

Research Article**Studies on antimicrobial activity of root of *Annona Squamosa* L. And
Withania Somnifera L.****Hashmi Habib Ahemad Habib Mubarak¹,
S. S. Bodke² and P. V. Pawar³****Article Info**^{1,2}Department of Botany and Horticulture, Yeshwant Mahavidyalaya, Nanded (M.S.) India³Department of Botany and Horticulture, Madhavrao Patil ACS College, Palam, (M.S.), India*Corresponding Author:
ssb26891@gmail.com

Received: 09/05/2025

Accepted: 11/06/2025

Published: 17/06/2025

DOI:

10.5281/zenodo.15687081

Publisher's Note: IJABR Press stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.**Copyright:** ©2025 by the author(s). Licensee IJABR Press, India. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution- Share Alike (CC BY -SA) license.**Abstract**

The Indian folk medicine practice is based on the use of plant extracts. Many phytochemicals like alkaloids, flavonoids, steroids, phenolics, terpenoids and saponins are isolated from plant roots. The plant and plant products are known for their therapeutic uses from thousands of years. In folk medicine it has been used as diuretic, Antipyretic, antidiabetic and analgesic agent.

The *Annona squamosa* L. and *Withania somnifera* L. of the family Annonaceae and Solanaceae respectively were studied for antibacterial properties against pathogenic bacteria. During the present study Aqueous and Methanolic extract of roots of *Annona squamosa* L. and *Withania somnifera* L. were used. Root extracts were evaluated for their antibacterial activity. The antibacterial study was carried out by disc diffusion method against the pathogens *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Salmonella typhi*, *Proteus vulgaris*, *Pseudomonas aeruginosa* and *Escherichia coli*.

The maximum zone of inhibition was observed in methanol extract as compared to aqueous extract for each bacterium. The methanolic extract of roots of the test medicinal plants showed the zone of inhibition against the test bacteria *S.typhi* (12 mm), *K. pneumoniae* (15 mm), *Proteus vulgaris* (22 mm), *Pseudomonas aeruginosa* (18 mm), *Staphylococcus aureus*(14 mm) and *Escherichia coli* (12 mm).The present study demonstrates that the selected medicinal plants have antibacterial potential.

Keywords: *Annona squamosa* L., *Withania somnifera* L., *K. pneumoniae*, *Proteus vulgaris* and antimicrobial activity.

Introduction

The medicinal plants are the herbal remedies in which one or more of its parts contain substances that can be used for therapeutic purposes. During the present studies the medicinal plants namely *Annona* and *Withania* were selected for their antibacterial activity studies.

It is clear from the literature that the plant roots contain many chemical compounds like alkaloids, tannins and coumarins. It is also clear from the literature that these compounds have high pharmaceutical values. They serve as protective mechanism to the plants. They are found to be stored in the wood and bark of the roots. The compounds like tannins are found to be resistant to proteolytic enzymes [1]. They are found to be used in the Indian system of medicine as astringent to intestinal tract. The roots are proved to be good astringent and can be used in diarrhea, dysentery, ulcers, piles and fissures. They contain starch and claims for food values. Considering these facts studies were undertaken for antibacterial properties of roots of *Annona squamosa* L. and *Withania somnifera* L. belonging to the family Annonaceae and Solanaceae respectively.[8-12]

Higher plants produce hundreds and thousands of diverse chemicals compounds with different biological activities[2]. The antimicrobial compounds produced by plants are active against plants and human pathogenic microorganisms[3].

Ashwagandha roots are a constituent of over 200 formulations in Ayurvedha, Siddha and Unani medicine, which are used in the treatment of various physiological disorders[4,5]. *Ashwagandha* is widely claimed to have sedative, rejuvenative and life prolonging properties. It is also used as a general energy-enhancing tonic known as *Medharasayana*, which promotes learning and a good memory and in geriatric problems[6,7].

Material and Methods

The roots of the test plants were collected from the Botanical garden of Yeshwant Mahavidyalaya Nanded. The collected roots were washed with water and shed dried. The dried roots were powdered in grinder and were stored in air tight containers for further use. The Preliminary phytochemical screening was performed according to Johansen (1940) and Harborne (1984).

Preparation of Plant extracts:

The roots of the test plants (250gms) were extracted with methanol solvents (450ml) separately by using soxhlet apparatus for eight hours at a temperature not exceeding the boiling point of the respective solvent. The obtained extracts were filtered by using Whatman No.1 filter paper and then concentrated under vacuum at 40°C, dried in desiccator and packed in sterile plastic bottles.

Aqueous extract was made by cold maceration method. 50gm of powdered material mixed with 500ml of distilled water and kept for seven days at room temperature. The extract obtained from water was filtered through Whatman filter paper No.1 and residue water content was evaporated (4°C) with heating mantle.

Preparation of test samples:

The test samples of the root extract were prepared in DMSO (Dimethyl Sulfoxide).

Phytochemical Analysis: The phytochemical screening of methanol extract was done to identify the phytoconstituents by their colour reaction (Evans, 1997).

Inoculum Preparation: The microorganisms are pre-cultured in nutrient broth overnight in a rotary shaker at 37°C, centrifuged at 10,000 rpm for five minutes. The pellet was suspended in double distilled water and cell density was standardized spectrophotometer at 660nm.

Antibacterial Activity:

The methanol root extract of the test plants were tested by disc diffusion method (Anonymous, 1996). Different concentration of the extracts (100µg/ml) was prepared by reconstituting with methanol. The test microorganisms were seeded in to respective medium by spread plate method. 10µl with the 24Hrs culture of bacteria was grown separately in the nutrient broth. After solidification the filter paper discs (5mm diameter) impregnated with the extract were placed on test organism-seeded plates. Streptomycin sulphate (10µg/ml) was used as

negative control. The antibacterial assay plates were incubated at 37°C for 24 hrs. The diameter of the inhibition zones were measured in millimeters.

Experimental Results

Phytochemical Analysis: The roots of the test plants were collected shade dried and powdered. The powder is used to prepare extract in methanol and water (Aqueous extract) and screened for qualitative phytochemical analysis. The results are presented in table-1.

Table-1: Phytochemical Analysis

Sr.No.	Phytochemicals	<i>Annona squamosa</i>		<i>Withania somnifera</i>	
		Methanol	Aqueous	Methanol	Aqueous
1	Tannin	+	+	+	+
2	Saponin	+	+	+	+
3	Terpenoid	+	+	+	+
4	Phenolic compounds	+	+	+	+
5	Flavonoids	+	+	+	+
6	Alkaloids	+	+	+	+

(+ Represents the presence of phytochemicals)

From the results presented in table-1 it is clear that the roots of both the test plants showed the presence of tannins, saponins, terpenoids, phenolic compounds, flavonoids and alkaloids.

The table presents a qualitative analysis of six phytochemicals in *Annona squamosa* and *Withania somnifera* using both **methanolic** and **aqueous** extracts.

Key Findings:

- All tested **phytochemicals—tannins, saponins, terpenoids, phenolic compounds, flavonoids, and alkaloids—**were **present (+)** in both plant species and in **both extraction solvents** (methanol and water).
- This uniform presence across all samples indicates that **both plants are rich in diverse bioactive compounds**, regardless of the solvent used.

The methanolic and aqueous extracts of *Annona squamosa* and *Withania somnifera* contain a broad spectrum of phytochemicals, which likely contribute to their observed antimicrobial and therapeutic properties. This supports their potential use in herbal medicine and natural drug development.

Photo Plates

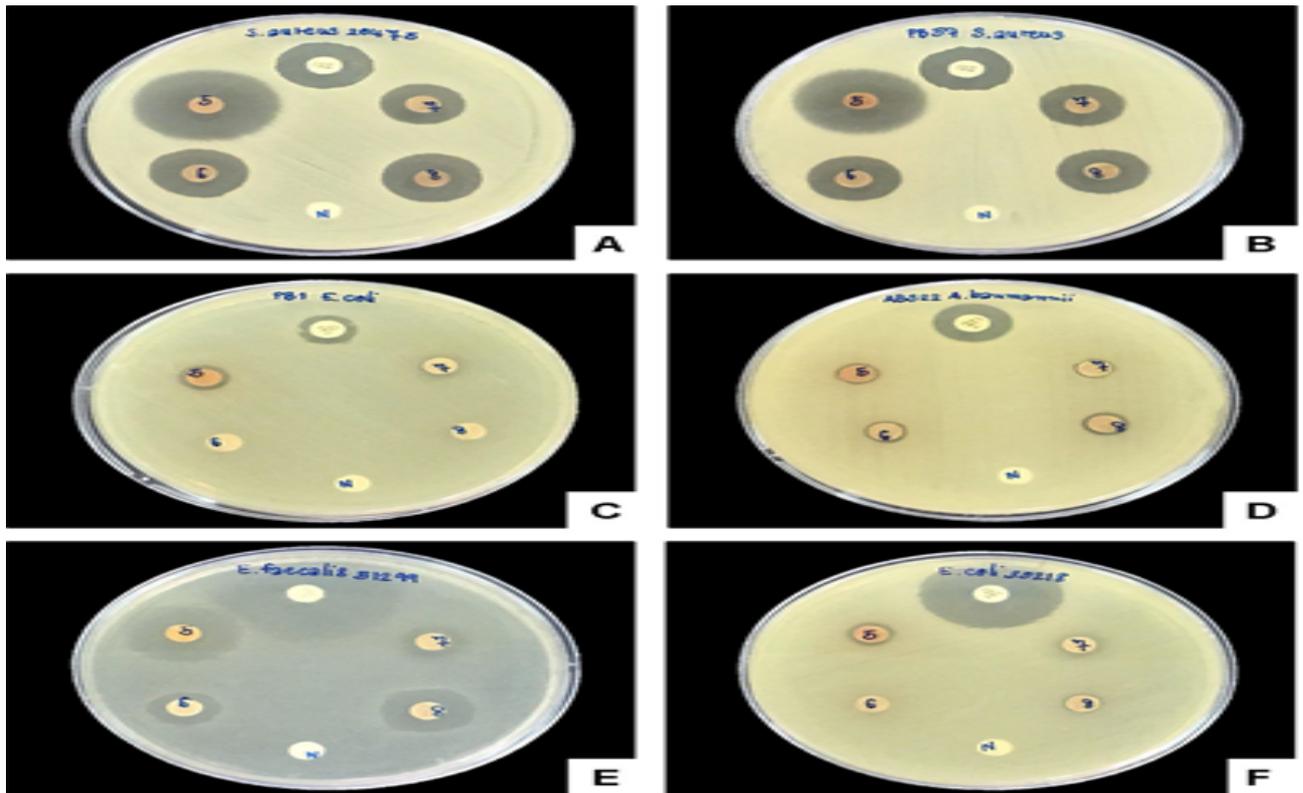


Plate-1: Antibacterial Activity of Root of *Annona squamosa* L. in different solvents

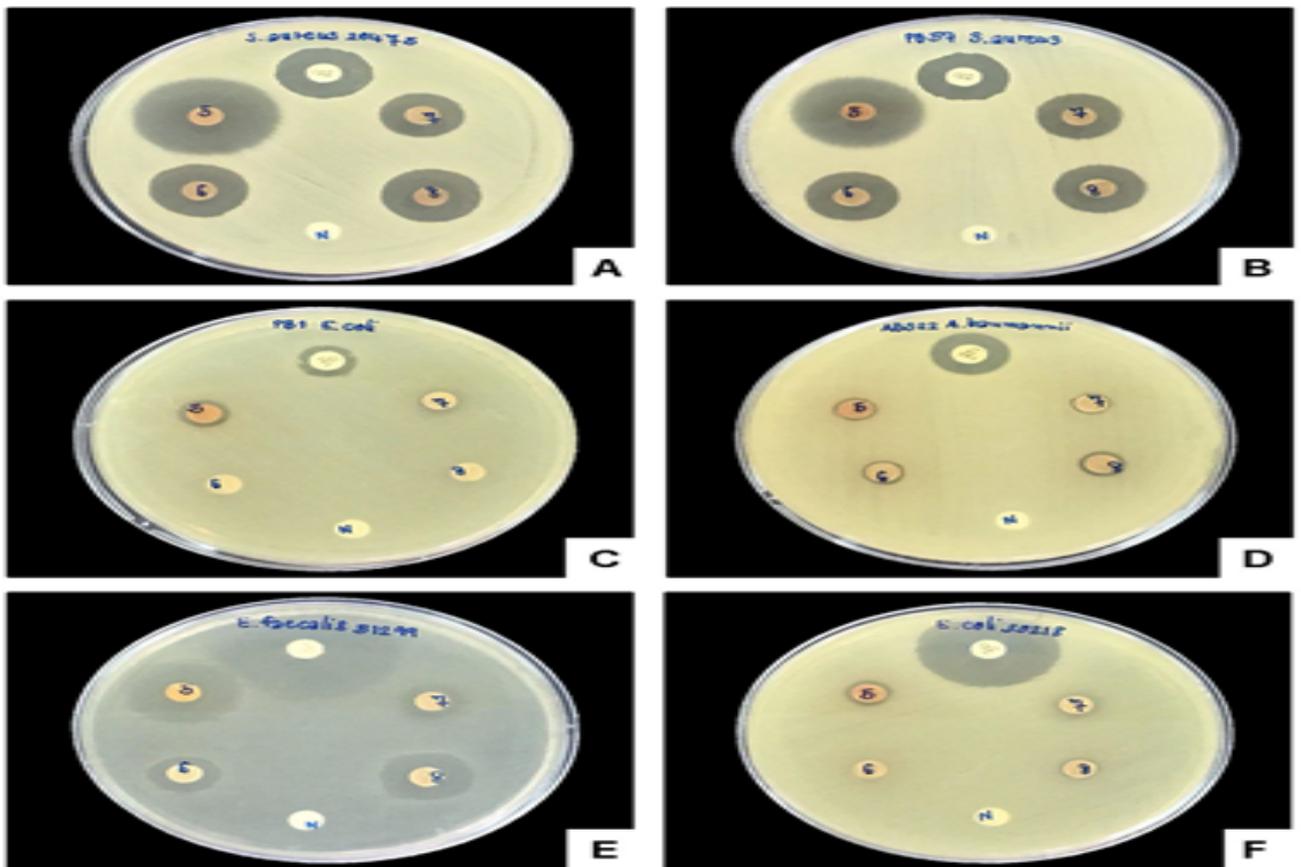


Plate-2: Antibacterial Activity of Root of *Withania somnifera* L. in different solvents

Antimicrobial Activity

During the present studies the results obtained revealed that the roots of the test plants have potential antibacterial activity against the human pathogenic bacteria as *Klebsiella pneumonia*, *Staphylococcus aureus*, *Salmonella typhi*, *Proteus vulgaris* and *Pseudomonas aeruginosa*, *Escherichia coli*. The methanolic extract showed significant antimicrobial activity as compared to the aqueous extract. The results are presented in table-2.

From the results it is clear that the highest antibacterial activity recorded in *P. vulgaris* (22 mm), *P. aeruginosa* (18 mm), *K.pneumoniae* (15 mm), *S.aureus* (14 mm), *S.typhi* (12 mm), and *E. coli*. (12 mm) for the plant *Annona squamosa* root extracts.

The results obtained for the second plant *Withania somnifera* were also significant and showed antimicrobial property as *P. vulgaris* (20 mm), *P. aeruginosa* (18 mm), *K.pneumoniae* (16 mm), *S.aureus* (13 mm), *S.typhi* (12 mm), and *E. coli*. (12 mm) .It was interesting to note that the results showed significant antibacterial activity in methanolic root extracts as compared to the aqueous root extracts. The results are presented in table-2.

Table-2: Antibacterial activity of methanol and aqueous extract (100 µg/ml) of *Annona squamosa* L. and *Withania somnifera* L. by disc diffusion method.

Human pathogenic bacteria	Zone of Inhibition* (mm)				
	<i>Annona squamosa</i> L.		<i>Withania somnifera</i> L.		Streptomycin sulphate (10 µg/ml)
	Methanol	Aqueous	Methanol	Aqueous	Negative Control
<i>S.aureus</i>	14	8	13	10	24
<i>S.typhi</i>	12	6	12	8	21
<i>K.pneumoniae</i>	15	9	16	7	18
<i>P. vulgaris</i>	22	5	20	6	23
<i>P. aeruginosa</i>	18	7	18	6	22
<i>Escherichia coli</i> .	12	8	12	10	16

The table presents the **zone of inhibition (in mm)** for six human pathogenic bacteria tested against extracts of *Annona squamosa* and *Withania somnifera* in both methanol and aqueous forms, compared with **streptomycin sulphate** (10 µg/ml) as the **positive control**, and a **negative control** (no inhibition).

Key Findings:

- **Methanolic extracts** of both plants showed **stronger antibacterial activity** than their aqueous counterparts.
- *Annona squamosa* (methanol) exhibited the **highest inhibition** against *Proteus vulgaris* (22 mm), followed by *Pseudomonas aeruginosa* (18 mm).

- *Withania somnifera* (methanol) was most effective against *Klebsiella pneumoniae* (16 mm) and *P. vulgaris* (20 mm).
- **Aqueous extracts** showed **milder activity**, with inhibition zones ranging mostly between 5–10 mm.
- **Streptomycin sulphate** consistently produced the **largest inhibition zones** (16–24 mm), confirming its superior antibacterial efficacy.

Conclusion:

Methanolic extracts of *Annona squamosa* and *Withania somnifera* demonstrate moderate antibacterial activity, particularly against *P. vulgaris* and *K. pneumoniae*, suggesting potential for use in herbal antimicrobial

therapies. However, their effects are significantly less potent than the standard antibiotic streptomycin.

Discussion

The plant material used as an alternative method to control pathogenic microorganisms and many components of plant products have been shown to be specially targeted against resistant pathogenic bacteria [11].

The present work explores the antimicrobial activity of medicinal plants *Annona squamosa* L. and *Withania somnifera* L. These plants are reported to have antibacterial, antifungal, anthelmintic properties [10]. The methanol root extract of the test plants showed the activity against all the six test microorganisms but the activity was very significant against *P. vulgaris* and *P. aeruginosa*. The methanol and aqueous extract of the medicinal plants were significantly active against *Staphylococcus* species.

The present study shows that the methanolic and aqueous extract of roots of both the test plants have the entire phytochemical constituent studied with negligible variation. Thus the significant activity against *P. vulgaris* and *P. aeruginosa* may be due to their phytochemical or secondary metabolites present in the medicinal plants.

Acknowledgement: None stated.

Conflict of interest: None to declare.

References

1. Bipul Biswas, Kimberly Rogers, Fredrick McLaughlin, and Dwayne (2013): Antimicrobial activities of leaf extracts of Guava (*Psidium guajava* L.) on two gram-negative and gram-positive bacteria, *Int. J. of Microbiology*. Article ID 746165. 7 pages.
2. Newman DJ, Cragg GM, Snader KM. The influence of natural products upon drug discovery. *Nat Prod Res*. 2000;17:215–34. doi: 10.1039/a902202c.
3. Yoganarasimhan N. Vol. 1. Bangalore:

- Interline Publishing Pvt Ltd; 1996. Medicinal plants of India.
4. Asthana R, Raina MK. Pharmacology of *Withania somnifera* (L.) Dunal-a review. *Indian Drugs*. 1989;26:199–205.
5. Singh S, Kumar S. Lucknow: Central Institute of Medicinal and Aromatic Plants; 1998. *Withania somnifera: The Indian Ginseng Ashwagandha*.
6. Nadkarni KM. Bombay: Popular Prakshan Limited; 1976. *Indian Materia Medica*; p. 1291.
7. Williamson EM. London: Churchill Livingstone; 2002. *Major Herbs of Ayurveda*; pp. 322–3.
8. Ghani A. (1990): *In Traditional Medicine*. Jahangirnagar University, Savar, Dhaka: 1990. p.15-40.
9. Gopalkrishnan Sarala, George Shibumon and PJ Benny (2010): Antimicrobial effect of *Punica grantum* on pyogenic bacteria. *J. of Pharma and Biomed Sci*. 3(6).
10. Mahesh B. and S Satish (2008): Antimicrobial activity of some important medicinal plant extract against plant and human pathogens. *World J. of Agri.Sci*. 4(S): 839-843.
11. Nostro A., L Cellini and S. Di Bartolomeo (2006): Effects of combining extracts (from propolis or *Zingiber officinale*) with clarithromycin on *Helicobacter pylori*. *Phytotherapy Research*. 20(3): 187-190.
12. Rastogi and Mehrotra (1993): *Imodium of Indian medicinal plants*. CDR (Lucknow). 2:496.