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Studies on *in Vitro* Antifungal Activity of *Berberis Aristata* Against Phytopathogenic Fungi

KEYWORDS: Antifungal activity, Plant extracts, Fruit pathogenic fungi, Minimum inhibitory concentration

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ABSTRACT *Plant extracts are being used to control the diseases since last several years. Extracts of the various plant parts like stem, root, leaves, fruit and seed are found to be effective against fruit pathogenic fungi. The in vitro studies have been performed by using agar well diffusion method to examine the antifungal activity of Berberis aristata. Both aqueous and alcoholic extracts of Berberis aristata were screened against phytopathogenic fungi viz. Alternaria alternata, Aspergillus flavus and Moucor rouxii. The aqueous extracts recorded effective antifungal activity against Alternaria alternata and Aspergillus flavus. These plant extracts can possibly be exploited in the management of fruit pathogenic fungi to prevent biodeterioration of fruits in an eco-friendly way.*

Introduction

Fungi are significant destroyers of fruits and grains during storage rendering them unfit for human consumption by retarding their nutritive value and often by producing mycotoxins (Martin *et al.*, 1999). In agriculture, the crop loss due to plant pathogens has become major concern. Increased usage of different chemicals based products to control these pathogens has resulted in problems like residual effect of chemicals in agri-based products, increased resistance for chemicals in target pathogens and environmental pollution. In recent years, secondary plant metabolites (Phytochemicals previously) with unknown pharmacological activities have been extensively investigated as source of medical agents.

Berberis aristata commonly known as "Daru haldi" and "chitra" is spinous shrub native to northern Himalaya region. The plant is widely distributed from Himalayas to Shrilanka, Bhutan and hilly areas of Nepal in Himalaya region (CSIR, 1985). It grows at the height of 2000-3000 meters especially in Kumaon and Chamba region of Himachal Pradesh. *Berberis aristata* is critically endangered species of Indian Himalaya due to extensively collection of roots for its berberine alkaloid. It is used in ayurvedic medicines from long time. Roots of plant *Berberis aristata* contains different types of alkaloids including berbamine, berberine, oxycanthine, epiberberine, palmative, dehydrocaroline jatrorhizine and columbamine, karachine, dehydrokarachin etc. The major alkaloid was isolated from *Berberis aristata* is berberine having yield of 2.23% (Chakravarti *et al.*, 1950).

The plant is used traditionally in wound healing (Biswas, 2003), skin and allergic disorders (Tripathi *et al.*, 1996), menohrrhahagia, diarrhea (Khanum and Gilanis, 2005) and affection of eye. The present study deals with the screening of antifungal activity of *Berberis aristata* against *Alternaria alternata, Aspergillus flavus* and *Moucor rouxii.*

Materials and Methods

Plant collection

The plant *Berberis aristata* was collected from the non-irrigated cultivated lands in and around Mandi District of Himachal Pradesh. The disease free and fresh plants of *Berberis aristata* were selected to carry out the following study.

Sterilization of plant material

Whole plant, stem, leaves and root samples were taken separately and washed under running tap water to remove the soil particles. They were washed with distilled water for three times. The plant material sterilized by addition of 2-3 drops of teepol for 3-4 minutes and washed thoroughly with distilled water two to three times. Surface sterilization was done with 70% alcohol for 5 minutes followed by washing with autoclaved distilled water. The plant samples were dried in the oven at temperature 60˚C-70˚C for 6 hours and ground into a fine powder and stored in air light containers.

Preparation of plant extracts of *Berberis aristata*

Both aqueous and alcoholic extracts of stems, leaves and roots were prepared. Five grams of each powder plant material was taken. To prepare the aqueous extract the plant material was soaked in 50 ml of DDW overnight whereas the ethanol extracts were made by soaking 5g of each plant material in 50 ml of alcohol overnight. The centrifugation of soaked material is done at 7000 rpm for 20 minutes. Supernatants were collected in separate centrifuge tubes. Aqueous extracts of stem, root, leaves and whole plant material were prepared whereas the alcoholic extracts were prepared for stem, root and leaves only. All the plant extracts were stored at 4˚C for further use.

Antifungal activity

Test microorganism

The test fungi were isolated from the rotten grapes, orange and tomato. The identification of the fungus cultures was done by adopting standard methods (Clark G., 1981) and the pure cultures were maintained at 32°C by subsequent sub culturing on SDA medium **(**Sabouraud Dextrose Agar) HiMedia.

Determination of antifungal activity of *Berberis aristata* **extracts**

Antifungal activity of extracts against test fungi *i.e. Alternaria alternata, Mucor rouxii* and *Aspergillus flavus* was evaluated by measuring inhibition zone diameter surround-

Volume : 5 | Issue : 6 | June 2015 | ISSN - 2249-555X

ing each well. Antifungal activity was screened by agar well diffusion method (Perez *et al.*, 1990). Twenty five microliters of fungal suspension was added on the SDA media plates and was spread uniformly using a flame sterilised glass spreader. Using a sterile cork borer a well was made in the centre of the SDA media plates. Hundred microliters of *Berberis aristata* extracts were added carefully into the well using a micropipette. Triplets were prepared for each alcoholic and aqueous extracts against each fungus. The SDA plate with 100µl of autoclaved distilled water in the well was used as control in all the experiments. Petriplates were incubated at 35°C for 48-72 hours.

Determination of MIC

The antifungal activities of the *Berberis aristata* extracts against the tested fungi- *Alternaria alternata* , *Mucor rouxii* and *Aspergillus flavus* was evaluated by measuring the inhibition zone diameter (millimetres) surrounding each agar well. Minimum Inhibitory Concentration was calculated by using different concentrations of best performing plant extract (aqueous root extract) i.e. 25%, 50%, 75% and 100% against most sensitive fungus. Triplicates of plates were prepared on each occasion and all experiments were repeated three times. The standard deviation was calculated using conventional methods. The results were represented as mean values ± standard deviation.

RESULTS

The antifungal activity was determined by measuring the diameter of zone of inhibition recorded. Both aqueous and alcoholic extracts tested at 100ul concentrations against fungi *Alternaria atlernata, Aspergillus flavus* and *Moucor rouxii*.

Results were reported as positive (+) if there is inhibition of growth and negative (-) if there is no inhibition of growth. The aqueous extracts of roots, stem and leaves showed (+) results against *Alternaria alternata* and aqueous whole extract of *Berberis aristata* showed (-) results against *Alternaria alternata*. The alcoholic roots and stem extracts showed (+) results against *Alternaria alternata.* Only aqueous leaves extract showed (+) results against *Aspergillus flavus* and all other aqueous and alcoholic extracts showed (-) results against *Aspergillus flavus*. All the aqueous and alcoholic extracts of *Berberis aristata* were found to be ineffective against *Moucor rouxii*. Aqueous extracts recorded effective antifungal activity against *Alternaria alternata* and *aspergillus flavus*. The alcoholic extracts were found to be uneffective against *Aspergillus flavus and Moucor rouxii*. Among aqueous extracts of stem, leaves and root, root extract recorded significant antifungal activity against the fungus *Alternaria alternata* followed by leaves and stems extracts respectively. *Alternaria alternata* found highly susceptible to aqueous extracts of *Berberis aristata,* where as *Moucor rouxii* showed no results with aqueous as well as alcoholic extracts. All the aqueous extracts of *Berberis aristata* showed inhibition zones against *Alternaria atlernata.* Aqueous root extract exhibited antifungal activity against *Alternaria alternata* and the inhibition zone of diameter was around 19.66 ± 2.51 mm to be recorded which was maximum. Aqueous stem extract and aqueous leaves extract exhibited antifungal activity against *Alternaria alternata* and showing diameter of zone of inhibition viz. 9.66 ± 2.30 mm and 10.00 ± 3.46 mm respectively. Only aqueous extract of leaves exhibited effective antifungal activity against *Aspergillus flavus* and showing diameter of zone of inhibition viz. 7.33 ± 0.57 mm. All other alcoholic as well as aqueous plant extracts were found to be ineffective against *Aspergillus flavus.* Alcoholic extracts of leaves and roots showed zone of inhibition having diameter 3.00 ± 1.00 mm and 2.83 ± 0.28 mm which was significantly at par with each other. Where as no zone of inhibition was reported in alcoholic stem extract and aqueous whole plant extract of *Berberis aristata.* "Table1 about here'.

TABLE1. Antifungal activity of different extracts of *Berberis aristata* against tested fungi

Name of fungus	Inhibition zone at concentration 100mg/ml in milimetre						
	Aqueous extract				Alcoholic extract		
	Whole	Roots	Stem	Leaves	Roots	Stem	Leaves
Alternaria alternate	-	19.66 ± 2.51	9.66 ± 2.30	10.00 ±3.46	3.00 ± 1.00	-	2.83 ±0.28
Aspergillus flavus	-	-	-	7.33 ± 0.57	-	-	-

Aqueous root extract produced maximum inhibition zone against *Alternaria alternata.* So the MIC was determined for the aqueous root extract. The aqueous root extract exhibit MIC at all the tested concentration 25%, 50%, 75% and 100% against *Alternaria alternata.* The minimum inhibitory concentration found was at 25% and the zone of growth inhibition was 8.00 ± 1.73 mm which further increase with increase in concentration of plant extract. The zone of inhibitions reported at 50% and 75% were 12.33 ± 0.57 mm and 15.33 ± 0.57 mm respectively.

TABLE 2. Antifungal activity in terms of MIC of aqueous root extract of *Berberis aristata* against fungus *Alternaria alternata*

S.No.	Concentrations of Root extract	Inhibition zone in mm
1	25%	8.00 ± 1.73
2	50%	12.33 ± 0.57
3	75%	15.33 ± 0.57
4	100%	19.66 ± 2.51

Discussion

Pre and post harvest bio-deterioration and spoilage of fruits, vegetables and grains produce due to infestation by insects and microorganisms may cause losses upto 100%. Excessive usage of pesticides in agriculture to overcome the pre-harvest and post harvest problem was resulted in many toxic epidemics. Synthetic fungicides, such as, thiabendazole, imazalil and sodium ortho-phenyl phonate (Poppe *et al.*, 2003) has been used traditionally to control the postharvest disease, but their excessive use complemented with high cost, residues in plants, and development of resistance, has left a negative effect on human health and the environment (Paster and Bullerman, 1988). Thus there is urgent need to search for alternative method for prevention of biodeterioration of fruits and grains during storage without any toxicity to the consumer.

Biologically active plant derived pesticides are expected to play an increasingly significant role in crop protection strategies. Exploitation of naturally available chemicals from plants, which retards the reproduction of undesirable microorganisms, would be a more realistic and ecologically

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sound method for plant protection and will have a prominent role in the development of future commercial pesticides for crop protection strategies, with special reference to the management of plant diseases (Verma and Dubey, 1999, Gottlieb *et al.*, 2002). Numerous scientific investigations are carried out to study the antimicrobial effect of *Berberis aristata* in the last decade. However, the research data reporting the antifungal nature of *Berberis* species is still limited. So the objective of current research was to explore the antifungal potential of aqueous and alcoholic plant extracts on mycelia growth of tested fungi *i.e. Alternaria alternata, Aspergillus flavus* and *Mucor rouxii*.

In present study both aqueous and alcoholic extracts of *Berberis aristata* showed positive results against *Alternaria alternata*. *Berberis aristata* is used in ayurvedic medicines from long time. *Berberis aristata* contains proberberine and bis-isoquinoline and other different types of alkaloids. Since alkaloids are the secondary metabolites, whose function is known to be defensive to the plants. The antifungal activity of *Berberis* species may be due to presence of major Alkaloid berberine (Chunekar and Bhavaprakasha, 1982). The mechanism of action of the highly aromatic nearly planar quaternary structure of berberine is attributed to its ability to interact with DNA. The interaction is combination with the inhibition of protein biosynthesis (which is major mode of action of berberine) should be responsible for the observed cytotoxic effect (Cernakova *et al.*, 2010) because both targets are central to all living cells. Among aqueous extracts of stem, leaves and root, root extract recorded significant antifungal activity against the fungus *Alternaria alternata* followed by stems and leaves extracts. This is because the roots of *Berberis aristata* contains more amount of berberine *i.e.* 3.80% as compare to the stem bark i.e. 2.60% (Andola *et al.*, 2010). This is well reported fact that the berberine occurs in more amount in organ of the plants which grow in the absence of light *i.e.* roots and rhizomes (Cromvell, 1933).

The reduced effectiveness of alcoholic extract against test fungi could be attributed to the solubility and volatility of its phytochemical components and losses during the process of extraction especially in organic solvents. The MIC values were calculated for the aqueous of *Berberis aristata* extract which showed the increase in growth inhibition of test fungus with the increase in the concentration of plant extract. The crude extracts contain mixture of active and inactive compounds and MIC of less than 100µg/mL suggested strong antimicrobial activity (Webster *et al.*, 2008). In this research MIC value was of 250µg/mL which suggested the good antifungal activity of aqueous extract of *Berberis aristata* against *Alternaria alternata*.

Out of different extracts tried, only aqueous leaf extract showed positive result against *Aspergillus flavus*. Anubhuti *et al.*, 2011 reported the significant activity of aqueous and alcoholic extracts of *Berberis aristata* collected from different regions of India against *Aspergillus niger.* But none of the extracts showed any activity against *P.aureginosa* and *E.coli.* Both aqueous and alcoholic extracts of *Berberis aristata* were found to be ineffective against *Mucor rouxii.* This varied susceptibility to the plant extracts could be due to inherent physiological and morphological characteristics of species involved in the study.

Most of the Scientist worked on alcoholic and hydro-alcoholic extract of *Berberis aristata* to carried out antimicrobial activity against different pathogens (Singh *et al.*, 2007, Mathur *et al.,* 2011 and Shahid *et al.*, 2009) and found the maximum antifungal activity against different pathogens. We emphasize that we adopted different experimental approach testing both aqueous and alcoholic extracts separately rather than using hydro-alcoholic (50%) extract, and activities were performed on different fungi. Furthermore, we noticed better antifungal activity by aqueous extracts in comparison to alcoholic extracts. Singh *et al.*, 2007 found lower spectrum of antifungal activity since they used the hydro-alcoholic mixture and thereby decreasing the concentration of aqueous extracts .Variations in other reports from our results also expected since variable concentrations of berberine already been reported in species of *Berberis* collected from different altitude and suggested probably as a result of adopted response to altitudinal gradient in addition to genetic characters (Chandra and Purohit, 1980).

The present investigation clearly reveals the antifungal nature of *Berberis aristata* and suggested that this plant could be exploited in the management of diseases caused by these fungi in plants and animals..

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