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ANTIBACTERIAL ACTIVITY OF *ANNONA SQUAMOSA* SEED EXTRACT

Biba V. S[1]; Jeba Malar P.W[2] ; and Remani P[1]
[1]Division of Cancer Research, Regional Cancer Centre, Thiruvananthapuram, Kerala, India. 695011
[2]Centre for Marine Science and Technology, Manonmaniam Sundaranar University, Rajakkamangalam, Kanyakumari, Tamil Nadu, India.629502.
Email: vsbiba@gmail.com

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Abstract

Many Annonaceae plants and their extracts have been extensively used in folk medicine as pesticides, antimicroial agents. the aim of this study was to evaluate the antibacterial activity of the chloroform against human pathogenic gram negative and gram positive bacterial strains (*Escherichia coli, Salmonella typhi, Klebseilia pnemonia, Proteus mirabilis Staphylococcus aureus, Bacillus subtillis*) by broth dilution technique. The chloroform extract showed remarkable antibacterial activity in gram negative and gram positive bacteria. The results from these investigations suggest that the chloroform extract of *Annona squamosa* seeds may be used as anti-infective agents.

Keywords: *Annona squamosa,* antibacterial activity.

Introduction

Antimicrobial drugs either kill microbes (microbiocidal) or prevent the growth of microbes (microbiostatic). Disinfectants are antimicrobial substances used on non-living objects or outside the body. Microorganisms remain unrivalled in their ability to produce bioactive small molecules for drug development. Microorganisms have developed resistance to many antibiotics and as a result, immense clinical problem in the treatment of infectious diseases has been created. The resistance of the organisms increased due to indiscriminate use of commercial antimicrobial drugs commonly used for the treatment of infectious disease. This situation forced the researchers to search for new other antimicrobial substance from various sources including medicinal plants (Bauer et al., 1996). Plants are rich in a wide variety of secondary metabolites, such as tannins, terpenoids, alkaloids, and flavonoids, which have been found *in vitro* to have antimicrobial properties (Lewis and Ausubel *et al* 2006). The effects of plant extracts on bacteria have been studied by a very large number of researchers in different parts of the world

(Ates and Erdogrul, 2003; Reddy et al., 2001). Much work has been done on ethnomedicinal plants in India (Negi etal, 1993; Rai, 1989).The characteristics of the plants that inhibit microorganisms and are important for human health have been researched in laboratories since 1926 (Erdogrul, 2002). This study was conducted to evaluate the antimicrobial activity of the extract of *Annona squmosa seeds.*

Preparation of seed extract

The seeds of *Annona squamosa* were collected from Thiruvananthapuram district, Kerala state, authenticated by the taxonomist and a voucher specimen TBGT 57051 has been kept in the herbarium of Jawaharlal Nehru Tropical Botanical Garden and Research Institute, Palode, Thiruvananthapuram. The shade dried and pulverized seeds of *Annona squamosa* were used for soxhlet extraction in a soxhlet apparatus using chloroform as the solvent and concentrated by using rotatory evaporator.

Microorganisms used

The microbial strains used were identified strains and obtained from the National Chemical Laboratory (NCL), Pune, India. The bacterial strains studied include gram negative bacteriae *Escherichia coli* (ATCC 8739)*, Salmonella typhi* (ATCC 23564)*, Klebsiella pnemoniae* (NCIM 2719)*, Proteus mirabilis* (NCIM 2040) and gram positive bacteriae (*Bacillus subtilis* (ATCC 6633), *Staphyllococcus aureus* (ATCC 23564).

Microbial culture conditions

The bacterial cultures were maintained in Nutrient agar plates. Overnight cultures were used in all experiments by inoculating a single colony of each type of culture in respective 5 ml Nutrient broth and incubating at 37^0C for 18-24 hours in a rotory incubator.

Assay of antibacterial activity by Broth dilution technique

Inhibitory Concentration of (*Annona squamosa* chloroform extract) ASCH towards gram negative bacteria and gram positive bacteria were determined using the broth dilution technique. A loopful of bacterial culture was taken from an overnight culture and was inoculated into 1 ml of nutrient broth. The inoculated culture was kept in a rotary shaker at 37^0C for 2 hours at 120 rpm and the organisms were seeded for the experiment once the optical density (O.D) reached 0.3 at 650 nm. 100 µl of the culture was added to all the wells of a 96 well microtitre plate. Different concentrations (10µg/ml to 60µg/ml) of the ASCH dissolved in 1ml DMSO were serially diluted and added in triplicates into the cell culture. The negative control was maintained by treating the bacterial cells with media

containing 10% DMSO. The plated cells were incubated at 37^0C for 48 hours. After the incubation period, optical density was read at 620 nm in a multiwell plate reader. Minimum inhibitory concentration i.e. 50 % death of organisms were calculated. The experiments were done in quadruplicates.

Statistical analysis

The results are represented as the mean±SD. The data was analysed by using Excel and Easy plot software

Results

Inhibition of the growth of microorganisms

The results revealed that at highest concentration of ASCH (60µg/ml) the inhibitory effect on gram negative bacteria *Escherichia coli, Salmonella typhi. Proteus mirabilis Klebseilia pnemonia, Staphylococcus aureus and Bacillus subtilis* were 64+0.95, 53.5±1, 56±1.29, 63±.75, 47±0.95, 60.75±1.1 respectively at 48 hours. Likewise, the lowest concentration of ASCH (10µg/ml) showed inhibitory effect of 36±1.4%, 35±1.2%, 37±0.9%, 48.5±0.8%, 34±0.8% and 40±1.1% respectively. (Fig.1 to Fig.6).

Minimum inhibitory concentration of ASCH on gram negative bacteria *Escherichia coli, Salmonella typh,. Klebseilia pnemonia, Proteus mirabilis and* gram positive bacteria *Staphylococcus aureus, Bacillus subtilis* were found to be 33.1, µg/ml, 50 µg/ml, 13.6 µg/m, 37.7 µg/ml,51 µg/ml,16 µg/ml respectively (Fig.1b – Fig.6b).

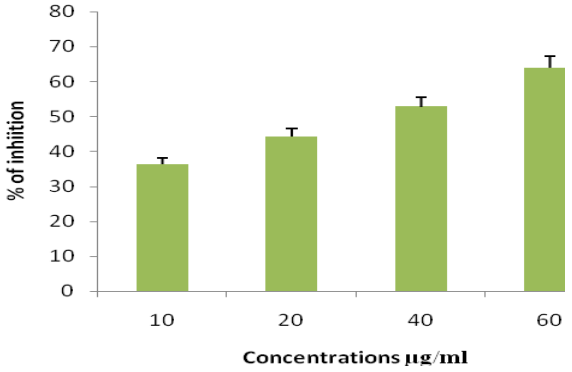

Fig.1 Inhibitory effect of ASCH on *E.coli* .

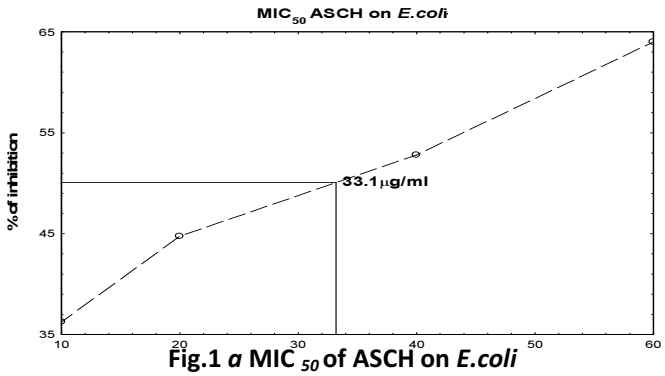

Fig.1 *a* MIC $_{50}$ of ASCH on *E.coli*

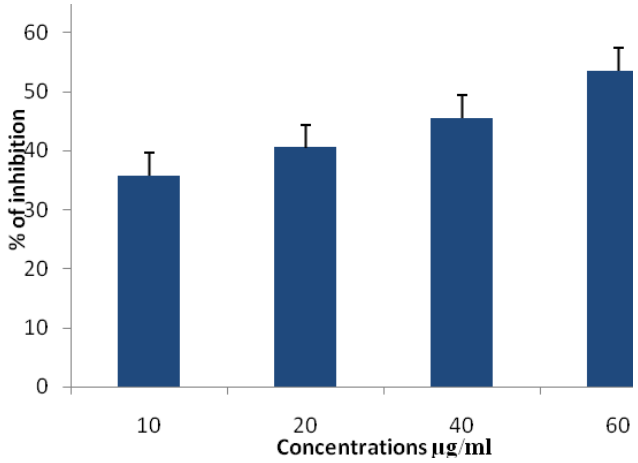

Fig.2 Inhibitory effect of ASCH on *Salmonella typhi*.

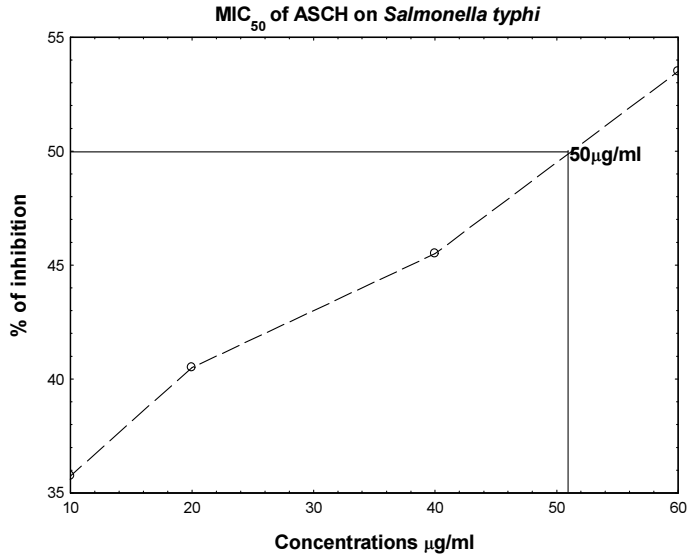

Fig.2 *a* MIC $_{50}$ of ASCH on *Salmonella typhi.*

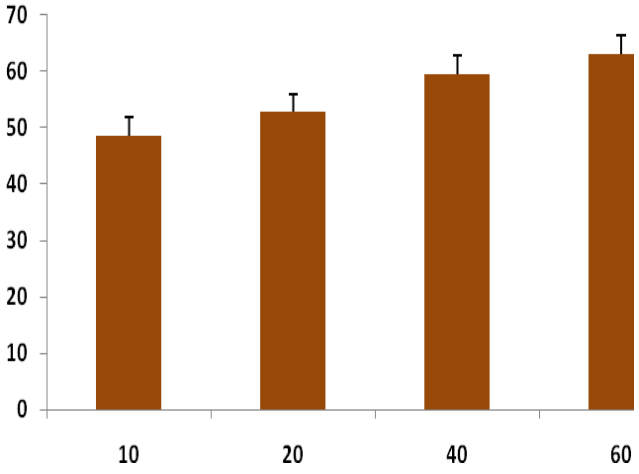

Fig.3 Inhibitory effect of ASCH on *Klebseilia pneumonia*.

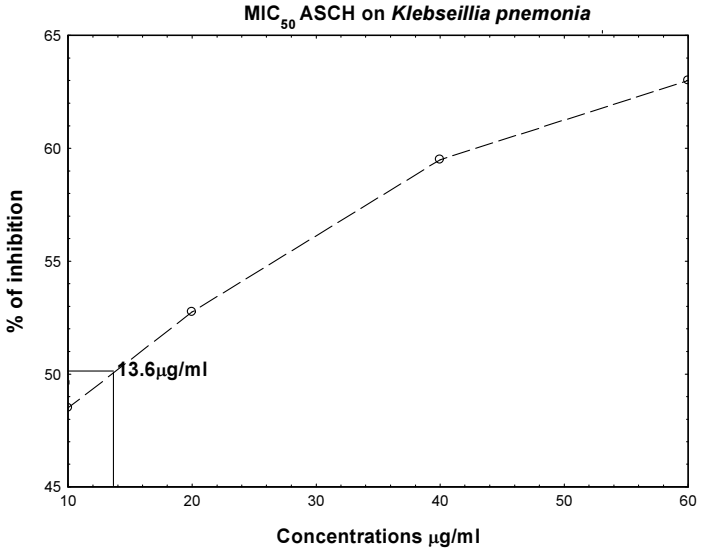

Fig.3 *a* MIC $_{50}$ of ASCH on *Klebseilia pneumonia.*

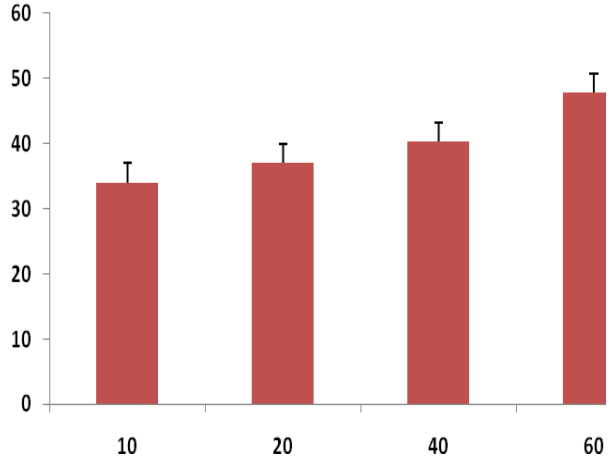

Fig.4 Inhibitory effect of ASCH on *Staphylococcus aureus*.

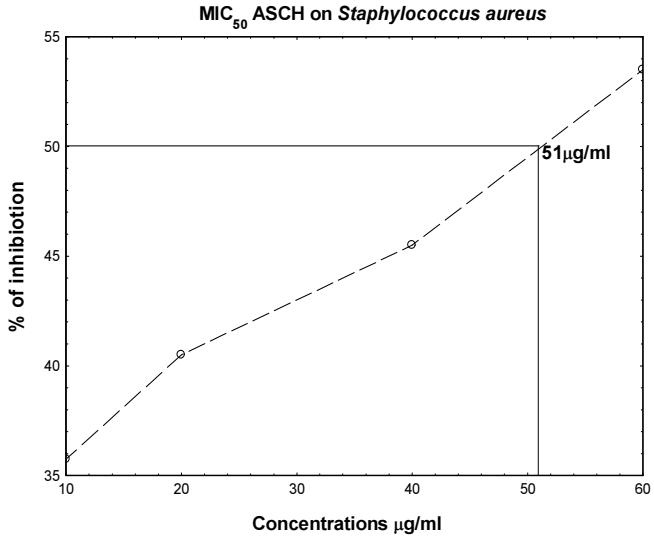

Fig.4 *a* MIC $_{50}$ of ASCH on *Staphylococcus aureus.*

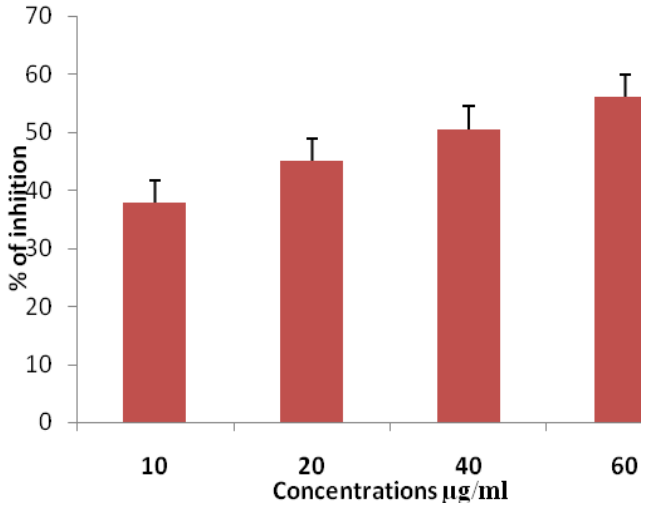

Fig.5 Inhibitory effect of ASCH on *Proteus mirabilis*.

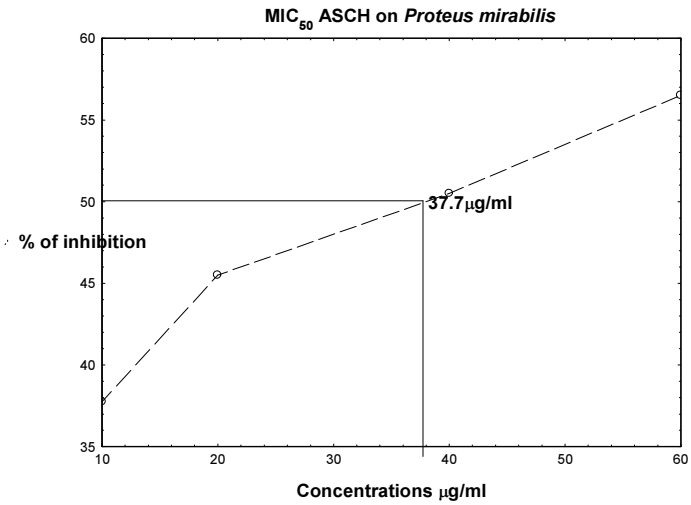

Fig.5 *a* MIC $_{50}$ of ASCH on *Proteus mirabilis*

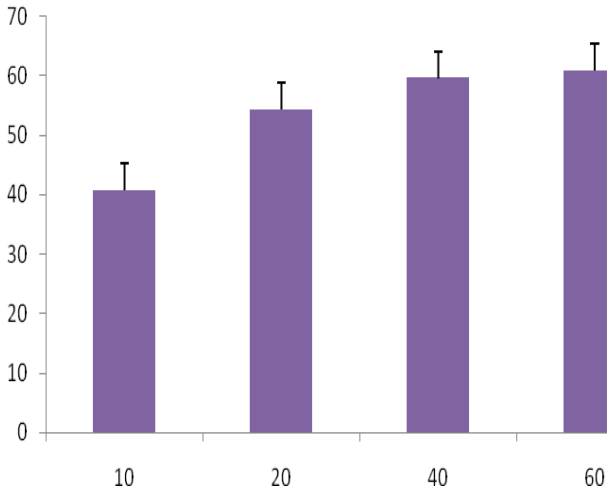

Fig.6 Inhibitory effect of ASCH on *Bacillus subtilis*.

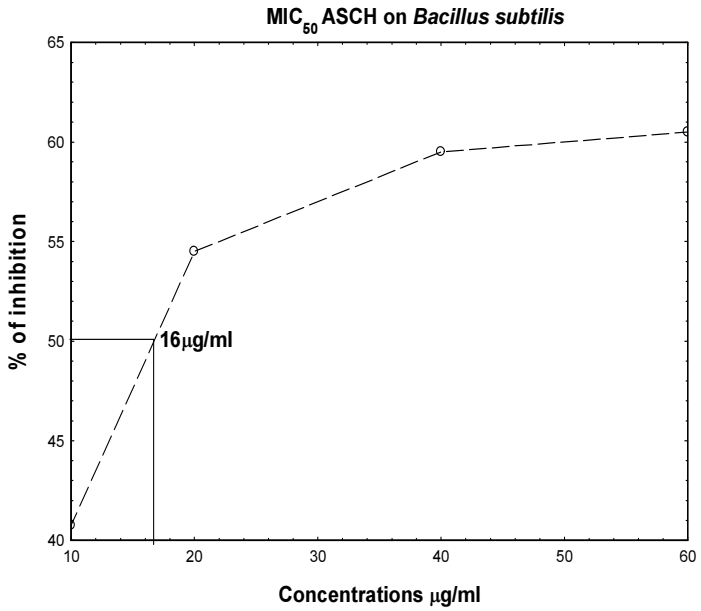

Fig.6 *a* MIC *50* of ASCH on *Bacillus subtilis.*

Discussions

In our study the results of Broth dilution assay indicated that the ASCH was effective in inhibiting gram negative *Escherichia coli, Salmonella typhi. Klebseilia pnemonia, Proteus mirabilis* and gram positive *Staphylococcus aureus, Bacillus subtilis.* It was also noticed that the inhibitory effect of ASCH was found to be increased with the concentration. Earlier studies by Jayshree *et al* ashowed antibacterial activity to the leaves of *Annona squamosa* (Jayshree *et al* 2008). Beena and sobhana (2006) had reported the antibacterial activity of *Annona squamosa* fruit pericarp. Antimicrobial agents are very important in the treatment of cancer to prevent the secondary infections. Many phytochemicals acting as antimicrobial agents in the plant's defence are likewise active against human pathogenic organisms, and various studies report on the antimicrobial activities of crude plant extracts (Rojas *et al* 2003). There are several investigators have proved the antimicrobial potentiality of some *Annonaceae* members. *A. squamosa* contains flavanoids which express strong antibacterial activity (Chavan *et al* 2010). Padhi *et al* 2011 reported antibacterial activity on *Annona squamosa* leaves extracts. Methanol extract of *Annona squamosa* 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 by Kachhawa B S *et al* (2012). This study encourages the use of herbal extract and demonstrated that folk medicine can be used as effective modern medicine to combat pathogenic microorganisms. The discovery of a potent remedy from plant origin will be a great advancement in bacterial infection therapies.

Conclusion

In concusion ASCH extract have great potential as antimicrobial compounds against microorganisms and that they can be used in the treatment of infectious diseases caused by microorganisms. This study suggests that the chloroform extract isolated from the seed of *Annona squamosa* is a promising candidate to be exploited further to develop as pharmacologically active agents. Further investigation with purified fractions is required to predict the exact mechanism of action.

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Corresponding author:

P. Remani*,

Email: *vsbiba@gmail.com*