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EVALUATION OF ANTIFUNGAL EFFECT ON AQUEOUS- ETHANOLIC EXTRACT OF *BARK of ANNONA SQUAMOSA L. (Annonaceae)* STEM.

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Abstract—*Annona Squamosa, a multipurpose, drought resistant evergreen tree commonly known as "Custard apple" belonging to family Annonaceae, is gaining lot of importance for its therapeutic potentials. Various part of tree has been used in traditional folkloric medicine. The crushed leaves are sniffed to overcome hysteria and fainting spells; they are also applied on ulcer and wounds and a leaf decoction is taken in case of dysentery. This research aim at testing the antifungal activity of Aqueous+ ethanolic extract of Annona squamosa stem bark extract against Fusarium equiseta, Aspergillus flavus, Alternaria alternate in Potato Dextrose Agar (PDA). Qualitative phytochemical screening showed presences of essential oil, phenolic compounds, alkaloids, Glycosides, tannins, amino acid, steroids. The antifungal activity of Aqueous + ethanolic extract of Annona squamosa stem bark was evaluated by employing various concentration (2- 8mg).All the concentration of stem bark extract inhibited the fungal growth. Among different doses, the diameter of inhibition zone ranged from 3 to 18 mm in various fungal species. Hence, the results of the present investigations indicate the Annona squamosa stem bark extract possess antifungal activity that can be exploited as an ideal treatment for future fungal disease.*

Keywords*: Annona squamosa, Antifungal activity, Potato Dextrose Agar (PDA), Inhibition zone, Phytochemical screening.*

1. INTRODUCTION

Infectious diseases represent a critical problem to health and they are one of the main causes of morbidity and mortality worldwide (Laredo JV *et al*., 1995). Fungi are the fifth most common pathogens after *Entero bacteriaceae, Staphylococcus aureus, Pseudomonas aeruginosa* and Coagulase-negative staphylococci. During the past several years, there has been an increasing incidence of fungal infections due to a growth in immunocompromised population such as organ transplant recipients, cancer and HIV/AIDS patients. The numbers of multi-drugs resistant microbial strains with reduced susceptibility to antibiotics are continuously increasing. The small number of drugs available for their treatment, most of them fungi static and emerging resistance permanently encourages the search for alternatives and led us to find them among low cost and low toxicity traditional therapies and natural products (Knobloch K *et al*., 1989).

The genus name "Annona" is from the Latin word "anon" meaning "yearly produce", (referring to the production of fruits of the various species in this genus) contains approximately 2300 known species (Audrey Leatemia J, 2004). Species name squamosa refers to the knobbly appearance of the fruit. Annona, a drought resistant tree or shrub, is widely distributed throughout the tropics and do well in hot and relatively dry climates such as those of the low-lying interior plains of many tropical countries (Agroforestry Database 4.0). The curative properties of medicinal plants are perhaps due to the presence of various secondary metabolites. The extraction of different parts of Annona squamosa in different solvents revealed the presences of alkaloids, flavonoids, phenols, carbohydrate, saponin, sterols and tannins (Agrawal et al., 2012; Ashok et al., 2010).

2. IDENTIFIED MEDICINAL PROPERTIES

18-acetoxy-entkaur-16-ene isolated from petroleum ether extract of custard apple bark exhibited analgesic and anti-inflammatory activity (Chavan et al., 2011), similarily Methanolic extract of stem bark of Annona squamosa possesses the in vitro antimicrobial activity against Bacillus coagulans and Escherichia coli bacteria of gram-positive and gram-negative strain (Kachhawa et al., 2012). Yadava et al., 2011 reported that antiulcer properties are also presented in plant which is found through cold restraint, pyloric ligation, aspirin, alcohol induced gastric ulcer and histamine induced duodenal ulcer models and further confirmed through in vitro assay of H+ K+-ATPase activity and plasma gastrin level.

3. MATERIALS AND METHODS

Plant material

Plant material was collected from Banswara Rajasthan. Plant was authentified by department of Botany, University of Rajasthan whose number is **RUBL 21059** and a voucher

specimen was deposited in the botany department, Rajasthan University Jaipur.

Preparation of Plant extract

After drying Plant material is crushed and powdered, the dried powder is subjected to soxhelet apparatus for 48 hours in solvent (water+ Ethanol).The residual extract thus obtained treated as experimental drug for the study.(Yield Value =12%)

Phytochemical Analysis of Extract: The extract was subjected for phytochemical investigations by qualitative chemical tests. Standard phytochemical methods were used to test for the presence of saponins, alkaloids, tannins, anthraquinones, cardiac glycosides, glycosides, amino acid & protein and flavonoids.

Fungal strains: Human pathogen Aspergillus flavus and two plant pathogens (Fusarium equiseti, Altenaria alternate) were used

Drug used*:* Cotrimazole was used as reference standard for antifungal studies

In-vitro antifungal susceptibility

The procedure for the Potato dextrose agar (PDA) method. PDA powder was dissolved in distilled water to a final concentration of 39 g/liter and then sterilized at 121°C for 15 min. The sterilized PDA solution was placed in a water bath, and the temperature was cooled to and maintained at 55 to 60°C. The antifungal agent stock solutions were mixed with the PDA solution to produce a series of different final concentrations as 2%, 6% and 8%. Drug-free agar containing only 1% DMSO was used as a positive control and without inoculation one plate used as negative control. The mixtures of antifungal agent and PDA solutions were poured directly into the plates. After the plates were cooled to room temperature, freshly made fungal suspension (5 ×103 to 2 ×104/ml) was inoculated onto the agar plate. The plates were incubated aerobically at 35°C for 7 days and then measure the growth of the fungi on the plate. Diameter of zone of inhibition was measured using zone reader and given (Table 3).

4. RESULT AND DISCUSSION

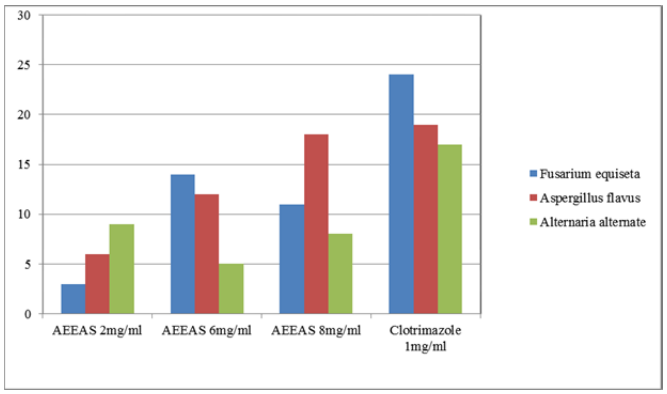
The result of the present study indicate that Aqueous+ethanolic extract of *Annona Squamosa Bark* showed antifungal activity against *Fusarium equiseta,Aspergillus flavus, Alternaria alternate* in different concentration. It shows maximum inhibition zone 12mm, 14mm and 18mm in different dose respectively (Table-3).

Table 1: Phytochemical screening of Aqueous+ethanolic extract of *Annona squamosa stems Bark extract*

Name of components	Name of chemical tests	Observation
Tannins and Phenolic Compounds	5% FeCl3 Solution	Deep blue-black ppt.
	Dil. Iodine Solution	Transient red color
Test of glycosides		
Cardiac glycosides	Legal test	Pink colour
Anthroquinone glycoside	Brontrager test	No significant change in Colour
protein and amino acid Compounds	Biuret test	No significant change in colour
Test of Steroid	Salkowshi reaction	Chloroform layer appear, red and blue
Flavonoids	Shinoga test	Pink Colour
Test of alkaloids	Dragendraff's test	Orange-brown Ppt

Table 2: Phytochemical screening of Aqueous+ethanolic extract of *Annona squamosa Bark extract*

S. NO	NAME OF TESTS	RESULTS
1	**Tannins and Phenolic compounds**	**+ve**
2	**Glycosides**	**+ve**
	Cardiac Glycosides	**+ve**
	Anthraquinone Glycosides	**-ve**
3	**Protein and Amino Acids**	**-ve**
4	**Test of steroid**	**+ve**
5	**Flavonoid**	**+ve**
6	**Alkaloids**	**+ve**



Graph 1: Zone of inhibition of stem bark extract of *Annona squamosa* for antifungal activity.

***AEEAS-Aqueous ethanolic extract of Annona squamosa**

Table 3:-Zone of inhibition of stem bark extract of *Annona squamosa* for antifungal activity.

	Zone of Inhibition (In mm)			
Fungus	**AEEAS 2mg/ml**	**AEEAS 6mg/ml**	**AEEAS 8mg/ml**	**Clotrimazole 1mg/ml**
Fusarium equiseta	**3**	**14**	**11**	**24**
Aspergillus flavus	**6**	**12**	**18**	**19**
Alternaria alternate	**9**	**5**	**8**	**17**

Qualitative phytochemical screening showed presence of phenolic compound, glycosides, steroids, alkaloids. Presence of constituents like flavonoids, tannin in the extract is likely to be responsible for the antimicrobial activity and might be due to presence of some active secondary metabolite in the plant. It may help in the discovery of new chemical classes of antibiotics that could serve as selective agent for the maintence of human health. Further investigations should be carried out in finding other activities.

5. CONCLUSION

Aqueous-Ethanolic extract of Annona squamosa possess significant antifungal activity against selective pathogens. Further studies aim at isolation and purification of active phytoconstituents. There is a need to test the in-vivo activity of the extract apart from the effect on many other fungi. This plant is an ideal candidate in the research for new bioactive phytocompound suggesting that a more extensive biological and chemical bioassay guided fractionation is required in order to isolate and characterize such bioactive compound.

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