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RESEARCH ARTICLE

A COMPARATIVE STUDY OF ANTIFUNGAL ACTIVITY OF ETHENOLIC LEAF AND SEED EXTRACTS OF ANNONA SQUAMOSA L.

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Abstract

A comparative study of antifungal activity of ethanolic extracts of the leaves and seeds of Annonasquamosa L. (A plant of Annonaceae Family, commonly known as custard apple-sitafal) were evaluated against four fungi namely, Rhizopusnigricans, Aspergillusniger, Culvularialunata and Cladosporiumcladosporioides using agar well and disc diffusion method. Maximum inhibition was found with 50mg/ml concentration of ethanolic seed extracts against all the tested organisms under investigation. The study suggests that the seeds of A. squamosa are promising source for the development of suitable phytomedicine having fungicidal properties in place of commonly and widely used chemical antifungal agents.

Introduction:-

The populations of human being living in the developing countries mostly rely on traditional medicine for their primary health care needs. Similarly, plant materials are also used to prevent crop from harmful micro-organisms. Thus, the characterisation of the chemical composition and the study of the antimicrobial behaviour of the medicinal plants may provide us the basis for development of herbal drugs and phytomedicinal agents. 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 numerous reasons that people of world using plants for medication. This includes treatment and improvement of health after herbal treatment through medicinal plants, reasonable cost of the drugs, no any side effect, non availability of synthetic drugs particularly in the rural areas 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 150 genera over 1500 species are *Annona*, with about 100 species, genera, the species of *Annonasquamosa*is a small evergreen tree reaching 6-12 feet tall, is commonly found in deciduous forests, cultivated throughout India and other countries. It is commonly called as custrard apple or sarifa or sitafal, it is native of West Indies. Sitafal is tradionally used for the treatment of epilepsy, dysentery, cardiac problem, worm infection, constipation, haemorrhage, antibacterial infection, dysuria, fever, and ulcer. However, *A. squamosa* seed cotyledon have been found to have antimicrobial and anti-insecticidal properties but the comparative account of antimicrobial and anti insecticidal properties of root, leaf and seed cotyledon are not well reported. In view of the above, in present investigation, a comparative study of fungicidal properties of ethanolic extracts of leaves and seed cotyledons of *A. squamosaL*. is presented.

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Materials And Methods:-

Collection of plant material

The leaves of *A. Squamosa L.* were collected from park of Lucknow and seeds were purchased from the market of Lucknow, India and brought to laboratory. The collected leaves and seeds were thoroughly washed with tap water and then rinsed with sterile distilled water. The leaves of plant were shed dried for week whereas seed was dried in oven at 50^0C for 36 hours before grinding in electric mixer. The powder materials were kept in airtight glass bottles. This stock powder were used for further extraction [6[.

Extraction of plant material by Soxhlet apparatus

The solvents with varied polarity solubilise plant biomolecules differently due to difference in their polarities. Considering it's polarity,ethanol was preferred to be used as solvent for extraction. Activity of extracted material was determined using agar diffusion method [6,7].

5.00 gm of each dried and ground leaves and seeds were placed in different whattman cellulose extraction thimble (43mm x 123mm) with single thickness. Samples were extracted in a Soxhlet extraction system using 150 ml of solvent in each case. The heating power was set to two cycles/hour so that six cycles of extraction were achieved within 03 hours (Fig.3). Rotary vacuum evaporator used for evaporation of solvent from crude and completely dried in atmospheric oven at low temperature. High temperature was avoided to minimize the component degradation [6,8]. All extracts were then stored at room temperature. The stock solutions of the extracts were prepared in dimethyl sulfoxide (DMSO). The extracts were further diluted with DMSO to prepare a series of solutions containing 10, 20, 30, 40 and 50 mg/ml concentrations for the investigation of inhibition of growth of fungal species. The fungal species were taken in petriplates and extracts of different dilutions were applied on them. Control treatment was done without using any plant extract in petriplate. The range of activity of extract was determined by dilution method and activity was calculated by slandered formula that we have incorporated in material and method part. Percentage inhibition of fungi growth by the leaf extracts was calculated using formula [6,9].

$$FG= \{(Dc-Dr)/ Dc\}x100$$
$$FGI= 100-FG$$

Where-

FG= fungal growth in %
Dc= Diameter of control (mm)
Dr=Diameter of test (mm)
FGI= Fungal Growth Inhibition in %

Agar-well diffusion method

A loopfull of the inoculums suspension of two sets of pure 04 cultured identified fungal organism were spread uniformly on the solidified sterile culture media (PDA) in the Petri plates for uniform distribution of the organism. Using a sterile cork borer a well of 0.5 cm was made in the media and in each well, plant extracts were filled to allow the diffusion of plant extracts in the media. The Petri plates were incubated at for 24 hours at 30^oC temperature and the observations were recorded as diameter of inhibitory zone in mm. Well in agar plate filled with sterile distilled water was used as control in all the experiments [6,10]. All experiments were performed in triplicates and the mean inhibition percentages are presented in the observation table.

Disc diffusion method

Disc diffusion method was followed by taking sodium pentachlorophenate as standard antibiotic for fungi. High potency bio-discs (Himedia) were prepared and placed on the lawn spreaded agar. After 2 days incubation at 26^oC for all 04 identified fungi viz.Rhizopusnigricans, Aspergillusniger, Culvularialunata and Cladosporiumcladosporioides the plates were examined and the minimum inhibitory concentrations were measured.

Results And Discussion:-

In the present investigation, four fungal cultures were tested to determine the antifungal activity of ethanolic extract of *A. Squamosa leaves and seeds*. The FGI values given in tables -1 are the mean of the three observations. The ethenolic leaf extract revealed maximum inhibition at 50 mg/ml in *Culvularialunata* (11 mm) followed by *Rhizopusnigricans*, (9 mm), *Cladosporiumcladosporioides*(8 mm) *and A.niger*(7 mm). Maximum inhibition for seed

extracts was observed at 50mg/ml concentration in Culvularialunata (11 mm), followed by A.niger (9 mm) and Cladosporiumcladosporioides (9 mm) but no effect was observed on Rhizopusnigricans. The standard sodium pentachlorophenate at 100 µg /ml showed highest inhibition in Cladosporiumcladosporioides (17 mm), followed by Aspergillusniger(15 mm), Rhizopusnigricans (13 mm), and Culvularialunata (13 mm). Among the plant materials, the seed extracts showed better inhibition as compared to leaf extract (Table-1).

This study shows that ethanol seed extracts inhibited the growth of Aspergillusniger, Culvularialunata and Cladosporiumcladosporioideseffectively and no antifungal effect was observed on Rhizopusnigricans. Other such studies also support this finding [11]. The leaf extracts were active against all the tested fungi. There were marked differences between the antifungal activities of the plant leaf & seed extracts and those of the pure antifungal drugs (Sodium pentachlorophenate). Such significant differences normally present when crude (unpurified) plant extracts are compared with pure drug that are already in clinical use [12]. The plant extracts having potential antifungal activities preferably be used for prevention and conservation of cultural properties as chemical agents normally used for such work are not eco-friendly. In this investigation, the inhibition values are almost similar and highest at 50mg/ml concentration of both ethanol leaf and seed extracts for Culvularialunata and is comparable to the effect of sodium pentachlorophenate solution on the same species. These results are almost similar to the observations of other researchers [13].

Table 1:-Antifungal activity of ethanolic crude extracts of *Annonasquamosa*leaves and seed cotyledons.

Samples	Conc. of Extract (mg/ml)	Fungal Growth Inhibition (%)			
		Rhizopusnigricans	*Aspergillusniger*	*Culvularialunata*	*Cladosporiumcladosporioides*
Leaf Extract	10	0	0	0	3
	20	7	6	7	5
	30	9	5	8	7
	40	8	6	8	8
	50	9	7	11	8
Seed Extract	10	0	0	6	0
	20	0	6	9	5
	30	0	7	12	7
	40	0	9	12	9
	50	0	9	12	9
Control	0	0	0	0	0
Sodium pentachlorophenate	100 µg/ml	13	15	13	17

Conclusion:-

In the present study, the extracts of *AsquamosaL* leaves and seed cotyledons are found to have wide range of antifungal activity, which confirms such earlier reports. The extracts of *A. squamosa* seed cotyledons have relatively better antifungal property. The observations reported in this paper may be useful for developing suitable phytomedicinal agents for preventive conservation, especially for cultural properties, paper and allied materials etc.

a. b.

c d

Fig 3:-a-d Extraction of leaf and seed.

Fig 1:-Antifungal activity of ethenolic crude extracts of Annonasquamosa leaves.

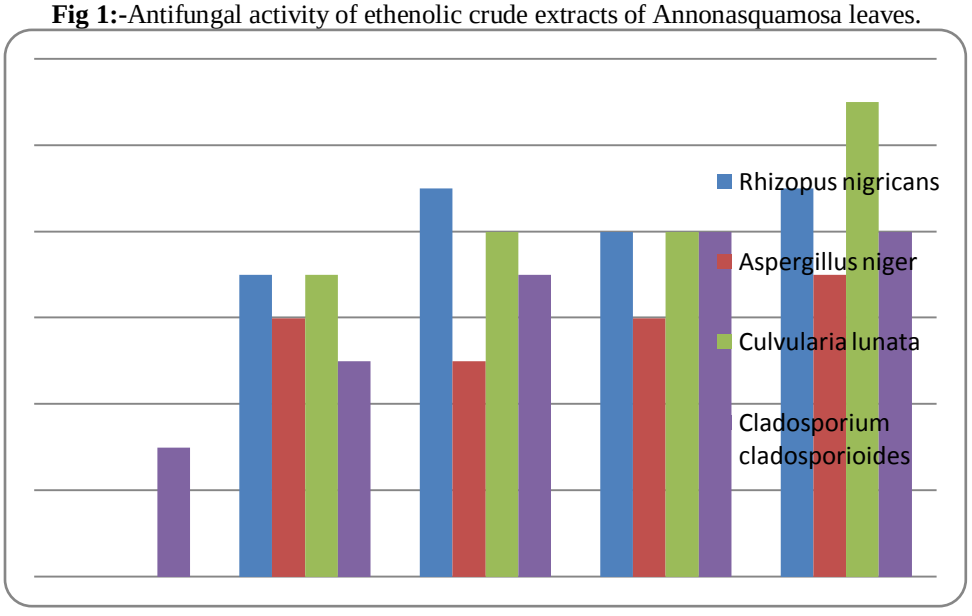
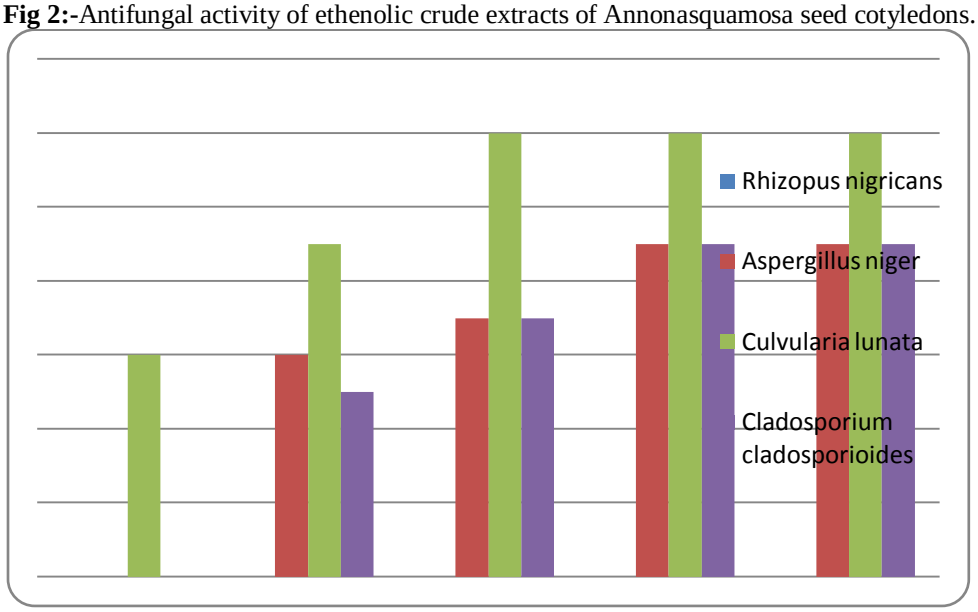


Fig 2:-Antifungal activity of ethenolic crude extracts of Annonasquamosa seed cotyledons.



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