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FUNGICIDAL POTENTIAL OF PURIFIED ALKALOIDS - SOLASODINE AND CAULOPHYLLUMINE-A FROM *SOLANUM MAURITIANUM* SCOP. AGAINST SOME PHYTOPATHOGENIC FUNGI

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ABSTRACT

Solanum mauritianum a shrub or small tree branched to form a rounded canopy. It is spineless and densely pubescent with sessile to long-stalked stellate hairs. The green berries ripen to yellow fruit which are globose. The fruit are borne in compact terminal clusters. The plant possesses the precursor for the synthesis of corticosteroid drugs from the unripe fruit or leaves of the plant. In this scenario, the present study aims the fungicidal potential of purified alkaloids - solasodine and caulophyllumine-A from *Solanum mauritianum* Scop. against selected phytopathogenic fungi such as *Alternaria alternata, Fusarium oxysporum, Albugo candida, Rhizoctonia solani,* and *Cercospora arachidicola* at concentrations of 0, 0.25, 0.5, 1, 1.5 and 2 and 3 (mg/l). Solasodine and caulophyllumine-A showed different degrees of fungicidal activity against the tested pathogens. When compared with the control, the highest fungicidal activity (MIC) was recorded for solasodine at a concentration of 0.15 mg/l against *F. oxysporum* while, caulophyllumine-A displayed antifungal property in the range 0.25 to 1mg/l. Alkaloids inhibited spore germination and mycelial growth significantly substantiating their fungicidal potentials. Extensive studies are warranted to trace the mode of action of the alkaloids as antifungal agent against the tested phytopathogenic fungi.

KEYWORDS: phytopathogenic fungi, *Solanum mauritianum,* solasodine and **caulophyllumine-A, inhibitory concentrations, spore germination assay.**

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INTRODUCTION

The plants belonging to Solanaceae possess several medicinal properties and are widely used for the treatment of various illnesses such as inflammations, chronic skin ailments, diarrhea etc. Phytopathogenic fungus is a group of disease causing organisms that are responsible for destructive diseases in edible crop plants and creates massive economic loss in the production of vegetables and fruits. Recent studies have proven that many higher plants possess various constituents which are effective against the control of these disease causing pathogens and are also proved to be ecofriendly when compared to the chemical fungicides. Generally, crops are protected from phytopathogenic fungi by the application of chemical fungicides. Currently, the uses of these synthetic chemicals are restricted due to their harmful effects on human health and environment.[1] Medicinal plants possess pool of bioactive secondary metabolites that are most likely responsible for the high specificity against pathogenic microorganisms.[2] These pathogenic fungi are nowadays causing serious problems in the field of agriculture, reduces the nutritional values and shelf life of fruits and vegetables.[3] Thus, for reducing the negative effects of these pathogens the environment friendly natural plant products are recommended. Plant extracts of many higher plants have been reported as bactericidal, fungicidal and insecticidal under *in vitro* and *in vivo* trails. [4, 5] In this juncture, the present study was attempted to evaluate the antifungal effect of purified solasodine and caulophyllumine-A from *Solanum mauritianum* Scop. against selected phytopathogenic fungi.

MATERIALS AND METHODS

Alternaria alternate (KAUM1456), *Fusarium oxysporum*(KAUM1234),*Albugo candida*(KAUM1977), *Rhizoctonia solani* (KAUM1786), and *Cercospora arachidicola* (KAUM145) are the fungal strains isolated from natural diseased plants. The samples were collected from Microbiolgy department, Kerala Agricultural University, Vellayani, Trivandrum, Kerala. All fungi were cultured on Potato Dextrose Agar plates and incubated at 28°C for one week. The isolated fungi were identified using mycelial and spore feature. [6, 7]

Extraction and purification of alkaloids

The plant material *Solanum mauritianum* Scop. (UCB3211) (40 g) shade dried and subjected to continuous soxhlet extraction using non polar to polar solvents (petroleum ether, chloroform, ethyl acetate, ethanol and water). Dragendorff's reagent test revealed the presence of more alkaloids in chloroform and ethyl acetate fractions. 100% chloroform yielded bluish coloured fraction. Subsequently, it was then eluted using ethyl acetate and lyophilised and again subjected to column chromatography for further purification. Bluish and yellowish green fractions were eluted out of the column using ethyl acetate and ethanol respectively. These fractions were further reloaded at the top of freshly packed column for purification. Petroleum ether and Chloroform mobile phase in the ratio 4:1 yielded highly purified bluish fraction. The elution time for bluish fraction was 120 h. Residual weight of the fraction was calculated after drying was 21.3 mg/g. Similarly, chloroform and ethyl acetate (6:4) solvent combination after 168 h resulted purified yellowish fraction. The residual weight was noted as 16.9 mg/g. Purity of the samples was first checked with FTIR. FTIR spectral peaks revealed the functional groups associated with alkaloids. Using proton NMR absorptions peaks, the identity and structure of the compounds were confirmed. Bluish coloured fraction was caulophyllumine-A and yellowish green fraction was identified as solasodine.

Fungicidal activity

The well diffusion assay was used to determine the fungicidal effect of solasodine and caulophyllumine-A against the selected strains. Using a sterile cork borer 50 mm diameter punch wells were prepared on the set plates. Sia and Yim protocol was used for the preparation of sabouraud dextrose agar plate i.e., 0.5 McFarland standardized fungal suspension was swabbed over the surface of the plate.[8] The wells were loaded separately with 25 µl of solasodine, caulophyllumine-A(0.125 and 0.5 mg.ml), 0.1 mg/ ml of fluconazole (positive control) and dimethyl sulfoxide (DMSO) as negative control. The whole sets were incubated for 24 h at 37°C. Zones of inhibition were measured after 24 h in terms of millimeters.[1] All experiments were carried in triplicates.

Determination of minimal inhibitory and fungicidal concentration of solasodine, caulophyllumine-A (MIC, MFC)

The MIC of solasodine, caulophyllumine-A was performed using the broth macro-dilution assay.[9] It was carried using four different concentrations (0, 0.25, 0.5, 1, 1.5 and 2 mg/l) by applying series of dilutions in sabouraud dextrose broth. 100 µl of standardized fungal inoculum (adjusted to 0.5 McFarland) of overnight culture was introduced into every tube, and incubated at 37° C 12 h. MIC refers the lowest concentration of a compound that inhibited the visible growth of microbe after overnight incubation. The minimal fungicidal concentrations (MFCs) of solasodine, caulophyllumine-A were determined by inoculating MIC dilutions onto sabouraud dextrose agar plates and incubated at 37° C for 24 h. After incubation, growth of the fungi on solid media indicated that particular concentration of the compound was able to kill the cells, and so the MFCs were assigned to the lowest concentration that resulted in no growth upon sub-culturing.

Spore germination assay

0.2 mL of different concentrations of solasodine and caulophyllumine-A (0.1, 0.25, 0.5, 1, 1.25, 1.5, 2, 2.5. 3 mg /mL) was mixed with 0.2 mL of the fungal spore suspension (appx. 10^6 spores/mL). The mixture was placed on fresh glass slides which were incubated in moisture chamber at 28º ±1°C for 24 h. At the end of the incubation period, each slide was fixed with lacto-phenol-cotton blue stain and observed under the binocular microscope (×40) for analyzing spore germination. Control refers spores without alkaloid was also tested in the similar way. About 200 spores were

counted and the % of spore germination was analyzed in comparison with the control.

Statistical analysis

All analysis was performed in triplicate and was subjected to analysis of variance using the statistical analysis software. Comparisons among means were made using Duncan's multiple range tests.

RESULTS AND DISCUSSION

Natural plant-derived fungicides comprise wide variety of molecules as alternatives to synthetic drugs. Even though, many synthetic antifungal agents have been reported, pathogens are constantly developing resistance to these drugs.[10] Currently, attempts have been made to screen indigenous herbal products against these infectious agents as safer and potential microbicidal drugs. Fungicidal activities of solasodine and caulophyllumine-A in terms of zone of inhibition were investigated against *Alternaria alternata, Fusarium oxysporum, Albugo candida, Rhizoctonia solani,* and *Cercospora arachidicola* using the concentrations 0.125 & 0.5 mg/g (Table 1). Serial dilutions of solasodine and caulophyllumine-A were tested. Zone of inhibitions were remarkable and comparable with fluconazole. Solasodine produced significant inhibition zones i.e., 4.8 and 28 mm. Caulophyllumine-A, showed moderate activity with inhibition zone diameter ranged between 4 and 23 mm. Negative control revealed poor activity with zone of inhibition diameter 0.1-0.15 mm. The tested fungal strains showed susceptibility against the alkaloids i.e., an inverse relationship was noticed between alkaloids and fungal growth i.e., reduced growth of selected fungal species against the increasing concentrations of the alkaloids. The highest antifungal activity was recorded for solasodine when compared with caulophyllumine-A. Synthetic fungicide fluconazole showed remarkable inhibition than the tested alkaloids. The linear growth of *Alternaria alternata, F. oxysporum, A. candida, R. solani,* and *C. arachidicola* continued to decrease with inoculation at different concentrations of the tested alkaloids. The alkaloids at 2 mg/ml concentration, continued to be the most effective inhibitor of *F. oxysporum* compared with the positive control (data not shown). The MIC and MFC were further analyzed and displayed in the table 2 &3. Solasodine showed significant MIC value against *F. oxysporum* (0.15 mg/ml) than others. In the present study, the MIC values of caulophyllumine-A was intermediate i.e., between 0.25-1.5 mg/ml. *R. solani* was more susceptible against caulophyllumine-A than solasodine. MIC value or susceptibility of the fungi against solasodine was in the order *Fusarium oxysporum, Albugo candida, Alternaria alternata, Rhizoctonia solani* and *Cercospora arachidicola*. For caulophyllumine-A the order was *Rhizoctonia solani, Fusarium oxysporum, Albugo candida, Alternaria alternate, Cercospora arachidicola*. The values of MFC for solasodine ranged from 0.2 to 1.25 mg/ ml. But in the case of caulophyllumine-A the values ranged from 0.4 to 2.0 mg/ml. Although the alkaloids showed varying levels of activity against all the tested fungi, the solasodine was found to be more significant than caulophyllumine-A. This may be due to the variations in the chemistry of the molecule. Further, the ability of alkaloids to complex with extracellular and soluble protein or to form complex with fungal cell wall are the other possibilities of its fungicidal potential. Dhamgaye revealed antifungal mechanisms of alkaloids from *Glaucium oxylobum* in terms of leaching of cellular minerals like iron, metabolic disruptions, calcineurin dependent core stress response pathways, shifting the metabolic flux towards fermentation and ROS generation leading to apoptosis.[11, 12] Cretton evaluated antifungal activity of quinoline alkaloids from *Waltheria indica.*[13] Zhang reported isoquinoline alkaloids from *Litsea cubeba* with antibacterial, antifungal and cytotoxic impacts.[14] Freiesleben[15]reviewed the differential mode of action of alkaloids i.e., naphtoquinone alkaloids show different mechanisms such as interference with fungal cell wall, mRNA transcription and also possibly inhibition of protein synthesis.[15, 16] Futher, the anthraquinone depolarize mitochondrial membrane potential and inhibition of energy-dependent efflux pumps. Compounds that inhibit efflux pumps can possibly be used in combination with other antifungal drugs to inhibit drug resistance in bacteria. In the present study, solasodine and caulophyllumine-A also showed differential potentialities through various mechanisms. Alkaloids like vincristine and vinblastin act only via disrupting microtubulin network in cell division process.[17] The spore inhibition assay of solasodine and caulophyllumine-A revealed remarkable reduction in spore germination against all the tested concentrations and duration compared to synthetic fungicide. Similarly, the synergic effect produced 100% inhibition of germination with in 4 h than given separately (Table 4 & 5). Alkaloids N-methylhydrasteine hydroxylactam and 1-methoxyberberine chloride is isolated from *Corydalis* longipes.[18] The mixture of the two compounds showed potential inhibitory effect on spore germination than given separately. Results of the present study related with the differential resistance among fungal species would support the directed toxicity hypothesis. Thus, the present results allow consideration of solasodine as an excellent alternative for treatment against pathogenic fungi.

Table 1

Fungicidal Potentiality Interms Of Zone Of Inhibition (Mm) Using Disc Diffusion Assay Of Solasodine & Caulophyllamine-A Against Selected Fungi

Solasodine	**0.125 (mg/ml)**	**0.5 (mg/ml)**
Alternaria alternate	7 ± 0.05	22 ± 0.45
Fusarium oxysporum	12.5 ± 0.02	28 ± 0.7
Albugo Candida	4.8 ± 0.07	21 ± 0.09
Rhizoctonia solani	6.3 ± 0.01	20 ± 0.05
Cercospora Arachidicola	5 ± 0.2	17 ± 0.03
Caulophyllumine-A		
Alternaria alternate	6 ± 0.05	20 ± 0.45
Fusarium oxysporum	6.5 ± 0.02	21 ± 0.7
Albugo Candida	5 ± 0.07	20 ± 0.09
Rhizoctonia solani	7.5 ± 0.01	23 ± 0.05
Cercospora Arachidicola	4 ± 0.2	16 ± 0.03
Fluconazole	18.5 ± 0.002	

Table 2

Antifungal activity of solasodine against selected fungi

	MIC (mg/ml)	**MFC (mg/ml)**	**MIC/MFC**
Alternaria alternate	0.25 ± 0.05	0.5 ± 0.45	0.5
Fusarium oxysporum	0.15 ± 0.02	0.2 ± 0.7	0.75
Albugo Candida	0.2 ± 0.07	0.5 ± 0.09	0.4
Rhizoctonia solani	0.4 ± 0.01	0.75 ± 0.05	0.53
Cercospora Arachidicola	0.5 ± 0.2	1.25 ± 0.03	0.4
Fluconazole	0.125 ± 0.002	0.2 ± 0.008	0.63

Table 3

Antifungal activity of caulophyllumine-A against selected fungi

	MIC (mg/ml)	**MFC (mg/ml)**	**MIC/MFC**
Alternaria alternate	1.0 ± 0.05	2.0 ± 0.45	0.5
Fusarium oxysporum	0.5 ± 0.02	1.5 ± 0.7	0.33
Albugo Candida	0.75 ± 0.07	1.5 ± 0.09	0.5
Rhizoctonia solani	0.25 ± 0.01	1.5 ± 0.05	0.16
Cercospora Arachidicola	1.5 ± 0.2	0.4 ± 0.03	3.75
Fluconazole	0.125 ± 0.002	0.2 ± 0.008	0.63

Table 4

Inhibition of fungal spore germination in Sabauroud Dextrose Agar (SDA) in microtitre plates in presence of increasing concentrations of solasodine (mg/ml) of extract at 30 ± 2°C

Fungi	**0.1**	**0.25**	**0.5**	**1**	**1.25**	**1.5**	**2**	**2.5**	**3**
Alternaria alternata	43	68	89	98	100	100	100	100	100
Fusarium oxysporum	88	100	100	100	100	100	100	100	100
Albugo candida	67	84	91	97	100	100	100	100	100
Rhizoctonia solani	80	98	100	100	100	100	100	100	100
Cercospora arachidicola	20	39	57	79	95	100	100	100	100
Fluconazole	20	79	100	100	100	100	100	100	100

Table 5
Inhibition of fungal spore germination in Sabauroud Dextrose Agar (SDA) in microtitre plates in presence of increasing concentrations of caulophyllumine-A (mg/ml) of extract at 30 ± 2°C

Fungi	0.1	0.25	0.5	1	1.25	1.5	2	2.5	3
Alternaria alternata	29	52	65	88	100	100	100	100	100
Fusarium oxysporum	42	76	82	94	100	100	100	100	100
Albugo candida	35	49	60	70	82	82	100	100	100
Rhizoctonia solani	80	98	100	100	100	100	100	100	100
Cercospora arachidicola	20	39	57	68	81	81	92	100	100
Fluconazole	20	79	100	100	100	100	100	100	100

CONCLUSION

The increasing need for novel phytochemicals for fungal chemotherapy in medicine has led to high throughput screening of different plant sources for antifungal activity. This study demonstrated that solasodine and caulophyllumine-A of *S. mauritianum* leaves have different degree of fungicidal activity. Antifungal activity of *S. mauritianum* was significant. Therefore, it can be concluded that alkaloids of S. *mauritianum* may serve as potential source of antifungal agent. Further studies are planned for over-production of secondary metabolites via plant cell cultures since intact plants often produce small amounts of the desired metabolites.

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