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IN VITRO ANTIFUNGAL ACTIVITY OF *Launaea nudicaulis* (Hook.f.)AGAINST THE TEST PATHOGEN *Colletotrichum falcatum* WENT.

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ABSTRACT

50 % Hydroethanolic extract of different locality available plant path of 7 Asteraceous plants were evaluated for their antifungal activity against the test pathogen *colletrotrichum falcatum* went. The leaf extract of *Launaea nudicaulis (Hook.f.)* exhibited maximum fungi toxicity. The leaf extract was further fractionated in different organic solvents and antifungal activity was recorded only in petroleum ether fraction. Minimum inhibitory concentration of petroleum ether fraction was worked out and it was found 1.2 micro g/ml. At this MIC the fraction was found fungicidal.

KEYWORDS: Antifugal Activity, Hydrothanolic Extract, Asteraceous Plant, *Launea nudicaulis, Colletotrichum folcatum.*

Plants develop a variety of chemicals inside them as their defensive mechanism to resist the natural antigen they face. There are several synthetic fungicides available in market but several of them have been proved carcinogenic teratogenic or they cause a variety of side effects in plants or in human being. In contrast the fungicides of plant origin are easily biodegradable and they do not cause undesired side effects in living beings. A lot of word has been done in recent years for the investigation of antifungal activity substances in plants. (2, 9 & 10). A number of Asteraceous plants have been found by various workers which contain antifungal compounds (1 &13). In the present investigation 50% hydrothanolic extract of some locally available Asteracous plant part have been evaluated for their antifungal activity against the test pathogen *collectotrichum falcatum* went the causal organisms of red rot of sugarcane. Only the extract of the leaf of *Launaea nudicaulis (Hook.f.)* was found 100% antifungal. It was fractionated in different organic solvent by differential solubility method. Only the petroleum ether fraction was found to contain antifungal constituents in it and minimum inhibitory concentration was worked out.

Different available parts of Asteraceous plants were locally collected and identified with the help of Ph.D. thesis on Azamgarh (3 &12). 50 % hydrothanolic extract of plants parts was made by crashing 5gm plant material in 25 ml of 50% ethanol (v/v) and the mixture was left whole night for the maximum extraction of compound in solution. The mixture was filtered through double layered cheese cloth. This extract was assayed for antifungal activity against the test pathogen *colletotrichum falcatum* went by the modified paper disk technique. Czapek Dox Agar medium was prepared and sterilized at 15lb/inch pressure at 121^0C for 30 minutes in as autoclane and then at the rate of 500 mg/fiter ciprofloxauine was added to it and mixed thoroughly for prevention of bacterial contamination during experimentation.

colletotrichum falcatum went the causal organism of Red Rot of Sugarcane, was lab cultured from the infected leaves of sugarcanes, collected from the field. The diseases spots were surface sterilized by 0.01% solution of mercuric chloride ($HgCl_2$) and the out spots were thoroughly washed repeatedly by sterile water to remove the traces of Hgcl2. The out pieces, containing disease spots, were aseptically transferred to the surface of potato dextrose agar medium in a petriplate. The pathogen spared over the surface of the medium in a week. This pathogen was identified on the basis of microscopic examination of conidia and after confirming the identifications the pathogen was reinoculated on presterilized petridish containg Czapek Doz Agar medium. This medium is suitable for mycelia growth of the pathogen where as Potato Dextrose Agar Medium facilitates sporulation of the pathogen.

2 ml of the hydroethanolic extract of each sample was impregnated in an Whatman No.01 paper disk (Diameter 19nm.) by repeatedly dipping and drying the disk under a running hair drier with help of a sterilized forceps. These disks were designated as “Treatment disks” for preparing control disks the Whatman No.01 paper disks were impregnated similarly with the same

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amount of 50% ethanol. These assay disks were aseptically transferred to the centre of pretridishes (diameter 70nm.) containing 10 ml. of Czapek Dox Agar medium / plate. A mycelial disc (diameter 5mm.) cut from the periphery of a 7- day old culture of the test pathogen, was aseptically inoculated upside down to the centre of each assay disk in 'treatment' as well as 'control' sets. The plated were incubated at room temperature for 6 days and observation were recorded on the 7th day. Unless mentioned otherwise, the experiment sets were kept in triplicates and repeated twice throughout the course of the present investigation. Colony diameter of the test fungus in 'treatment' and 'control' sets were measured in mutual perpendicular direction and fungi toxicity was recorded in terms of percent mycelial inhibition, calculated as per formula-

%mycelial inhibition = 100 x (dc-dt)/dc

Where,

dc = mean colony diameter of control and

dt = mean colony diameter of 'treatment'

The results are given in Table – 1.

Table 1: Investigation of antifungal activity in some Asteraceous plants against *Colletotrichum falcatum* Went.

Sl. No.	Plants	Plant Part	% Mycelial inhibition
01.	*Blumea lacera* D.C.	Whole Plant	39.47
02.	*Ohrysanthemum indicum* L.	Leaf Flower	14.44 11.11
03.	*Edipta alba* Linn.	Whole Plant	46.42
04.	*Parthenium hysterophorus* L.	Whole Plant	52.26
05.	*Tegetes erecta* Linn.	Leaf Flower	20.85 14.44
06.	*Tridex procumbans* Linn.	Whole Plant Leaf	11.11 57.14
07.	*Launea nudicaulis* Hook. F.	Leaf	100.00

The hydroethanolic extract of active plant part were fractionated by 'Differential Solubility Method'. It is the general principle that different organic solvents have different polarity i.e. efficiency to dissolved specific compounds into them. On the basis of the principle, the hydroethanolic extract of active plants part was treated with different organic solvents one by one. The non-polar solvents were used first and then other solvents were used in increasing order of polarity i.e. in the order of petroleum ether, benzene, corbon tetrachloride, choloform, acetone and finally methanol to fractionate the compounds in different fraction. All the fraction were tested for antifungal activity against the test pathogen by the usual modified paper disk technique and the result were tabulated in table – 2.

Table 2: Antifungal Activity in different fraction of fruit (The Active Plant Part) of *Launea nudicaulis*

Sl. No.	Different fraction of the active plant part	% myceliel inhibition
1.	Petroleum Ether	100
2.	Benzene	0
3.	Carbon Tetrachloride	0
4.	Chloroform	0
5.	Acetone	0
6.	Methanol	0

To prepare different concentration of the constituents of the active fraction, the solvent of the active fraction was evaporated at room temperature, the reminder was weighed on a chemical balance and again dissolved in a known volume of the solvent (10% methanol) to get a known concentrate solution of the active fraction. It was further diluted in measured amount of sterilized Czepek Dox Agar medium (as per req.)to make different concentration of the active fraction and each concentration was subjected to antifungal bioassay by the 'Poisoned Food Technique' adopted by Grover and Moore (1962). The data on % myceliel inhibition were recorded in Table – 3.

Table 3: Effect of various concentration of the active fraction on percent myceliel inhibition of the test fungus.

Concentration mg/liter	% myceliel inhibition
2.0	100.00
1.8	100.00
1.6	100.00
1.4	100.00
1.2	100.00
1.0	94.00

Out of different parts of 7 plants screened, the hydroethanolic extract of the leaf *Launea nudicaulis* Hook. F. exhibited 100% antifungal activity against the test pathogen. It is useful to screen all the available plant parts to obtain the knowledge about the distribution of fungitoxic factor in a plant. This may further be helpful in deciding the hervestation of a particular plant part with maximum activity without eradicating the whole plant. The use of 50% ethanol has been made in the present work which ensures extraction of maximum compounds (ethanol and water have maximum polarity) as well as facilitates further purification of active(4). Aqueous extract juices may lose their efficacy due to degradation of active constituents by continued enzymatic activity.

Several workers first isolated different compounds from the plants and them their antimicrobial activity was tested in vitro (6, 9 & 11). Another aspect is that some workers first tested the antimicrobial activity of plants and of they were found antimicrobial then fractionation was done and each fraction was assayed against the test pathogen to find out the active fraction (7 & 14). In the present investigation the hydroethanolic extract of *Launea nudicaulis* leaf was found to contain antifungal activity and it was fractionated by different solubility method. After fractionation of hydroethanolic extract in different organic solvent each fraction assayed against the test pathogen. Only the petroleum ether fraction was found to possess antifungal activity.

Minimum inhibitory concentration (MIC) of some active fraction compounds has been studied by several workers and found to vary considerably. In the present investigation MIC of the active petroleum ether fraction was found 1.2 micro g/ml. the variations in the MICs of various fraction may either be due to the differences in the chemical composition of the active constituents or due to differences in resistance of different test fungi. In the present study the active fraction was proved to be fungicicial at MIC.

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