

Research Article

PHYTO CHEMICAL SCREENING AND EVALUATION OF ANTI-FUNGAL ACTIVITY ON THE SEED EXTRACTS OF *ANNONA SQUAMOSA* (L) AND *MANILKARA ZAPOTA* (L.)

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ABSTRACT

The aim of the study is to evaluate the antifungal activity of extracts of two plant species against the fungi *Aspergillus niger* and *Candida albicans*. The plants were selected on the basis of their reported ethnobotanical uses. Hydro alcoholic extract of the seeds of *Annona squamosa* (L) and *Manilkara zapota* (L.) are investigated for antifungal activity against *Aspergillus niger* and *Candida albicans*. The hydro alcoholic extract showed broad spectrum of significant activity on all the two microorganisms by producing zone of inhibition. The extracts of *Annona squamosa* (L). and *Manilkara zapota* (L.) could be considered as potential sources of antifungal compounds for treating fungal infections. These extracts exhibited momentous activity, even at very lower concentrations.

Keywords: *Annona squamosa* Seeds, *Manilkara zapota* Seeds, Anti-Fungal Activity, Hydro Alcoholic Extract, *Aspergillus niger*, *Candida albicans*

INTRODUCTION

Annona squamosa (L) belongs to Annonaceae family, commonly called as custard apple. Literatures of many research works prove that every parts of *A.squamosa* has medicinal property. *Manilkara zapota* (L.) commonly known as Sapodilla or Sofeda belongs to the family Sapotaceae. It has been used in the indigenous system of medicine for the treatment of various ailments. From the ethnobotanical studies we found that both are having good antifungal activity and can be used as anti-fungal agents. The present study was aimed at screening Hydro alcoholic extracts of two different plant seeds viz. *Manilkara zapota* L (Sapotaceae), *Annona Squamosa* L (Annonaceae), for their antifungal activity. From our literature survey we came to know that very few works have been carried out on seeds for other activity. So we are taking seeds of *Manilkara zapota* and *Annona Squamosa* for determining antifungal activity against the fungi like *Aspergillus niger* and *Candida albicans* (Dang et al., 2011; Pandey et al., 2014; Nandhakumar and Indumathi, 2013).



Figure 1: Seeds of *Annona squamosa* (L) and *Manilkara zapota* (L.)

MATERIALS AND METHODS

Collection of Plant Material

The plant material was collected from the plant *Annona squamosa* (L) and *Manilkara zapota* (L.) Figure 1. Which are collected during the month of August at Sekuru, Guntur (Dist) of Andhra Pradesh. Then, it was authenticated by Dr SM. Khasim, professor, Department of Botany and Microbiology, Acharya Nagarjuna University, Nagarjuna nagar, Guntur.

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Preparation of the Plant Material

The seeds were collected, rinsed with tap water and air dried under shade for about 14 days and made to fine powder by means of blender. The powder was kept in an air tight bottle until needed for use (Panda et al., 2011).

Preparation of Extracts

Required amount of the powdered sample was soaked in 100 ml of the solvent contained in a 500 ml of sterile conical flask and enclosed with a cotton wool. It was then plugged and wrapped with aluminium foil and shaken vigorously.

The mixture was then allowed to stand for 24 hrs. The mixture was then filtered using a clean muslin cloth and then whatman filter paper. The filtrate was then evaporated to dryness at 20°C. The percentage yield was calculated (yield 5.8%). For the preparation of dilutions of crude extracts for antifungal activity assay, the extracts were reconstituted by dissolving in the DMSO (Vidyasagar and Shivakumarsingh, 2013).

Microorganisms

Aspergillus niger and *Candida albicans* were obtained from the microbiology laboratory and were stored in a refrigerator.

Reference and Control

The reference like Flucanazole was used as the standard for all fungal species. The Control experiment consists of a plate of solidifying agar onto which was inoculated pure solvent with microorganism mixed in a 1:1 portion.

Phytochemical Screening of the Plant Material

Phytochemical screening was done on the powdered plant material for the presence of different bioactive components such as tannins, carboxylic acids, glycosides, alkaloids, saponins are depicted in Table 2.

Antifungal Activity

The Antifungal activities of these extracts were tested against *Aspergillus niger* and *Candida albicans*. The antifungal sensitivity pattern for the isolates was studied by the disc diffusion method (Vijay and Sriram, 2010).

Methodology

Potato dextrose agar media was made and the plates were swabbed with the cultures of respective fungi grown in nutrient broth. Sterile discs of 6mm diameter were impregnated with 25µl each extract separately.

Blank disc impregnated with DMSO was used as negative control and discs of Fluconazole (30 µg) as positive control.

The plates were then incubated at 37°C for 24 hrs. The antifungal activity was assessed by measuring the zone of inhibition.

The relative antifungal activity of the extract was calculated by comparing its zone of inhibition with the standard drugs (Chanda and Nagani, 2010; Mital and Sumitra, 2012; Bhargavi et al., 2013). Inhibition was recorded by measuring the diameter of inhibition zone at the end of 24h in Figure 2b.

RESULTS AND DISCUSSION

The results obtained in the study are depicted in Table 1 and figure 2 which shows the growth of inhibition produced by the seed extracts of on 2 species of *Annona squamosa* (L), and *Manilkara zapota* (L.) on fungi.

These activities can be referred based on their zone of inhibition. The seed extracts of *Annona squamosa* L., and *Manilkara zapota* (L.) was found to be highly active against *Aspergillus niger* and *Candida albicans*.

The results of the plant extract against various fungi were in concordant with positive control (Fluconazole). The study revealed that the hydro alcoholic extracts of both the species possess good anti-fungal activity. To our surprise the standard Flucanazole didn't show any zone of inhibition against the fungus *Aspergillus niger* and the test species showed a significant activity against the fungus.

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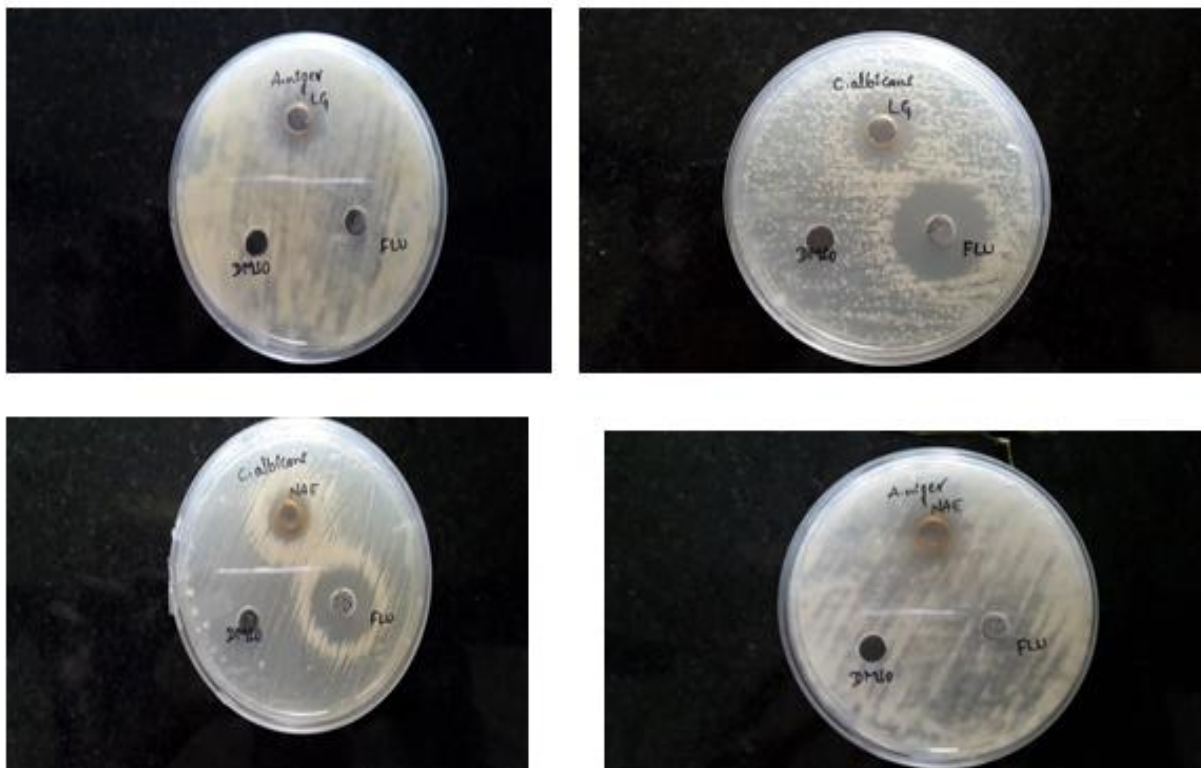


Figure 2: The Extract of Two Plant Species Producing Zone of Inhibition on Different Microorganisms

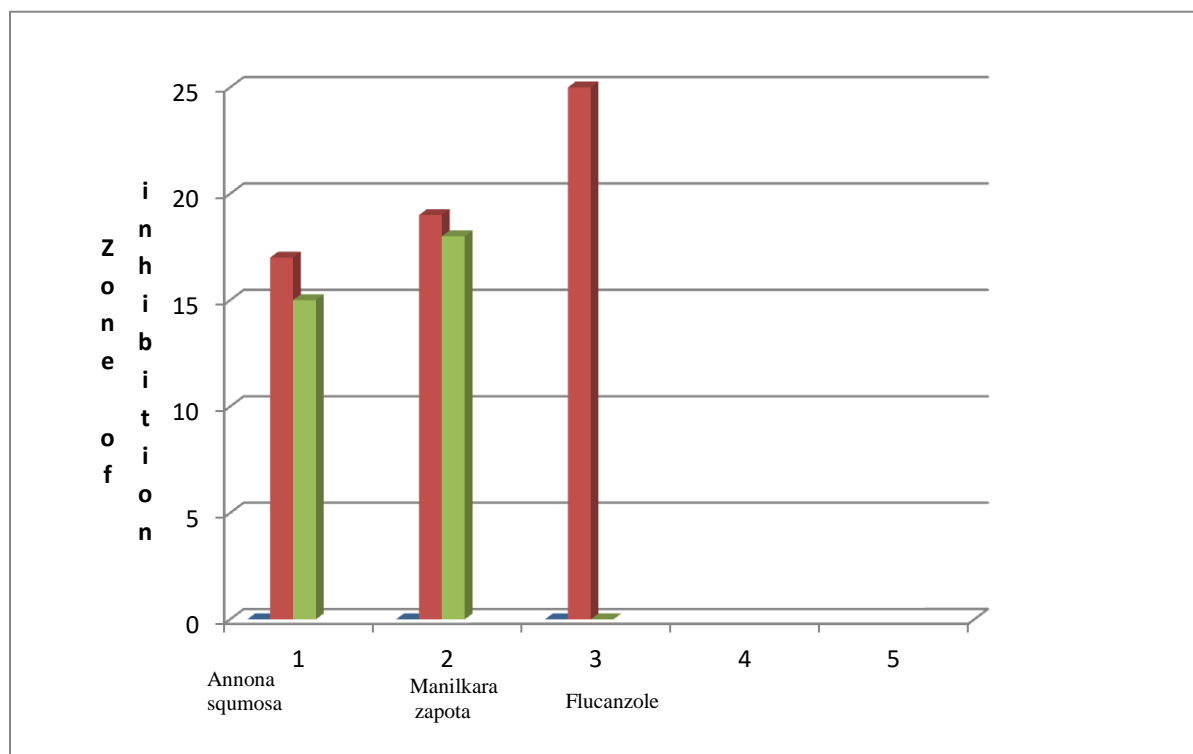


Figure 2b: Growth Inhibition of Crude Plant Extracts on the Selective Fungi

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Table 1: Zone of Inhibition of Two Plant Seeds against the Microorganisms

S. NO	Name of Plant Extract	Name of the Fungi	DMSO (Negative Control)	Hydroalcoholic Extract (250µg/ml)	Hydroalcoholic Extract (500µg/ml)	Flucanazole (Positive Control)
1	<i>Annona squamosa</i> L	<i>Candida albicans</i>	NZI	17	19	25
2	<i>Manilkara zapota</i> L	<i>Aspergillus niger</i>	NZI	15	18	NZI

NZI: No zone of inhibition observed

Conclusion

From the above study it is evident that the seeds of *Annona squamosa* (L.), and *Manilkara zapota* (L.) have significant anti-fungal action and can be used as extensive anti-fungal agent. Further this study promotes finding the minimum inhibitory concentration and to isolate the compound responsible for the action.

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