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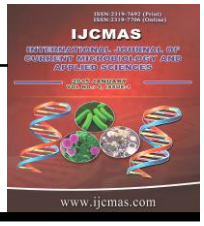


**Original Research Article**

# Antifungal activity of *Acorus calamus* against *Fusarium oxysporum f.* sp. *lycopersici*

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Antifungal, Zone of inhibition, MIC, *Acorus calamus*, paper disc diffusion assay

**A B S T R A C T**

Knowing the tremendous antimicrobial and antifungal potential of medicinally important plants, *Acorus calamus* used in Indian folklore medicine was chosen to screen its antifungal activity. Rhizome extracts of the plant were prepared in organic solvents namely methanol, ethanol petroleum ether and in aqueous solution as well and tested against fungal pathogens *Fusarium oxysporum f. sp. lycopersici* by paper disc diffusion assay. Zone of inhibition were obtained against *Fusarium oxysporum f. sp. lycopersici* in case of all the solvents and aqueous extracts too. The solvent concentration with highest antifungal activity recorded was (29.5mm) acetone extract at its 1000 mg/ml concentration against *Fusarium oxysporum f. sp. lycopersici*. Similarly ethanol, aqueous and petroleum ether extracts were also effective. Rest of the plant extracts exhibited moderate to minimal antifungal activity. MIC was also determined for each solvent.

## Introduction

Nature has been a source of medicinal components since the beginning of the civilization (Nostro *et al.*, 2000), and an impressive number of valuable drugs have been isolated from natural sources. Most of them play a very important role in herbal health care. Approximately more than 80% population depends on herbal medicinal system for their primary health. Plant products also have an important role in health care system of remaining 20% who reside in developing countries (WHO, IUCN, WWF, 1993). In developed countries medicinal plants are extremely important, about 119 chemicals derived from plant species can be considered as important drug in use (Mullholland, 2000).

A medicinal plant is any plant which in one or more of its organ contains active substances which are called as secondary metabolites and are used in therapeutic purposes. Plants have an almost limitless ability to synthesize aromatic substances, most of which are phenols or their oxygen substituted derivatives (Geissman, 1963). Most are secondry metabolites, of which at least 12,000 have been isolated, a number estimated to be less than 10% of total (Schultes, 1978). These active compounds

are however a most important part of plants defense mechanism. These compounds are very effective against various microorganisms, insects and herbivores naturally. Like some terpenoids provides a particular odor to its native plant, Tanins and quinons are responsible for plant pigment formation. Many compounds are basically flavoring in nature (e.g., the terpenoid capsaicin from chili peppers), and some other spices and herbs used by humans to season food items. Plants active compounds are secondary metabolites originated in plants as a result of their normal metabolic activity. It is very likely that the actual numbers of secondary metabolites (SMs) or bioactive compounds in the plant kingdom would exceed 100,000 structures (Wink, 2006)

Himalaya is well known for its plants. The diversity amongst its plants is grand. Plants are not only used for local demands but also used in exploiting various drugs. Himalayan region is subdivided into various belts. The Indian Himalayan region alone supports about 18,440 species of plants (Angiosperms: 8000 spp., Gymnosperm: 44 spp., Pteridophytes: 600 spp., Bryophytes: 1736 spp., Lichens: 1159 spp. and Fungi: 6900 spp.) of which about 45% are having medicinal properties. According to Samant *et al.* (1998) out of the total species of vascular plants, 1748 species are medicinal. Uttarakhand is a storehouse of rich variety herbs, medicinal and aromatic plant species.

In present study medicinal plant has been selected to know its antifungal activity. The description of this plant species is as follows;

*Acorus calamus* is a tall perennial wetland monocot of the Acoraceae family. *A. calamus* is found in eastern and tropical southern Asia including Japan and Taiwan (Bown 1988; Rost 1979). Sweet flag, *Acorus calamus* L., is an uncommon but widespread, semi-aquatic plant of aquatic habitats in temperate to subtemperate regions. An herbaceous perennial, sweet flag has long, erect, narrow, aromatic leaves ascending from a branched, underground rhizome. Internally the rhizome is whitish pink in color and pleasantly aromatic, smelling of citrus, although it has a bitter taste. The inflorescence consists of a leaf-like spathe and a spike-like spadix, produced from the middle of the spathe which is densely covered with yellow and green flowers.

The major chemical constituents of the essential oils of sweet flag are phenylpropanes, mono-terpenes, and thermolabile sesquiterpenoids (Rost, 1979; Bos, 1979). Methyleugenol, cis-methylisoeugenol, 3-asarone, geranylacetate, f-farnesene, shyobunone, epishyobunone, isoshyobunone, and an unknown constituent are the most abundant chemical compounds constituting about 20% of the essential oil (Rost, 1979; Bos, 1979).

Fusarium wilt disease has ever been the most destructive plant diseases in history (Halila and Strange, 1996). All members of *F. oxysporum* are successful saprophytes. Some isolates induce root-rot and vascular diseases on specific hosts (Olivain *et al.*, 2003). *Fusarium oxysporum f. sp. lycopersici (Fol)* is responsible for important crop losses in the tomato fields (Benhamou *et al.*, 1998).

## Materials and Method

Plant samples (cutted rhizome) were air dried under shade at room temperature for 2 weeks, then ground to fine powder using a laboratory mill. Powders were packed in air

tight bags, weighed and stored in the dark. Organic and water extracts were prepared for each sample. Organic extracts were prepared by extracting sample successively with acetone, ethanol and petroleum ether. The resultant extract was weighed and stored in airtight sample bottles. For the water extracts, plant powder of sample was soaked in distilled water. All the extracts were oven evaporated till complete dryness. Plant extracts were second time extracted with DMSO. 200 mg of each extract was weighed into a sterilized sample bottle and dissolved in DMSO (Sigma) to make a concentration of 200 mg/ml.

Microorganism used in this study *Fusarium oxysporum f.sp. lycopersici* was obtained from Microbial Type Culture Collection MTCC, Institute of microbial technology, Chandigarh. Fungus culture (MTCC 1755) was maintained in Potato sucrose agar medium for an optimum pH of 6.8.

The fungal spore suspension was prepared by the addition of a loopful of fungal spores in a 5 ml of sterile distilled water and 1 ml Tween 20. Then fungal spore suspension was mixed well in aseptic conditions and spread evenly on the petriplate containing 20 ml of solidified potato sucrose agar.

**Antimicrobial activity**

In Paper disc method Some amount of Potato Sucrose Agar (PSA) was dispersed in petridishes and allowed to solidify. A micropipette will be used to introduce 0.5 ml. spores on agar medium and spread with glass rod spreader under sterile conditions. Sterilized discs (6 mm, Whatmann No. 1 filter paper) will be prepared by soaking in different concentrations of the extracts ie, 250, 500, 750, 1000 mg/ml for 6 hour.The discs will be then removed and allowed to dry. To assay for antifungal activity various discs impregnated with different concentrations of the extracts will be placed on the fungal spore or mycelium with the help of sterilized forceps. The petridishes incubated at 35˚ C for 48 h. Antifungal activity will be determined by measurement of the zone of inhibition around the discs after the period of incubation.

In microbiology, Minimum Inhibitory Concentration (MIC) is the lowest concentration of an antimicrobial that will inhibit the visible growth of a microorganism after overnight incubation. Minimum Inhibitory Concentrations are important in diagnostic laboratories to confirm resistance of microorganisms to an antimicrobial agent and also to monitor the activity of new antimicrobial agents. A lower MIC is an indication of a better antimicrobial agent. A MIC is generally regarded as the most agents against an organism. MICs can be determined by agar or broth dilution methods.

Experiments were performed in the laboratory of L.S.M.G.P.G. college Pithoragarh.

## Result and Discussion

The antifungal activity was observed *in vitro* under paper disc diffusion method. The crude extracts of the selected plant had inhibitory effects on test organism *Fusarium oxysporum f.sp. lycopersici.* It was evaluated that out of all the extracts including aqueous too the acetone extract was most effective with inhibition zone of 29.5 mm at 1000 mg/ml concentration. Its 250 mg/ml concentration showed no inhibition zone, while 500 and 750 mg/ml concentrations were moderately effective with inhibition zone of 11.5 and 16.6 mm respectively.

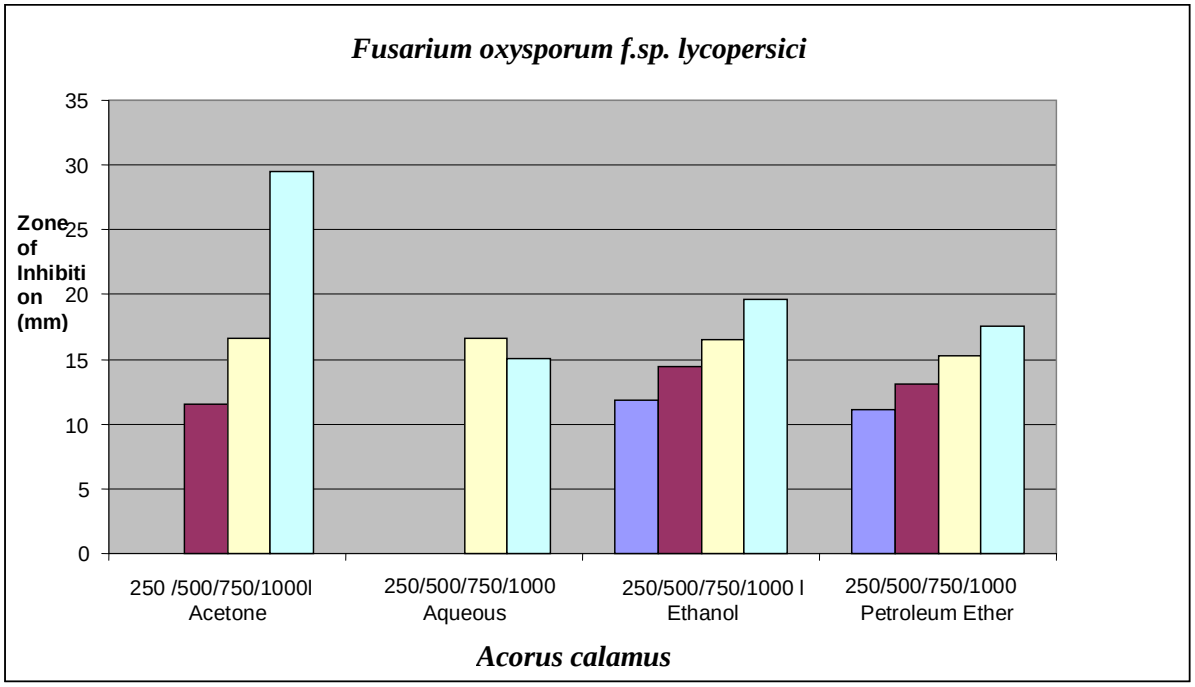
The aqueous extract was also effective. Its

250 mg/ml and 500 mg/ml concentrations were not effective with no zone of inhibition over fungus. But its 750 mg/ml concentration was significantly effective by 16.6 mm inhibition zone. The 1000 mg/ml concentration was moderately effective by 15.1 mm inhibition zone. The ethanol extracts of *A. calamus* was active with inhibition zone diameters of 11.8 mm at 250 mg/ml. The 500mg/ml concentration was effective with 14.4 mm. 16.5 mm inhibition zone was obtained at 750 mg/ml and with leading inhibition zone of ethanol extract fraction 19.6 mm zone was observed at 1000 mg/ml concentration.

**Table.1** Antifungal activity of *Acorus calamus* against *Fusarium oxysporum f. sp. lycopersici*

| ***Acorus calamus*** | **Inhibition zone (mm) against *Fusarium oxysporum f. sp. lycopersici*** | | | |
|---|---|---|---|---|
| **Concentrations(mg/ml)** | **Acetone** | **Aqueous** | **Ethanol** | **P.E.** |
| **250** | 0±0 | 0±0 | 11.8±0.8 | 11.1±1.2 |
| **500** | 11.5±1.1 | 0±0 | 14.4±0.7 | 13.05±2.2 |
| **750** | 16.6±1.2 | 16.6±1.2 | 16.5±1.2 | 15.3±2.0 |
| **1000** | 29.5±3.2 | 15.1±1.3 | 19.6±2.2 | 17.6±2.1 |
| **Control** | 0 | 0 | 0 | 0 |
| **MIC (mg/ml)** | 500 | 750 | 250 | 250 |

Results are the mean of four replications ± S.D.



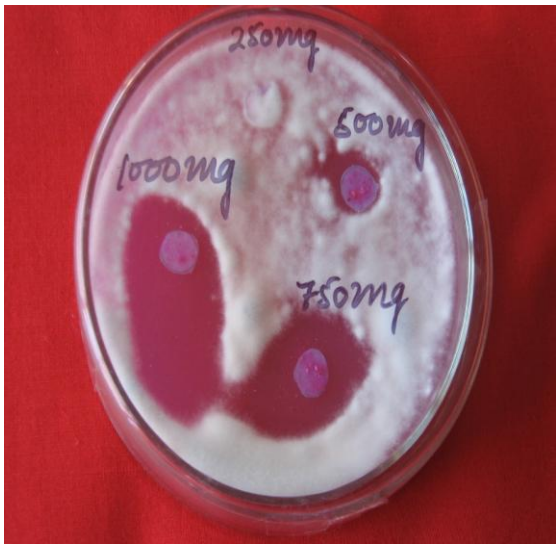



**Figure.2** Antifungal activity of acetone extract of *Acorus calamus*


**Figure.3** Antifungal activity of aqueous extract of *Acorus calamus*


**Figure.4** Antifungal activity of ethanol extract of *Acorus calamus*


**Figure.5** Antifungal activity of petroleum ether extract of *Acorus calamus*

Petroleum ether fraction was evaluated by measuring 11.1 mm zone of inhibition at its 250 mg/ml concentration. Zone of inhibition was observed 13.05 mm and 15.3 mm at its 500 and 750 mg/ml concentration. Leading inhibition zone at 1000 mg/ml concentration was recorded as 17.6 mm. The antifungal study evaluated that 250, 500, 750 and 1000 mg/ml concentrations of *Acorus calamus* possess significant antifungal activity against *Fusarium oxysporum f. sp. lycopersici* shown in Table 1. The acetone extract at its 1000 mg/ml concentration shows highest antifungal activity. Acetone extract showed its MIC value at 500 mg/ml concentration. Aqueous extract was significantly effective with 750 mg/ml

MIC value. The MIC value of ethanol extract was observed at 250 mg/ml and petroleum ether was similarly effective with MIC value of at its 250 mg/ml concentration.

*Fusarium oxysporum f.sp. lycopersici* can cause severe losses in tomatos. It has been concluded from the present research that certain plant extracts are the source of cheap and effective fungicide of *Fusarium oxysporum*, also they don't have human and environmental health implications. So some plant extracts such as *Acorus calamus* could be a good replacement for fungicide

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