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### IN VITRO ANTIMICROBIAL ACTIVITY AND PHYTOCHEMICAL ANALYSIS OF THE LEAVES OF *Annona muricata*



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#### ABSTRACT

The antibacterial effect of the leaves of *Annona muricata* was evaluated on bacterial strains like *Staphylococcus aureus* ATCC29213, *Escherichia coli* ATCC8739, *Proteus vulgaris* ATCC13315, *Streptococcus pyogenes* ATCC8668, *Bacillus subtilis* ATCC12432, *Salmonella typhimurium* ATCC23564, *Klebsiella pneumonia* NCIM No.2719 and *Enterobacter aerogenes* NCIM No. 2340. The solvents used for the extraction of plants were water and methanol. The in vitro antibacterial activity was performed by agar cup method. The most susceptible Gram-positive bacteria was *B. subtilis* and *S. aureus* while the most susceptible Gram-negative bacteria was *K. pneumoniae* and *P. vulgaris*. The significant antibacterial activity of active extracts was compared with the standard antibiotic, streptomycin (100 ppm). The results obtained in the present study suggest that *Annona muricata* can be used in treating diseases caused by the test organisms.

**Key Words:** Medicinal plants, antibacterial activity, aqueous extract, methanol extract.

#### INTRODUCTION

Infectious diseases are the leading cause of death world-wide. Antibiotic resistance has become a global concern (1). The clinical efficacy of many existing antibiotics is being threatened by the emergence of multidrug-resistant pathogens (2). Many infectious diseases have been known to be treated with herbal remedies throughout the history of

mankind. Natural products, either as pure compounds or as standardized plant extracts, provide unlimited opportunities for new drug leads because of the unmatched availability of chemical diversity. There is a continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action for new and re-emerging infectious diseases (3). Therefore, researchers are increasingly turning their

attention to folk medicine, looking for new leads to develop better drugs against microbial infections (4). The increasing failure of chemotherapeutics and antibiotic resistance exhibited by pathogenic microbial infectious agents has led to the screening of several medicinal plants for their potential antimicrobial activity (5,6). India is a varietal emporium of medicinal plants and is one of the richest countries in the world in regard to genetic resources of medicinal plants. It exhibits a wide range in topography and climate, which has a bearing on its vegetation and floristic composition. Moreover, the agro-climatic conditions are conducive for introducing and domesticating new exotic plant varieties (7). In recent years, secondary plant metabolites (phytochemicals), previously with unknown pharmacological activities, have been extensively investigated as a source of medicinal agents (8). Thus, it is anticipated that phytochemicals with adequate antibacterial efficacy will be used for the treatment of

bacterial infections (9). Since time immemorial, man has used various parts of plants in the treatment and prevention of various ailments (10). The aim of this study was to evaluate the activity of the leaves of *Annona muricata* against several Gram-positive and Gram-negative bacterial strains in vitro.

#### **OBJECTIVE:**

The main objectives of the present research work are phytochemical analysis of the leaves of *Annona muricata* and evaluation of antibacterial activity of the leaves extract of the same by agar cup method.

#### **MATERIALS AND METHODS:**

##### **Collection and Identification of Plant Material**

Fresh plant/plant parts were collected randomly from Kanyakumari, TamilNadu. The taxonomic identities of these plants were confirmed by *Flora of the Presidency of Madras* (Gamble J.S. 1925). Fresh plant material was washed under running tap water, air dried and then homogenized to fine powder and stored in airtight bottles. Preliminary Phytochemical Analysis Qualitative phytochemical analysis of the crude powder of the plant collected was determined as follows: Tannins (200 mg plant material in 10 ml distilled water, filtered); a 2 ml filtrate + 2 ml FeCl<sub>3</sub>, blue-black precipitate indicated the presence of Tannins. Alkaloids (200 mg plant material in 10 ml methanol, filtered); a 2ml filtrate + 1% HCl + steam, 1 ml filtrate + 6 drops of Dragendroff reagent, orange precipitate indicated the presence of respective alkaloids. Saponins (frothing test: 0.5 ml filtrate + 5 ml distilled water); frothing persistence indicated presence of saponins. Cardiac glycosides (Keller-Kiliani test: 2 ml filtrate + 1 ml glacial acetic acid + FeCl<sub>3</sub> + conc. H<sub>2</sub>SO<sub>4</sub>); green-blue color indicated the presence of cardiac glycosides. Steroids (Liebermann-Burchard reaction: 200 mg plant material in 10 ml chloroform, filtered); a 2 ml filtrate + 2 ml acetic anhydride + conc. H<sub>2</sub>SO<sub>4</sub>. blue-green ring indicated the presence of terpenoids. Flavonoids (200 mg plant material in 10 ml ethanol, filtered); a 2 ml filtrate + conc. HCl + magnesium ribbon pink-tomato red color indicated the presence of flavonoids (11).

##### **Extraction of Plant Material**

10g of air dried powder of sample was added to 10 times of solvent like water or methanol. The sample was kept in dark for 48hrs. for maceration with intermittent shaking. 48h later, the solution was filtered through filter paper & the filtrate was collected which contains the extracted material/ constituents.

## Bacterial Strains

In vitro antimicrobial activity was examined for aqueous and methanol extracts from the leaves of *Annona muricata*. Microorganisms were obtained from the National Chemical Laboratory (NCL), Pune, India. Amongst eight microorganisms investigated, three Gram-positive bacteria were *Staphylococcus aureus* ATCC29213, *Streptococcus pyogenes* ATCC8668, *Bacillus subtilis* ATCC12432, while five Gram-negative bacteria were *Enterobacter aerogenes* ATCC2340, *Escherichia coli* ATCC8739 and *Klebsiella pneumoniae* NCIM2719, *Proteus vulgaris* ATCC13315, *Salmonella typhimurium* ATCC23564. All the microorganisms were maintained at 4°C on nutrient agar slants.

## Media Preparation and Antibacterial Activity

The antimicrobial assay was performed agar cup method (12) for both solvent extracts. The molten Mueller Hinton agar was inoculated with 100 µl of the inoculum ( $1 \times 10^8$  cfu/ml) and poured into the Petri plate (Hi-media). For agar cup method, a well was prepared in the plates with the help of a cork-borer (10 mm). 200 µl of the test compound was introduced into the well. The plates were incubated overnight at 37 °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 zone diameter.

## RESULTS AND DISCUSSION:

The antibacterial activity of the leaves of *Annona muricata* extracts was assayed in vitro by agar cup method against 8 bacterial species. Table 2 summarizes the microbial growth inhibition of both aqueous and methanol extracts of the screened plant species. The

aqueous extract showed antibacterial activity; the other aqueous extracts did not show any antibacterial activity. On the other hand, methanol extracts of almost all the plants exhibited antibacterial activity towards all bacterium. The maximum antibacterial activity was shown by following organisms against methanol extract. The methanol extracts of the investigated plants showed maximum antibacterial activity than aqueous extract.

Preliminary phytochemical analysis revealed the presence of secondary metabolites like tannins, steroids, cardiac glycosides, etc. were present in trace amounts in the leaves (Table 1). It is not surprising that there are differences in the antimicrobial effects of plant species, due to the phytochemical properties and differences among species. The potential for developing antimicrobials from higher plants appears rewarding as it will lead to the development of a phytomedicine to act against microbes. Plant-based antimicrobials have enormous therapeutic potential as they can serve the purpose with lesser side effects that are often associated with synthetic antimicrobials (Iwu et al., 1999). Continued further exploration of plant-derived antimicrobials is needed today. 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. However, the present study of in vitro antimicrobial evaluation of the leaves of *Annona muricata* forms a primary platform for further phytochemical and pharmacological studies.

It was found that the methanolic extract of the leaves was effective against *Staphylococcus aureus*, *Proteus vulgaris*, *Klebsiella pneumoniae* & *Bacillus subtilis*. The minimum inhibitory concentration was found to be 6000 ppm for *Staphylococcus aureus*, *Proteus vulgaris*, *Klebsiella pneumoniae*, *Bacillus subtilis* while 8000 ppm for *Escherichia coli* & *Enterobacter*

*aerogenes*. The Comparative antibacterial activity between Methanolic extract of *Annona muricata* and standard antibiotic Streptomycin was studied. The Methanolic extract showed significant antibacterial efficacy as compared to the standard antibiotic, Streptomycin. (Refer Table No. 1 & 2)

Strepto: Streptomycin.

The beneficial effects of treatment can be achieved with the treatment with the leaves of *Annona muricata* in various bacterial infectious diseases like Pneumonia, Diarrhoea, Urinary tract infection, & even some skin disease.

In conclusion, *Annona muricata* extract possess a broad spectrum of activity against a panel of bacteria responsible for the most common bacterial diseases. These promissory extracts open the possibility of finding new clinically effective antibacterial compounds.

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#### REFERENCES:

1. Westh H, Zinn CS, Rosdahl VT et al. An international multicenter study of antimicrobial consumption and resistance in *Staphylococcus aureus* isolates from 15 hospitals in 14 countries. *Microb Drug Resist* 10: 169-176, 2004.

2. Bandow JE, Brotz H, Leichert LIO et al. Proteomic approach to understanding antibiotic action. *Antimicrob Agents Chemother* 47: 948-955, 2003.

3. Rojas R, Bustamante B, Bauer J et al. Antimicrobial activity of selected Peruvian medicinal plants. *J Ethnopharmacol* 88: 199-204, 2003.

4. Benkeblia N. Antimicrobial activity of essential oil extracts of various onions (*Allium cepa*) and garlic (*Allium sativum*). *Lebensm-Wiss u-Technol* 37: 263-268, 2004.

5. Colombo ML, Bosisio E. Pharmacological activities of *Chelidonium majus* L (Papaveraceae). *Pharmacol Res* 33: 127-134, 1996.

6. Iwu MW, Duncan AR, Okunji CO. New antimicrobials of plant origin. In: Janick J. ed. *Perspectives on New Crops and New Uses*. Alexandria, VA: ASHS Press; 1999: pp. 457-462.

7. Martins AP, Salgueiro L, Goncalves MJ et al. Essential oil composition and antimicrobial activity of three Zingiberaceae from S. Tome e Principe. *Planta Med* 67: 580-584, 2001.

8. Krishnaraju AV, Rao TVN, Sundararaju D et al. Assessment of bioactivity of Indian medicinal plants using Brine shrimp (*Artemia salina*) lethality assay. *Int J Appl Sci Eng* 2: 125-134, 2005.

9. Balandrin MF, Kjocke AJ, Wurtele E et al. Natural plant chemicals: sources of industrial and mechanical materials. *Science* 228: 1154-1160, 1985.

10. Tanaka H, Sato M, Fujiwara S. Antibacterial activity of isoflavonoids isolated from *Erythrina variegata* against methicillinresistant

Staphylococcus aureus. Lett Appl Microbiol 35: 494- 498, 2002.

11. Oguyemi AO. In: Sofowora A. ed. Proceedings of a Conference on African

Medicinal Plants. Ife-Ife: Univ Ife; 1979: pp. 20-22.

12. Bauer AW, Kirby WMM, Sherris JC et al. Antibiotic susceptibility testing by a standardized single disk method. Am J Clin Pathol 45: 493-496, 1966.

## TABLES AND FIGURES:

**Table I:** Preliminary phytochemical analysis of the leaves of *Annona muricata*.

Tests	Leaves extract		Inference
	Aqueous extract	Methanol Extract	
Reducing sugars, Fehling's test	+	+	Carbohydrates Present
Starch, Iodine test	-	-	Polysaccharides Absent
Test for Steroids	+	+	Steroids Present
Keller-Killani test	+	+	Cardiac Glycosides Present
Dragendorff's test	-	-	Alkaloids Absent
Test for Saponin	-	-	Saponins Absent
Borntrager's test	-	-	Anthraquinone Glycoside Absent
Ferric chloride test	+	+	Tannins Present
Test for Phenolics	-	-	Phenols Absent
Test for Flavonoids	-	-	Flavonoids Absent

+ = Presence of constituent

- = Absence of constituent

**Table I:** Preliminary phytochemical analysis of the leaves of *Annona muricata*.

Name of organism	Zone of inhibition (mm)								
	Aqueous extract of leaf				Methanol extract of leaf				Strepto.100 ppm
	6000	7000	8000	9000	6000	7000	8000	9000	
	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	
<i>Bacillus subtilis</i>	-	-	17	19	<b>15</b>	18	19	20	10
<i>Proteus vulgaris</i>	-	-	19	17	<b>14</b>	15	15	17	10
<i>Staphylococcus aureus</i>	-	-	22	18	<b>15</b>	16	18	21	10
<i>Klebsiella pneumonia</i>	15	16	23	17	<b>15</b>	16	17	19	10
<i>Salmonella typhimurium</i>	-	-	18	17	<b>14</b>	15	16	17	-
<i>Escherichia coli</i>	-	-	24	17	-	-	<b>14</b>	17	10
<i>Enterobacter aerogenes</i>	-	-	20	-	-	-	<b>13</b>	16	10
<i>Streptococcus pyogenes</i>	-	-	-	-	<b>14</b>	15	16	18	10