tests for alkaloids, flavonoids, carbohydrates, glycosides, saponins, tannins, Terpenoids, Proteins and Anthraquinone were performed according to the procedure described by Harborne et al. (1973). Mayers test, Wagner's test for Alkaloids, Shinodas test for flavonoids, Benedicts test, Molisch's test for carbohydrates, Keller-Killani test for cardiac glycosides, Froth test for saponins, Lead acetate test for tannins, Salkowski test for terpenoids, Ninhydrin and Biuret test for protein and Ammonia test for anthraquinone were performed.

### Quantitative analysis

The amount of starch was quantitatively analyzed by Anthrone method (Hedge et al., 1962). Estimation of protein was quantitatively analyzed by Lowry's method (Lowry et al., 1951). Estimation of iron was quantitatively analyzed by Ramsay method (Ramsay 1953). Estimation of calcium was quantitatively analyzed by Clarke and Collip method (McLien, et al., 1934). Estimation of phosphorous was quantitatively analyzed by Fiske-subbarow method (Fiske et al., 1925). Estimation of ascorbic acid was quantitatively analyzed by Roe and Kuether method (Natelson, 1971). Estimation of Vitamin A was quantitatively analyzed by Barker and Frank method (Natelson, 1971). The activity of catalase enzyme activity was

determined by Sinha method (Sinha, 1972). The activity of superoxide dismutase was analyzed and the absorbance was read at 420nm using green filter immediately (Kakkar et al., 1984). The antioxidant activity of the plant extracts was assessed on the basis of the radical scavenging effect of stable 1, 1-diphenyl 2 picryl hydroxyl (DPPH), free radical activity (Benzie et al., 1996). The phenol content was quantitatively analyzed by means of Singleton method (Singleton et al., 1965).

### Antibacterial Activity Assay

Among the variety of microorganisms that are available, *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Klebsiella* were taken for the assay of antibacterial activity. The bacterial strains were obtained from the Department of Microbiology, Sri Sankara Arts and Science College, Enathur.

### Results

The present study reveals that *Annona muricata* plant shows the presence of phytochemical constituents like alkaloids, flavonoids, carbohydrates, glycosides, proteins, saponins, tannins, terpenoids and anthraquinones in different solvent extracts as shown in Table 1.

The quantitative analysis of aqueous extract of *Annona muricata* reveals that the seed contains higher protein content when compared to leaf and the leaf contain the higher phenol content when compared to the seed as shown in the Table 2.

**Table.1** Qualitative Phytochemical screening of Aqueous, Methanol and Ethanol extract of *Annona muricata*

S. No.	Test / Leaf Extract	Water	Methanol	Ethanol
1.	<b>Test for Alkaloids</b> a)Mayer's test b)Wagner's test c)Dragendorff's test	- - -	+ + -	+ + -
2.	<b>Test for flavonoids</b> a)Shinoda's test b)Alkaline reagent test	+ -	+ -	+ -
3.	<b>Test for carbohydrates</b> a)Benedict's test b)Molisch's test	+ +	+ +	+ +
4.	<b>Test for glycosides</b> a)Borntrager's test b)Keller – Killani test	- +	- +	- +
5.	<b>Test for Proteins</b> a)Ninhydrin test b)Biuret test	+ +	+ +	+ +
6	<b>Test for saponins</b> a)Froth test b)Lead acetate test	+ +	+ +	+ +
7	<b>Test for Tannins</b> a)Ferric chloride test b)Lead acetate test	- +	- +	- +
8	<b>Test for Terpenoids</b> a)Salkowski test	+ -	- -	- -
9	<b>Test for Anthraquinones</b> a)Ammonia test	+ -	- -	- -

**Table.2** Quantitative analysis of aqueous extract of *Annona muricata*

S.No	Parameters	Aqueous extract	
		Leaf (mg %)	Seed (mg %)
1	Protein	8.60	36.66
2	Phenol	134.28	45.60

**Table.3** Non- Enzymatic Antioxidant components of *Annona muricata*

S.No	Antioxidant components	Aqueous extract	
		Leaf (mg %)	Seed (mg %)
1	Vitamin – C	66.60	22.23
2	Vitamin – E	6.68	26.68

**Non-Enzymatic Antioxidant components of *Annona muricata*:**

The non-enzymatic antioxidant components are Vitamin-C and Vitamin-E. The aqueous extract of *Annona muricata* reveals that the leaf contains higher Vitamin C content when compared to seed and the seed contain higher Vitamin E content when compared to leaf as shown in Table 3.

**Table.4** Enzymatic Antioxidant Components of *Annona muricata*

S.No	Antioxidant components	Aqueous extract	
		Leaf	Seed
1	SOD	37.5U/mg	83.4U/mg
2	Catalase	255µmol	68µmol

**Enzymatic Antioxidant Components of *Annona muricata***

The enzymatic antioxidant components of *Annona muricata* are superoxide dismutase and catalase. The aqueous extract of seed contains higher superoxide dismutase content than the leaf. The aqueous extract of leaf contains higher catalase content when compared to the seed as shown in the Table 4.

**Table.5** Quantitative analysis of ethanol and aqueous extract of *Annona muricata*

S.No	Substances	Ethanol extract			Aqueous Extract
		Leaf (Mg %)	Root (Mg %)	Bark (Mg %)	Seed (Mg %)
1	Phosphorous	128.00	32.00	16.00	8.00
2	Iron	1.07	0.52	0.33	0.90
3	Calcium	3.00	22.00	13.00	5.00
4	Carbohydrate	7.31	1.23	0.45	11.02

**Table.6** Free radical scavenging activity of Methanolic activity of *Annona muricata*

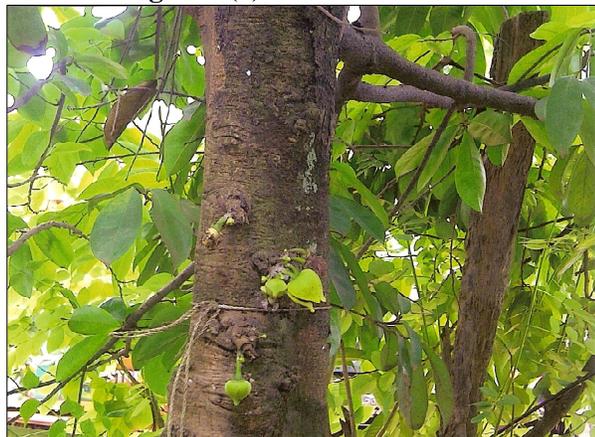
Sample	Concentration	% DPPH scavenging activity	
		Leaf	Root
Methanolic extract (5% dry plant powder in Methanol)	Plant extract (1: 8) dilution	25	25
	Plant extract (1: 4) dilution	43	37
	Plant extract (1: 2) dilution	75	68
	Crude Plant extract	87	87
Standard Ascorbic acid	100 mg/ml	87	87

**Table.7** Antibacterial activity of *Annona muricata*

S.No	Name of the organisms	Ethanol extract					
		Leaf		Bark		Root	
		Control*	Test*	Control*	Test*	Control	Test*
1	<i>Pseudomonas aeruginosa</i>	9	18	10	10	9	11
2	<i>Staphylococcus aureus</i>	11	18	-	11	-	10
3	<i>Klebsiella pneumonia</i>	11	15	10	15	10	10
4	<i>Bacillus subtilis</i>	-	10	12	14	11	13
5	<i>E.coli</i>	9	15	10	16	11	15

\*Zone of inhibition in mm

**Figure.1(a)** *Annona muricata*



**Figure.1(b)** *Annona muricata*



### **Quantitative analysis of ethanol and aqueous extract of *Annona muricata***

The ethanolic extract of leaf contains higher amount of Calcium and Iron. The ethanolic extract of root contains higher amount of Calcium and the aqueous extract of seed contains the higher amount of Carbohydrate content as shown in the Table 5.

### **Antibacterial Activity**

Antibacterial activity of *Annona muricata* was seen against to the several organisms namely *Pseudomonas*, *Staphylococcus*, *Klebsiella*, *Bacillus* and *E. coli*. The ethanol leaf extract showed maximum activity against *Pseudomonas* and *Staphylococcus*. The ethanolic bark extract showed maximum activity against *E. coli*. The ethanolic root extract shows maximum activity to the *E.coli* as shown in the Table 7.

### **Discussion**

Alkaloids are naturally occurring chemical compounds containing basic nitrogen atoms. They often have pharmacological effects and are used as medications and recreational drugs (Rhoades, 1979). Flavonoids enhance the effects of Vitamin C and function as antioxidants. They are also known to be biologically active against liver toxins, tumors, viruses and other microbes (Korkina et al., 1997). Plant terpenoids are used extensively for their aromatic qualities. They play a role in traditional herbal sonedies and are under investigation for Antibacterial, Antineoplastic and other Pharmaceutical functions (Yamunadevi et al., 2011). Tannins have shown potential Antiviral, Antibacterial and Antiparasitic effects. Saponins cause hemolysis of red blood

cells (Winter et al., 1993). Cardiac glycosides are drugs used in the treatment of congestive heart failure and cardiac arrhythmia. Most phytochemicals have antioxidant activity and protect our cells against oxidative damage and reduce the risk of developing certain types of cancer. The antibacterial activity was screened because of their great medicinal properties towards the pathogenic organisms. The medicinal plant *Annona muricata* showed good antibacterial activity against several organisms like *Staphylococcus aureus*, *Pseudomonas*, *Bacillus*, *Klebsiella*, and *E. coli* as supported by previous studies.

### **Conclusion**

The present study conclusively demonstrates that *Annona muricata* is a good source of various phytochemicals like alkaloids, flavonoids, carbohydrates, glycosides, saponins, tannins, Terpenoids, Proteins and Anthraquinone. Enzymatic and non-enzymatic antioxidant activity was also demonstrated, which may be useful in prevention of cancer. The antibacterial activity of *Annona muricata* was clearly shown by the present study against various test organisms like *Staphylococcus aureus*, *Pseudomonas*, *Bacillus*, *Klebsiella*, and *E. coli*. All these preliminary reports warrant an in depth analysis of the usefulness of *Annona muricata* as miracle drug against various ailments.

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