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Research Article

Antifungal Potential of *Polyalthia longifolia* against *Rhizoctonia solani*

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Abstract

Use of synthetic fungicides in inappropriate manner have negative effects on whole ecosystem with a possible carcinogenic risk on human health so there is a strong need to search a strategy which are environmentally safe as well as an economically viable. In this continuation present study reveals the antifungal activity of partially purified extracts of *Polyalthia longifolia* leaf against *Rhizoctonia solani* which is responsible for black scurf disease of potato. Partially purified extracts of *P. longifolia* leaf were prepared by hot extraction method in different organic solvents. Among all the organic solvents used for extraction, maximum percent extractive value was obtained with petroleum ether fraction which was 13.25%. Acetone were used for solubility of extract because acetone did not show any toxic effect on test pathogen. All partially purified fractions were subjected for phytochemical screening by standard methods. Maximum metabolites were found in petroleum ether, acetone and aqueous fractions. Poison food technique was used for determination of antifungal activity. Among all fractions, maximum inhibition of test pathogen was showed by petroleum ether fraction which is 81.25%. MIC and MFC were performed by two fold serial dilution method. 0.625 mg/ml was found as MIC and 1.25 mg/ml was MFC. Mancozeb and Bavistin were used as standard fungicides. Results suggest that Petroleum ether fraction have maximum secondary metabolites which are the reason of maximum inhibition of pathogen so we can conclude that *Polyalthia longifolia* leaf either in the form of extract or powder can be used as ecofriendly herbal biocontrol agent against *Rhizoctonia solani*.

Keywords: *Antifungal Activity; Hot Extraction; Poison Food Technique; MIC; MFC*

Introduction

The today's need to control plant diseases because most of the synthetic fungicides leave toxic substance which impact adverse effect on environment [1,2] and increases the resistant pathogen populations. So, to overcome this global safer measures have to develop which will be economically viable and free from the dependency on the synthetic agrochemicals for plant disease control [3-6]. Secondary metabolites present in the plants are well known source of natural antifungal agents because they are truly natural so do not leave any toxic substance on plants, animals, soil and on complete ecosystem. They are novel environmentally safer compounds which are a treasure of nature which can be used separately or can be mixed all together to form an herbal bioformulation as they are plant based bioproducts.

Secondary metabolites have been isolated in the form of plant extracts, essential oil and studied antifungal activity by several workers [7,8] and people are using these strategy for biological control of plant diseases and arrange biological control programs [9-11]. Biological

control has the potential to manage plant disease which associated with various phenomenon Plant extracts is usually prepared with cold and hot extraction methods [12-15]. Ethanol or Methanol is universal solvents which use for preliminary screening of active principles of plants. Crude extract is used for screening of antifungal activity of plant material which is followed by hot extract which is prepared by successive extraction method [16].

Partially purified extracts are used for studies of various phytochemicals, responsible for antimicrobial activity. The medicinal and antimicrobial activities of plant species are due to the phytochemical constituents present in it. Plants are full of secondary metabolites viz. tannins, terpenoids, alkaloids, and flavonoids [17,18] and they have well known antimicrobial properties. Antifungal agents are natural products which have always been found a better treatment control of fungi [19]. Antimicrobial properties are very well known factor of plants since ancient time. Many literatures have reported about the inhibitory effect of plants and their control of plant diseases. In the present study antifungal *Polyalthia longifolia* leaves were detected against *Rhizoctonia solani*.

Polyalthia longifolia is a lofty evergreen plant, native to India, commonly planted due to its effectiveness in alleviating noise pollution. Many common names are given to *P. longifolia* but most common name is ashoka, "the buddha tree" [20]. Its growth is symmetrical pyramidal with pendulous branches and long narrow lanceolate leaves. The tree is known to grow over 30 ft in height. This plant is known for its antibacterial, antioxidant, anti-inflammatory, anti-cancer, hepato-protective and anti-hyperglycemic activity [21] but there is little or no information about the antifungal activity of *P. longifolia* against plant pathogenic fungi *R. solani* which is our test pathogen therefore, the main object of our study is to determine the antifungal activities of *P. longifolia* partially purified extracts using *in vitro* antimicrobial screening methods.

Materials and Methods

Collection and identification of plant material

The healthy infection free leaves of *Polyalthia longifolia* were collected from the campus of University College of science, Mohanlal Sukhadia University, Udaipur, Rajasthan, India. The herbarium specimen was identified by Dr. Amit Kotia, In charge Herbarium, Department of Botany, and University college of Rajasthan, Jaipur, India.

Preparation of extract

Test Plant material leaves were dried in shade at room temperature and grounded with electric grinder. The ground material was passed through sieve of mesh size 60 to obtain a fine powder which was used to prepare partially purified extract by hot extraction method.

Hot extraction

In this extraction method continuous extraction of powdered plant material in soxhlet apparatus with various organic solvents were used for separation of different phytochemical constituents [22,23]. Solvent series used for successive separation was non-polar to polar i.e.

Petroleum ether → Benzene → Chloroform → Acetone →Alcohol→ Methanol → Water.

In this method the dried extract and fractions were weighed and their percent extractive value was also calculated percentage by the following formula given below.

Percent extractive value

$$\text{Percent extractive} = \frac{\text{Weight of dried extract}}{\text{Weight of dried plant material}} \times 100$$

Phytochemical study of *Polyalthia longifolia* leaf extract

Various fractions obtained by hot extraction of leaves of test plant were than subjected to qualitative test for the identification of phytochemical constituents.

Inhibitory activity of solvent against test pathogen

Besides the solubility, inhibitory activity of solvent is also an important requirement for the selection of suitable solvent. Best solvent should have good solubility as well as less toxicity. To ascertain less toxic solvent all the solvents examined in solubility testing were assayed for finding inhibitory effect on test pathogen using poison food technique.

Assay of antifungal activity of partially purified extracts

The inhibitory activity of partially purified extracts of *Polyalthia longifolia* was assayed by poison food technique [24,25]. 10 mg/ml concentration of stock solution was used for antifungal activity. 2 ml of stock solution was mixed with 18 ml molten sterile culture medium and this mixture was poured into pre-sterilized petri-plates (9 cm diameter) and allowed to solidify at room temperature. In the control set no extract was used. The plates were then incubated at 28 ± 2°C for seven days. Culture control and acetone control were also maintained along with test samples. Antifungal activity was measured as a function of increase in growth of 6 mm disc of inoculum.

The average diameter of the fungal colonies was measured on the seventh day of incubation and percentage of mycelial growth inhibition was calculated by the following formula given below:

Percent Mycelial growth inhibition = gc-gt/gc×100

Where:

gc = Growth of mycelia colony after incubation period in control set subtracting the diameter of inoculum's disc

gt = Growth of mycelia colony after incubation period in treatment set subtracting the diameter of inoculum's disc.

Estimation of minimum inhibitory concentration (MIC) of selected plant extract

Minimum inhibitory concentration (MIC) was determined by broth dilution method [26,27]. Potato dextrose broth (PDB) was used for determining inhibitory activity. 20 mg/ml concentration of extract was prepared for stock solution. Two fold serial dilution method was used for the preparation of 10 mg/ml to 0.019 mg/ml concentration from the stock solution and subsequently autoclaved. The final concentration was serially diluted with sterile potato dextrose broth medium to attain final concentrations. All these tubes were than respectively inoculated with 10 µl of spore suspension (1×10^6 spores/ml) and incubated at 27 ± 2°C for 72h. One tube containing extract free autoclaved medium was used as control. Three replicates of each concentration were maintained and experiment was repeated thrice.

Estimation of MFC of selected plant extract

A loopful of fungal biomass from all of the above mentioned tubes containing 9 ml broth medium and MIC other concentrations respectively were streaked onto the extract free PDA slants and incubated at 27 ± 2°C for 72h. Presence or absence of growth was observed after respective incubation time (7th day). Appearance of growth indicates that the extract concentration is just fungistatic and absence of growth indicates that extract concentration is fungicidal.

Statistical analysis

All experiments were performed in triplicates (n = 3) and the data were presented as the mean ± standard deviation.

Results and Observations

The percent extractive values of partially purified leaf fractions in various organic solvents are given in table 1. Petroleum ether fraction gave maximum percent extractive value of 13.25% whereas alcohol, acetone, benzene, chloroform, acetone and aqueous fraction of *Polyalthia longifolia* gave 4.8, 4.5, 4.2 and 0.7 extract value respectively.

S. No.	Type of Extract	Weight of Dried extract (gm)	% Extractive Value
1.	Petroleum ether fraction	2.65	13.25
2.	Benzene fraction	0.84	4.2
3.	Chloroform fraction	0.4	2
4.	Acetone fraction	0.9	4.5
5.	Alcohol fraction	0.96	4.8
6.	Aqueous fraction	0.14	0.7

Table 1: *Percent extractive value of different partially purified fractions of Polyalthia longifolia leaf extract.*

Results of phytochemical testing of *Polyalthia longifolia* leaves extract obtained in different solvents are given in table 2. Alkaloids, phytosterols, carbohydrate, saponins and flavanoids are present on petroleum ether fraction. In benzene only saponins and flavanoids are present. In chloroform fraction only saponins are present and rest of all absent. In acetone fraction, alkaloids, phytosterols and volatile oil are absent and remaining metabolites are present. In alcohol fraction only phytosterol and volatile oil are present. In aqueous fraction, phytosterols and tannins are absent and remaining metabolites are present.

S. No.	Fractions	Alkaloids	Phytosterols	Volatile oil	Tannins	Carbohydrate	Saponins	Flavonoids
1.	PE	+	+	-	-	+	+	+
2.	Benzene	-	-	-	-	-	+	+
3.	Chloroform	-	-	-	-	-	+	-
4.	Acetone	-	-	-	+	+	+	+
5.	Alcohol	-	+	+	-	-	-	-
6.	Aqueous	+	-	+	-	+	+	+

Table 2: *Phytochemical screening of various fractions of Polyalthia longifolia leaf extract.*

+ ve: Present, - ve: Absent.

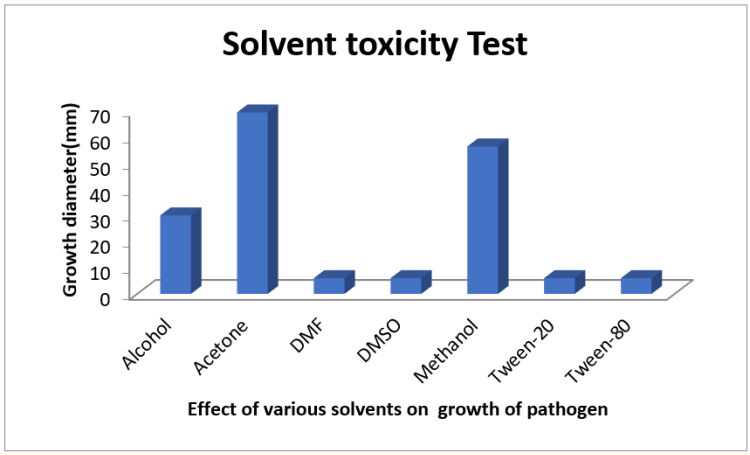

Figure 1: *Effect of various solvents on pathogen growth.*

Antifungal activity of partially purified extract

Results of antifungal activity of partially purified extract fraction of *Polyalthia longifolia* leaf and standard chemical fungicides against *R. solani* are given in figure 2. Amongst comparative inhibitory activity of mancozeb and bavistin, mancozeb was significantly active against *R. solani.* Significant inhibition of *R. solani* was observed with all partially purified fractions but petroleum ether fraction showed maximum inhibition with 81.25% followed by acetone (73.33%) Chloroform fraction (65.83%), benzene fraction (64.92%), Ab.alcohol (62.92%) and aqueous fraction (30.83%).

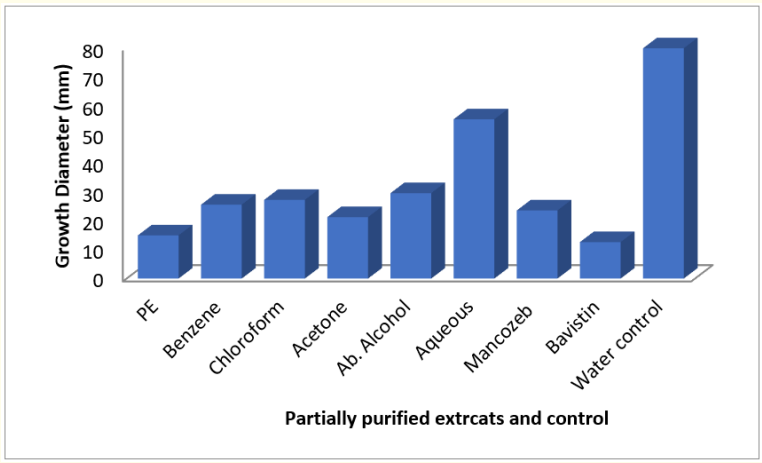

Figure 2: *Antifungal activity of partially purified extract of Polyalthia longifolia.*

MIC and MFC of petroleum ether fraction

Petroleum ether fraction was used for further studies as this fraction was found to be most effective. MIC and MFC are presented in table 3. 0.019 mg/ml to 10 mg/ml of petroleum ether extract was assayed and MIC was found to be 0.625 mg/ml for *Alternaria solani* and MFC for this fungus was observed at 1.25 mg/ml.

S. No.	Test pathogen	MIC (mg/ml)	MFC (mg/ml)
1.	*Rhizoctonia solani*	0.625	1.25

Table 3: *MIC and MFC of petroleum ether fraction of Polyalthia longifolia leaf extract.*

Discussion

Chemical fungicides impose the adverse effect on all living and non living objectives present on earth. Hence, there is a great need for natural antifungal belonging to plants which can be acted on new targets with fewer side effects [28].

The traditional use of plants as medicines provides the basic knowledge for selecting plant ingredients either in the form of essential oils/extracts/powder. It is important to investigate scientifically validate and investigate the plants which have been used in traditional medicines as potential sources of novel antimicrobial compounds [29]. Also, the resurgence of interest in natural therapies and increasing consumer demand for effective, safe, natural products means that quantitative data on plant oils and extracts is required [30,31]. Various secondary metabolites are responsible for antimicrobial potential [32-36].

Secondary metabolites were found effective against many pathogenic bacteria, fungi and viruses and even found active against drug resistant microorganisms [37]. Plant extracts and pure compounds have shown antimicrobial activity against pathogens can be used as nutraceuticals and in food preservation [38-40].

The poison food technique was used for the assay of antifungal activity depends on the inhibition of microorganism growth as an indication of activity which is measured as a function of diameter of the growth zone [41]. The dilution methods indicate the activity in terms of MIC and MFC also. Hence, in the present study MIC of plant extracts and essential oils against the test fungi was done by macro broth two fold serial dilution techniques [42].

Rhizoctonia solani is the common soil inhibiting plant pathogenic fungus which causes black scruff disease in potato [43]. Natural chemicals and their use for integrated plant protection is one of the focuses of research workers all over the world. The results of the present investigation are clear indication for plant extracts to control fungal pathogen [44].

Antimicrobial screening with various organic solvents because the primary metabolites are soluble in water whereas secondary metabolites are soluble in organic solvents [45]. These extracts are studied to search for various compounds, responsible for antimicrobial activity.

Results of the present study suggested that petroleum ether extract gave best activity against *R. solani*. It can be due to better solubility of the active molecules like terpenoids, flavanoids, tannins in petroleum ether as compared to water. [46] reported that extract prepared with an organic solvent exhibits better antimicrobial activity. Stronger extraction capacity directly proportion to produce greater number of active constituents responsible for antimicrobial activity [47-49].

Conclusion

Antifungal activity of partially purified extracts of *P. longifolia* leaf against *R. solani* was studied by poison food technique and broth dilution method. Petroleum ether fraction showed maximum inhibition against *R. solani*. This indicates the probably active compound is present in this fraction. On the basis of results obtained it can be concluded that partially purified petroleum ether fraction of *Polyalthia longifolia* leaf showed maximum inhibition against *Rhizoctonia solani* as compare to standard fungicides. This was used to develop an eco-friendly natural fungicide which will not leave any toxic substance in the environment

Conflicts of Interest

There are no conflicts of interest. As this is my original research work.

Research Involving Human Participants and/or Animals

In this study no use of any kind of animals for the experimental purpose.

Authors Contribution Statement

Ms. Poonam Meena perceived the idea, carried out the research study, evaluated the results and drafted the manuscript. Dr. Tripta Jain guided to Ms. Meena in conducting this research study and also reviewed and approved the manuscript.

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