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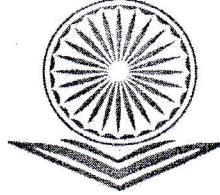
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12. Antifungal Activity and Phytochemical Analysis of *Adhatoda Vasica* against Two Species of *Fusarium*

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Abstract

In the present study, the various fractions of stem of *Adhatoda vasica* were evaluated for two species of *Fusarium* such as *Fusarium oxysporum*, *Fusarium proliferatum*. All isolated fractions are tested for phytochemical analysis. The ethanolic and ethyl acetate fractions showed maximum antifungal activity against both species of *Fusarium*. Ethyl acetate and ethanolic fractions of stem of *Adhatoda vasica* showed presence of alkaloids, tannins, saponins and glycosides.

Introduction

Fusarium is a widespread and very common soil-borne pathogen causing important diseases in a diverse host range either as primary or secondary invaders. They can cause diseases such as crown rot, stalk rot, head blight, and scab on cereal and grains. It is also responsible for vascular wilts on a wide range of horticultural crops such as tomato, root rots in beans, peanuts, soybean and other diseases. Several *Fusarium* species have also been studied extensively because the mycotoxins they produce, which are secondary metabolites that cause different physiological and pharmacological responses in plants and animals. *Fusarium* species are widely distributed in soil, roots and aerial plant tissues, plant debris, and other organic substrates. They are common in tropical and temperate regions and have also been found in deserts, alpine and arctic areas. Plant infection by *Fusarium* can occur at all developmental stages, from germinating seeds to mature vegetative tissues, depending on the host and *Fusarium* species involved (1). The medicinal plant have the large amount of phytochemicals, secondary metabolites which contributes the defence mechanism of plant. Microorganisms and fungal mycelium are not able to invade the plant due to presence of variety of phytochemicals alkaloids, flavonoids, saponins, tannins etc.

Materials and Methods

Plant material

The plant *Adhatoda vasica* (Acanthaceae) was collected from Visnupuri area, Dist. Nanded and plant identified and authenticated by Taxonomist, Department of Botany, Yeshwant Mahavidyalaya, Nanded-431602, Maharashtra.

Preparation of Plant Fractions

The stem of *Adhatoda vasica* was collected made small pieces and shade dried. Using electric grinder the dried stem was made powder. The fine powder of plant was the fractioned by Soxhlet apparatus using different solvents ethanol, ethyl acetate and petroleum ether. The obtained fraction finally concentrated and used for further antifungal and phytochemical analysis.

Preliminary Phytochemical Analysis

Different solvent stem fractions of *Adhatoda vasica* were screened for phytochemical analysis such as alkaloids, tannins, saponins and glycosides using standard protocol (2).

Fusarium Culture

In the present investigation, the two species of *fusarium* (*Fusarium oxysporum*, *Fusarium proliferatum*) were obtained from culture collection Centre, National Chemical Laboratory, Pune, Maharashtra. The *fusarium* cultures were repeatedly subcultured using potato dextrose agar and incubated at 37°C.

Antifungal Assay

Fungal fragments, precultured in mycelial growth medium, were placed in the center of PDA plates, after which the cultures were incubated for 96 h at 25 °C in the dark. After incubation, spores were isolated from cultures growing in PDA. Spore concentrations were then adjusted to 2×10^4 spores/mL in potato dextrose broth after which 80 µL was added to the wells of sterile 96-well flat-bottomed microtiter plates along with 20 µL of fraction. Several wells were kept untreated as a control to monitor fungal growth. Plates were incubated in the dark at 25 °C for 24 h before hyphal growth was determined by measuring optical density at 595 nm using a microtiter plate Elisa reader (3). Each test was performed in duplicate. Percentages of inhibition were then calculated.

Results and Discussions

The phytochemical analysis of different solvent fraction of *Adhatoda vasica* showed positive test of ethanolic and ethyl acetate fraction towards alkaloids, tannins, saponins and glycosides. The petroleum ether fraction showed absence of saponins and glycosides in *Adhatoda vasica*.

The results of preliminary phytochemical analysis of different fraction of *Adhatoda vasica* are shown in Table 1.

The percent inhibition of growth of two species of *Fusarium* were tested. The results of antifungal activity of all fractions are summarised in Table 2. Ethanolic fraction (72%) against *Fusarium oxysporum*, (78%) *Fusarium proliferatum* and ethyl acetate fraction *Fusarium oxysporum* (69%), *Fusarium proliferatum* (74%) showed maximum *fusarium* growth inhibition against both the species. The petroleum fraction of *Adhatoda vasica* showed least inhibition of growth *Fusarium oxysporum* (32%), *Fusarium proliferatum* (27%). The *fusarium* growth inhibition also tested with Chloramphenicol (25 µg) for comparison. Among the tested plants ethanolic and ethyl acetate extract effective towards the inhibition of both the species of *fusarium*.

Considering the need for an alternative eco-friendly approach to control the phytopathogen, it was believed to be worthwhile to screen the antifungal effects of locally available flora. The results of this study are indicating the differential activities of the plant extracts on the mycelium growth of *Fusarium* species because many of these extracts have strong inhibition against the mycelium growth of the test fungi and a definite potential for new effective fungicide exists. Among the different plants screened the ethanolic and ethyl acetate fractions of *Adhatoda vasica* shown a maximum inhibitory effects, which might be due to the presence of some antimicrobial secondary metabolites in the plant samples and it possess several medicinal properties (4, 5). Hence the spray of the ethanolic and ethyl acetate fractions of *Adhatoda vasica* plant on the infected plant may give protection against pathogenic organisms like *Fusarium* species. The extract of selected plants contains different types of phytochemicals like alkaloids, saponins, tannins, glycosides showed various medicinal properties (6, 7). There are several reports published that two or more combination of plant extract may enhance the activity that may be due to the combination of two or more phytochemicals and the synergistic effect observed on the pathogenic organisms. The several species of *Fusarium* are spreading wildly and directly and indirectly destroy the food grains plants, oil seeds plants, vegetables and various fruit plants. There are several bio-control methods similarly the various plant extract treatment will also provide a huge plat form for preparation of natural prevention of several of plant pathogens.

Conclusion

The ethanolic and ethyl acetate fractions of stem of *Adhatoda vasica* investigated have considerable antifungal activity. The bioactive compounds like namely alkaloids, tannins saponins, glycosides, are present in selected plants. Further studies are needed with these

medicinal plants to isolate, characterize and elucidate the structure of the bioactive compounds of the plant extracts which are responsible for the antifungal activities.

Table 1. Preliminary phytochemical analysis of stem fraction of *Adhatoda vasica*

Sr. No.	Phytochemical Test	Stem fraction of <i>Adhatoda vasica</i>		
		Ethanol Fraction	Ethyl acetate fraction	Pet. Ether fraction
1	Alkaloids	++	++	++
2	Tannins	++	++	++
3	Saponins	++	++	--
4	Glycosides	++	++	--

Table 2. Antifungal activity of plant fraction (100 µg) on two *Fusarium* species

Sr. No.	Fractions of <i>Adhatoda vasica</i>	Percent inhibition of growth	
		<i>Fusarium oxysporum</i>	<i>Fusarium proliferatum</i>
1	Ethanol fraction	72	78
2	Ethyl acetate fraction	69	74
3	Petroleum ether fraction	32	27
4	Chloramphenicol (25 µg)	81	85

References

1. Armstrong GM and Armstrong JK (1950). Biological races of *Fusarium* causing wilt of cowpeas and soybeans. *Phytopathology* 40, 181-193.
2. Yadav RNS, Agarwala M (2011) Phytochemical analysis of some medicinal plants. *Journal of Phytology* 3(12), 10-14.
3. Hadian S (2012). Antifungal Activity of Some Plant Extracts against Some Plant Pathogenic Fungi in Iran. *Asian journal of experimental biological sciences* 3 (4), 714-718.
4. Minz S, Samuel C O, Tripathi S C (2012). The Effect of Plant Extracts on the Growth of Wilt Causing Fungi *Fusarium oxysporum*. *Journal of Pharmacy and Biological Sciences* 4(1), 13-16.
5. Bhardwaj SK (2012). Evaluation of Plant Extracts as Antifungal Agents Against *Fusarium solani* (Mart.) Sacc. *World Journal of Agricultural Sciences* 8 (4), 385-388.
6. Ross JP (1965). Effect of medicinal plants on *Fusarium* species in highly saline soil. *Phytopathology* 55, 361-364.
7. Hirrel MC (1983). Sudden death syndrome of soybean: A disease of unknown etiology. *Phytopathology* 73,501-502.