

## SCREENING OF STEM BARK METHANOL EXTRACT OF *ANNONA SQUAMOSA* FOR ANTIBACTERIAL ACTIVITY

KACHHAWA J.B.S., SHARMA N., TYAGI, S., SHARMA K.K.\*

Molecular Developmental Biology Laboratory, Department of Zoology, M.D.S. University, Ajmer 305009 Rajasthan, India.

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### ABSTRACT

*Annona squamosa* is a medicinal plant belonging to family *Annonaceae*. For the preliminary study, methanol extract of stem barks has been used to check the efficacy of antibacterial activity against *Bacillus coagulans* and *Escherichia coli* bacteria of gram-positive and gram-negative strain respectively using disc diffusion method. Laboratory screening of methanol extract of *A. squamosa* has given encouraging results which indicating their potential uses in the control of selected pathogenic bacteria.

**Keywords:** Antibacterial activities, *Annona squamosa*, *Bacillus coagulans*, *Escherichia coli*, Disc diffusion assay

### INTRODUCTION

Increasing prevalence of multidrug resistant of many microbes has revealed exploration of alternative antimicrobial agents. Medicinal plants have become the focus of intense study in terms of validation of their traditional uses through determination of their actual pharmacological effects. Synthetic drugs are not only expensive but have more side effects. Therefore, there is a need to search new antimicrobial agents from the plant source to control microbial infections.

### MATERIAL AND METHODS

#### Plant materials

*Annona squamosa* L. (*Annonaceae*) commonly known as "Custard apple" in English and "Sharifa" in Hindi is a native of West Indies and is cultivated throughout India, mainly for its edible fruit (Morton 1987). Different parts of *A. squamosa* are used in folkloric medicine for the treatment of several disorders and beneficial for cardiac diseases, diabetes, hyperthyroidism and cancer (Shirwaikar *et al.*, 2004); it also showed antifertility, antitumor and antidiabetic activities in mice and rats (Rao and Shah, 1988; Gupta *et al.*, 2005). The plant is said to show varied medicinal effects, including insecticidal, anti-ovulatory and abortifacient (Damaseeno *et al.*, 2002).

*A. squamosa* is traditionally used for the treatment of epilepsy, dysentery, worm infestation, constipation, hemorrhage, dysuria, fever, thirst, ulcers and also as an abortifacient (Vohora *et al.*, 1975; Asolkar *et al.*, 1992; Yoganarasimhan, 2000). Alkaloids present in *A. squamosa* leaves have proved to have antioxidant activity (Shirwaikar *et al.*, 2004). Raj *et al.*, (2009) have been evaluated hepatoprotective activity against diethylnitrosamine induced liver injury in mice.

Bullatacin is one such compound that possessed antitumoral and pesticidal activity *in vivo* (Ahmmadsahib *et al.*, 1993). *A. squamosa* seeds possessed potent proapoptotic characteristics for several human tumour cells; an ethanol extract of the leaves and stem is reported to have anticancer activity (Farrell, 1975; Pardhasaradhi *et al.*, 2005). It also possess chemopreventive and antilipidperoxidative efficacy against experimental carcinogenesis in male golden Syrian hamsters (Suresh *et al.*, 2006).

A benzo-oxyquinazoline alkaloid, samoquasine A (Morita *et al.*, 2000), has been isolated from the seeds of *Annona squamosa*. Two major alkaloids were isolated from the root of *A. squamosa*, liriodenine (I) and oxoanalogine (II), belongs to the group of oxoaporphines (Pérez-Amador *et al.*, 2004).

#### Preparation of plant extracts

The fresh stems of *Annona squamosa* were collected from Jhalawar district of Rajasthan (India) and was identified (Voucher No. RUBL-

19931) from Department of Botany, University of Rajasthan, Jaipur. The stem bark were shade dried, powdered and subjected to soxhlet extraction in 100% v/v methanol (60-80°C) for 30-36 hours. The residual extract thus obtained treated as experimental drug for the present study.

#### Test microorganism

The antimicrobial activity was individually tested against Gram-positive bacteria i.e. *Bacillus coagulans* and Gram-negative bacteria i.e. *Escherichia coli*. Both test strains were maintained on nutrient agar (Hi-media Laboratory Pvt. Ltd., Mumbai, India) and were sub-cultured every two weeks. The bacteria *B. coagulans* was obtained from Sporlac tablets and *E. coli* was procured from Institute of Microbial Technology, Chandigarh, India

#### Preparation of the bacterial inoculum

A loop full of the test organism was inoculated on the nutrient broth and incubated for 24 hours at 37°C. 10<sup>6</sup> cfu/ml bacterial cells with O.D. 1 were taken to study the antibacterial activity

#### Antibacterial activity assay

The disc diffusion method was adopted to test the antibacterial activity where Ciprofloxacin was used as a standard drug to compare the results of experimental plant.

#### Paper disc diffusion method

The disc diffusion method was used to determine the growth inhibition of bacteria by the plant extract. Discs containing different concentration (200, 100, 50 and 25 mg/ml) of dissolved plant extract and prepared by using sterile Whatman filter paper No. 1 (6 mm in diameter). The discs were dried at 50°C. Overnight cultures of each of bacterial isolates was diluted with sterile normal saline to give inoculum size of 10<sup>6</sup> cfu/ml. Nutrient agar medium was prepared, sterilized, cooled and poured in to sterile petri dishes to a depth of 4 mm about 25 ml/plate to solidify. Pure cultures of the test organism were used to inoculate the petri dishes. This was done by spreading the inocula on the surface of the prepared nutrient agar plate using sterile cotton swabs which have been dipped in the diluted suspension of the organism. The discs were then aseptically placed evenly on the surface of the inoculation and gently pressed down to ensure contact using a pair of forceps. The plates were finally incubated at 37°C for 18-24hrs. The plates were examined after 24 hrs for clear zone of inhibition. All measurements were taken in mm.

### RESULT AND DISCUSSION

Results obtained for the antibacterial test performed on different concentrations of methanol extract of *Annona squamosa* are presented in figure 1 and 2. Various concentrations of methanol extract showed broader spectrum of antibacterial activity being

active to both gram positive and gram negative organisms. 100mg/ml concentration showed maximum antibacterial activity against *Bacillus coagulans* and *Escherichia coli* with MIC of 8mm. The

broad spectrum antibacterial activity of the plant extract possibly due to identified alkaloids further confirms its use as a health remedy (Pérez-Amador et al., 2004).

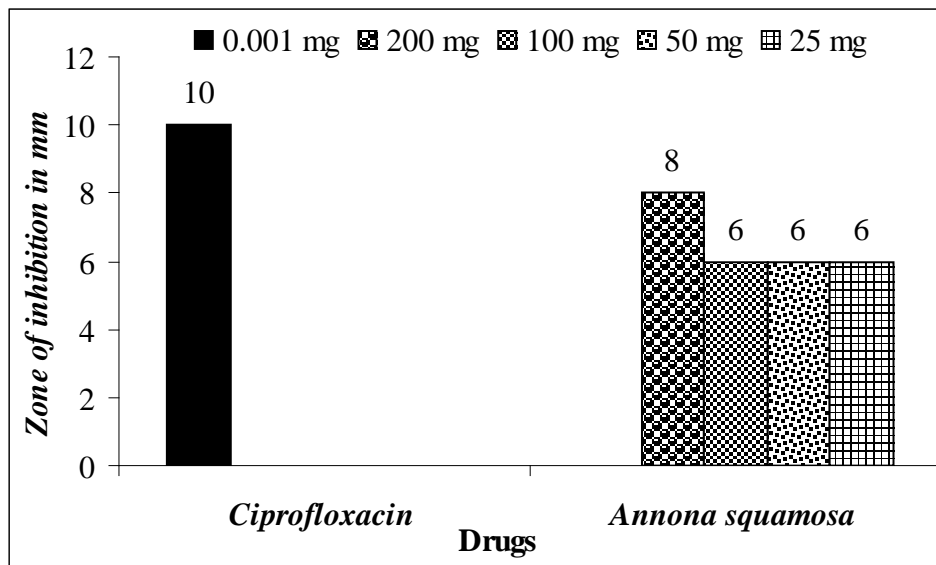


Fig. 1: Antibacterial activity of different concentrations of methanol extract of *Annona squamosa* against *B. coagulans*

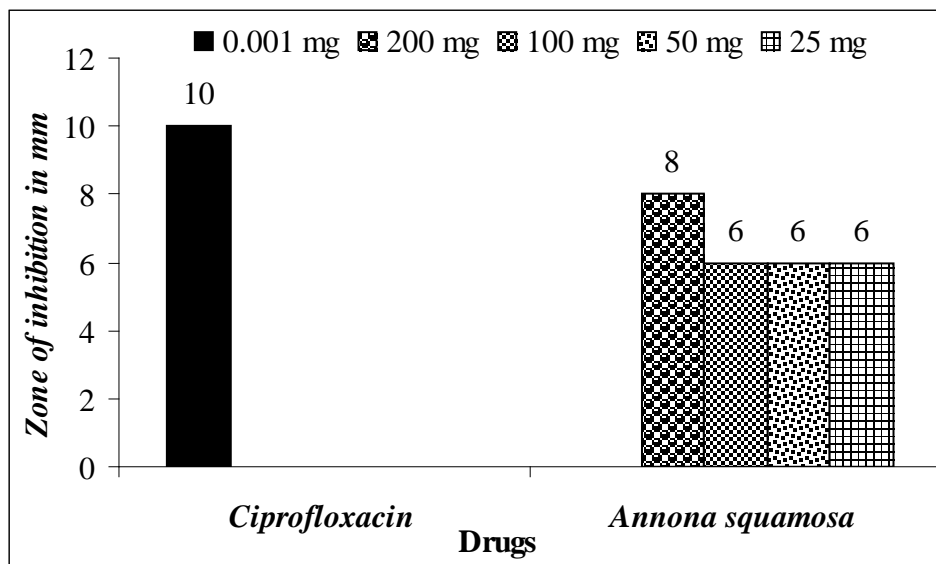


Fig. 2: Antibacterial activity of different concentrations of methanol extract of *Annona squamosa* against *Escherichia coli*

## CONCLUSION

In the current investigation the stem bark methanol extract of *A. squamosa* was found to be active on test bacteria. Bioactive substances from this plant can therefore be employed in the formulation of antimicrobial agents for the treatment of various bacterial diseases.

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