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SCREENING THE ANTIFUNGAL ACTIVITY OF *ANNONA SQUAMOSA* LEAF EXTRACTS AGAINST DIFFERENT FUNGAL STRAINS.

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ABSTRACT **Background and aim:-** The present study is set to screen the antifungal activity using different solvent extracts of *A.squamosa* leaf (Hydroethanolic, petroleum ether, ethyl acetate and chloroform) against 5 different fungal strains.

Materials and methods:- The present study was been carried out during June, 2016 to December, 2016. *Annona squamosa* leaf extracts were obtained and all the isolates were subjected to well diffusion method on Sabrouds Dextrose agar.

Results:- our prospective study showed that, among the four types of *Annona squamosa* leaf extracts ie., hydroethanolic, petroleum ether, ethyl acetate and chloroform extract. Ethyl acetate was very efficient when tested against five fungal strains including ***Aspergillus niger, Candida albicans, Aspergillus fumigatus,Penicillium chrysogenum*** and **Mucor sp.** Thus, the plant has some kind of an antifungal activity, which could be effectively used against the fungal infection.

Conclusion:- *Annona squamosa* leaf extract confirms the traditional and the possible therapeutic uses of the plant. Therefore, pharmacological validation is necessary to isolate and characterize their active compounds. Moreover, these plants extract should be further investigated *in vivo* to better understand their safety, efficacy and properties in order to explore concealed areas and their practical clinical application, which can be used for the welfare of the mankind.

KEYWORDS : *A.squamosa,* antifungal activity, Hydroethanolic extract, Ethyl acetate.

INTRODUCTION:-

Due to indiscriminant use of commercial antimicrobial drugs, which are commonly used in the treatment of infectious diseases has given rise to the multi-drug resistant pathogenic microorganism. This has become a worldwide issue and hence, scientists have to search for new antimicrobial substances from various sources to overcome this issue. These antimicrobial substances could be a source from medicinal plants, which serve as a good source for antimicrobial agents in the past and present (Karaman *et al.,2013)*.The World Health Organisation has also recognized the importance of traditional medicine and has created strategies, guidelines and standards for botanical medicine.

Apart from the English medicine, still many population depend on traditional and regional medicine for psychological and physical health problems (Rabe and Van Stoden, 2000). Researchers have identified a number of compounds in the mainstream medicine and are derived from "ethnomedical" plant sources (Fabricant and Farnsworth, 2001). Thus, by creating a fusion of ancient wisdom and modern science, India can create world class products.

Our present study is based on the antifungal activity of custard apple's (sugar fruit) leaf extract. *Annona squamosa* (Annonaceae), commonly known as the custard apple is a native of West Indies. But the cultivation is present throughout India, because of its edible nature (Annie shirwaikar et al 2004). This plant is attributed with the medicinal properties that include anti-fertility and anti-tumour activities which were observed in mice and rats. The young leaves of *Annona squamosa* were used extensively due to its anti-diabetic activity by the tribal men in Aligarh district in Uttar Pradesh. Its leaves were used as the insecticidal and antispasmodic agents that were used in the treatment of rheumatism and painful spleen.

The plant was reported traditionally to possess analgesic, anti-inflammatory, anti-pyretic, anti-ulcer and antiseptic activities. With this background information, the present work was carried out from June 2016 – December 2016 to perform studies on antifungal activity in 4 different leaf extracts viz., hydroethanolic, petroleum ether, ethyl acetate and chloroform of *Annona squamosa*.

MATERIALS AND METHODS:-

Antifungal screening:

Antifungal agents work by exploiting differences between mammalian and fungal cells to eliminate the fungal organism without dangerous effects on the host. Antifungal activity of the *Annona squamosa* was determined, using the agar well diffusion method .The fungal test organisms used were ***Aspergillus niger, Candida albicans, Aspergillus fumigatus,Penicillium chrysogenum*** and **Mucor sp** . For determination of antifungal activity agar-well diffusion method was followed in which the spore suspension with agar at 45°C and allowed to set. Wells (4.6mm in diameter) were cut in a similar way as for the antibacterial activity with a sterile borer and 60µl extract solutions were delivered into them. The plates were incubated at 28°C for 3 days after which diameter of zones of inhibition (DIZ) were measured. Amphotericin B was used as positive control.

RESULTS AND DISCUSSION:-

Antifungal Screening:

The results obtained for screening of antifungal activity have been tabulated. Ethyl acetate extract exhibited effective inhibition against all the fungal strains tested with zone of inhibition ranging from 4.5 mm (*Mucor*), 5 mm (*A.fumigatus*), 6 mm (*A.niger, Candida albicans*), and 6.5 mm (*Penicillium*) respectively. Chloroform extract was found to affect the growth of *Candida albicans* and *Penicillium* with zone of inhibition 4 mm and 6 mm respectively. The moderate or less antifungal affect of petroleum ether was found against all the tested fungi except *Penicillium chrysogenum* with 4 mm.

All fungal strains were found to be sensitive with standard antifungal agent, Amphotericin B. Dimethyl sulfoxide was used as the negative control which shows no zone of inhibition against tested fungi. Herbal remedies used in traditional medicine provide an interesting and largely unexplored source for the development of new drugs. Drug resistance is a serious global problem and spread of resistance poses additional challenges for clinicians and the pharmaceutical industry. Hence, there is need to screen medicinal plants for promising biological activity

Plants are reservoir of valuable bioactive chemical constituents. In particular, the flavonoids were reported to be responsible for anti-microbial activity associated with some ethanomedicinal plants (Perez C *et al*., 1994). The increasing emergence of antibiotic resistance has deviated the attention of researchers towards the medicinal herbs in search of new and non-toxic drugs (Mbaveng AT *et al*., 2008). Therefore, this study was carried out to evaluate the antifungal activity of the various extracts of *Annona squamosa* leaf.

Antifungal effect of *Annona squamosa* leaf extracts was found to be active against the fungal strains in accordance to ethyl acetate extract which was much effective. In conclusion, *Annona squamosa* leaf extract confirms the traditional and the possible therapeutic uses of the plant. Therefore, pharmacological validation is necessary to isolate and characterize their active compounds. Moreover, these plants extract should be further investigated *in vivo* to better understand their safety, efficacy and properties in order to explore concealed areas and their practical clinical application, which can be used for the welfare of the mankind.

Fig. 1 – *Candida albicans* showing zone of inhibition

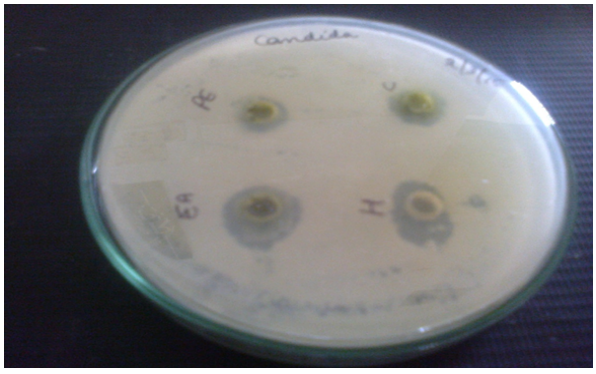
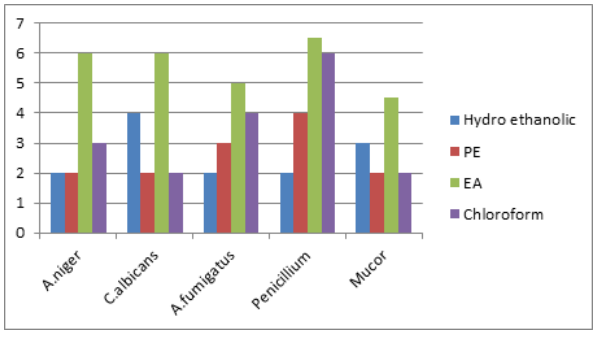


Table 1:- Antifungal activity of *A. squamosa* leaf extracts

Extract/ Drug (50µg)	Diameter of zone of inhibition (mm)				
	A.niger	*Candida albicans*	*A.fumigatus*	*Penicillium Chrysogenum*	*Mucor*
Hydro ethanolic	2 ± 0.76	4 ± 0.78	2 ± 0.67	2 ± 0.69	3 ± 0.75
Petroleum Ether	2 ± 1.03	2 ± 1.09	3 ± 0.96	4 ± 0.99	2± 1.01
Ethyl acetate	6 ±0.59	6 ± 0.65	5 ± 0.75	6.5 ± 0.78	4.5 ± 0.68
Chloroform	3 ± 2.57	2 ± 2.45	4 ± 2.11	6 ± 0.97	2 ± 1.95
Amphotericin (100 µg)	18 ± 0.79	14 ± 0.84	16 ± 1.01	18 ± 1.19	15 ± 0.98

*Values are mean + SD of triplicates

Graph 1:- Depicting anti-fungal activity of different *A.squamosa* leaf extracts.



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