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Antifungal Activity of 50% Aqueous-Ethanolic Extract of Leaves of *Calotropis procera* R.Br.

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Abstract- The analysis of antifungal activity of aqueous-ethanolic extract of leaves of *Calotropis procera* R.Br. against *Botrytis cinerea, Rhizopus oryzae, Fusarium oxysporum* was carried out in disc method. The zone of inhibition produced by the crude 50% aqueous ethanolic leaf extract against sensitive fungi was examined. The results obtained showed that 50% aqueous ethanolic leaf extract of *Calotropis procera*, has inhibitory effect on the growth of isolates. The effect exhibited by ethanolic extract of leaves was significant. 50% aqueous-ethanolic extract of leaves of *Calotropis procera* R.Br. was partitioned sequentially in petroleum ether, benzene and chloroform and tested for antifungal activity. It was observed that sample obtained from chloroform portion showed most inhibitory effect. Samples obtained from petroleum ether and benzene portion also showed significant inhibitory effect on the growth of isolates.

Index Terms- Antifungal activity, *Calotropis procera* R.Br. *Botrytis cinerea*

I. Introduction

Calotropis procera belongs to the family Asclepiadaceae, is a soft wooded, evergreen perennial shrub. It is a xerophytic erect shrub, growing widely throughout the tropical and sub-tropical regions of Asia and Africa. This plant is popularly known because it produces large quantity of latex. Medicinal plants always remained the major sources of traditional medicine worldwide (Goyal et al, 2011). It is clear that the plant kingdom harbours an inexhaustible source of effective phytocompounds valuable in the management of phytopathogenic disease. It is evident that the use of the plants, plant extracts and pure compounds isolated from natural sources has always provided a foundation for modern pharmaceutical compounds (Murti et al, 2012). Pharmacologically substances such as calotropon , calotropin , uscharine , calotoxin , calctin , uscharidin and calotropagenin etc. have been reported from the leaves and latex of *Calotropis procera* (Rohit Sharma et.al,2012). Antimicrobial activity of *Calotropis procera* was previously screened (Kareem et al., 2008; Varahalarao Vadlapudi et al., 2004). An antibacterial cardenolide, proceragenin, active against gram positive and gram negetive bacteria has also been reported (Nargis Akhtar, Abdul Malik, March 2000). The present study was undertaken to assay the antifungal activity of 50% aqueous ethanolic extract of *Calotropis procera* against *Botrytis cinerea, Rhizopus oryzae,* and *Fusarium oxysporum*. Antifungal activity was screened by agar cup method (Perez et al., 1990; Alade and Irobi et al., 1993; Abioye et al., 2004).

II. MATERIAL AND METHODS

1.Plant material:

Calotropis procera leaves were collected from Bamanpukur, Sree Mayapur, Nadia during the month of June – July 2013. The plant material was identified at the field using standard keys and descriptions.

2. Method of extraction:

Solvent – 50% aqueous ethanol, petroleum ether, benzene, chloroform

Method – Maceration

Procedure:

The collected plant leaves were sun dried and powdered with electric blender. 100 gm. of the powered plant material was soaked in 1L. of 50% aqueous ethanol for seven days. Material was filtered first with a muslin cloth and then with filter paper. Residue was repeatedly soaked with 50% aqueous ethanol and filtered until the extract became colourless. Samples obtained from 50% aqueous ethanolic extract was concentrated in vacuum evaporator. The residual mass collected and dissolved in propylene glycol. The sample then applied for antifungal assay to find antifungal activity. 50% aqueous ethanolic extract obtained from dried powdered plant material of *Calotropis procera* was partitioned sequentially in petroleum ether, benzene and chloroform. Each fraction thus obtained was concentrated in vacuum evaporator. The residual mass were collected separately and dissolved in propylene glycol. The three samples were subjected to antifungal assay to locate any antifungal activity of these samples.

3. Collection of fungal strain :

The reference strains used in the antifungal assays were: *Fusarium oxysporum* (MTCC No. 1755), *Botrytis cinerea* (MTCC No. 2104) and *Rhizopus oryzae (*MTCC No. 8784). All the three fungal strains were procured from the plant biochemistry, molecular biology & advance plant physiology research laboratory, Department of Botany, University of Kalyani, West Bengal, India. Test fungal strains were maintained on PDA medium (Ph -6.6) slants at 29^0 C for antifungal assay.

4. Fungicidal Assay:

Antifungal activity was screened by agar cup method (Perez et al., 1990;Alade and Irobi et al., 1993; Abioye et al., 2004). At first the PDA medium was poured into the sterile petriplates and kept into the Laminar Air Flow cabinet until the medium became solidified. Next the test fungal cultures were evenly spread over the media by sterile cotton swabs. Then by using sterile cork borer, wells of 9 mm were made in the medium. 100ul of each samples having different concentration were applied to the petri plates containing PDA medium. Plates containing pure culture of *Botrytis cinerea* and *Rhizopus oryzae* were incubated at 29^0 C for 48- 72 hours. In case of *Fusarium oxysporum* incubation was done at 29^0 C for 18-20 days. After the incubation period was over, all the plates were observed for the formulation of inhibition zone and to locate antifungal nature of the samples. The zone of inhibition was measured by using millimetre scale. One control set was prepared by Appling pure cultures of fungus strains to petriplates containing PDA medium. These observations were made triplicate applying sample concentration / fungus combination.

III. RESULT AND DISCUSSION

TABLE 1: Antifungal effect of 50% aqueous ethanolic leaf extract of *Calotropis procera*

Concentration of sample (mg / ml)		Diameter of inhibition zone against fungus (in mm)			Minimum inhibitory concentration (MIC)
		Botrytis cinerea(MTCC No. 2104)	*Rhizopus oryzae* (MTCC No. 8784)	*Fusarium oxysporum* (MTCC No. 1755)	
Control /0		-	-	-	
5		-	-	-	
10		-	-	-	
15		-	-	-	
20	Mean Std.Dev	2.14 0.06	-	-	20mg/ml in *Botrytis cinerea*
25	Mean Std.Dev	7.33 0.07	3.11 0.02	2.11 0.02	25 mg/ml in *Rhizopus oryzae* and *Fusarium oxysporum*
30	Mean Std.Dev.	8.83 0.02	4.25 0.05	5.31 0.02	
35	Mean Std.Dev.	10.16 0.02	8.11 0.02	9.18 0.02	
40	Mean Std.Dev.	19.23 0.02	18.08 0.07	14.23 0.02	
45	Mean Std.Dev.	27.10 0.10	21.20 0.08	19.86 0.07	
50	Mean Std.Dev.	38.10 0.08	29.11 0.02	25.18 0.05	
SE CD at 5% level		±0.04 0.08	±0.03 0.06	±0.02 0.05	

The observed values are expressed as mean ± standard deviation (std.dev.).

All the values expressed in the table are statistically analysed by ONE WAY ANOVA TEST in respect of control set and found significantly different at 5% level ($p< 0.05$). Control set showed no inhibition zone.

TABLE 2: Determination of antifungal activity of the sample obtained from petroleum ether extract

Concentration of sample (mg / ml)		Diameter of inhibition zone against fungus (in mm)			Minimum inhibitory concentration(MIC)
		Botrytis cinerea	*Rhizopus oryzae*	*Fusarium oxysporum*	
Control /0		-	-	-	
5		-	-	-	
10		-	-	-	
15		-	-	-	
20		-	-	-	
25		-	-	-	

30	Mean Std.Dev.	3.59 0.03	-	-	30mg/ml in *Botrytis cinerea*
35	Mean Std.Dev.	6.05 0.04	4.29 0.04	3.85 0.14	35 mg/ml in *Rhizopus oryzae* and *Fusarium oxysporum*
40	Mean Std.Dev.	8.76 0.14	6.03 0.02	5.48 0.24	
45	Mean Std.Dev.	11.71 0.06	9.98 0.02	7.35 0.10	
50	Mean Std.Dev.	14.93 0.03	12.07 0.05	10.62 0.07	
55	Mean Std.Dev.	16.46 0.38	18.18 0.06	17.24 0.04	
60	Mean Std.Dev.	21.53 0.26	23.13 0.03	20.12 0.02	
SE CD at 5% level		±0.11 0.23	±0.02 0.04	±0.07 0.14	

The observed values are expressed as mean ± standard deviation (std.dev.).

All the values expressed in the table are statistically analysed by ONE WAY ANOVA TEST in respect of control set and found significantly different at 5% level (p< 0.05). Control set showed no inhibition zone.

TABLE 3: Determination of antifungal activity of the sample obtained from benzene extract

Concentration of sample (mg / ml)		Diameter of inhibition zone against fungus (in mm)			Minimum inhibitory concentration(MIC)
		Botrytis cinerea	*Rhizopus oryzae*	*Fusarium oxysporum*	
Control /0					
5		-	-	-	
10		-	-	-	
15		-	-	-	
20		-	-	-	
25	Mean Std.Dev.	-	-	3.97 0.01	25 mg/ml in *Fusarium oxysporum*
30	Mean Std.Dev.	4.60 0.09	3.29 0.03	6.60 0.02	30 mg /ml in *Botrytis cinerea* and *Rhizopus oryzae*
35	Mean Std.Dev.	7.45 0.11	7.30 0.02	8.97 0.04	
40	Mean Std.Dev.	10.26 0.07	10.58 0.03	12.14 0.03	
45	Mean Std.Dev.	14.16 0.06	14.12 0.02	15.19 0.07	
50	Mean Std.Dev.	17.24 0.02	19.21 0.00	20.14 0.03	
55	Mean Std.Dev.	20.14 0.04	23.14 0.03	25.64 0.02	
60	Mean Std.Dev.	25.67 0.04	27.58 0.03	29.12 0.02	
SE CD at 5% level		±0.04 0.08	±0.01 0.03	±0.02 0.05	

The observed values are expressed as mean ± standard deviation (std.dev.).

All the values expressed in the table are statistically analysed by ONE WAY ANOVA TEST in respect of control set and found significantly different at 5% level (p< 0.05). Control set showed no inhibition zone.

TABLE 4: Determination of antifungal activity of the sample obtained from chloroform extract

Concentration of sample (mg / ml)		Diameter of inhibition zone against fungus (in mm)			Minimum inhibitory concentration(MIC)
		Botrytis cinerea	*Rhizopus oryzae*	*Fusarium oxysporum*	
Control /0		-	-	-	
5		-	-	-	
10		-	-	-	
15		-	-	-	
20	Mean Std.Dev.	5.97 0.01	3.16 0.02	-	20 mg/ml in *Botrytis cinerea* and *Rhizopus oryzae*
25	Mean Std.Dev.	12.96 0.02	8.23 0.03	2.08 0.02	25 mg/ml in *Fusarium oxysporum*
30	Mean Std.Dev.	18.56 0.02	12.54 0.03	6.15 0.02	
35	Mean Std.Dev.	23.28 0.05	15.11 0.01	10.18 0.08	
40	Mean Std.Dev.	30.15 0.04	23.52 0.02	16.14 0.02	
45	Mean Std.Dev.	38.14 0.01	29.11 0.01	21.25 0.03	
50	Mean Std.Dev.	43.58 0.03	34.17 0.02	30.21 0.10	
55	Mean Std.Dev.	48.27 0.07	40.07 0.07	36.09 0.05	
60	Mean Std.Dev.	54.21 0.07	45.26 0.13	42.14 0.02	
SE CD at 5% level		±0.02 0.05	±0.03 0.07	±0.03 0.07	

The observed values are expressed as mean ± standard deviation (std.dev.).

All the values expressed in the table are statistically analysed by ONE WAY ANOVA TEST in respect of control set and found significantly different at 5% level ($p < 0.05$). Control set showed no inhibition zone.

Table 1 showed minimum inhibitory concentration (MIC) of 50% aqueous ethanolic leaf extract of *Calotropis procera* against *Botrytis cinerea* (20mg/ml), *Rhizopus oryzae* (25mg/ml), and *Fusarium oxysporum* (25mg/ml). Table 2 showed that phytocompounds obtained from petroleum ether extract have most inhibitory effect against *Botrytis cinerea* having MIC value 30mg/ml followed by *Rhizopus oryzae and Fusarium oxysporum* having MIC value 35mg/ml. From table 3 it is clear that active principles obtained from benzene extract showed better inhibition against *Fusarium oxysporum* having MIC value 25 mg/ml followed by *Botrytis cinerea* and *Rhizopus oryzae* having MIC value 30 mg/ml. Table 4 clearly indicates that compounds obtained from chloroform extract have most inhibitory effect against *Botrytis cinerea* and *Rhizopus oryzae* having MIC value 20 mg/ml followed by *Fusarium oxysporum* having MIC value 25mg/ml.

Control set showing no inhibition zone in *Rhizopus oryzae*

50% aqueous ethanolic extract showing inhibition zone in *Rhizopus oryzae* (50mg/ml)

Control set showing no inhibition zone in *Botrytis cinerea*

50% aqueous ethanolic extract showing inhibition zone in *Botrytis cinerea* (50mg/ml)

Control set showing no inhibition zone in *Fusarium oxysporum*

50% aqueous ethanolic extract showing inhibition zone in *Fusarium oxysporum* (50mg/ml)

IV. CONCLUSION

Table 1 showed antifungal effect of 50% aqueous ethanolic leaf extract against *Botrytis cinerea, Rhizopus oryzae* and *Fusarium oxysporum.* From this it can be concluded that 50% aqueous ethanolic leaf extract of *Calotropis procera* may be used as potent fungicide in agricultural purpose. Further purification of the 50% aqueous ethanolic extract using different solvents such as petroleum ether, benzene and chloroform was done. It was observed that samples obtained from these solvent extract showed potent antifungal activity. Samples obtained from

chloroform extract showed most inhibitory effect against all fungi having minimum inhibitory concentration 20mg/ml against *Botrytis cinerea* and *Rhizopus oryzae* and 25 mg/ml against *Fusarium oxysporum.* So it can be concluded that samples obtained from chloroform extract of *Calotropis procera* R.Br. may be used to control agricultural damage caused by *Botrytis cinerea, Rhizopus oryzae* and *Fusarium oxysporum.*.

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