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EVALUATION OF *IN VITRO* ANTIFUNGAL POTENTIAL OF *RAUVOLFIA SERPENTINA (L). BENTH. EX KURZ.* AGAINST PHYTOPATHOGENIC FUNGI

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ABSTRACT
Rauvolfia serpentina L. Benth. ex Kurz. (Apocynaceae) commonly called as sarpgandha is an important medicinal plant, mainly known for its various phytochemicals. The main objective of present study was to evaluate the antifungal activity of *Rauvolfia serpentina* L. against phytopathogenic fungi *i.e. Alternaria alternata, Aspergillus flavus* and *Mucor rouxii*. Aqueous extract of whole plant, stem and roots of *Rauvolfia serpentina* L. were prepared and antifungal activity was studied with the help of agar well diffusion assay. The aqueous root extract of *Rauvolfia serpentina* L. showed significant higher antifungal activity against *Alternaria alternata* and *Aspergillus flavus* than the other extracts under study. The present investigation clearly reveals the antifungal nature of *Rauvolfia serpentina* L. and suggests the exploitation of this plant against pest management of various plants and animal.

KEY WORDS: *Rouvolfia serpentina*, phytopathologic fungi, *in vitro* antifungal activity.

INTRODUCTION

Pathogenic fungi are the main infectious agents in plants, causing alterations during developmental stages including post harvest. In fruits and vegetables, there is a variety of fungal genera causing quality problems related to aspect, nutritional value, organoleptic characteristics, and limited shelf life (Agrios, 2004).The most important fungi causing post harvest diseases include *Aspergillus flavus*, *Alternaria* spp. and *Rhizopus stolonifer* (Ogawa *et al.*, 1995). Many fruits are prone to damage caused by insects, animals, early splits and during mechanical harvesting.This damage predispose the fruits to the wound invading pathogen *Aspergillus flavus* and other fungi, that causes decay on stored fruits. *Aspergillus flavus* can pose a health problem; especially it produces aflatoxin, a group of toxic, carcinogenic compound (Diener *et al*., 1987, Plamubo *et al*., 2006 and Wilson and Payne, 1994). Generally phytopathogenic fungi are controlled by synthetic fungicides, such as, thiabendaole, imazalil and sodium ortho- phenyl phonate (Poppe *et al.*,2003) however, the use of these is increasingly restricted due to harmful effects of pesticides on human health and the environment (Harris *et al.*, 2001). Environmentally friendly plant extracts agent have shown to be great potential as an alternative to synthetic fungicide (Janisiewicz and Korsten, 2002 , Zhang and Zheng, 2005). Plant extract are least expensive and cause less health hazards. Several higher plants and their constituents have shown success in plant disease control and are proved to be harmless and non phytotoxic unlike chemical fungicide. Medicinal plants were used as excellent antifungal agents because it posses a variety of chemical constituent is nature recently much attention has directed towards extracts and biologically active compounds isolated from popular plant species. In recent years, secondary plant metabolites (Phytochemicals) previously with unknown pharmacological activities have been extensively investigated as source of medical agents. Tropical plant *Rauvolfia serpentina* L. Benth ex. Kurz commonly known as sarpgandha is an important medicinal plant of Indian subcontinent and South East Asian countries (Bhatt *et al*., 2008, Dey and De, 2010). It has been reported to contain 50 indole alkaloids that are mainly localized in the root bark and are of great pharmaceutical interest. Reserpine is a potent alkaloid first isolated from this plant which is being widely used as an anti hypersensitive drug (anonymous, 2003). The root extract of this plant is very useful in disorder of gastrointestinal tract *viz*. diarrhea, dysentery and cholera and colic. So, the present work was carried out to evaluate the *in vitro* antifungal potential of *Rauvolfia serpentina* L. against *Aspergillus flavus, Mucor rouxii and Alternaria alternata*.

MATERIALS & METHODS

Plant collection

The whole plant of *Rauvolfia serpentina* L. was collected from district Mandi Himachal Pradesh to carry out the following studies. The plant was identified at Department of Botany, GGDSD College, Sec- 32, Chandigarh.

Sterilization of plant material

Whole plant leaves, flowers, stem, were kept under running tap water to remove the adhered soil particles. The plant material sterilized by addition of 2-3 drops of teepol for 3-4 minutes and washed thoroughly with distilled water 2-3 times. Surface sterilization was done with 70% alcohol for 5 minutes followed by washing with autoclaved distilled water. The plant sample was dried in the oven at 60◦C to 70◦C for 6 hrs.

Preparation of aqueous plant extracts of *Rauvolfia serpentina* L.

Surface sterilized stem, leaves and roots of *Rauvolfia serpentina* L. were taken and crushed in sterilized pestle and mortar. The volume was made 100ml by addition of sterilized distilled water. Homogenized tissue was centrifuge at 7000rpm for 20 minutes. The supernatant was collected in another centrifuge tube and filter sterilized by Whatman filter paper No. 1 followed by storage in sterile capped bottles under refrigeration conditions (4◦C) for further use.

Isolation of fungi

For the purpose of antifungal evaluation, the test fungi were isolated from the rotten grapes, orange and tomato. The above fruits were kept under unrefrigerated conditions and were allowed to rot for 10 days. Rotten grapes, orange and tomato were surface sterilized using 70% alcohol for 4-5 minutes followed by washing with distilled water 2-3 minutes. The test fungus was inoculated on Sabouraud Dextrose Agar by streaking method. Identification of fungus culture was done by method of (Clark, 1981) and pure cultures were maintained at 32◦C by subsequent sub culturing on SDA media **(**Sabouraud Dextrose Agar (Hi Media).

Determination of antifungal activity

Antifungal activity of extract against test fungi *i.e. Alternaria, Mucor rouxii* and *Aspergillus flavus* was evaluated by agar well diffusion method (Perez *et al.*, 1990). 25µl of fungal suspension was added on the plates having SDA and was spread uniformly using a flame sterilized glass spreader. Using a sterile cork borer a well was created in the center of the SDA Petri plates. 100µl of *Rauvolfia serpentina* L. extracts was added carefully into the well using a micropipette. Triplets were prepared for each aqueous extract against each fungus. The SDA plate with 100µl of autoclaved distilled water in the well was used as control. All the Petri plates were sealed using parafilm and incubated in an incubator at 32ºC for 48- 72 hours.

Determination of Minimum inhibitory concentration

After antifungal activity the plant extract that showed best result were selected and further used for determination of MIC. MIC was calculated by using different concentrations of the root extract of *Rauvolfia serpentina* L. *i.e*. 50%, 75% and 100% against most sensitive fungus. Antifungal activity of root stem and whole plant extract of *Rauvolfia serpentina* L. against test fungi *i.e. Alternaria alternata, Mucor rouxii* and *Aspergillus flavus* was evaluated by measuring inhibition zone diameter surrounding each well. Results were reported as (+) if there is inhibition of growth and (-) negative if there is no inhibition of growth.

Statistical analysis

All experiments on "Evaluation of *in vitro* antifungal activity of *Rauvolfia serpentina* L. Benth. ex Kurz. against fungi" was conducted in triplets and statistical analysis were done by using conventional method and presented as mean ± standard deviation.

RESULTS & DISCUSSION

The objective of current endeavor was to study the *in vitro* antifungal activity of *Rauvolfia serpentina* (L). Benth. ex Kurz. against phytopathogenic fungi. *Alternaria alternata, Mucor rouxii* and *Aspergillus flavus* are causal agent of post harvest diseases of plants as reported by Eckert and Sommer, 1967 and Adaskaveg and Sommer, 2001. Post harvest diseases account to about 50% losses in fruits stored in poor storage condition especially under high humidity. They are posing a major threat to the agriculture industry (Agrios, 2005). *Aspergillus flavus* can pose a health problem; especially it produces aflatoxin, a group of toxic, carcinogenic compounds Use of chemical fungicides is common in fruit rot disease management but they often result problems of toxic residue. Synthetic fungicides, such as, thiabendazole, imazalil and sodium ortho-phenyl phonate has been used traditionally to control the post harvest diseases, but their excessive use complemented with high costs, residues in plants, and development of resistance, has left a negative effect on human health and the environment (Paster, and Bullerman,1988, Bull *et al*.,1997). Environmentally friendly plant extracts agents have shown to be great potential as an alternative to synthetic fungicides. Recently, the antimicrobial activity of some higher plant products that are biodegradable and safe to human health (Kumar *et al*., 2008) has attracted the attention of microbiologists in the control of plant disease. The use of these products for the control of post harvest pathogens of fruits is still limited. Considering the rich diversity of plants, it is expected that a screening and scientific evaluation of plant extract for their antifungal activity may provide new antifungal substances. The purpose of selecting *R. serpentina* as test plant was because of its wide demand in pharmaceutical industry (Roja and Roja, 1996).The purpose of our study is to test the possibility of using *R. serpentina* aqueous extract to control or inhibit the pathogen causing post harvest diseases in fruits. Tested fungi were isolated from rotten fruits *i.e.* tomato, grapes and orange. On the basis of their cultural and morphological characteristics fungi identified as *Alternaria alternata, Mucor rouxii* and Aspergillus *flavus*. Table 1 shows that all the plant extract *i.e.* whole plant extract, root and stem extract were effective against *Alternaria alternata* where as root and stem extract was effective against *Aspergillus flavus* and *Mucor rouxii* respectively. Root extract of *R. serpentina* recorded significant antifungal activity against *Alternaria alternata* and *Aspergillus flavus.* No positive results were reported in whole plant extract against these two tested fungi *i.e. Aspergillus flavus* and *Mucor rouxii*.

Among the three tested fungi, maximum zone of growth inhibition 11.60 ± 5.00 mm was reported against the fungi *Alternaria alternata* by the root extract of *Rauwolfia serpentinta* L. at the concentration of 100mg/ml followed by whole plant extract and stem extract [Table 2]. Minimum Zone of growth inhibition found was 6.60 + 3.21mm formed by whole plant extract. The adequate activity of the root extract was found against *Aspergillus flavus*. The zone of growth inhibition obtained was 7.00 ± 3.05mm. Several phytoconstituents like flavanoids, phenolics and polyphenols, tannins, terpenoids, sesquiterpene *etc*. are effective antimicrobial substances against a wide range of microorganisms. Presence of saponins and flavonoids like compounds showed the

justified use of extracts from *R. serpentina* plant extract. Presence of high level of indole alkaloids *i.e.* reserpine, ajmaline and yohimbine in the root extract of *R. serpentina* may be responsible for the observed antifungal activity. Cowan, 1999 reported the presence of ajmaline, reserpine and yohimbine in root extract.

TABLE 1: Effect of aqueous extracts of different parts of *Rauvolfia. Serpentina* on *in vitro* growth inhibition of tested fungi

S.NO	Name of fungi	Root extract	Stem extract	Whole plant extract
1.	*Alternaria alternata*	+	+	+
2.	*Aspergillus flavus*	+	_	_
3.	*Mucor rouxii*	_	+	_

TABLE 2: Antifungal activities in terms of zone of inhibition of aqueous extracts of *Rauvolfia. Serpentina* against tested fungi

S.No	Name of fungi	Zone of inhibition in millimeter at concentration 100mg/ml		
		Root extract	Stem extract	Whole plant extract
1.	*Alternaria alternata*	11.60 ± 5.00	4.00 ± 1.73	6.60 ± 3.21
2.	*Aspergillus flavus*	7.00 ± 3.05	0.00± 0.00	0.00± 0.00
3.	*Mucor rouxii*	0.00± 0.00	2.50 ± 0.57	0.00± 0.00

Presence of high level of indole alkaloids, phenolic and tannin constituents of *R. serpentina* may also elicit antifungal activity as found in many medicinal plants with mechanism of action characterized by cell membrane lysis, inhibition of protein synthesis, proteolytic enzymes and microbial adhesions. Like our reports Deshmukh *et al.,* 2012 also found the best results of root extract of *Rauvolfia serpentina* L. against *Salmonella typhi*. Root extract of *R. serpentina* showed no results against *Mucor rouxii*. No zone of growth inhibition was reported in stem and whole plant extract at this concentration against the fungi *Aspergillus flavus*. Out of three extracts tested, the stem extract of *Rauvolfia serpentina* L. was found to be effective against *Mucor rouxii* where as it was effective against *Aspergillus flavus* as well as *Alternaria alternata*. The mechanism of action of stem extract of *Rauvolfia serpentina* L. on *Mucor rouxii* is unknown but many alkaloids such as yohimbine, ajmaline, ajmalicine known to disrupt the DNA of filamentous fungi. There might be presence of other potent alkaloids which are not yet revealed. The antifungal activity against *Mucor rouxii* may be suspected due to presence of ajmaline and yohimbine like unknown alkaloids (Siddiqui and Siddiqui, 1931). Diameter of zone of growth inhibition was 2.50± 0.57mm where as root and whole plant extract were ineffective against this pathogenic fungi. As reported above the root extract showed the best results against the tested fungi so we have calculated the MIC of root extract against them. Table 3 shows that all concentrations (50%, 75%, and 100%) of root extract of *R. serpentina* have antifungal activity against *Alternaria alternata* and *Aspergillus flavus.* Root extract shows the lowest MIC 3.00± 1.00mm against *Aspergillus flavus* at 50% concentration. The antifungal activity of the extracts was enhanced by increase in the concentration of the extract. Our results were similar to the Zahid *et al.,* 2012. They reported that higher concentrations of 40, 50 and 60% aqueous extract of *F. vulgare* markedly enhanced fungal biomass production of *Macrophomina phaseoli, Rhizocotina solani* and *Fusarium moniliforme* at all the harvest intervals.

TABLE 3: Antifungal activity in terms of MIC of aqueous root extracts of *Rauvolfia. Serpentina* against fungus *Alternaria alternata* and *Aspergillus flavus*

S.NO.	Name of Tested fungi	Zone of inhibition at different concentration of root extract in millimeter (mm)		
		50%	75%	100%
1.	*Alternaria alternata*	6.60 ± 3.21	7.30 ± 4.61	11.60 ± 5.00
2.	*Aspergillus flavus*	3.00 ± 1.00	5.00 ± 1.15	7.00 ± 3.05

CONCLUSION

Biological control had attained importance in modern agriculture to curtail the hazards of intensive use of chemicals for pest and disease control . Accordingly, the observed efficacy of aqueous root extract of *Rauvolfia serpentina* L. tested in the present study against rottening fungi *i.e. Alternaria alternata* and *Aspergillus flavus* explores the possibilities of controlling fungal pathogenesis by using plant extract and highlights on results encouraging the possible application in agriculture after field investigations.

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