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Screening of some medicinally important plants for their antifungal activity against *Fusarium oxysporum* and *Aspergillus parasiticus*

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ABSTRACT

Considering the tremendous antimicrobial and antifungal potential of medicinally important plants, four important plants used in Indian folklore medicine were chosen to screen their antifungal activity. Leaf extracts of the plants viz. *Solanum torvum*, *Adhatoda vasica*, *Terminalia chebula* and *Asparagus racemosus* were prepared in organic solvents namely methanol, ethanol and methanol-ethanol (1:1) and were tested against fungal pathogens *Fusarium oxysporum* and *Aspergillus parasiticus* by agar well diffusion assay. Irrespective of the extraction solvent used all the plants extracts showed certain degree of antimicrobial activity against *Aspergillus parasiticus*. Zone of inhibition were obtained against *Fusarium oxysporum* in case of methanolic and ethanolic extracts only. The plants with highest antifungal activity recorded were *Solanum torvum* and *Adhatoda vasica* against *Fusarium oxysporum*. Similarly *Asparagus racemosus* and *Adhatoda vasica* were most effective against *Aspergillus parasiticus*. Rest of the plant extracts exhibited moderate to minimal antifungal activity.

Keywords: Antifungal activity, zone of inhibition, agar well diffusion assay.

1. Introduction

From ancient to the present world, the plant kingdom has rendered a variety of herbal medicines. Ancient literature and scriptures show the importance of medicinal properties of plants and their use since time immemorial. Uses of folk medicine and ethno botanical practices have been more prevalent and fruitful in small isolated villages and native communities across developing countries^[1], as they confer valuable therapeutic properties^[2]. With the increase use of pharmaceutical medicines and its better prospects one more threat has come that is the ability of the microbes to develop more resistance against them. As compared to modern medicine traditional medicines from different plants are cheaper and in addition medicines obtained from plant products have proved to be more effective in terms of their broad range effect^[3, 4]. Some of these plants are known to have anti-inflammatory, antineoplastic drugs; antiviral, antifungal, antibacterial anti-arrhythmic, antihypertensive properties^[5, 6]. The increasing concerns to the global health community are the evolution of new strains of disease causing pathogens and a steep rise in spread of antibiotic resistance. Our ability to effectively treat diseases is dependent on the development of new pharmaceuticals, and one potential source of novel drugs is traditional medicine^[7]. Now-a-days, plants have been exploited as a powerful and potential source for medicinal drugs^[8]. Herbal drugs are mainly focused as an alternative source against manifestations caused by various micro-organisms due to the increasing resistance of existing antimicrobial agents. Synthetic drug are also available but microbes were easily resist or overcome on it. The ethno-botanical wealth of developing countries like India and others having esteem richness in biodiversity and varied environmental conditions needs to be harnessed in terms of their availability and immense medicinal and economic importance^[9]. In quest of new antifungal agents depends greatly on part of ethno-botanical information and ethno-pharmacologic survey^[10]. At present knowledge, plant extracts having amply of antimicrobial activity, yet minimal scientific study has been carried out in the area of medicinal plants having antifungal properties^[11].

Due to notable medicinal importance of *Adhatoda vasica*, *Solanum torvum*, *Terminalia chebula* and *Asparagus racemosus* a preliminary study was conducted to unearth the antifungal activity of these plant species against pathogens such as *Fusarium oxysporum* and *Aspergillus parasiticus*.

2. Material and methods

2.1 Experimental Section

All the chemical reagents used were procured from Merck specialties private limited, Mumbai. Glassware used in the investigation was of Borosil grade. The media (Potato Dextrose Agar) for microbial culture was obtained from Hi-Media Pvt. Limited, Bombay, India.

2.2 Plant Material

Fresh disease free leaves of the plant species viz. *Adhatoda vasica*, *Solanum torvum*, *Terminalia chebula* and *Asparagus racemosus* were collected from Nashik, Maharashtra (Table I). Voucher specimens of all the samples were deposited in the herbarium of Plant biotech department, College of Agricultural Biotechnology, Nashik, Maharashtra.

2.3 Preparation of extract

Thoroughly washed mature disease free leaves of all the test plants were shade dried. Further the dried leaves were powdered with the help of a blender. Twenty-five grams of the powder was extracted with ethanol, methanol and ethanol-methanol (1:1) separately by refluxing at regular intervals for the next twenty four hours. All extracts were subjected to centrifugation at 10000 rpm for 15 min and the supernatant obtained was collected carefully^[16]. The extract were preserved at 5 °C in airtight brown bottle until further use. All the extracts were subjected to antifungal activity against the test fungi viz. *Fusarium oxysporum* and *Aspergillus parasiticus*.

2.4 Collection of fungi and Maintenance of Culture

Fungal inoculum for *Fusarium oxysporum* and *Aspergillus parasiticus* culture were obtained voluntarily from Microbiologia laboratories. The cultures were maintained on Potato Dextrose Agar (PDA) plate at 27 °C^[17].

2.5 Antifungal Activity

Antifungal activity was screened by agar well diffusion method^[18]. The PDA medium was poured in to the sterile petriplate and allowed to solidify. The fungal Inoculums were seeded on PDA medium. Then wells (5 mm) were made in the medium using sterile cork borer. 200 µl of each of the extracts were transferred into separate wells. The plates were incubated at 27 °C for 72 hrs. After incubation they were observed for the presence of clear inhibition zone around the well indicating antifungal activity. For each treatment three replicates were maintained and the zone of inhibition was measured in millimeters.

3. Result and Discussion

In the present study all the four evaluated medicinal plant leaf extracts exhibited potential antifungal activity against *Fusarium oxysporum* and *Aspergillus parasiticus*. In the agar well diffusion method, different zone of inhibition level depicting varying level of antifungal activity was observed (Table 2). For ethanolic leaf extracts, *Adhatoda vasica* showed highest activity (11.1±0.25) against *Fusarium oxysporum* with *Terminalia chebula* showing the minimal activity (7.1±0.50) and moderate activity in case of

Asparagus racemosus and *Solanum torvum*. Further for methanolic extracts *Adhatoda vasica* showed the highest activity (9.8±0.15) and least activity (7.1±0.20) was observed for *Asparagus racemosus*. No zones of inhibition were observed in case of Ethanol Methanol (1:1) extract for all the four plants specimens against *Fusarium oxysporum*. (Figure 1)

In case of activity against *Aspergillus parasiticus* the ethanolic leaf extract of *Adhatoda vasica* showed highest activity (9.1±0.62) whereas *Solanum torvum* was least effective (4.6±0.30). The Methanolic extract of *Asparagus racemosus* showed highest activity (6.8±0.3) and *Solanum torvum* showed the least activity (5.2±0.20) against *Aspergillus parasiticus*. With Ethanol Methanol (1:1) extracts, *Terminalia chebula* showed the highest activity (9.2±0.10) and least activity (3.6±0.20) was observed in case of *Adhatoda vasica*. (Figure 2)

Microbial infectious diseases are the leading cause for fatal death worldwide. Evolution of multidrug resistant microbes and their rapid propagation is a major concern across the globe. In addition to it the side effects and high costs of modern medicine has led to thinking towards herbal and ayurvedic medicines. Amongst microbe's fungi, on several occasions act as opportunist pathogen having capability to cause disease in multi hosts both in plants and animals^[19]. The fungal pathogens adopt a parasitic life style with ability to recognize and penetrate specific hosts. The two selected test fungal pathogens infest both plants and animals. *Aspergillus parasiticus* is a mold which produces aflatoxin, a potent liver carcinogen and *Fusarium oxysporum* is a mycelial fungus and an evident human pathogen. In present study the ethanolic and methanolic leaf extracts showed varied zone of inhibition against fungal pathogens thus indicating antifungal activity against the tested fungi viz. *Aspergillus parasiticus* and *Fusarium oxysporum*. Ethanolic extracts as reflected in the results showed highest zone of inhibition as compared to that of methanolic ones. However ethanol methanol (1:1) extracts when tested against *Fusarium oxysporum* showed no effect. There have been previous reports of completely randomized results for the antifungal potential of aqueous and polar extracts of *Hagenia abyssinica*, *Allium sativum*, *Phytolacca dodcandera*, *Croton macrostachyus*, *Maesa lanceolata*, *Eucalyptus globules*, *Eucalyptus citriodera* and *Lippia adoensis* plants species *in vitro* and *in vivo* against *Colletotrichum kahawae*. It may be attributed to the inhibitory effect of the extracts which in turn depends upon the type of plant species used, method of extraction and time of application of the extracts. However the present investigation affirms that the crude leaf extract of *Adhatoda vasica*, *Solanum torvum*, *Terminalia chebula* and *Asparagus racemosus*, have antifungal properties. These plant species thus can be investigated on large-scale for isolation of the potent metabolites having immense drug value. These plants can be an important source of potentially useful compounds for the development of new therapeutic agent^[20] combating against various fungal pathogens in particular *Aspergillus* and *Fusarium* species. Further, agro-industrial technologies need to be applied for the cultivation and processing of these plants to yield beneficial herbal medicines.

3.1 Tables and Figures

Table No. 1

Plant name	Family	Common name	Habit	Plant part used	Medicinal Importance/Properties
<i>Asparagus racemosus</i> Willd	Asparagaceae	Shatavari	climber	Leaves, roots	Beneficial in female infertility, increases libido, enhances folliculogenesis and ovulation, acts as postpartum tonic, cures leucorrhoea and menorrhagia ^[12] .
<i>Terminalia chebula</i> Retz.	combretaceae	Harad	Tree	Fruit, leaves	Antioxidant, antidiabetic, antibacterial, antiviral, antifungal, anticancerous, antiulcer, antimutagenic, wound healing properties ^[13] .
<i>Solanum torvum</i> sw.	Solanaceae	Turkey berry	prickly shrub	Fruits, leaves	Possess antihypertensive, antioxidant, cardiovascular, anti-platelet aggregation activities, anti-microbial activity, sedative, digestive, hemostatic and diuretic properties ^[14] .
<i>Adhotoda vasica</i> Nees	Acanthaceae	Vasaka	shrub	leaves, bark, fruit, flower	Cures respiratory disorders, control both internal and external bleeding, possesses antispasmodic, expectorant and blood purifying qualities, Skin Diseases ^[15] .

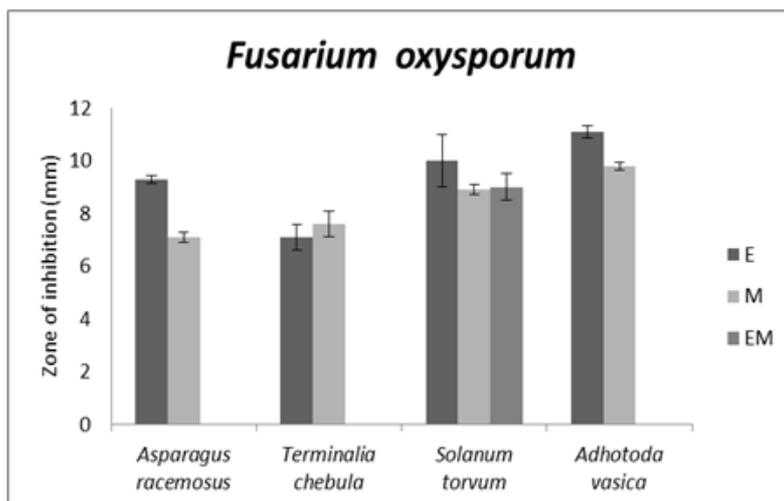


Fig 1: Antifungal activity against *Fusarium oxysporum*.

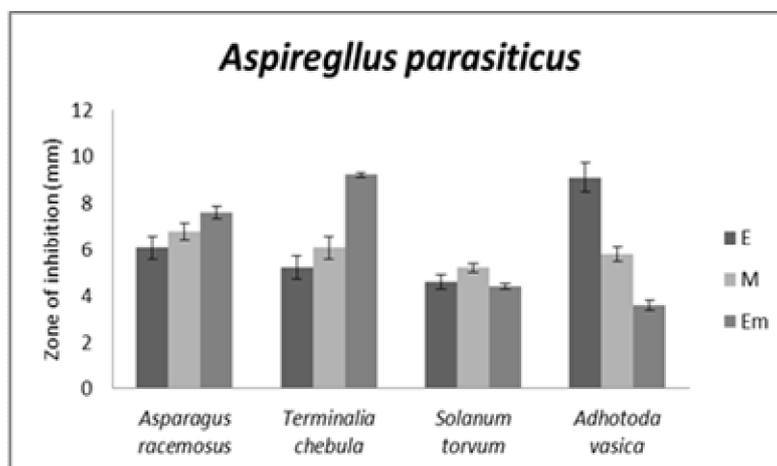


Fig 2: Antifungal activity against *Fusarium oxysporum*

Table No. 2

Medicinal Plants	Zone of Inhibition (mm)		
	Leaves Extract	Fungal Species	
		Fusarium oxysporum	Aspergillus parasiticus
Asparagus racemosus	Ethanol	9.3 ±0.15	6.1±0.50
	Methanol	7.1 ±0.20	6.8±0.35
	EM	-	7.6±0.25
Terminalia chebula	Ethanol	7.1 ±0.50	5.2±0.50
	Methanol	7.6 ±0.50	6.1±0.50
	EM	-	9.2±0.10
Solanum torvum	Ethanol	10.2±1.0	4.6±0.30
	Methanol	8.7±0.20	5.2±0.20
	EM	9.5±0.50	4.4±0.12
Adhotoda vasica	Ethanol	11.1±0.25	9.1±0.62
	Methanol	9.8±0.15	5.8±0.30
	EM	-	3.6±0.20

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