



Research Article

Antibacterial Study of Leaf Extract of *Annona* Species

Nirmala Patel ^{1*}, RS Chauhan ²

^{1,2} Department of Botany, PMCOE, Sanjay Gandhi Smriti Govt. Autonomous PG College, Sidhi, Madhya Pradesh, India

Corresponding Author: *Nirmala Patel

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Abstract

Plants have been one of the important sources of medicine since the beginning of human civilization. Demand for medicinal plants, health products, medicines, and food is increasing. supplements and cosmetics. *Annona* species is a versatile tree that produces edible fruit. Source of pharmaceutical and industrial products. Used as an antioxidant, antidiabetic, and liver disease drug. Protective, cytotoxic, genotoxic, anti-tumor, anti-lice, etc. The purpose of this article is to Examination of chemical components contained in crude leaf extract of *Annona* species. Particular attention is paid to their pharmacological actions. Qualitative phytochemical screening was performed. Use crude leaf extract in three different solvents such as water, alcohol, and chloroform. Phytochemical analysis of the extract revealed glycosides, alkaloids, oils, saponins, and Flavonoids. A comparison of the antibacterial activity of dried leaf extracts of *Annona* species was carried out. Two Gram-negative bacterial strains were evaluated: *Escherichia coli* and *Pseudomonas*. Agar is used to detect aeruginosa and two clinical fungal pathogens, namely *Candida albicans* and *Aspergillus niger*. Cup method. *Annona* species leaf extracts were found to have higher antibacterial activity than that antifungal effect. The results show that the leaves are a rich source of valuable primary nutrients and secondary metabolites exhibiting antimicrobial activity.

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1. INTRODUCTION

Since ancient times, humans have explored nature, particularly plants, in search of new discoveries. medicines, which has led to the use of a large number of medicinal plants with medicinal properties. properties for the treatment of various diseases [1]. According to a WHO study, 80% Populations living in developing countries rely solely on traditional medicine for their health needs. Primary health care needs, most of which involve the use of plant extracts [2]. Plant research continues, mainly with the aim of discovering new secondary plants. Metabolites or phytochemicals that are non-essential nutrients obtained from plants. It exerts many protective functions for human consumers.

Annona squamosa is a small evergreen tree belonging to the Annonaceae family. It is common in India and is native to the West Indies and South America. Various parts *Annona squamosa* is used in traditional medicine to treat various diseases [3]. It is mainly grown in gardens for its fruit and ornamental purposes. This plant is commonly called custard apple in English, sharifa in Hindi and seafaram in Telugu. in India [4].

It is considered beneficial for cardiac disease, diabetes hyperthyroidism and cancer. The root is considered as a drastic purgative [5]. An infusion of the leaves is considered efficacious in prolapsusani of children. The crushed leaves are sniffed to overcome hysteria and fainting spells. A leaf decoction was taken in the case of dysentery [6]. Leaves are used as poultice over boils and ulcers. The ripe fruits of this plant are applied to malignant tumors to hasten suppuration. The dried unripe fruit powder is used to destroy vermin. The seeds are acrid and poisonous. Powdered seeds serve as fish poison and insecticides. A paste of seed powder has been applied to the head to kill lice. It is also used for destroying worm in the wound of cattles [7].

Phytochemical screening is a method of identifying or identifying components. A property that is readily exploitable in plants for biological activity or ethnomedical use. Herbs antibiotics have great therapeutic potential because they can achieve this goal at low cost. Side effects often associated with synthetic antibiotics [8].

Thus it is anticipated that phytochemicals with adequate antibacterial efficiency can be used for the treatment of bacterial infections [9]. Antioxidants and antimicrobial properties of various extracts from many plants have recently been of great interest in both research and in food industry, because of their possible use as natural additives to replace synthetic antioxidants and antimicrobials with natural ones [10]. Thus medicinal plants play an important role in the development of newer drugs because of their effectiveness, less side effects and relatively low cost when compared with synthetic drugs [11]. The present study aims in exploring the phytochemical constituents, antibacterial and antifungal properties of the crude leaf extracts of *Annona squamosa*.

2. MATERIALS AND METHODS

Collection and Extraction of Plant Materials

The fully matured fresh leaves of *Annona squamosa* were collected from Ratahara area in Rewa district (Fig. 1-4). The

leaves were washed thoroughly, shade dried and finely powdered. The dried powdered leaves were extracted with three different solvents such as water, acetone and chloroform. For aqueous extraction, ten grams of the powdered leaves was mixed with 100ml distilled water, boiled for two hours and filtered. Whereas acetone and chloroform extracts were prepared by mixing ten grams of powdered leaf samples with 100ml of each solvent separately in mechanical shaker for 48 hours at room temperature. Extracts were filtered, concentrated, dried and were stored in the refrigerator at 4 °C for future use.



Fig 1: Plant of *Annona squamosa*



Fig 2: Leaves of *Annona squamosa*



Fig 3: Flowers of *Annona squamosa*



Fig 4: Fruits of *Annona squamosa*

Extraction of Plant Material by Soxhlet Apparatus

In the current research, the leaves and seeds of *Annona squamosa* were first gathered and dried in sunlight. Following the drying process, the raw materials were ground into a fine powder using a mixer grinder. A total of 12.5 grams of shade-dried leaf powder and 12.5 grams of shade-dried seed powder were measured. These two powders were combined, and 25 grams of the mixture was extracted successively with 250 ml of methanol in a Soxhlet extractor for a duration of 48 hours. The methanol extract was then concentrated and stored in an airtight bottle in the refrigerator for subsequent antimicrobial testing.



Fig 5: Methanolic Leaf and Seed extract of *Annona squamosa*.

Phytochemical Analysis

The prepared plant extracts were analysed for the presence of alkaloids, glycosides, saponins, proteins, aminoacids, fixed oils, phenolic compounds, tannins, flavonoids, gum and mucilages etc. [12].

Preparation of Plant Extract for Antimicrobial Analysis

The collected leaves were washed thoroughly in tap water, shade dried and finely powdered. Ten grams of powdered leaf samples were mixed with 100ml of ethanol and kept in mechanical shaker for 48 hours at room temperature (Fig. 6-9). Extracts were then filtered, concentrated and dried. The

extracted powder was dissolved in 10% dimethyl sulfoxide (DMSO) and stored in refrigerator at 4 °C.



Fig 6: *Bacillus subtilis*

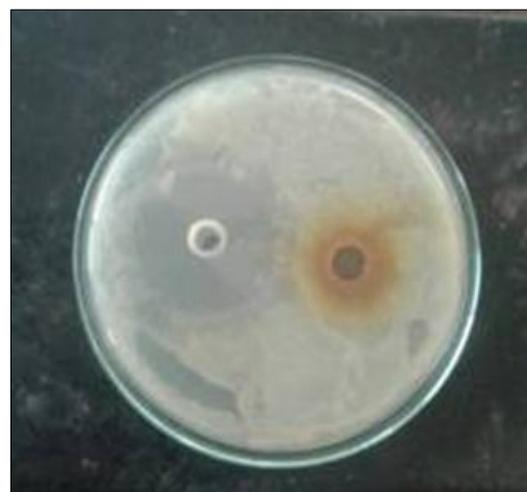


Fig 7: *Staphylococcus aureus*



Fig 8: *E. coli*



Fig 9: *Pseudomonas aeruginosa*

Antibacterial Activity

Antibacterial activity was carried out against two selected gram negative pathogens (such as *Escherichia coli* and *Pseudomonas aeruginosa*). The strains used for the present study were obtained from Biogenix Research centre, Valiyavila, Trivandrum. In order to access the biological significance and ability of the plant part, the minimal inhibitory activity was determined by Agar cup method.

Petriplates containing 20 ml of Muller Hinton medium were seeded each with 24 hr old culture of bacterial strains such as *E.*

coli and *P. aeruginosa*. Wells of approximately 10 mm diameter was bored using a well cutter and 25 μ l, 50 μ l and 100 μ l of the extracts were added to the well from a stock concentration of 0.1g/1ml. The plates were then incubated at 37 $^{\circ}$ C for 24 hours. Antibacterial activity was assayed by measuring the diameter of the inhibition zone in millimeters formed around the wells [13]. Gentamycin (standard antibacterial agent, concentration: 20 mg/ml) was used as a positive control.

Antifungal Activity

Antifungal activity was also determined by Agar cup method. Potato Dextrose agar plates were prepared and overnight grown isolates of fungi such as *Candida albicans* and *Aspergillus niger* were swabbed. Wells of approximately 10mm diameter was bored using a well cutter and extracts of 25 μ l, 50 μ l and 100 μ l concentrations were added and the zones of inhibition were measured after overnight incubation which were then compared with that of standard antibiotics. Clotrimazole was used as a positive control.

3. RESULTS AND DISCUSSION

Phytochemical Analysis

Table 1 represent the various phytochemical constituents present in the leaf extracts of *Annona squamosa*. The phytochemical studies of all the three extracts conclude that acetone and water extracts of leaf samples had more positive results for glycosides, oils, saponins and flavonoids.

Table 1: Phytochemical analysis of *Annona squamosa* leaf extracts

Phytochemicals	Glycosides	Phytosterols	Alkaloids	Oils	Saponins	Phenols	Flavonoids
Water	+	-	-	+	+	+	+
Acetone	+	+	-	+	+	-	+
Chloroform	+	+	-	+	+	-	-

+: Present - : Absent

Preliminary phytochemical analysis revealed the presence of six compounds (Table 1) viz. flavanoids, glycosides, oils, saponins, phenolics, gum and mucilage. With acetone and chloroform extracts flavanoids, glycosides, phytosterols, oils and saponins were present but alkaloids, proteins, aminoacids, phenols and flavanoids were found to be absent. Traditionally saponins have been extensively used as detergents, pesticides as well as molluscides, in addition to their industrial application such as foaming, surface active agents etc. and also found to have beneficial health effects [14]. Chloroform leaf extract of the plant contain an active constituent Annotemoyin. Flavonoids isolated from aqueous extract of *Annona squamosa* (L) exhibits antimicrobial activity. The plant is reported to contain glycosides, alkaloids, saponins, flavonoids, tannins, carbohydrates, phenolic compounds and phytosterols by previous workers.

Patel *et al* (2008) [15] also reported the presence of 4-(2-nitroethyl 1)-1-6-((6-o- β -Dxylopyranosyl- β -D-glucopyranosyl)-oxy) benzene, Anonaine, Benzyltetrahydroisoquinoline, Borneol, Camphene, Camphor,

car-3-ene, Carvone, β -Caryphyllene, Eugenol, Farnesol, Geraniol, 16- Hetriacontanone, Hexacontanol, Higemamine, Isocorydine, Limonine, Linalool acetate, Methylheptenone, p-(hydroxybenzyl)-6,7-(2- hydroxy,4-hydro)isoquinoline, n-Octacosanol, a- Pinene, b-Pinene, Rutin, Stigmasterol, β -Sitosterol, Thymol, n-Triacontanol etc and absence of alkaloids, proteins, amino acids etc. in the leaf extract of *Annona squamosa*.

Anti-Bacterial Activity

Antibacterial activity of *Annona squamosa* (L) (leaf ethanol extract with DMSO) was assayed *in vitro* by agar cup method against clinical isolates of *E. coli* and *P. aeruginosa*. The given Table shows the microbial growth inhibition of ethanolic leaf extracts of *Annona squamosa* (L) among the varying concentration of leaf extracts, higher concentration exhibited maximum antibacterial activity against the two clinical isolates of *E. coli* and *P. aeruginosa*. Table 2 shows the zone of inhibition formed by the extracts against the bacterial strains on Muller Hinton agar.

Table 2: Zone diameter of inhibition of ethanolic leaf extract of *Annona squamosa*

Test organisms	Zone of inhibition in mm			Positive
	Concentration of leaf extracts Control			
	25	50	100	
<i>E. Coli</i>	Nil	11	17	30
<i>P. aeruginosa</i>	Nil	Nil	15	30

The sequence of antibacterial activity of leaf extract against *E. coli* exhibited no activity in 25 μ l but produced 11 mm and 17 mm zones of inhibition in 50 μ l and 100 μ l concentrations respectively (Table 2). Whereas the sequence of antibacterial activity of plant extract against *P. aeruginosa* showed no activity in both 25 μ l and 50 μ l respectively but produced a 15 mm inhibition zone in 100 μ l concentration (Table 2). Antibacterial activity was expressed at varying degrees with the difference in concentration. Higher concentration of the leaf extract shows highest antibacterial activity. The result obtained might be considered sufficient for further studies for isolation and identification of active principle and for the evaluation of possible antimicrobial activity of other extracts from other parts of *Annona squamosa*.

It was supported by Cosentino *et al.*, (1999) [16] and stated that the extracts from other parts of *Annona squamosa* are used against microbial infections due to the presence of secondary metabolites such as phenols, essential oils, terpenoids, alkaloids and flavanoids. Flavanoids were present in *Annona squamosa* which was earlier studied by Kotkar *et al.*, (2001) [17] and reported that flavanoids expose strong antibacterial activity.

Antifungal Activity

The antifungal activity of *Annona squamosa* (leaf ethanol extract) was assayed *in vitro* by agar cup method against clinical isolates of *C. albicans* and *A. niger*. The given Table shows the microbial growth inhibition of ethanolic leaf extracts of *Annona squamosa*.

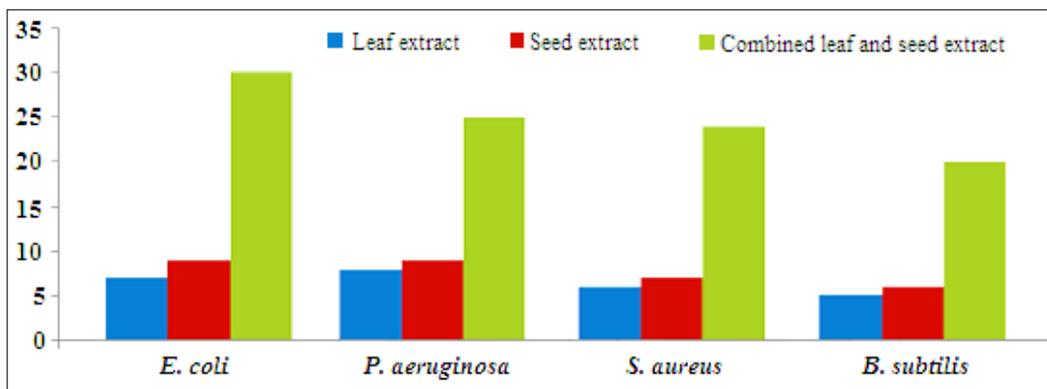
The sequence of antifungal activity of leaf extract against *C. albicans* was 11 mm in 100 μ l, but no activity was found in 25 μ l and 50 μ l concentrations respectively (Table 3). *Annona squamosa* leaf extract was found to have no inhibitory activity against *A. niger* at all the three different concentrations.

Table 3: Zone diameter of inhibition of ethanolic leaf extract of *Annona squamosa*

Test organisms	Zone of inhibition in mm			Positive
	Concentration of leaf extracts Control			
	25	50	100	
<i>C. albicans</i>	Nil	Nil	11	25
<i>A. niger</i>	Nil	Nil	Nil	25

In the present study the plant exhibited better antibacterial activity than antifungal activity. In literature it has been indicated that the antibacterial activity is exhibited by the different chemical agents present in the extract including essential oils, flavanoids, terpenoids and other compounds of phenolic nature or free hydroxyl group which are classified as active antimicrobial compounds. These findings can form the basis of further studies to isolate active phytochemicals, elucidate them against wider range of bacterial strains with the goal to find new therapeutic principles.

The present study reveals that the leaf extracts of *Annona squamosa* were active against *E. coli* and *P. aeruginosa* than fungi. Anti fungal activity were found to be negligible when compared to antibacterial activity (Fig. 10). The results of the study supports to a certain degree, the usage of traditional medicinal plants in human and animal disease therapy and reinforce the concept that ethno botanical approach to screening plants as potential sources of bioactive substances is successful [18]. The aqueous extract generally exhibits a high degree of antibacterial activity which seems to confirm the traditional therapeutic claims of this plant [19]. These results suggest the presence of either good antibacterial potency or high concentration of an active principle in the extract.

**Fig 10:** Statistical representation of Comparative antibacterial activity of methanolic extracts of *Annona squamosa* leaf, seed, combined leaf and seed extract

4. CONCLUSION

Medicinal plants were the potent source of human health due to the active phytochemical compounds that is responsible for its various pharmacological activities. On the basis of the results

obtained in the present study conclude that *Annona squamosa* are rich in phytochemical constituents and showed antimicrobial properties. The results of the phytochemical screening of the leaf extracts of samples varied, while some of

the components were present, some were absent. It was observed that most of the components were present in aqueous extracts. In *Annona squamosa* glycosides, oils and saponins were present in all the three solvents. The presence of various secondary metabolites such as glycosides, phytosterols, alkaloids, oils, saponins, phenols and flavanoids confirmed the antimicrobial efficacy against selected pathogens.

The present study highlights the possible use of *Annona squamosa* leaf extracts as a source of antioxidants and as antibacterial agents that can be used to prevent enteric diseases. The study showed that the results of extraction yield, total phenolic and flavonoid compounds and bioactivity tests varied depending on the type of solvent being used. The study revealed that the leaves of *Annona squamosa* contain a considerable quantity of phenolic-flavonoid compounds that were found to be the major contributor for their antioxidant and antibacterial activities. Hence it can be concluded that the leaves of *Annona squamosa* would direct to the establishment of some compounds that could be used to invent new and more potent anti-microbial drugs of natural origin. Future research should be addressed on the application of using *Annona squamosa* leaves as natural remedier and to protect against the enteric diseases.

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About the corresponding author



Nirmala Patel is affiliated with the Department of Botany at PMCOE, Sanjay Gandhi Smriti Government Autonomous PG College, Sidhi, Madhya Pradesh, India. Her academic work focuses on botanical sciences, contributing to teaching, research, and the advancement of plant science education while supporting students in developing scientific understanding and research skills.