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Research Article

A COMPARATIVE ANTIMICROBIAL ACTIVITY OF METHANOLIC ROOT, LEAF, SEED COTYLEDON EXTRACTS OF *ANNONA SQUAMOSA* L.

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ABSTRACT

Annona squamosa L., belongs to the family Annonaceae, commonly known as custard apple. A comparative antimicrobial activity of methanolic root, leaf and seed cotyledons extracts of *A. squamosa* were evaluated against four fungi namely, *Trichophyton rubrum, Aspergillus niger*, *Aspergillus flavus.* (filamentous) and *Candida albicans* (dimorphic fungi) and three bacteria, *Bacillus subtilis* (Gram positive), *Escherichia coli* and *Serratia marcurs* (Gram negative) *using* agar well diffusion method. Maximum inhibition was found with 40mg/ml concentration of methanolic root and seed cotyledon extracts against all the tested organisms under investigation. The minimum inhibitory concentrations were determined by disk diffusion method. Preliminary phytochemical tests were carried out using crude extracts. The study suggests that the root and seed cotyledon of *A. squamosa* are promising in the development of phytomedicine for antimicrobial properties.

Keywords: *Annona squamosa* L., Seed cotyledon, A comparative antimicrobial activity.

INTRODUCTION

According to the WHO survey 80% populations living in the developing countries rely almost exclusively on traditional medicine for their primary health care needs. Exploration of the chemical constituents of the plants & pharmacological screening may provide us the basis for developing the leads for development of novel agents. In addition, herbs have provided us some important life saving drugs used in the armamentarium of modern medicine. However among the estimated 2,50,000-4,00,000 plant species, only 6% have been studied for biological activity and about 15% have been investigated phytochemically [1,2]. The Therapeutic efficacy of many indigenous plants, for various diseases has been described by traditional herbal medicinal practitioners[3,4]. There are several reasons that people use plants for medication. This includes improvement of health after herbal treatment, low cost of the drugs, non availability of synthetic drugs particularly in the rural areas, where available were either fake or expired drugs and in some cases the people are more accustomed to and comfortable with traditional healing [5]. Annonaceae is one of the biggest families, which comprising about 130 genera over 2000 species are *Annona*, with 150 species, genera, the species of *Annona squamosa* is a small evergreen tree reaching 6-8 meters (20-26 ft) tall, is commonly found in deciduous forests, cultivated through out India and other countries. It is commonly called as custrard apple, it is native of West Indies. The plant is tradionally used for the treatment of epilepsy, dysentery, cardiac problem, worm infection, constipation, hemorrhage, antibacterial infection, dysuria, fever, and ulcer. It also has antifertility, antitumor and abortifacient properties[6-9]. Several activities has been studied on the plant of *Annona squamosa* like antimutagenic[10], Anthelmintic[11], Scavenging[12], Antidiabetic[13], licicidal[14], Hepatoprotective[15], Antithyroid[16], Antigenotoxic[17],Antiplasmodial[18], Molluscicidal[19], Analgisic activity[20]and Antimicrobial activity[21]. However seed cotyledon antimicrobial activity and a comparative antimicrobial study of root, leaf and seed cotyledon still not reported. With the importance of above literature survey, in our present investigation we here in report the comparative antimicrobial activity of methanolic root, leaf, seed cotyledon of *A squamosa* L.

MATERIALS AND METHODS

Collection of plant material

The root, leaves, seed cotyledon of *A. squamosa* were collected in fresh bags from around Gulbarga University, Gulbarga, Karnataka, India and brought to laboratory. The collected plant materials were initially rinsed with distilled water to remove soil and other contaminants and dried on paper towel in laboratory at 37 ± 2ºC for week.

Extraction of plant material by soxhlet apparatus

The root, leaf and seed cotyledon materials after drying were ground in a grinding machine in the laboratory then 25g of shade dried powder was weighed and extracted successively with methanol in soxhlet extractor for 48h. The methanol extracts were concentrated under reduced pressure and preserved in refrigerator in airtight bottle for further use.

Test microorganisms

Four fungal cultures *Trichopyton rubrum, Aspergillus niger, Aspergillus flavus*, *Candida albicans* three bacterial cultures *Bacillus subtilis, Escherichia coli, Serratia marcurs* were used in the present study, all the tested strains were obtained from Department of Microbiology, Gulbarga University, Gulbarga, Karnataka, India. Bacterial cultures were grown in nutrient broth (Himedia, M002) at 37ºC and maintained on nutrient agar slants at 4ºC, fungal cultures were grown in potato dextrose broth at 28 ºC and maintained on potato dextrose agar slants at 4ºC.

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 5 days old culture strain. The fungal strains were suspended in a saline solution (0.85% NaCl) and adjusted to a turbidity of 0.5 Mac Farland standards (108 CFU/ml). 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. 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 37ºC. After incubation for 48h, the plates were observed for zones of inhibition. The diameter zone of inhibition was measured and expressed in millimeters. Dimethyl formamide (DMF) was used as a negative control. The experiments were conducted in triplicates. The same method was followed for testing antibacterial activity using nutrient agar medium incubated at 37ºC for 18h.

Disc diffusion method (MIC)

Disc diffusion method was followed by taking Ketoconazole as standard antibiotic for fungi and Streptomycin sulphate for bacteria. High potency bio-discs (Himedia) were prepared and placed on the lawn spreaded agar. After 2 days incubation at 26 ±2º C for *T. rubrum, C. albicans, A. niger, A. flavus* and 18h of incubation at a specific temperature 36 ± 2 º C for *B. subtilis, E. coli, S. marcurs,* the

plates were examined and the minimum inhibitory concentrations were measured.

RESULTS AND DISCUSSION

In the present investigation three bacterial species and four fungal cultures were tested to determine the antifungal and antibacterial activity of methanolic extract of *A. squamosa*. The values given in tables -1 and 2 are the mean of the three observations.

Methanolic root extract showed maximum of 16.00mm inhibition in *Candida albicans* at 40mg/ml followed by *Trichophyton rubrum* (12mm), *Aspergillus niger* (11mm) and *Aspergillus flavus* (11mm). The leaf extract revealed maximum inhibition at 40mg/ml in *Candida albicans* (11mm) and *A.flavus* (11mm), followed by *T.rubrum* (10mm), *A.niger* (8mm). Maximum inhibition seed extract was also observed at 40mg/ml concentration in *A.flavus* (11mm) and *T.rubrum* (11mm), followed by *A.niger* (10mm) and *C.albicans* (9mm). The standard ketoconazole at 100 µg /ml showed highest inhibition in *A.flavus* (23mm), followed by *C.albicans* (21mm) and followed by *T.rubrum* (19mm), *A.niger* (19mm). Among the plant materials used root extract showed maximum inhibition (Table-1).

Methanolic root extract showed maximum of 17.00 mm inhibition in *Bacillus subtilis* at 40mg/ml followed by *Escherichia coli* (10mm), and *Serratia marcurs* (8mm). The leaf extract revealed maximum inhibition at 40mg/ml in *E. coli*(7mm), followed by *B. subtilis* (6mm) and *S. marcurs* (6mm). Maximum inhibition in seed extract was also observed at 40mg/ml concentration against *E. coli* (12mm) followed by *S. marcurs* (8mm) and *B. subtilis* (7mm). The standard streptomycin sulphate at 100 µg/ml showed highest activity against *E. coli* (20mm), followed by *B. subtilis* (16mm), *S. marcurs* (16mm). Among the plant materials used root and seed extracts showed maximum antibacterial activity (Table. 2).

The negative control used DMF could not show inhibition against all the tested fungal and bacterial strains. The MIC values (in mg/ml concentration per disk) of antifungal and antibacterial activity were summarized in figure 1 & 2.

Preliminary phytochemical screening results of root, leaf and seed cotyledon of *A. squamosa* is summarised in Table.3.

The root extract showing positive results of flavonoid and steroid tests whereas showing negative results of tannin, alkaloid and oil tests. All the test were present in the leaf extract accept oil. The Seed cotyledon extract exposing positive results of tanins, steroids, and oil, negative in alkaloids and flavonoids.

Our study shows that methanol root extracts inhibited the growth of *C. albicans*, *A. niger*. Finding report also supported by other scientists those reported that the methanol leaf extract of *A. squamosa* to be active against *A. niger* and *C. albicans* [22]. While the root extract was active against all the tested fungi including bacteria *B. subtilis,* the seed cotyledon extract was highly inhibited *E coli, B subtilis,* moderately inhibited tested fungal *strains* is not reported.

From agar well diffusion, disk diffusion method obtained that there were marked differences between the activities of the plant extract and these of the pure antifungal drugs (Ketoconazole). Such significant differences normally present when crude (unpurified) plant extracts are compared with pure drug that are already in clinical use [23,] in our invistigation methanol leaf extract at 40mg/ml concentration highly inhibited *A. flavus,* is similar to root extract results. The similar types of result were observed [24]. The seed cotyledon extract at 40mg/ml concentration inhibited *T. rubrum* is near to root extract result. Methanol leaf extract maximum inhibited fungi are *Candida albicansis (is* near to root extract result), *A. flavus* and bacteria are *B. subtilis, E. coli. A. squmosa* leaves contain flavonoids which expose strong antibacterial activity [25]. Volatile compounds of this plant were also studied for its antimicrobial activity [26].

In our investigation alkaloids are present in leaf extract[27], Steroids present in all the tested extracts[4]. Tannins and steroids are absent in root, leaf extracts the similar results were reported[12,] volatile oil is present in seed cotyledon[28].

Table 1: Antifungal activity of methanolic crude extracts of *Annona squamosa* roots, leaves and seed cotyledon.

Samples	Conc. of extract (mg/ml)	Diameter of inhibition zone in mm			
		T.rubrum	*C.albicans*	*A.niger*	*A.flavus*
Root extract	5	-	8	-	-
	10	8	11	8	7
	20	9	12	8	9
	40	12	16	11	11
Leaf extract	5	-	-	-	-
	10	8	7	5	8
	20	8	10	7	9
	40	10	11	8	11
Seed extract	5	6	-	5	6
	10	8	-	7	8
	20	9	7	8	9
	40	11	9	10	11
Control (DMF)		-	-	-	-
Ketoconazole	100 µg,/ml	19	21	19	23

T. rubrum - Trichophyton rubrum, C. albicans - Candida albicans, A. niger - Aspergillus niger, A. flavus - Aspergillus flavus, -- No zone inhibition

Table 2: Antibacterial activity of methanolic crude extracts of *Annona squamosa* root, leaves and seed cotyledon.

Sample	Conc. of extract (mg/ml)	Diameter of inhibition zone in mm		
		B.subtilis	*E. coli*	*S.marcurs*
Root extract	5	8	-	-
	10	10	7	6
	20	11	8	7
	40	17	10	8
Leaf extract	5	-	-	-
	10	5	5	4
	20	5	7	5
	40	6	7	6
Seed extract	5	-	-	-
	10	5	7	5
	20	6	9	6
	40	7	12	8
Control (DMF)		-	-	-
Streptomycene	100 µg,/ml	16	20	16

B. subtilis-Basillus subtilis, *E. coli -Escherichia coli*, *S. marcurs - Serratia marcurs*, -- No zone inhibition

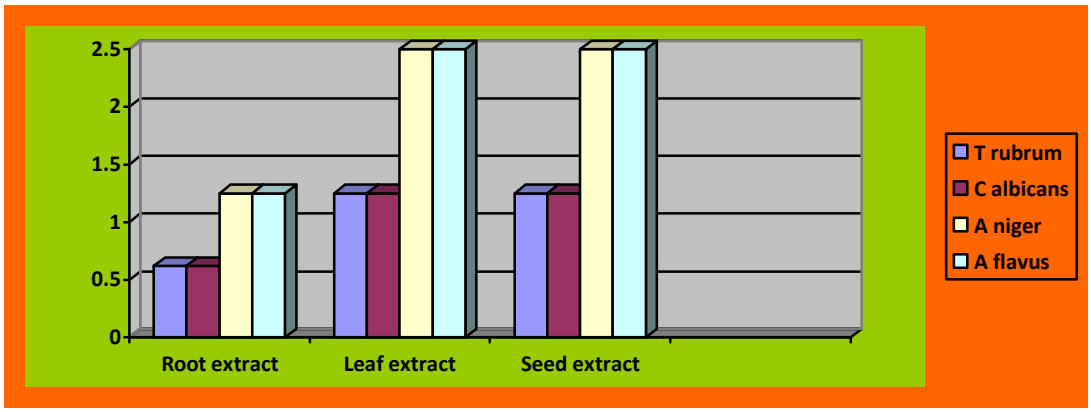

Fig. 1: MIC values (in mg/ml) of antifungal activity of methanolic extracts of *Annona squamosa* roots, leaves and seed Cotyledon.

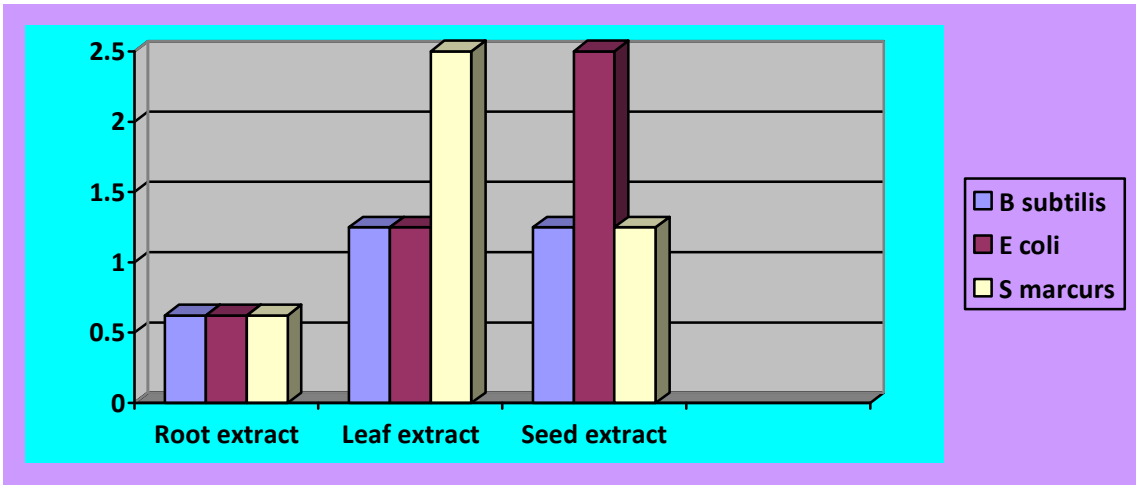

Fig. 2: MIC values (in mg/ml) of antibacterial activity of methanolic extracts of *Annona squamosa* roots, leaves and seed cotyledon.

Table 3: Preliminary phytochemical screening of extracts* of *Annona squamosa* L. root, leaf and seedcotyledon.

Test(s)	Methanolic crude extracts		
	Root	Leaf	Seed cotyledon
Tannin			
Ferric chloride	-	+	+
Alkaloid			
Dragendroff's	-	+	-
Mayer's	-	-	-
Flavonoid			
Shinoda	+	+	-
NaOH	-	+	-
Steroid			
Salkowski's	+	+	+
Liebermann Bur chard	+	+	+
Oils	-	-	+

*Prepared in methanol; " +" Present; "-"absent.

CONCLUSION

In the present study, based on previous reports we have found that among the three extracts of *A squamosa*, root and seed cotytledon extracts showed wide range of antifungal and antibacterial activity. Further investigations should be carried out in finding other activities of the extracts of root and seed cotyledon.

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