

Evaluation of Antifungal Activity of Some Plant Extractives

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Nidiry: Antifungal activity of plant extractives

***In vitro* antifungal activity of thirty two extractives of seven plants obtained using hexane, ethyl acetate and methanol were tested against the mycelial growth of *Colletotrichum gloeosporioides*. Among these extractives, ethyl acetate extractives of onion seeds, chilli seeds and chilli pericarps and methanol extractives of chilli seeds and chilli pericarps exhibited more than 20 % mycelial growth inhibition at a concentration of 0.5 % level. Methanol extractives of *Solanum viarum* berries and ethyl acetate extractive of soybean leaves exhibited more than 20 % mycelial growth inhibition at a concentration of 1.0 % level. Thin layer chromatographic bioautography showed that hexane and ethyl acetate extractives of onion seeds, chilli seeds and chilli pericarps exhibited spore germination inhibition of *Cladosporium cucumerinum* at a dose of 1.0 mg.**

Key words: Antifungal activity, plant extractives, *Colletotrichum gloeosporioides*, *Cladosporium cucumerinum*

Investigations regarding the antifungal activity of plant extractives are important because they give insight into the defence mechanism evolved by plants against the attack of fungal pathogens. Such plant extractives and compounds with antifungal activity can be used against human, animal and plant pathogens and for post-harvest preservation, provided they possess suitable properties and sufficient amounts can be extracted from natural sources. Alternatively, structure elucidation of natural antifungal compounds helps in the synthesis of the compounds per se or analogues with improved physical and biological properties. Quantitative evaluation of such compounds in crop plants can facilitate the screening of plants for the development of resistant varieties.

In this paper the evaluation of thirty-two extractives of plant materials namely onion seeds, onion leaves, *Gloriosa superba* seeds, *Bougainvillea spectabilis* (green leaves, white bracts and pink bracts), chilli seeds, chilli pericarps, *Solanum viarum* berries *Albizia amara* leaves and soybean leaves obtained using hexane, ethyl acetate and methanol for their antifungal activity against the mycelial growth of *Colletotrichum gloeosporioides* is being discussed. Evaluation of the extractives of onion seeds, chilli seeds and chilli pericarps against the spore germination of *Cladosporium cucumerinum* by

Thin Layer Chromatography (TLC) bioautography is also being discussed.

The plant materials except soybean leaves were collected from the farms of ICAR-Indian Institute of Horticultural Research (IIHR), Hessaraghatta, Bangalore, India or purchased from the local market. Soybean leaves were obtained from ICAR-Indian Institute of Soybean Research, Indore, Madhya Pradesh, India. The plant materials were dried at 60° and subsequently powdered.

The powdered plant materials were extracted using a Soxhlet apparatus first with hexane, then with ethyl acetate and finally with methanol. In the case of *Gloriosa superba*, methanol extraction was done immediately after hexane extraction. The respective extractives were obtained by completely distilling out the solvents on a water bath and were tested for antifungal activity.

Colletotrichum gloeosporioides ITCC 4573 was used for the poisoned food technique and *Cladosporium cucumerinum* IMI 249540 was used for TLC

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bioautography. Antifungal activity of hexane, ethyl acetate and methanol extractives was studied by observing the mycelial growth inhibition of *Colletotrichum gloeosporioides* by poisoned-food technique[1]. Per cent mycelial growth inhibition was calculated using the formula $P=(C-T/C)\times 100$, where C is the mycelial diameter of the control and T that of treated ones, after giving due adjustment of the mycelial diameter of the inoculum. The values given in Table 1 are the average of two replications. For TLC bioautography,

conidial suspension of *Cladosporium cucumerinum* in the nutrient solution suggested by Homan and Fuchs^[2].

The results of *in vitro* antifungal activity of thirty-two extractives of seven plants against the mycelial growth of *Colletotrichum gloeosporioides* are presented in Table 1. Among these extractives, ethyl acetate extractives of onion seeds, chilli seeds and chilli pericarps and methanol extractives of chilli seeds and chilli pericarps exhibited more than 20 % mycelial

TABLE 1: ANTIFUNGAL ACTIVITY OF PLANT EXTRACTIVES FOR THE MYCELIAL GROWTH INHIBITION OF *Colletotrichum gloeosporioides*

Plant	Plant part	Solvent used for preparation of extractive	Concentration (%)	Percent mycelial growth inhibition
Onion (<i>Allium cepa</i>)	Seeds	Hexane	0.5	0
Onion (<i>Allium cepa</i>)	Seeds	Ethyl acetate	0.5	22.5
Onion (<i>Allium cepa</i>)	Seeds	Methanol	0.5	10
Onion (<i>Allium cepa</i>)	Leaves	Hexane	1	0
Onion (<i>Allium cepa</i>)	Leaves	Ethyl acetate	1	10
Onion (<i>Allium cepa</i>)	Leaves	Methanol	1	6
Chilli (<i>Capsicum annuum</i>)	Seeds	Hexane	1	0
Chilli (<i>Capsicum annuum</i>)	Seeds	Ethyl acetate	0.5	46.4
Chilli (<i>Capsicum annuum</i>)	Seeds	Ethyl acetate	1	54.6
Chilli (<i>Capsicum annuum</i>)	Seeds	Methanol	0.5	25
Chilli (<i>Capsicum annuum</i>)	Seeds	Methanol	1	38.1
Chilli (<i>Capsicum annuum</i>)	Pericarps	Hexane	1	0
Chilli (<i>Capsicum annuum</i>)	Pericarps	Ethyl acetate	0.5	21.4
Chilli (<i>Capsicum annuum</i>)	Pericarps	Methanol	0.5	20.7
Chilli (<i>Capsicum annuum</i>)	Pericarps	Methanol	1	43.6
<i>Gloriosa superba</i>	Seeds	Hexane	0.5	0
<i>Gloriosa superba</i>	Seeds	Methanol	0.5	0
<i>Bougainville spectabilis</i>	Green leaves	Hexane	0.5	0
<i>Bougainville spectabilis</i>	Green leaves	Ethyl acetate	0.3	10
<i>Bougainville spectabilis</i>	Green leaves	Methanol	0.5	0
<i>Bougainville spectabilis</i>	White bract	Hexane	0.5	0
<i>Bougainville spectabilis</i>	White bract	Ethyl acetate	0.3	7
<i>Bougainville spectabilis</i>	White bract	Methanol	0.5	0
<i>Bougainville spectabilis</i>	Pink bract	Hexane	0.5	0
<i>Bougainville spectabilis</i>	Pink bract	Ethyl acetate	0.3	7
<i>Bougainville spectabilis</i>	Pink bract	Methanol	0.5	0
<i>Solanum viarum</i>	Berries	Hexane	1	0
<i>Solanum viarum</i>	Berries	Ethyl acetate	1	31.2
<i>Solanum viarum</i>	Berries	Methanol	1	31.2
<i>Albizzia amara</i>	Leaves	Hexane	1	0
<i>Albizzia amara</i>	Leaves	Ethyl acetate	1	0
<i>Albizzia amara</i>	Leaves	Methanol	1	0
Soybean (<i>Glycine max</i>)	Leaves	Hexane	1	0
Soybean (<i>Glycine max</i>)	Leaves	Ethyl acetate	1	25
Soybean (<i>Glycine max</i>)	Leaves	Methanol	1	20

Note: Percent mycelial growth inhibition was calculated using the formula $P=(C-T/C)\times 100$, where C is the mycelial diameter of the control and T that of treated ones. The values given are the average of two replications.

TABLE 2: ANTIFUNGAL ACTIVITY OF EXTRACTIVES OF ONION SEEDS, CHILLI SEEDS AND CHILLI PERICARPS AGAINST THE SPORE GERMINATION OF *Cladosporium cucumerinum* BY TLC BIOAUTOGRAPHY

Plant	Plant part	Solvent used for preparation of the extractive	Inhibition at a dose of 1.0 mg (Direct bioautography without prior elution)	Rf value of inhibition spot after elution with EtOAc
Onion (<i>Allium cepa</i>)	Seeds	Hexane	+	0.49
Onion (<i>Allium cepa</i>)	Seeds	Ethyl acetate	+	0.49
Onion (<i>Allium cepa</i>)	Seeds	Methanol	-	0.79
Chilli (<i>Capsicum annuum</i>)	Seeds	Hexane	++	0.72
Chilli (<i>Capsicum annuum</i>)	Seeds	Ethyl acetate	+	0.62, 0.72
Chilli (<i>Capsicum annuum</i>)	Seeds	Methanol	-	0.72 (faint)
Chilli (<i>Capsicum annuum</i>)	Pericarps	Hexane	++	0.72
Chilli (<i>Capsicum annuum</i>)	Pericarps	Ethyl acetate	+	0.62, 0.72
Chilli (<i>Capsicum annuum</i>)	Pericarps	Methanol	-	0.72 (faint)

Note: Observation on *Cladosporium cucumerinum* inhibition taken after an incubation of 4 d; Thickness of the plate=0.5 mm; Diameter of the spot 1.2 cm; + indicates inhibition; ++ indicates conspicuous inhibition; - indicates no inhibition

growth inhibition at a concentration of 0.5 % level. Methanol extractives of *Solanum viarum* berries and ethyl acetate extractive of soybean leaves exhibited more than 20 % mycelial growth inhibition at a concentration of 1.0 % level. Hexane extractives of onion seeds, chilli seeds and chilli pericarps did not show activity against the mycelial growth of *Colletotrichum gloeosporioides*. The extractives of onion leaves, *Albizia amara* leaves, *Bougainvillea spectabilis* (green leaves, white bracts and pink bracts) and seeds of *Gloriosa superba* also did not show any significant activity.

Chemical nature of some antifungal compounds present in the presently investigated plants has been reported by earlier workers. An antimicrobial protein has already been isolated from onion seeds^[3]. But it is doubtful whether the antifungal activity of ethyl acetate extractive presently reported is due to this protein since ethyl acetate extractive has only moderate polarity. Antifungal activity of capsaicin present in chilli has been established^[4]. Antifungal activity of glycoalkaloids of *Solanum viarum* has been reported^[5]. Antifungal activity of isoflavones^[6] in soybean leaves and glyceollins^[7] in infected soybean leaves also has been reported.

The results presented in Table 1 shows that the hexane extractives of onion seeds, chilli seeds and chilli pericarps do not show activity against the mycelial growth of *Colletotrichum gloeosporioides* even at a high concentration of 1 % level. But Table 2 shows that these extractives show activity against the spore germination of *Cladosporium cucumerinum* on TLC plate. A similar trend was observed in the earlier

studies reported from this institute in the cases of watermelon seeds and tomato seeds^[8,9]. Most probably the fatty acid esters present in the hexane extractives of these vegetable seeds (watermelon, tomato, onion and chilli) have an antagonistic effect on the antifungal compounds as far as mycelial growth inhibition of *Colletotrichum gloeosporioides* is concerned. But this antagonistic effect of fatty acid esters is not effective in case of activity against the spore germination of *Cladosporium cucumerinum*. It is noteworthy that the ethyl acetate extractives of onion seeds, chilli seeds and chilli pericarps exhibit activity against the mycelial growth of *Colletotrichum gloeosporioides* and spore germination of *Cladosporium cucumerinum* on TLC plate. Direct bioautography of the methanol extractives of onion seeds, chilli seeds and chilli pericarps on TLC plate without prior elution with ethyl acetate did not exhibit any inhibition. This is most probably due to the presence of some carbohydrates or peptides present in the methanol extractives which promote the growth of *Cladosporium cucumerinum*. But if TLC plates spotted with methanol extractives are previously eluted with ethyl acetate and subsequently sprayed with the inoculum, inhibition zones are seen upon incubation.

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