

Research article

# Phytochemical Screening and Antimicrobial activities of *Annona muricata* (L) leaf extract.

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## ABSTRACT

The antibacterial and phytochemical activities of methanolic and aqueous leaf extract of *Annona muricata* was evaluated on *Staphylococcus aureus*, *Escherichia coli* *Streptococcus pyogenes*, *Bacillus subtilis*, *Salmonella typhimurium* and *Klebsiella pneumonia*. The antibacterial activity was done using agar cup method. The most inhibited gram positive bacteria were *Bacillus subtilis* and *Staphylococcus aureus* at minimum inhibitory concentration of 400mg/ml with zone diameter of  $19.5 \pm 0.5$ m and  $20.5 \pm 0.5$ m, while the most inhibited gram-negative bacteria was *Escherichia coli* at minimum concentration of 200mg/ml,  $16.5 \pm 0.5$ m. *Klebsiella pneumonia* was inhibited at almost every concentration of the methanolic extract. Both extracts showed antibacterial properties but the methanolic extract was more effective as it inhibited a wide range of organism at varying concentrations. There was a significant difference ( $P < 0.05$ ) between the methanolic and aqueous extract. The phytochemical screening of methane and aqueous extracts of the leaf revealed the presence of Steroids, Alkaloids, Saponnins, Tannins, Flavonoid, and Cardiac glycosides. Antibacterial activity of extracts was compared with the standard antibiotic, streptomycin (100mg/ml). The results obtained in the present study suggest that *Annona muricata* can be used as an antibacterial substance. **Copyright © AJBCPS, all rights reserved.**

**KEY WORDS:** Antimicrobial, Phytochemical screening, *Annona muricata*, Inhibition

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## INTRODUCTION

*Annona muricata* is a member of the family of custard apple tree called annonaceae and a species of the genus *Annona* known mostly for its edible fruits *Annona muricata* produces fruits that are usually called sour sop due to its slightly acidic taste when ripe (9).

The fruit is juicy, acidic, whitish and aromatic with abundant seeds, the average weight of 1000 fresh seeds is 470g and has an average oil content of 24% (9). When dried for three days in 60°C the average seed weight was 322g and were tolerant of the moisture extraction showing no problems for long-term storage under reasonable conditions. The creamy and delectable flesh of the fruit consist of 80% water, 1% protein, 18% carbohydrates and fair amount of vitamins B, B2 and C, potassium and dietary fiber (17).

Its flavour is described as a combination of strawberry and pineapple with sour citrus flavour contrasting with an underlying creamy flavour reminiscent of coconut or banana (16).

*Annona muricata* has been used as a folkloric herbal medicine in many regions throughout the world. It is considered to be antispasmodic and emetic. A decoction of *Annona muricata* leave is used to kill bed bug and head lice to reduce fever. This can be taken orally or added to bathing water also has the same effect (8). The crushed fresh leaves are also applied on skin eruption for faster healing. A poultice of young *Annona* leaf is applied on the skin to alleviate rheumatism and other skin infection like eczema. When applied during the healing of wounds result in less or no skin scars (8).

The decoction can also be used as wet compress on swollen feet and other inflammations. The juice of the fruits is taken orally as herb remedy of arthritis, haematuria and liver ailments. Pulverizing the *Annona* seed and mixing it with soap and water is used as effective spray against caterpillar. The *Annona* leaves are placed inside pillow or placed on top of the mattress to induce a good night sleep (8).

All parts of the *Annona* tree are used in natural medicine in the tropic including the bark, leave, root, fruit and seeds. Different properties and uses are attributed to the different parts of the tree. Generally, the fruit and fruit juice are taken to eliminate worms and parasites, cool fever, increase mother's milk after child birth, and is as an astringent for diarrhea and dysentery (7). The crushed seeds are used against internal and external parasites, head lice and worms (9). The dark, leaves are considered sedative and antispasmodic (7).

In the Peruvian Andes, a leaf tea is used for catarrh, and the crushed seed is used to kill parasites. In the Peruvian Amazon, the bark, root and leaves are used for diabetes and as a sedative and antispasmodic. Indigenous tribes in Guyana use a leaf and /or bark tea as a sedative and heart toxic. In the Brazilian Amazon, a leaf tea is used for liver problems and the oil of the leaves and unripe fruit is mixed with olive oil and used externally for neuralgia, rheumatism, and arthritic pain, (17). In Jamaica, Haiti and the West Indies, the fruit and/or fruit juice is used for fever, parasites and diarrhea (17; 6).

Annona produces natural compounds in its leaf and stem, bark, and fruit seeds. Three separate research have confirmed that these chemicals have significant antitumorous properties and selective toxicity against various types of cancer cells (without harming healthy cells) publishing eight clinical studies on their findings, (4). Many of the acetagenins have demonstrated selective toxicity to tumors cells at very low dosage – as little as one part per million. Four studies were published in 1998 which further specify the chemicals and acetogenins in annona which are demonstrating the strongest anticancerous, antitumorous and antiviral properties. In a 1997 clinical study, novel alkaloids found in annona fruits exhibited antidepressive effect in animal (4; 13).

Phytochemicals are found in fruits, vegetable, grains, legumes and green tea. Some common foods that contain high amount of phytochemicals include garlic, onion, broccoli, cabbage, carrots, tomatoes, vegetable oil, strawberries, lemon and pepper (22). These phytochemicals appears to have disease fighting properties when isolated from natural sources and put in a pill form (10).

Many different ways of approach may be followed in the search for plant and plant products of pharmaceutical and chemical interest. A very small percentage of the world's flora has been studied chemically in details (19). The significance of research in to medicinal plants is that it extends the knowledge of their biological constituent, their pharmaceutical activities and their therapeutic value, so that they can be used effectively in the treatment and prevention of diseases (19).

Over twenty laboratory studies, kept have shown this tree 10,000 times more powerful than Adriamycin, a commonly used chemotherapy (12)

In laboratory studies, Annona selectively hunts down and kills 12 different types of cancer cells, including breast, prostrate, lung, colon and pancreatic cancer. In view of the usefulness of this plant, there is a need for further research on its antimicrobial properties as well as the determination of its bio-active components (18)

The aim and objective of this research work is to screen the aqueous and alcoholic 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. And to determine the antimicrobial activities on some selected micro organisms.

## **MATERIAL AND METHODS**

### **Sample Collection**

The plant sample (leaves) of *Annona muricata* was collected at Ungwan Dosa layout Kaduna in July, 2010. The fresh plant material was washed under running tap water; air dried and then homogenized to fine powder and was stored in a jar.

### **Extraction of Plant Material**

10g of air dried powder of sample was added to 10 times of the solvents (water and methanol). The sample was kept in dark for 48 hours for maceration with intermittent shaking. After 48 hours, the solution was filtered through a filter paper and the filtrates were evaporated to dryness. The weight of recovered extracts were 4.8g with a dark green colour for methanol extract and 5.0g with a brown colour for aqueous extract They were collected and kept in a cool place (1).

### **Bacterial Strains**

In vitro antimicrobial activity was examined for aqueous and methanol extract from the leaves of *Annona muricata*. Microorganisms were obtained from the University of Abuja Teaching Hospital Gwagwalada, Abuja, Nigeria. Amongst six organisms investigated, three were gram positive bacteria; *Streptococcus pyogenes*, *Staphylococcus aureus*, *Bacillus subtilis* while three were gram negative bacteria; *Escherichia coli*, *Salmonella typhimurium*, *klebsiella pneumonia*. The entire microorganisms were maintained at 4<sup>0</sup>C on nutrient agar slants.

### **Media Preparation and Antibacterial Activity**

The antimicrobial assay was performed using agar cup method for both solvent extracts. The molten Mueller Hinton agar was inoculated with 100ml of the inoculums ( $1 \times 10^8$  cfu ml) and poured into Petri dishes for agar cup method, a well was prepared in the plate using a 3mm cork borer, 200ml of the test compound was introduced into the well and the plates were incubated for 24 hours at 37<sup>0</sup>C. Microbial growth was determined by measuring the diameter of zone of inhibition. For each bacterial strain, controls were maintained where pure solvents were used instead of the extract. The result was obtained by measuring the minimum inhibitory concentration.

### **Phytochemical Screening**

Quantitative phytochemical analysis of the crude powder of the plant collected was determined using methods described by (21; 20; 11; 2; 14 and 1).

## **RESULTS**

The phytochemical analysis revealed the presence of secondary metabolites like Tannins, Steroids, Cardiac glycoside; Alkaloids, saponins and Flavonoids were present in trace amount in the leaves except Anthraquinone (Table 3). It is not surprising that there are difference in the antimicrobial effects of plant species, due to the phytochemical properties and differences among species. It was found that, *Klebsiella pneumonia* was inhibited in all the concentrations of both extract (Tables 1 and 2), *Streptococcus pyogenes* did not show any inhibition with the aqueous extract (Table 2). *Staphylococcus aureus* had the highest zone of diameter of inhibition  $20.5 \pm 0.5$  at 400mg/ml of the methanolic extract, while *Bacillus subtilis* showed the highest zone diameter of inhibition  $18.5 \pm 0.5$  at 400mg/ml of aqueous extract.

**Table 1:** Zone diameter of inhibition of methanolic leaf extract on *Annona muricata*

Test Organisms	Concentration of Extract (mg/ml)				Positive control (streptomycin)mg/ml	Negative control (water)ml
	400	200	100	50		
	<b>Diameter of inhibition (m)</b>					
<i>Bacillus subtilis</i>	19.5 ± 0.5	18.5 ± 0.5	17.5 ± 0.5	14.5 ± 0.5	23.10 ± 0.5	0.00
<i>Staphylococcus aureus</i>	20.5 ± 0.5	17.5 ± 0.5	15.5 ± 0.5	14.5 ± 0.5	23.40 ± 0.5	0.00
<i>Klebsiella pneumonia</i>	18 ± 1.0	16.5 ± 0.5	15.5 ± 0.5	14.5 ± 0.5	21.80 ± 0.5	0.00
<i>Salmonella typhimurium</i>	16.5 ± 0.5	15.5 ± 0.5	14.5 ± 0.5	14.5 ± 0.5	23.50 ± 0.5	0.00
<i>Escherichia coli</i>	16.5 ± 0.5	13.5 ± 0.5	-	-	26.80 ± 0.5	0.00
<i>Streptococcus pyogenes</i>	17.25 ± 1.15	15 ± 1.0	14.5 ± 0.5	13.75 ± 0.15	22.40 ± 0.5	0.00

**Table 2** Zone diameter of inhibition of Aqueous leaf extract of *Annona muricata*

Test Organisms	Concentration of extract (mg/ml)				Positive control (streptomycin) mg/ml	Negative control (water)ml
	400	200	100	50 Diameter of inhibition (m)		
<i>Bacillus subtilis</i>	18.5 ± 0.5	16.75 ± 1.12	-	-	23.10 ± 0.5	0.00
<i>Staphylococcus aureus</i>	17.75 ± 0.13	20.5 ± 4.5	-	-	23.40 ± 0.5	0.00
<i>Klebsiella pneumonia</i>	16 ± 1.0	22.5 ± 0.5	15.25 ± 1.15	14.5 ± 0.5	21.80 ± 0.5	0.00
<i>Salmonella typhimurium</i>	16.5 ± 0.5	17.5 ± 0.5	-	-	23.50 ± 0.5	0.00
<i>Escherichia coli</i>	17.5 ± 0.5	24.5 ± 0.5	-	-	26.80 ± 0.5	0.00
<i>Streptococcus pyogenes</i>	-	-	-	-	22.40 ± 0.5	0.00

**Table 3:** Results of Phytochemical Screening of the Leaf of *Annona muricata*

Secondary metabolites	Aqueous Extract	Methanol Extract
Steroids	+	+
Cardiac glycoside	+	+
Alkaloid	+	+
Saponin	+	+
Anthraquinone	-	-
Tannin	+	+
Flavonoids	+	+

**Key:** + = Presence of Secondary Metabolite

- = Absence of Secondary Metabolite

## Discussion

The aqueous and methanolic extract showed antibacterial activity. The methanol extract of the investigated plants showed maximum antibacterial activity than aqueous extract. This is in line with previous work by (3).

Phytochemical analysis revealed the presence of secondary metabolites like tannins, steroids, cardiac glycosides, saponin, flavonoids and anthraquinone as reported by (13).

The determination of the identity of the bioactive compounds from the leaves of *Annona muricata* forms a primary platform for further phytochemical and pharmacological studies (5; 15).

The comparative antibacterial activity between methanolic and aqueous extracts of *Annona muricata* and the standard antibiotic streptomycin revealed that the methanolic extract showed significant ( $P < 0.05$ ) antibacterial efficacy and could compete with the standard antibiotic, streptomycin.

*Annona muricata* leaf extract can be employed in treatment of various bacterial infectious diseases like pneumonia, diarrhea, urinary tract infection and even some skin disease.

It was discovered in this study that *Annona muricata* extract possess a broad spectrum of activity against a panel of bacteria responsible for the most common bacterial diseases. These promissory extract open the possibility of finding new clinically effective antibacterial compounds. Further research is necessary to determine the identity of the antibacterial compounds from the leaves of *Annona muricata* and also to determine their full spectrum of efficacy. The present study of *in vitro* antimicrobial evaluation of leaves of *Annona muricata* forms a primary platform for further phytochemical and pharmacological studies.

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