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## Evaluation of antimicrobial activity of seed extracts of *Annona squamosa* L. and *Azadirachta indica*

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### Abstract

*Annona squamosa* L., and *Azadirachta indica* belongs to the family Annonaceae and Meliaceae commonly known as custard apple and neem respectively. A comparative antimicrobial activity of extracts of *A. squamosa* and *A. indica* were evaluated against five fungal culture arranged from the laboratory of CSIR-MTCC Chandigarh, India namely *Aspergillus niger*, *Rhizopus stolonifer*, *Curvularia lunata*, *Penicillium* sp. and *Cladosporium cladosporoides* using agar well diffusion method. Maximum inhibition was found with 40 mg/ml concentration of seed extracts against all the tested organisms under investigation. The minimum inhibitory concentrations were measured through disk diffusion method. The study suggests that the seed cotyledon of the said medicinal plants are promising in the development of phytomedicine for antifungal properties. This study also suggested that the seed of *Azadirachta indica* is more effective as a fungicide than *Annona squamosa* L.

**Keywords:** *Annona squamosa* L, *Azadirachta indica*, seed cotyledon, a comparative antimicrobial activity and antifungal properties

### Introduction

According to World Health Organization (WHO) various medicinal plants would be the great source to obtain a variety of drugs <sup>[1]</sup>. About more than 80% of individuals from developed countries use traditional medicine, which has compounds derived from various parts of medicinal plants <sup>[2]</sup>. The use of crude extracts of various parts of plants and their phytochemistry 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 <sup>[3]</sup>.

Neem (*Azadirachta indica*) 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 <sup>[4]</sup>.

*Annona squamosa* Linn. is (Custard apple) a small evergreen tree is cultivated throughout India for its fruits. 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. *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 <sup>[5]</sup>.

Keeping these facts in view this study was carried out to competitive evaluate antimicrobial activity of *Annona squamosa* (Custard apple) and *Azadirachta indica* (Neem) against pathogenic cultures of various fungi collected from the laboratory of CSIR-MTCC, Chandigarh, India. The present study is an attempt to find out the antimicrobial activities of above mentioned medicinal plants and also compared that activity with commonly available antibiotics.

### Materials and Methods

#### Extraction of plant material by soxhlet apparatus

Seeds of medicinal plants (Sitafal and Neem) were purchased from local market from Lucknow, India and evaluated for their antifungal activity through disc diffusion assay

technique. The 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. These crushed materials were extracted sequentially in ethyl acetate solvent with the help of Soxhlete apparatus approx 08 hrs per day for three days [6]. Resulting extracts were evaporated and concentrated to dryness using the rotary evaporator at 50 °C.

### Test microorganisms

Five fungal cultures *Aspergillus Niger*, *Rhizopus stolonifer*, *Curvularia lunata*, *Penicillium* sp. and *Cladosporium cladosporides* were used in the present study. All the tested strains/slant culture of fungi was obtained from the laboratory of CSIR-MTCC, Chandigarh, India. Pure fungal cultures were grown in potato dextrose broth at 28 °C [7] and maintained on potato dextrose agar slants at 4 °C.

### Agar-well diffusion method

The assay was conducted by agar well diffusion method. About 15 to 20 ml of potato dextrose agar medium was poured in the sterilized petri dishes and allowed to solidify. Fungal lawn was prepared using pure culture strain. 1 ml of fungal strain was spread over the medium using a sterilized glass spreader. Using flamed sterile borer, wells of 4 mm diameter were punctured in the culture medium [6, 7]. Required concentrations of serially diluted extracts (5, 10, 20 and 40mg/ml) were added to the wells. The plates thus prepared were left for diffusion of extracts into media for one hour in the refrigerator and then incubated at 37 °C. After incubation for 48h, the plates were observed for zones of inhibition. Disc diffusion method (MIC) was followed by taking antibiotic Streptomycin sulphat. The plates were examined and the minimum inhibitory concentrations were measured [8-10]. The diameter zone of inhibition was measured and expressed in millimeters. Di methyl sulfoxide (DMSO) with streptomycin sulphate was used as a negative control. The experiments were conducted in triplicates.

### Result and Discussion

In the present investigation five fungal cultures were tested to

determine the antifungal activity of ethyl acetate seed extract of *Annona squamosa* L and *Azadirachta indica*. The values given in tables -1 are the mean of the three observations. The ethyl acetate seed extract of *Annona squamosa* L showed maximum of 14.00 mm inhibition for *Curvularia lunata* with 40mg/ml followed by *Cladosporium cladosporides* (13 mm), *Aspergillus Niger* (13 mm), *Penicillium* sp (12 mm) and *Rhizopus stolonifer* (11 mm). The ethyl acetate seed extract of *Azadirachta indica* revealed maximum inhibition at 40mg/ml for *Curvularia lunata* (16 mm) followed by *Rhizopus stolonifer* (14 mm), *Cladosporium cladosporides* (13mm), *Aspergillus niger* (11mm) and *Penicillium* sp (11 mm). The standard streptomycin at 100 µg /ml showed highest inhibition in and *Penicillium* sp (23 mm), followed by *Rhizopus stolonifer* (21 mm) and *Cladosporium cladosporides* (21 mm), *Curvularia lunata* (20 mm) and *A. niger* (19 mm). The negative control used DMSO with streptomycin could not show inhibition against all the tested fungal strains. Our study shows that ethyl acetate seed extracts of *Annona squamosa* L and *Azadirachta indica* maximum inhibited the growth of *Curvularia lunata*. Finding report also supported by other scientists [11]. From agar well diffusion and disk diffusion method obtained that there were marked differences between the activities of the plant extract and the pure antifungal drugs (Streptomycin). Such significant differences normally present when crude (UN purified) plant extracts are compared with pure drug that are already in clinical use [7]. In our investigation ethyl acetate seed extract at 40mg/ml concentration highly inhibited *Curvularia lunata* is near to both seed extract results. Result interpretation was done on the basis of zone of inhibition created due to the antifungal properties of the plant extracts. 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. Plant based products/ extracts are cheaper alternatives to the development of synthetic drugs.

**Table 1:** Antifungal activity of ethyl acetate crude extracts seeds of *Annona squamosa* L and *Azadirachta indica*

Samples	Conc. of extracts (mg/ml)	Diameter of inhibition zone in mm				
		<i>Aspergillus niger</i>	<i>Rhizopus stolonifer</i>	<i>Curvularia lunata</i>	<i>Penicillium sp</i>	<i>Cladosporium cladosporides</i>
<i>Annona squamosa</i> L seed extract	5	--	--	6	--	--
	10	6	--	9	7	--
	20	9	7	11	10	9
	40	13	11	14	12	13
<i>Azadirachta indica</i> seed extract	5	--	8	8	--	--
	10	5	10	11	--	7
	20	7	11	13	9	10
	40	11	14	16	11	13
Control(DMSO)		--	---	--	--	--
Streptomycene	100 µg./ml	19	21	20	23	21

No inhibition zone

### Conclusion

Effects of two plant extracts (Sitafal and Neem) were observed on various fungal cultures. It was observed that both the extracts are effective for the culture of fungus but neem extract has shown maximum zone of inhibition which is the indicator of good anti-fungal property. It was also remarkable that sitafal extract also shown good antifungal activity against various cultures. To fine the observation solvent, DMSO and antibiotic discs were used as control. From this study it may

be concluded that the seed extract of *Azadirachta indica*, can be used as potent antifungal formulation in pharmaceutical and medicine industries in near future.

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