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Research Article

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In vitro inhibitory effects of *Annona glabra* on selected human pathogens

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ABSTRACT

Indian herbal medicines have served as major source of medicines for prevention and treatment of many diseases including microbial infections. Emergence of multidrug resistance has limited the therapeutic options for antibiotics in the world and monitoring resistance by bacteria on these antibiotics has become an important necessity. The use of traditional herbal medicines in crude or refined form may help in the treatment of microbial infections with two advantages, i.e. the cure is achieved and the chances of microbes becoming resistant are minimized. The herbal medicines have the advantage of not producing major side effects as is found in case of usual antibiotics. Therefore this study was undertaken to focus on the in vitro antimicrobial effects of two plants. Plant materials of Annona glabra were tested for their antibacterial activities on selected strains of bacteria namely, Bacillus cereus, Shigella flexneri and Pseudomonas aeruginosa. These activities were compared with standard antibiotic namely Broad spectrum antibiotics, Penicillin and Ampicillin. Antimicrobial activity was measured using the standard method of diffusion disc plates on agar and the MIC was calculated using dilution method. Our results clearly indicate that Annona glabra has antimicrobial properties.

Key words: Pathogens, *Pseudomonas aeruginosa*, *Shigella flexneri*, *Annona glabra*, Antibiotics.

INTRODUCTION

During the last decades infectious diseases have threatened the lives of millions of people around the world. Even though pharmaceutical industries have produced a number of new antimicrobial drugs in the past, resistance to these drugs by microorganisms has increased. In general, bacteria have the genetic ability to transform and acquire resistance to drugs used as therapeutic agents. The initial introduction of new medicinal agents into the health care system some times, requires information beyond that is recorded in libraries relying instead on reports available through traditions and healers within a society. Thus traditional medicinal practices, conserved over years by civilizations, can serve as an effective basis for the discovery and development of modern therapeutic drugs. [1, 2]
The development of novel, efficient and inexpensive drugs is of great importance. In a constant attempt to improve the quality of life, men have used plants as a source of food, shelter, clothing, cosmetics and for seeking relief from hardships of life. Some plants are known as medicinals, because they contain active principles. Knowledge and use of medicinal plants in various parts of the world contributed significantly to primary health care. For centuries, medicinal plants have been used all over the world for the treatment and prevention of various ailments, particularly in developing countries, where infectious diseases are endemic and modern health services and facilities are inadequate. Many potent drugs have been purified from medicinal plants which range from antibacterial, anti-malarial, anti cancer and anti diabetic. [3, 4, 5, 6]

The present study focuses on the antibacterial properties of *Annona glabra. Annona glabra* is a tropical fruit tree in the family Annonaceae. The trees grow to a height of around 10–12 m. They have thin, gray trunks and sometimes grow in clumps. The leaves are ovate to oblong with an acute tip, 8–15 cm long and 4–6 cm broad with a prominent midrib. The upper surface is light to dark green. The fruit is oblong to spherical and apple-sized or larger, 7–15 cm long and up to 9 cm diameter, and falls when it is green or ripening yellow. It disperses by floating to new locations, and it is food for many animal species such as wild boar. Reproduction begins after 2 years. A fruit contains 100 or more pumpkin-like seeds, about 1 cm. long. Although much pharmacological work on *Annona squamosa* is well documented there are scanty reports on

Annona glabra [7].Tsai and Lee, 2010, have characterized the acetylecholinestarase inhibitory constituents from *Annona glabra.*[8] Abdel-lateff *et al*, 2009, have reported the cytotoxicity of seed extracts of *Annona glabra* on brine shrimp and few cancer cell lines [9]. In the present study antibacterial activity of ethanolic leaf extracts of *Annona glabra* was undertaken.

EXPERIMENTAL SECTION

Plant Materials

Plant materials were collected from authorized Ayurvedic store. Both the plants were identified and authenticated by reputed botanist.

Extraction from plants

The plant materials were dried in shade and powdered in a mechanical grinder. The powder of the plant materials were initially de-fatted with petroleum Benzene (60^0C-80°C), followed by extraction with 1000 ml of ethanol by using a Soxhlet extractor for 72 hrs at a temperature not exceeding the boiling point of the solvent. The extract was filtered using Whattman filter paper (No.1) and then was concentrated and dried at 45°C for ethanol elimination, and the extracts were kept in sterile bottles, under refrigerated conditions until further use. The dry weight of the plant extracts was obtained by solvent evaporation and used to determine concentration in mg/ml. The extract thus obtained was directly used in the assay of antimicrobial activity.

Antibiotics

Broad spectrum antibiotics, Penicillin and Ampicillin were used as control drugs.

Bacterial Strains

The strains of microorganisms (*Bacillus cereus, Shigella flexneri and Pseudomonas aeruginosa* were used.

Experimental Section

Determination of Antimicrobial Activity

Antimicrobial activity was measured using the standard method of diffusion disc plates on agar and the MIC was calculated using dilution method (Kirby- Bauer method).

Dilution Methods

Dilution susceptibility testing methods were used to determine the minimal concentration of antimicrobial to inhibit or kill the microorganisms. This was achieved by dilution of antimicrobial in either agar or broth media. Antimicrobials are generally tested in log2 serial dilutions (two fold).

Broth Dilution Method

The Broth Dilution Method is a simple procedure for testing a small number of isolates, even single isolates.

Preparation of microorganisms for experiment

The pure cultures of organisms (*Bacillus cereus, Pseudomonas aeruginosa and Shigella flexneri)* were sub-cultured in nutrient broth. They were inoculated, separately, into nutrient broth and kept at 37°C for 24 hours. Then, they were kept at 4°C until use.

Growth Method

At least three to five well-isolated colonies, of the same morphological type, were selected from an agar plate culture of a particular microorganism. The top of each colony was touched with a loop, and the growth was

transferred into a tube, containing 4 to 5 ml of Nutrient broth medium. The broth culture was incubated at 35°C for 8 hours. After the incubation period broth culture became turbid.

Disc Diffusion Method:

(a) **Mueller-Hinton Agar Medium**

Mueller-Hinton Agar is considered to be the best for routine susceptibility testing of non- fastidious bacteria because it shows acceptable batch-to-batch reproducibility for susceptibility testing. Medium is transparent, so that the inhibition zone can be visualized clearly. It gives satisfactory growth of most non fastidious pathogens. A large body of data and experience has been collected concerning susceptibility tests performed with this medium.

Preparation of Mueller-Hinton Agar

Mueller-Hinton Agar was prepared from a commercially available dehydrated base according to the manufacturer's instructions. Immediately after autoclaving, it was allowed to cool in a 45 to 50°C water bath. The freshly prepared and cooled medium was poured into glass or plastic, flat-bottomed Petri dishes on a level, horizontal surface to give a uniform depth of approximately 4 mm. This corresponds to 60 to 70 ml of medium for plates with diameters of 150 mm and 25 to 30 ml for plates with a diameter of 100 mm. The agar medium was allowed to cool to room temperature and unless the plate is used the same day, stored in a refrigerator. Plates were used within seven days after preparation unless adequate precautions, such as wrapping in plastic, have been taken to minimize drying of the agar. A representative sample of each batch of plates was examined for sterility by incubating at 30 to 35°C for 24 hrs or longer.

Preparation of antibiotic stock solutions

Powders of the two antibiotics (Penicillin and Ampicillin) were brought from authorized medical shop. They were accurately weighted and dissolved in sterile distilled water in appropriate dilutions to yield the required concentrations. The stocks were kept in aliquots of 5 ml volumes and frozen at -20°C

Preparation of plant extracts solutions

The dried plant extracts were weighed and dissolved in sterile distilled water to prepare appropriate dilution to get required concentrations (1.0mg/ ml, 1.5mg/ ml, 2.0 mg/ ml, and 2.5 mg/ ml). They are kept under refrigeration.

Preparation of dried filter paper discs

Whattman filter paper (No.1) was used to prepare discs approximately 6 mm in diameter, which are placed in a Petri dish and sterilized in a hot air oven. After the sterilization, the discs were poured into the different concentration of broad spectrum antibiotics and into the prepared plant extract solutions and again kept under refrigeration for 24 hrs.

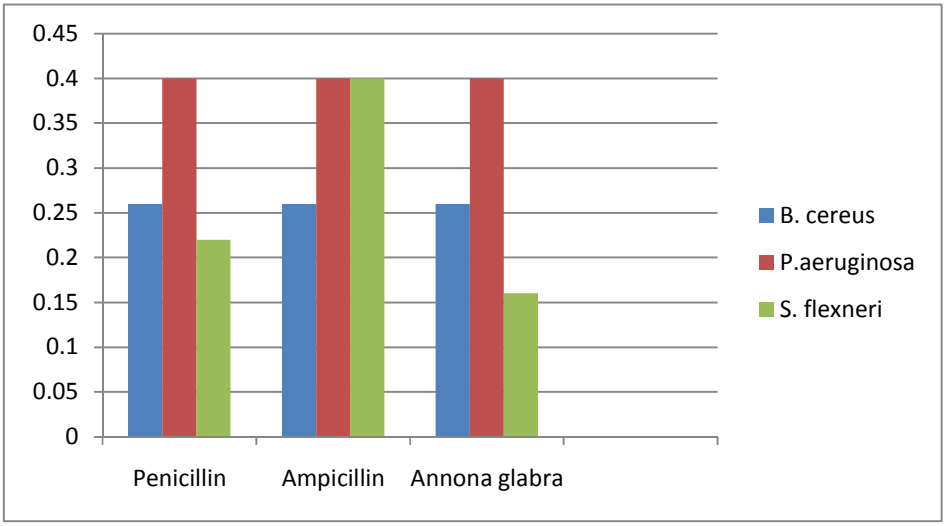
RESULTS AND DISCUSSION

Reading of Minimum Inhibition Concentration

Minimum inhibition concentration was expressed as the lowest dilution which inhibited growth judged by lack of turbidity in the tube, because very faint turbidity might be given by the inoculums itself. The inoculate tube was kept in the refrigerator overnight and was used as the standard for the determination of complete inhibition. Figure 1 indicates The Minimum Inhibitory Concentration (MIC) of Penicillin, Ampicillin and *Annona glabra* against *Bacillus cereus, Pseudomonas aeruginosa and Shegella flexneri*.

The plant extracts were found to be effective against the three selected bacterial species.

Figure 1. The Minimum Inhibitory Concentration (MIC) of Penicillin, Ampicillin and *Annona glabra* against *Bacillus cereus, Pseudomonas aeruginosa and Shegella flexneri*

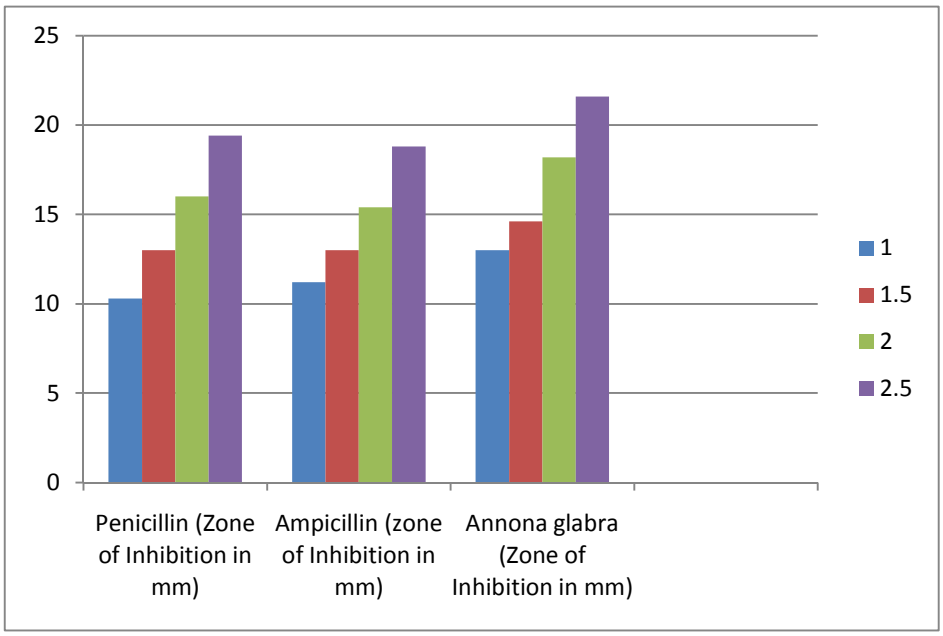


Reading Zone of Inhibition and Interpreting Results

After 16 to 18 hrs of incubation each plate was examined. Once the resulting zones of inhibition came uniformly circular and in a confluent lawn of growth, the diameters of the zone of complete inhibition (as judged by the unaided eye) are measured, including the diameter of the disc. Zones are measured to the nearest mm using a ruler, which was held on the back of the inverted Petri plate.

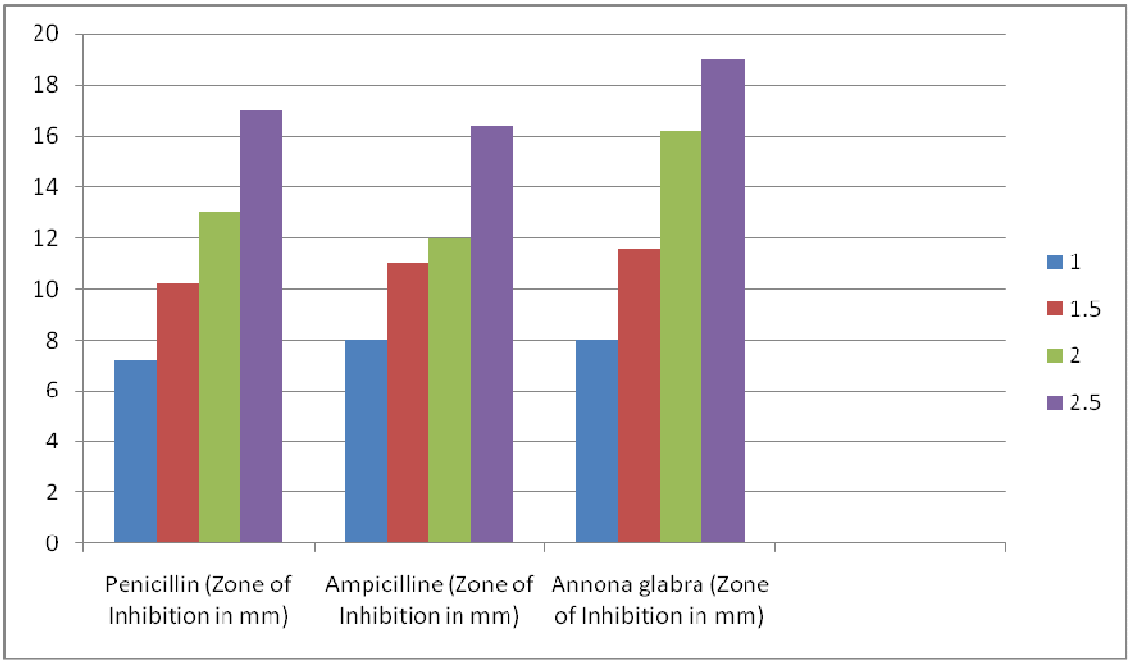
Clear inhibition zones indicated the presence of antimicrobial activity.

Figure 2. Indicates the zones of inhibition by *Bacillus cereus* against Penicillin, Ampicillin and *Annona glabra* extract at various concentrations



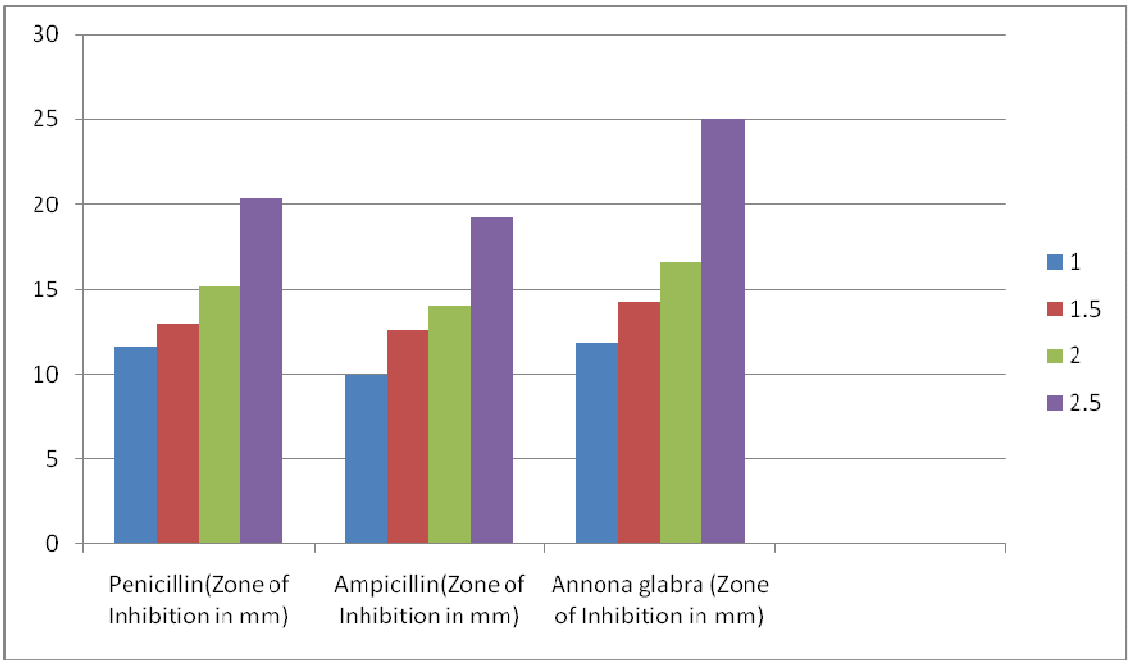
Group II, III and IV are compared with Group I, Group I, III and IV are compared with Group II.

Figure 3. Indicates the zones of inhibition by *Pseudomonas aeruginosa* against Penicillin, Ampicillin and *Annona glabra* extract at various concentrations



Group II, III and IV are compared with Group I, Group I, III and IV are compared with Group II.

Figure 4. Indicates the zones of inhibition by *Shigella flexneri* against Penicillin, Ampicillin and *Annona glabra* extract at various concentrations



Group II, III and IV are compared with Group I, Group I, III and IV are compared with Group II.

The use of medicinal plants in the world and especially in India, contribute significantly to primary health care. The antimicrobial medicinal plants are well documented by Shelef [10]. The results of different studies provide evidence

that some medicinal plants might indeed be potential sources of new antibacterial agent even against some antibiotic resistant strains was reported by Agbafore and Nwachukwu [11].

In the present study the results show that the extract from *Annona glabra* possess antimicrobial activities against *Bacillus cereus, Shigella flexneri* and *Pseudomonas aeruginosa.* The extract compared favorably with the standard antibiotics Penicillin and Ampicillin. The plant extract showed more activity than that observed by broad spectrum antibiotic activities. The MIC of *Annona glabra* was shown in Figure 2, Figure 3 and Figure 4. The standard Penicillin and Ampicillin had MIC values varying between 0.244 mg/ml and 0.488 mg/ml. The results indicated that the extract of *A. glabra* has stronger activity than that of standard antibiotics. Since ancient times, herbs and /or their essential oils have been known for their varying degrees of antimicrobial activities. [12, 13, 14, 15, 16, 17, 18] On the basis of the results obtained in the present study we conclude that the extract has significant antimicrobial activity. Further studies are on to isolate the active principles responsible for their antimicrobial activities.

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