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ORIGINAL RESEARCH ARTICLE

Study of Antifungal Property in the Crude Leaf Extract of *Ixora coccinea* against *Botrytis cinerea*

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ABSTRACT

This paper represents the antifungal property of the crude leaf extract of *Ixora coccinea* against *Botrytis cinerea*. First, the crude extract was evaluated to confirm the presence of antifungal potentiality of this plant. As the result was positive, we performed sequential solvent partitioning of the crude leaf extract of *Ixora coccinea* to locate which fraction actually carries the antifungal one. Diethyl ether fraction was found to contain some bioactive *phytochemical(s) that can be of ecofriendly use to control the spreading of Botrytis cinerea*.

Key words: *Phytochemicals*, Diethyl ether fraction, Active principles, *Ixora coccinea*, *Botrytis cinerea*.

1. INTRODUCTION

In India, different phytochemicals have been identified by various therapeutic purposes since from the era of Charak, Sushruta and Banbhatta. Over the years, various medicinal plants and their extracts have been reported to be effective in the treatment of diseases. Plants produce an enormous array of secondary metabolites, and it is commonly reasoned that a significant part of this chemical diversity serves to protect plants against plant pathogens. The problems of environmental pollution have stimulated investigations of alternative strategies for the control of pests and pathogens [1,2]. Apart from conventional fungicides and microbial biocontrol agents, plant products/extracts have found to be effective against a wide range of pathogens [3, 4]. Furthermore, plant product based biofungicides are systemic, specific in action, nonphytotoxic, cost effective and have poor environmental retention [5].

Ixora coccinea L. belongs to the family Rubiacae, is a common flowering shrub native to Asia including Bangladesh, Southern India, and Sri Lanka [6]. The flowers of *I. coccinea* are used in the treatment of dysentery, leucorrhoea, dysmenorrheal, haemoptysis, bronchitis and scabies [7]. The antimicrobial properties of the flower [8] and leaves [9] have also been reported.

Botrytis cinerea is an airborne plant pathogen with a necrotrophic lifestyle attacking over 200 crop hosts worldwide. Although there are fungicides for its control, many classes of fungicides have failed due to its genetic plasticity. It has become an important model for molecular study of necrotrophic fungi. *Botrytis cinerea* affects many plant species, although its most notable hosts may be wine grapes.

Present study involves the biological control of *Botrytis cinerea* using the crude leaf extract of *Ixora coccinea*. Present study incorporates some additional evaluation of *Ixora coccinea* as an ecofriendly biocontrol agent of *Botrytis cinerea*.

2. MATERIALS AND METHODS

2.1 Preparation of *Ixora coccinea* leaf extract:

500 g sun dried leaves of *Ixora coccinea* were ground to a fine powder and then extracted in 1.5 liter of 50% aqueous ethanol at room temperature for 7 days. The extract was filtered and concentrated under reduced pressure and a solid, dark brown residual solid (5 g) was obtained. The residual solid obtained was then subjected to sequential solvent partitioning for locating the antifungal property of the plant. As the crude extract was positive in antifungal assay, sequential solvent partitioning of the crude leaf extract of

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Ixora coccinea and identification of the antifungal fraction was performed.

2.2 Sequential solvent partitioning of the crude leaf extract of *Ixora coccinea* and identification of the antifungal fractions:

The extract was filtered and the filtrate was charcoalised. The charcoalised fraction was filtered repeatedly through Whatman No.42 filter paper and a clear brown filtrate was obtained. The filtrate was then successively partitioned over petroleum-ether (60-80ºc), diethyl ether and chloroform. Each fraction was collected separately, dried over anhydrous sodium sulphate and was concentrated under reduced pressure. A brown residual solid mass was obtained in each case. Four different concentrations (10 mg ml^{-1}, 20 mg ml^{-1}, 40 mg ml^{-1}, and 80 mg ml^{-1}) of the residue obtained from each of the solvent fraction were prepared and their antifungal property was evaluated. In each of the experiments a control set was maintained and MIC was calculated by proper measurement.

2.3 Preparation of sample solution:

The test solution was prepared by dissolving the dark brown residual mass in few drops of propylene glycol and then diluting with sterile water [10] in the concentration of 10 mg ml^{-1}, 20 mg ml^{-1}, 40 mg ml^{-1}, and 80 mg ml^{-1}. Few drops of propylene glycol diluted with sterile water were used as control. All the dilutions were sterilized by filtration using membrane filter (0.02 µ pore size).

2.4 Fungal strains:

Pure cultures of *Botrytis cinerea* was procured from MTCC (MTCC /06/7/4260, Code- 359), India.

2.5 Media preparation:

The potato tubers were peeled off and weighed for about 250 g tubers were chopped into small pieces into the sterile conical flask. After boiling the supernatant were collected and dextrose (20g) with agar (20g- Microbiology Grade) to dissolve the ingredients. The pH of the medium was adjusted to 6.8 – 7.0. The total volume of the medium was adjusted to one liter. Finally the medium was sterilized in autoclave at 121^0C for 17 minutes.

2.6 Antifungal assay:

Antifungal assay was done following the method of Kordali *et al*., 2005 [11]. The fungal cultures used for this assay were 3 days old. Fungal suspension was prepared to contain approximately 1 x 10^6 CFU ml^{-1}. An overnight broth culture was used to seed sterile molten PDA medium maintained at 45 °C. Small cylinders of agar were cut and scooped out using 7 mm sterile cork borer. 0.1 ml of test solutions of different above mentioned concentrations were loaded separately to each cup with the help of micropipette. Propylene glycol with sterile water was loaded to maintain control. The petridishes were sealed with a strip of paraffin and incubated at 37ºC ± 1ºC for 24 hrs. The test solutions were allowed to diffuse into the agar from the cup. After incubation, the diameter of the inhibition zone around the well, were measured in cm every day after the fungal growth. The final measurement was taken when the control reached the full size within the petridish. If a culture grew in an irregular shape, two or more measurements were made and average was recorded. From the growth diameter of the fungal colony, the percentage of inhibition and the effective concentration for colony growth inhibition was calculated.

2.7 Statistical analysis:

All data recorded in this experiment are statistically analyzed by using ANOVA (Analysis Of Variance), based on F-statistics [12, 13, 14] However, it does not indicate which specific group pair(s) are the ones where statistical difference occur. So, in this present experiment, Post Hoc Test was used in conjunction with ANOVA to determine which specific group pair(s) is statistically different from each other.

3. RESULTS

3.1 Antifungal screening of the crude extract:

It is interesting to note that from the investigation of screening for biological activity of *Ixora coccinea*, against *Botrytis cinerea*, the result obtained from table 1A, 1B and 1C confirmed the antifungal potentiality of the crude plant leaf extract of *Ixora coccinea*. The activity was studied in respect to its fungicidal property against phytopathogenic *Botrytis cinerea*. (**Table 1A**) shows that the crude extract of 10 mg ml^{-1} of *Ixora coccinea* was the minimum inhibitory concentration of *Botrytis cinerea* having inhibition percentage of 9.36. Inhibition percentage increases with the increase concentration of crude extract. At 80 mg ml^{-1} the inhibition percentage was 46.87. (**Table 1B & 1C**) shows statistical calculations and confirm the value to be significant at 5% level.

3.2 Antifungal screening of the different solvent fractions obtained from crude leaf extract:

The results from (**Table 2A**) shows that only the diethyl ether fraction (60°-80°C) possess the

antifungal activity. The concentration of this fraction at 10 mg ml^{-1} shows the diameter of inhibition zone was 1.1768. Here again, the diameter of inhibition zone increases with the increase concentration of crude extract. At 80 mg ml^{-1} the diameter of inhibition zone was 7.1312. (**Table 2B & 2C**) shows statistical calculations and confirm the value to be significant at 5% level.

Table 1A: Studies on antifungal efficiency of crude leaf extract of *Ixora coccinea* against *Botrytis cinerea* by cup diffusion method

Fungus taken	Treatment (mg ml^{-1})	Diameter of Inhibitory Zone (cm)	Inhibition (%)
Botrytis cinerea	Control	0.0000	0
	10	0.8796	9.36
	20	1.8299	21.78
	40	2.0583	23.13
	80	4.2189	46.87

Table 1B: One way ANOVA to calculate the statistical significance among the antifungal effects of different doses (10, 20, 40 and 80 mg ml^{-1}) of crude leaf extract with that of the control one

Analysis	F	Sig.
One way ANOVA Among Groups	3.381	p <0.05

Table 1C: Post Hoc Tests (Multiple comparisons), LSD

Dependant variable	Other variables	Mean Difference	Sig.
Control (without treatment)	Treated with 10 mg/ml	0.880	p <0.05
	Treated with 20 mg/ml	1.83	p <0.05
	Treated with 40 mg/ml	2.07	p <0.05
	Treated with 80 mg/ml	4.22	p <0.05
Treated with 10 mg/ml	Control (without treatment)	0.880	p <0.05
	Treated with 20 mg/ml	0.949	p <0.05
	Treated with 40 mg/ml	1.19	p <0.05
	Treated with 80 mg/ml	3.34	p <0.05
Treated with 20 mg/ml	Control (without treatment)	1.83	p <0.05
	Treated with 10 mg/ml	0.949	p <0.05
	Treated with 40 mg/ml	0.241	p <0.05
	Treated with 80 mg/ml	2.39	p <0.05
Treated with 40 mg/ml	Control (without treatment)	2.07	p <0.05
	Treated with 10 mg/ml	1.19	p <0.05
	Treated with 20 mg/ml	0.241	p <0.05
	Treated with 80 mg/ml	2.15	p <0.05
Treated with 80 mg/ml	Control (without treatment)	4.22	p <0.05
	Treated with 10 mg/ml	3.34	p <0.05
	Treated with 20 mg/ml	2.39	p <0.05
	Treated with 40 mg/ml	2.15	p <0.05

The antifungal effect of control dose and different doses of crude leaf extract was statistically measured by comparing each of them with the others with significant mean difference at 0.05 probability level with variance ratio F = 3.381 (p <0.05).

Table 2A: Antifungal screening of different solvent fractions obtained from crude leaf extract of *Ixora coccinea* against *Botrytis cinerea*

Fungus taken	Dose (mg ml^{-1})	Diameter of Inhibition Zone (cm)			MIC (mg ml^{-1})		
		PE	DE	Chl	PE	DE	Chl
Botrytis cinerea	Control	0	0	0	0	0	0
	10	0	1.1768	0		10	
	20	0	2.2119	0			
	40	0	5.2871	0			
	80	0	7.1312	0			

PE = Petroleum ether (60°-80°C) fraction, DE = Diethyl ether fraction, Chl = Chloroform fraction, MIC of PE = 10 mg ml^{-1}.

Table 2B: One way ANOVA to calculate the statistical significance among the antifungal effects of different doses (10, 20, 40 and 80 mg ml^{-1}) of Diethyl ether fraction of crude leaf extract with that of the control one

Analysis	F	Sig.
One way ANOVA Among Groups	1.007	p <0.05

Table 2C: Post Hoc Tests (Multiple comparisons), LSD

Dependant variable	Other variables	Mean Difference	Sig.
Control (without treatment)	Treated with 10 mg/ml	1.17	p <0.05
	Treated with 20 mg/ml	2.21	p <0.05
	Treated with 40 mg/ml	5.28	p <0.05
	Treated with 80 mg/ml	7.13	p <0.05
Treated with 10 mg/ml	Control (without treatment)	1.17	p <0.05
	Treated with 20 mg/ml	1.03	p <0.05
	Treated with 40 mg/ml	4.10	p <0.05
	Treated with 80 mg/ml	5.95	p <0.05
Treated with 20 mg/ml	Control (without treatment)	2.21	p <0.05
	Treated with 10 mg/ml	1.03	p <0.05
	Treated with 40 mg/ml	3.07	p <0.05
	Treated with 80 mg/ml	4.91	p <0.05
Treated with 40 mg/ml	Control (without treatment)	5.28	p <0.05
	Treated with 10 mg/ml	4.10	p <0.05
	Treated with 20 mg/ml	3.07	p <0.05
	Treated with 80 mg/ml	1.84	p <0.05
Treated with 80 mg/ml	Control (without treatment)	7.13	p <0.05
	Treated with 10 mg/ml	5.95	p <0.05
	Treated with 20 mg/ml	4.91	p <0.05
	Treated with 40 mg/ml	1.84	p <0.05

The antifungal effect of control dose and different doses of Diethyl fraction of crude leaf extract was statistically measured by comparing each of them with the others with significant mean difference at 0.05 probability level with variance ratio F = 1.007 (p <0.05).

4. DISCUSSION

From the above results it can be conclude that, the diethyl ether fraction of the crude extract of *Ixora coccinea* showed antifungal activity against *Botrytis cinerea*. Hence, *Ixora coccinea* can be identified as a source of antifungal compounds.

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REFERENCES

1. Kuc J., Immunization and its applicability for disease control. In: Chet, Y. (ed.), Innovative Approaches to Plant Disease control. Vol. I. New York: Wiley; 1987. pp. 225-272.
2. Lyon GD. Reglinski T and Newton AC., Novel disease control compounds: the potential to “immunize” plants against infection. Plant Pathol. 1995; 44: 407-427.
3. Amadioha AC., Controlling rice blast *in vitro* and *in vivo* with extracts of *Azadirachta indica*. Crop Protect. 2000; 19: 287-290.

4. Bowers JH and Locke JC. Effect of formulated plant extracts and oils on population density of *Phytophthora nicotianae* in soil and control of *Phytophthora* blight in the greenhouse. Plant Dis. 2004; 88: 11-16.
5. Singh DC., Scope of medicinal and aromatic plants in pest management. International symposium, allelopathy in sustainable agriculture, forestry and environment. New Delhi, September 6-8, 1994. pp. 68.
6. Ghani A., Medicinal plants of Bangladesh with chemical constituents and uses. Dhaka, The Asiatic Society of Bangladesh, 2003. pp. 267.
7. Anonymous., The wealth of India, A Dictionary of Indian Raw materials and Industrial Products. Vol. V, Council of Scientific and Industrial Research, New Delhi. 1959. pp. 91.
8. Latha PG, Abraham TK, Panikkar KR., Antimicrobial properties of *Ixora coccinea* L. Ancient Sci. Life. 1995; 14 : 286–290.
9. Annapurna J, Amarnath PVS, Amar Kumar D, Ramakrishna SV, Raghavan KV., Antimicrobial activity of *Ixora coccinea* leaves. Fitoterapia. 2003; 74: 291–293.
10. Mukherjee PK, Saha K, Giri, SN, Pal M and Saha BP. Antifungal screening of *Nelumbo nucifera* (Nymphaeaceae) rhizome extract. Ind. J. Microbiol. 1995; 35 (4): 327.
11. Kordali S, Kotan R, Mavi A and Cakir A., Determination of the chemical composition and antioxidant activity of the essential oil of *Artemisia dracunculus* L. and of the antifungal and antibacterial activities of Turkish *A. dracunculus, A.*
12. *absinthium* and *Santonicum* essential oil. J. Agric Food Chem. 2005; 53: 9452- 9458.
13. Tamhane AC., Multiple comparisons in model I one-way ANOVA with unequal variances. Comm Statist Theory Methods. 1977; 6: 15-32 Z.
14. Gabriel KR., A Simple Method of Multiple Comparisons of Means. Journal of the American Statistical Association. 1978; 73: 364.
15. Hoaglin DC, Welsch RE., The hat matrix in regression and ANOVA. American Statistician. 1978; 32: 17-22.