EXCELLENT PUBLISHERS

International Journal of Current Research in Biosciences and Plant Biology

ISSN: 2349-8080 Volume 2 Number 4 (April-2015) pp. 54-58

www.ijcrbp.com

Original Research Article

Evaluation of Antibacterial Activity of *Annona squamosa*, *Psidium guajava* and *Azadirachta indica* against Pathogenic Bacterial Cultures

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Abstract

Medicinal plants are part and parcel of human society to combat diseases, from the dawn of civilization. Plants are being considered as important source of medicine. There has growing demand for plant based medicines, health products, pharmaceuticals, food supplements, cosmetics etc. Drug resistance is a serious global problem, and spread of resistance poses additional challenges for clinicians and the pharmaceutical industry. Keeping these facts in view this study was carried out to evaluate antimicrobial Activity of *Annona squamosa* (Custard apple), *Psidium guajava* (Guava), *Azadirachta indica* (Neem) against pathogenic cultures of Gram positive and Gram negative bacterial strains. Plant samples were collected from road side plants at Gwalior (M.P) and evaluated for their antimicrobial activity through disc diffusion assay technique. Result was interpreted by measuring zone of inhibition. All the plant samples were treated by standard protocol and extracts were kept under the rotary evaporator at 50°C. Result interpretation was done on the basis of zone of inhibition created due to the antibacterial properties of the plant extracts which was compared with positive and negative control respectively. This study was an effort to prove the use of herbal medicines in the developed world because they are rich source of novel drugs and their bioactive principles form the basis in medicine, nutraceuticals, pharmaceutical intermediates and lead compounds in synthetic drugs. Screening medicinal plants for biologically active compounds offers clues to develop newer antimicrobial agents. These compounds after possible chemical manipulation provide new and improved drugs to treat the infectious diseases. Plant based products/ extracts are cheaper alternatives to the development of synthetic drugs.

Keywords

Corcyra cephalonica

Egg mortality

Glinus lotoides

Lethal concentrations

Phytochemicals

Introduction

According to World Health Organization (WHO) medicinal plants would be the best source to obtain a variety of drugs. About 80% of individuals from developed countries use traditional medicine, which has compounds derived from medicinal plants. The use of crude extracts of plants parts and phytochemicals, of known antimicrobial properties, can be of great significance in the therapeutic treatments. Many plants have been used because of their antimicrobial traits, which are due to the secondary metabolites synthesized by the plants. These products are known by their active substances like, phenolic compounds which are part of the essential oils, as well as in tanning. Use of herbal medicines in the developed world continue to rise because they are rich source of novel drugs and their bioactive principles form the basis in medicine, nutraceuticals, pharmaceutical intermediates and lead compounds in synthetic drugs (De and Ifeoma, 2002; Ncube et al., 2008).

Screening medicinal plants for biologically active compounds offers clues to develop newer antimicrobial agents. These compounds after possible chemical manipulation provide new and improved drugs to treat the infectious diseases (Natarajan et al., 2003, Shah et al., 2006). Plant based products/ extracts are cheaper alternatives to the development of synthetic drugs. Plants which are used for the study of antimicrobial activity are Neem (*Azadirachta indica*), Guava (*Psidium guajava*) and Sharifa (*Annona squamosa* Linn.).

Neem (*Azadirachta indica* A. Juss.) is perhaps the most useful traditional plant in India. Each part of the neem tree has some traditional property and is thus commercially exploitable. A. indica (Neem - leaf, bark and seed) are known to contain antibacterial, antifungal activities against different pathogenic microorganisms and antiviral activity against vaccinia, chikungunya, measles and coxsackie B viruses (Biswas et al., 2002; Talwar et al., 1997). Aqueous extract of Neem leaf extract has a good therapeutic potential as anti hyperglycemic and anti inflammatory agent (Bajaj and Srinivasan, 1999; Abu et al., 2008). Neem leaves has antibacterial properties and could be used for controlling airborne bacterial contamination in the residential premise (Saseed and Aslam, 2008; El- Mahmood et al., 2010).

Psidium guajava (guava) is evergreen shrub native to tropical America that has neutralized in South East Asia. Leaves of guava are reported to have antibacterial activity. Other parts of the plant have been used in traditional medicine to manage conditions like malaria, gastroenteritis, vomiting, diarrhea, dysentery, wounds, ulcers, toothache, coughs, sore throat, inflamed gums, and a number of other conditions (Abdelrahim et al., 2002, Lutterodt et al., 1992). There are bioactive components in the guava leaf that can fight against pathogens, regulate blood glucose levels, and can even aid in weight loss. The leaves of guava contain an essential oil rich in cineol, tannins, triterpenes, flavonoids, resin, eugenol, malic acid, fat, cellulose, chlorophyll, mineral salts, and a number of other fixed substances (Burkill et al., 1997; Ncube et al., 2008). Guava leaves (*Psidium guajava* L.) containing the active chemical compound saponins, flavonoids, tannins, eugenol and triterpenoids. Polyphenolic compounds are antibacterial compounds that can inhibit the growth of bacteria.

Annona squamosa Linn. is (Custard apple) a small evergreen tree is cultivated throughout India for its fruits. It belongs to family Annonaceae. It is known as custard apple, sugar apple, sweet apers in English, sharifa in Hindi, sitaphal in Telgue. 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). The plant is said to show varied medicinal effects, including insecticidal, anti-ovulatory and abortifacient. *A. squamosa* (Custard apple) 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). In the present study, the extracts of *Azadirachta indica*, *Psidium guajava* and *Annona squamosa* were tested against selective microbes for antimicrobial activity.

Materials and methods

Collection and extraction of plant materials

The fully matured fresh leaves of the fruit *Annona squamosa* (Custard apple), *Psidium guajava* (Guava), *Azadirachta indica* (Neem), were collected from Road side of Gwalior city. The leaf and seeds were washed thoroughly with tap water followed with sterilized

distilled water and shade dried for few days and then powered with the help of blender. Powder was dissolved in the solvents used for extraction: methanol and chloroform separately and was stored at 4°C. These crushed materials were extracted sequentially in chloroform and methanol with the help of Soxhlete apparatus approx 8hrs per day for three days. Resulting extracts in different solvents were evaporated and concentrated to dryness using the rotary evaporator at 50°C.

Preparation of culture media

Hilton Mueller Agar media was prepared by dissolving it in distilled water. Autoclave media at 121°C at15 lbs for 15 min for sterilization purpose.

Antibacterial activity assay

The agar-diffusion method was used to assess the antimicrobial activities. Antibacterial activity of aqueous and solvent extracts was determined by agar well diffusion method according to National Committee for Clinical Laboratory Standards (NCCLS). Plates are poured with about 20 ml of culture media in aseptic condition. Plates are allowed to solidify. After it spreading of microorganism is done with the help of inoculum with a sterile swab moistened with bacterial suspension. Once the spreading is done the culture plate is cut into two halves and the wells of 8 mm are punched in each half with the help of inoculum. Wells are filled with extracted plant material and allowed to diffuse at room temperature for 2 h. The plates were then incubated. Wells containing the same volume of DMSO (10%), chloroform, methanol, ethanol served as negative controls while standard antibiotic discs of gentamycine, penicillin were used as positive control. After incubation, the diameters of the growth inhibition zones were measured in mm.

Results and discussion

Effect of different plant extracts were observed on various bacterial cultures. It was observed that guava extract has shown no effect on both the culture of bacteria but neem has shown maximum zone of inhibition which is the indicator of good anti bacterial property of neem extract. It was also remarkable that shareefa neem extract also shown good antibacterial activity against both cultures. To fine the observation all solvents, DMSO and antibiotic discs were used as control.

Fig. 1: Effect of *Psidium guajava* extract on *Staphylococcus* culture

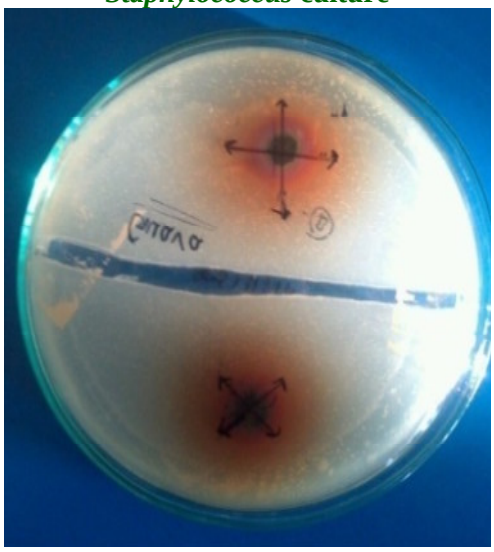


Fig. 2: Effect of *Azadirachta indica* and *Annona squamosa* leaf extract and DMSO (Control) on *Staphylococcus* culture

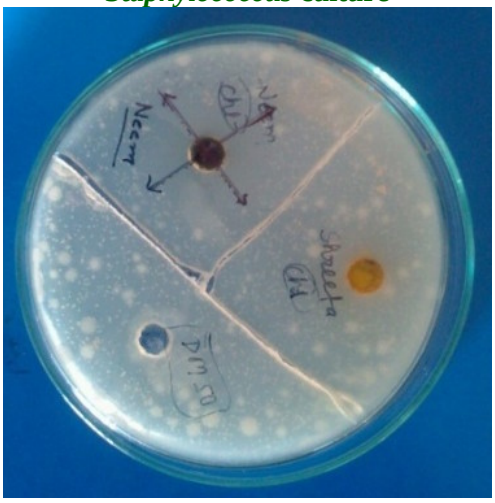


Fig. 3: Effect of antibiotics on bacterial culture.

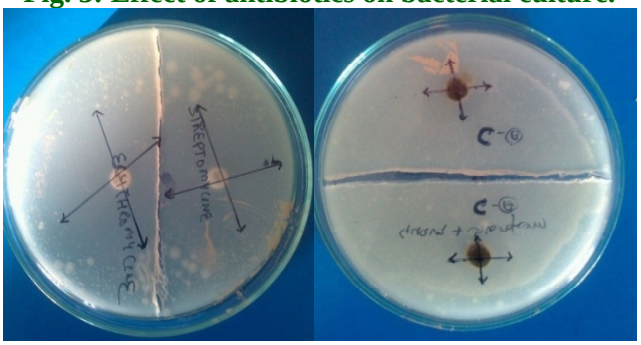
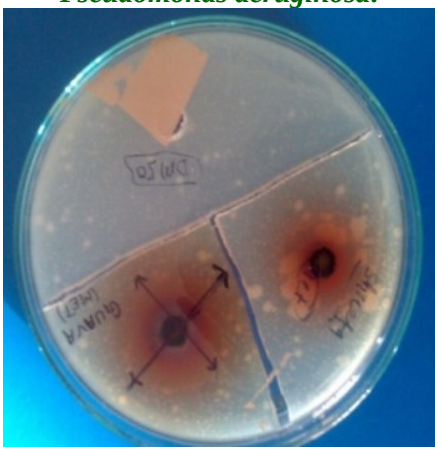


Fig. 4: Effect of *Annona squamosa* leaf on *Pseudomonas aeruginosa.*

Fig. 5: Effect of *Azadirachta indica* and *Psidium guajava* leaf extract and DMSO (Control) on *Pseudomonas aeruginosa*.



The experiments described above demonstrated that *Azadirachta indica* leaves extracts possess compounds with significant anti microbial activity. In addition to being a good anti microbial, further studies are needed to be done before reaching to any concrete conclusion. Leaves extract of *Psidium guajava* also shown an excellent effect against various bacterial cultures. Less effective extract evaluated was *Annona squamosa* during studies.

Neem leaves has antibacterial properties and could be used for controlling airborne bacterial contamination in the residential premise (Saseed et al., 2008, Mahmood et al., 2010). There are bioactive components in the guava leaf that can fight against pathogens, regulate blood glucose levels, and can even aid in weight loss. The leaves of guava contain an essential oil rich in cineol, tannins, triterpenes, flavonoids, resin, eugenol, malic acid, fat, cellulose, chlorophyll, mineral salts, and a number of other fixed substances (Burkill et al., 1997; Ncube et al., 2008). *A. squamosa* (Custard apple) 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). These clearly show that the presence of various bioactive components in the plants used in the study is responsible for the antimicrobial effect recorded in the present study.

Table 1. Antibacterial activity of various plant extracts.

Bacteria used	Extract (plant leaves + solvent)	Zone of Inhibition
Pseudomonas aeruginosa	*Annona squamosa* + Chloroform	28 mm
	Annona squamosa + Methanol	32 mm
	Psidium guajava + Chloroform	No Effect
	Psidium guajava + Methanol	No effect
	Azadirachta indica + Cholоroform	38 mm
	Azadirachta indica + Methanol	42 mm
Staphylococcus aureus	*Azadirachta indica* + Cholоroform	42 mm
	Azadirachta indica+ Methanol	39 mm
	Annona squamosa + Chloroform	38 mm
	Annona squamosa +Methanol	34 mm
	Psidium guajava+ Chloroform	16 mm
	Psidium guajava + Methanol	20 mm
Antibiotic	Streptomycine	38 mm
	Erythromycine	34 mm
	Zentamicin	42 mm
	Tetracycline	30 mm
	Penicillin	28 mm

Acknowledgement

We are sincerely grateful to the Dean, School of Life Sciences and HOD, Department of Life Sciences for providing us all necessary requirements for conducting this work.

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