

**ANTIMICROBIAL EFFECT OF *ANNONA SQUAMOSA*
ETHYL ACETATE EXTRACT****BIBA V. S¹; JEBA MALAR P.W² AND REMANI P*¹**¹Division of Cancer Research, Regional Cancer Centre, Thiruvananthapuram, Kerala, India. 695011²Centre for Marine Science and Technology, Manonmaniam Sundaranar University, Rajakkamangalam, Kanyakumari, Tamil Nadu, India.629502.**ABSTRACT**

Annona squamosa has been known to possess wide range of biological activities. The present study was focused on investigating the antibacterial property of the ethyl acetate extract from the seeds of *Annona squamosa* using broth dilution assay against several human pathogenic gram negative and gram positive bacterial strains. Minimum inhibitory concentration of *Annona squamosa* ethyl acetate extract (ASEA) on gram negative bacteria *Escherichia coli*, *Salmonella typhi*, *Proteus mirabilis*, *Klebsiella pneumoniae* were found to be 19.5 µg/ml, 20.6 µg/ml, 35 µg/ml, 61.5±0.9 and gram positive bacteria *Staphylococcus aureus*, *Bacillus subtilis* were found to be 67.8µg/ml, 75 µg/ml, 27.6 µg/ml respectively. Results showed that ethyl acetate extract showed antibacterial activity to all the gram negative and gram positive bacteria. This study suggests that ethyl acetate extract of *Annona squamosa* was used as an antimicrobial agent.

KEY WORDS: *Annona squamosa*, Antibacterial activity, Broth dilution technique, Ethyl acetate.**REMANI P**Division of Cancer Research, Regional Cancer Centre, Thiruvananthapuram,
Kerala, India. 695011

INTRODUCTION

Medicinal plants have been used to treat a number of diseases. Though the recovery is gradual, the therapeutic application of medicinal plant is becoming familiar because of its disability to principle side effects and antibiotic resistant microorganisms¹. 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 properties². Plants with possible antimicrobial activity should be tested against an appropriate microbial model to confirm the activity and to ascertain the parameters associated with it. The effects of plant extracts on bacteria have been studied by a very large number of researchers in different parts of the world^{3,4,5}. The selection of crude plant extracts for screening programs has the potential of being more successful in initial steps than the screening of pure compounds isolated from natural products⁶. The search for new anti-microbial agents from medicinal plants is even more urgent as the causative agents are developing an increasing resistance against many of the commonly used antibiotics^{7,8}. In traditional literature, it is found that *Annona squamosa* (Annonaceae) leaves were used as folk medicine for the treatment of wound in different parts of the world. This study was conducted to evaluate the antimicrobial activity of the ethyl acetate extract of *Annona squamosa* seeds.

MATERIALS AND METHODS

Microorganisms

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), *Proteus mirabilis* (NCIM 2040), *Klebsiella pneumoniae* (NCIM 2719), and gram positive bacteria *Staphylococcus aureus* (ATCC 23564), (*Bacillus subtilis* (ATCC 6633).

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 Botanic 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 ethyl acetate (ASEA) as the solvent and concentrated by using rotatory evaporator.

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°C for 18- 24 hours in a rotary incubator.

Assay of antibacterial activity by Broth dilution technique

Inhibitory concentration of *Annona squamosa* seed ethyl acetate extract (ASEA) towards gram negative bacteria and gram positive bacteria were determined using the broth dilution technique. A loop full 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°C 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 micro titre plate. Different concentrations (10µg/ml to 80µg/ml) of the ASEA 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. Penicillin (PN) was used as positive control against gram negative strains and Streptomycin (SN) against gram positive strains. The plated cells were incubated at 37°C 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 mean \pm SD. The data were analysed by using Excel and Easy plot software

RESULTS AND DISCUSSIONS**Inhibition of growth of microorganisms**

The results revealed that at highest concentration of ASEA (80 μ g/ml) the inhibitory effect on gram negative bacteria *Escherichia coli*, *Salmonella typhi*, *Proteus mirabilis* and *Klebseilla pnemonia* were 61.5 \pm 0.9, 61 \pm 1, 56.25 \pm 1.4 and 52.5 \pm 0.6 respectively (figure:1 – Figure 4). The gram positive bacteria *Staphylococcus aureus* and *Bacillus subtilis* were 50 \pm 0.8 and 58.5 \pm 1.1 respectively at 48 hours. (Figure 5 to Figure 6). The (80 μ g/ml) inhibitory effect of penicillin on gram negative bacteria: *Escherichia coli*, *Salmonella typhi*, *Klebseilla pnemonia*, *Proteus mirabilis* were 97.2, 96.5, 91, 83.5. The inhibitory effect of streptomycin (80 μ g/ml) on gram positive bacteria *Staphylococcus aureus*, *Bacillus subtilis* were 94 \pm 1.15 and 96 \pm 0.8 respectively. Minimum inhibitory concentration of ASEA on gram negative bacteria *Escherichia coli*, *Salmonella typhi*, *Proteus mirabilis*, *Klebseilla pnemonia*, and gram positive bacteria *Staphylococcus aureus*, *Bacillus subtilis* were found to be 19.5, μ g/ml, 20.6 μ g/ml, 35 μ g/ml, and 67.8 μ g/ml, 75 μ g/ml and 27.6 μ g/ml, respectively (Figure 7 - Figure 12). In our study the results of Broth dilution assay indicated that the ASEA was effective in inhibiting gram negative *Escherichia coli*,

Salmonella typhi, *Klebseilla pnemonia*, *Proteus mirabilis* and gram positive *Staphylococcus aureus*, *Bacillus subtilis* bacteria. It was also noticed that the inhibitory effect was also found to be increased in concentration dependent. Earlier studies by Jayshree *et al* also showed antibacterial activity to the leaves of *Annona squamosa*⁹. 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 extracts¹⁰. There are several investigators have proved the antimicrobial potentiality of some Annonaceae members. *A. squamosa* contains flavanoids which express strong antibacterial activity^{11,12}. 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 method¹³. This study of *Annona squamosa* ethyl acetate extract 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.

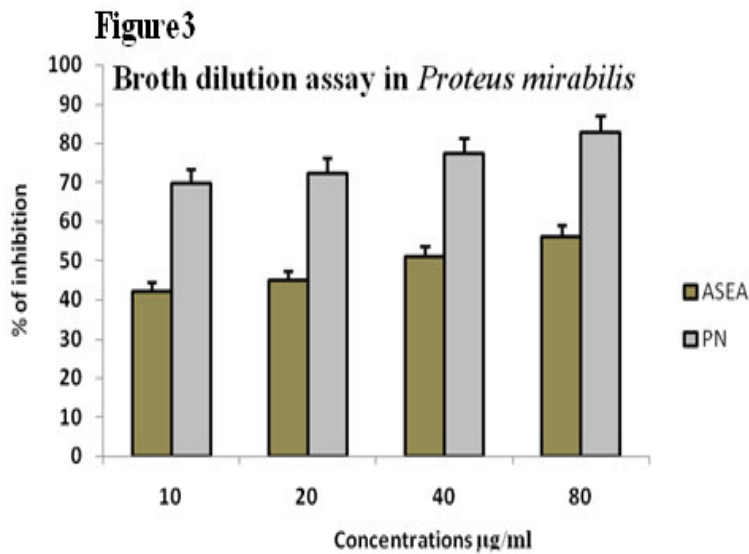
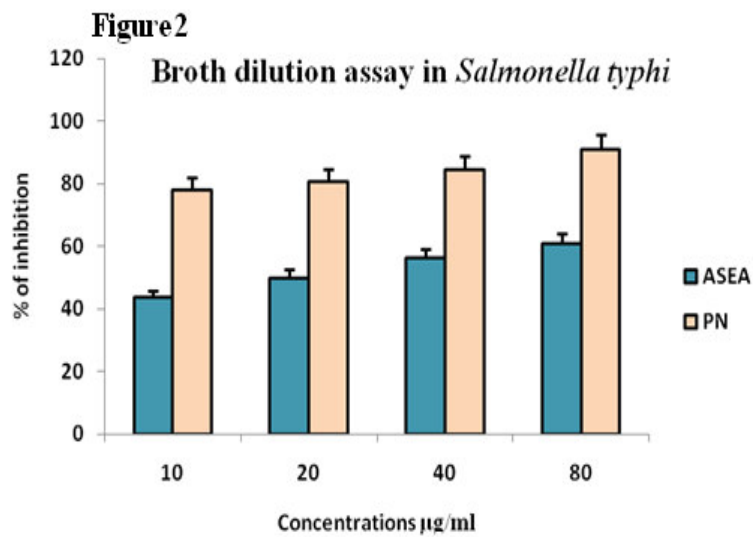
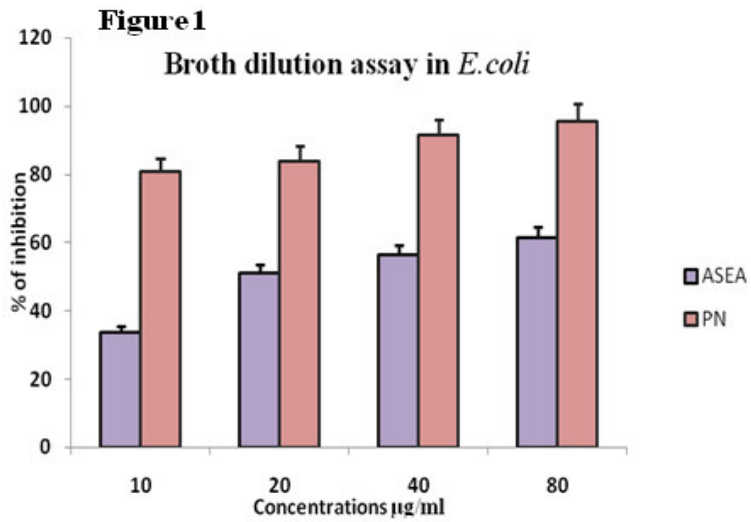


Figure 4

Broth dilution assay in *Klebsellia pneumonia*

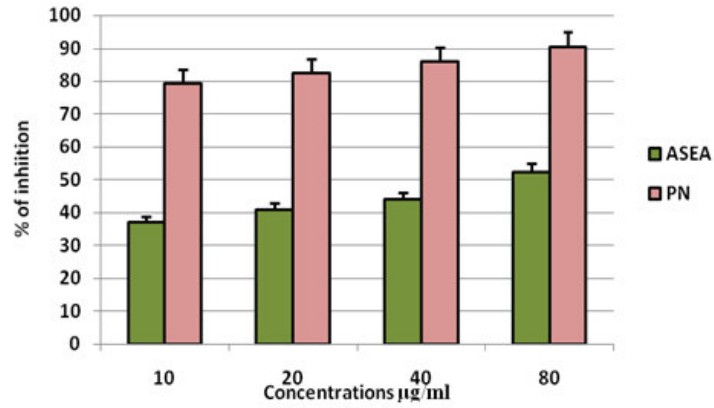


Figure 5

Broth dilution assay on *Staphylococcus aureus*

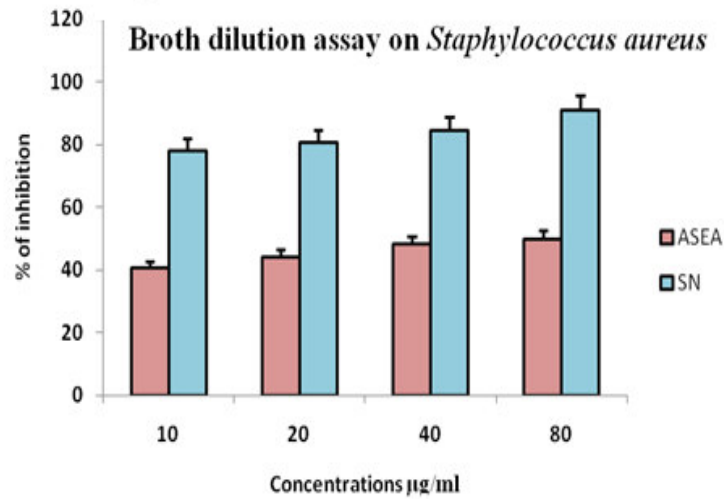
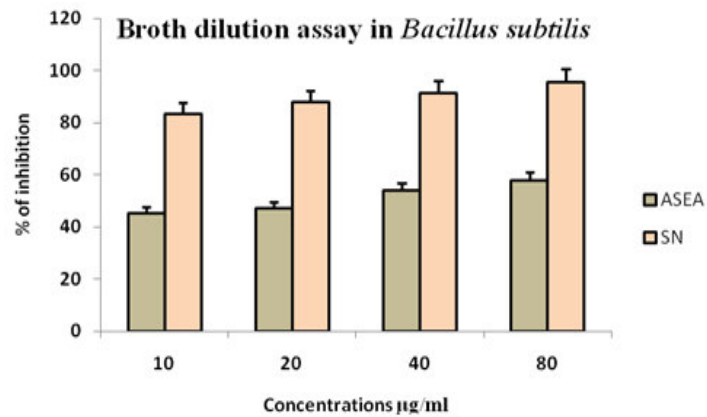
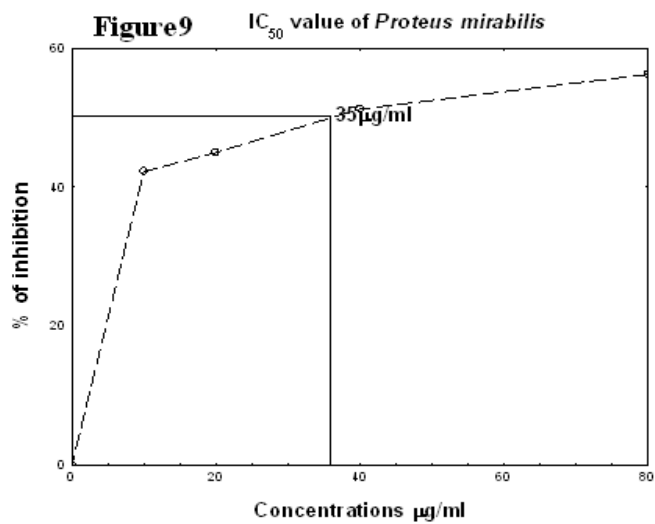
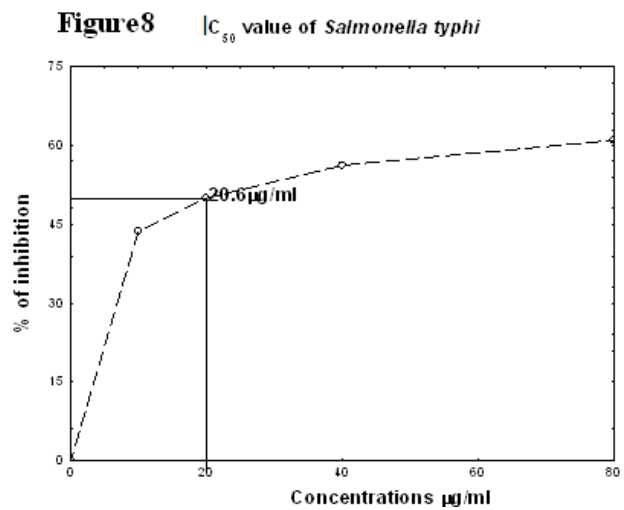
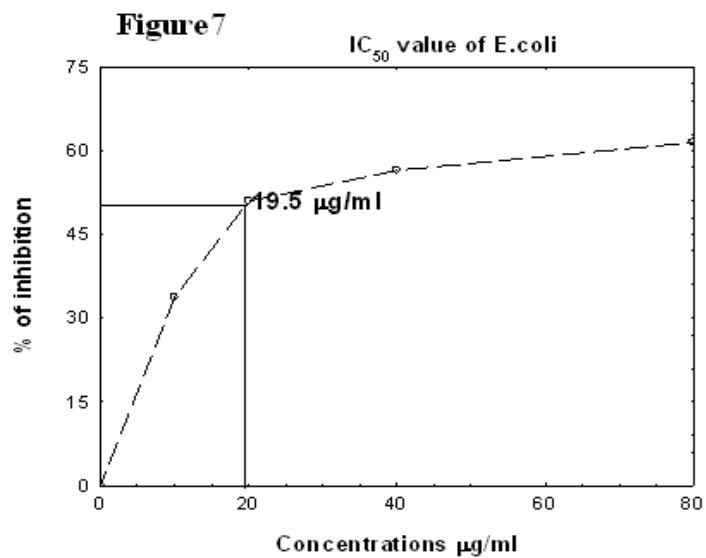
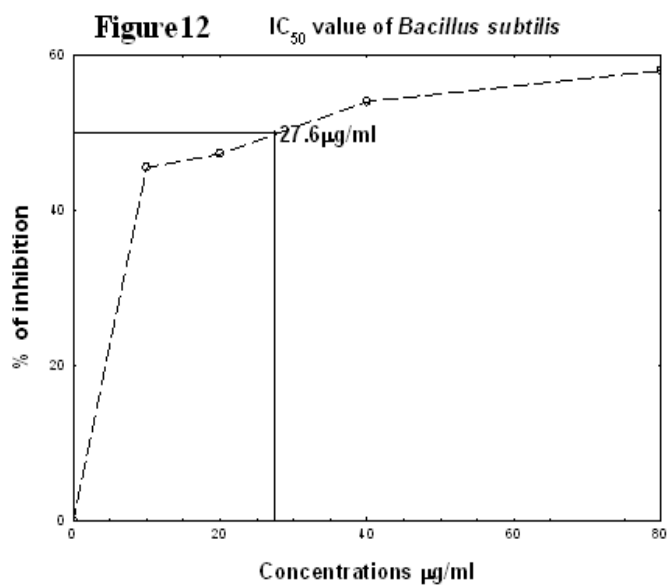
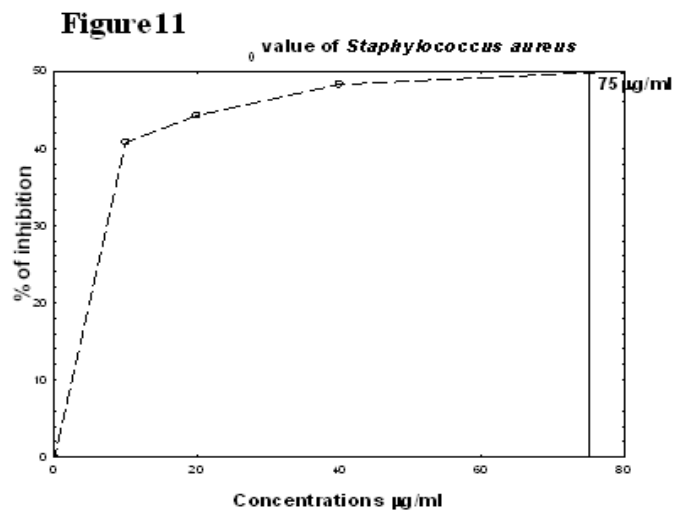
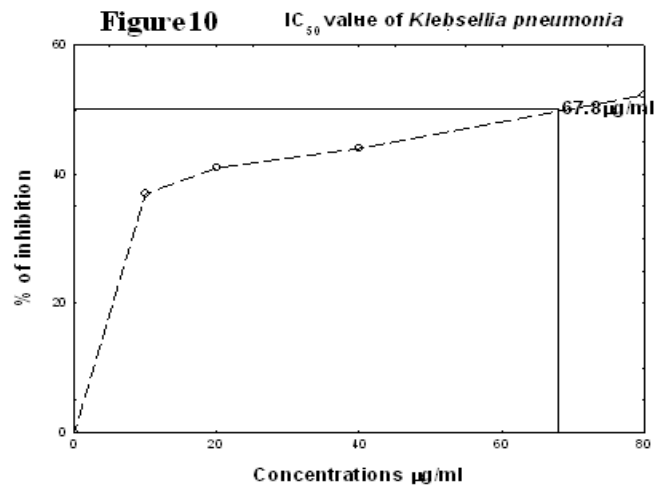


Figure 6

Broth dilution assay in *Bacillus subtilis*







CONCLUSION

This study can be concluded that *Annona squamosa* ethyl acetate extract have great potential as antimicrobial compounds against microorganisms and that they can be used in the treatment of infectious diseases caused by microorganisms. The core technologies used to discover microbial natural products have not evolved significantly over the past several decades, resulting in a shortage of new drug leads. This study suggests that the ethyl acetate extract isolated from the seeds of *Annona squamosa* is a promising candidate to be exploited further to develop as antimicrobial agents.

REFERENCES

1. Rawat RBS, Uniyal RC. National Medicinal plants Board committed for overall development of the sector. *Agro Bios Med Plants*. 1:12–16, 2003.
2. Lewis K, Ausubel FM., Prospects for plant-derived antibacterial. *Nature Biotechnology*. 24: 1504 – 1507, 2006.
3. Ates DA, Erdogrul OT, Antimicrobial activities of various medicinal and commercial plant extracts. *Turk J Biol*, 27: 157-162, 2003.
4. Erdogrul OT, Antibacterial activities of some plant extracts used in folk medicine *Pharmaceutical Biol* 40: 269 -273, 2002.
5. Reddy PS, Jamil K, Madhusudhan P., Antibacterial activity of isolates from *Piper longum* and *Taxus baccata*. *Pharmaceutical Biol*. 236- 238.resolution. *J. org. Chem*. 43: 2923-2925, 2001.
6. Kusumoto IT, Nakabayashi T, Kida H., Screening of various plant extracts used in ayurvedic medicine for inhibitory effects on human immunodeficiency virus type 1 (HIV-1) protease. *Phytotherapy Res*. 9:180-184, 1995.
7. Hart CA, Karriuri S Anti-microbial resistant in developing country. *British Medical Journal*. 317: 647–650, 1998.
8. Abebe D, Debella A, Urga K, Medicinal Plants and Other Useful Plants of Ethiopia, Camerapix Publishers International. 54–61, 2003.
9. Jayshree D. Patel and Vipin Kumar: *Annona squamosa* L: Phytochemical analysis and Antimicrobial Screening *Journal of pharmacy research*. 01/2008.
10. Rojas R, Bustamante B, Bauer J, Ferrandez I , Alban J and Lock O: Antimicrobial activity of selected Peruvian medicinal plants. *J. Ethnopharmacol*. 88: 199-204, 2003.
11. Chavan C.B, Shinde Hogade M, and S. Bhinge: Screening of *In-vitro* antibacterial assay of *Barleria prionitis* Linn. *J. Herb. Med. Toxicol*. 4: 197-200, 2010.
12. Padhi L.P, Panda S.K, Satapathy SN, Dutta S.K: Invitro evaluation of antibacterial potential of *Annona squamosa* .L and *Annona recticulatae* L. From Simpilipal Biosphere Reserve, Orissa, India. *Journal of Agricultural Technology*, 7:133-142, 2011.
13. Kachhawa JBS, Sharma N, Tyagi S, Sharma KK. Screening of stem bark Methanol extract of *Annona squamosa* for antibacterial activity. *International Journal of Current Pharmaceutical Research*. 4: 48-50, 2012.

Statistical analysis

The results are presented as the average and standard deviation of four experiments. The data was analysed by using Excel and Graph pad.

ACKNOWLEDGEMENT

The authors gratefully acknowledge Indian Council of Medical Research, Govt. of India (Grant No. 59/47/2011/ BMS/TRM) for financial support.

Conflict of Interest

The authors declare no conflict of interest.

