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Comparative study of the antimycotic activity of aqueous and ethanolic extracts of *Berberis lyceum* and *Rumex obtusifolius* against selected rot fungi

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Two medicinal plants, *Berberis lyceum* and *Rumex obtusifolius*, were screened for the presence of various phytochemicals and for their antifungal activity. The phytochemical tests carried out in the present study demonstrated the presence of phenols, alkaloids, tannins, flavonoids, quinones and terpenoids in the plant extracts. Therefore, ethanolic and aqueous extracts of these medicinal plants were evaluated for their antifungal activity against *Penicillium chrysogenum*, *Aspergillus niger*, *Cladosporium herbarum* and *Trichothecium roseum*, using the agar well diffusion method.

All the plant extracts at different concentrations showed significant antifungal activity against the tested fungi. Of the two plant extracts, *B. lyceum* showed stronger antifungal activity than *R. obtusifolius*. Ethanolic extracts of both plants showed stronger mycelial growth inhibition than aqueous extracts. The ethanolic extracts of *B. lyceum* showed the strongest antifungal activity against *Penicillium chrysogenum* (inhibition zone diameter of 41 mm) followed by the ethanolic extracts of *R. obtusifolius* against *P. chrysogenum* (inhibition zone diameter 39 mm).

Hence, it is concluded that these medicinal plants have a broad-spectrum antifungal activity and are a potential alternative to reduce various fungal pathogens.

Key words: plant extracts, phytochemicals, antimycotic effectiveness, minimum inhibitory concentration, inhibition zone.

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V provedené studii byl zjišťován obsah biologicky aktivních látek v léčivých rostlinách *Berberis lyceum* a *Rumex obtusifolius* a jejich účinnost proti houbám. Fytochemické testy prokázaly přítomnost fenolů, alkaloidů, taninů, flavonoidů, chinonů a terpenoidů v extraktech z uvedených rostlin. S využitím jamkové metody byla hodnocena antimykotická aktivita etanolových a vodných extraktů z těchto rostlin proti *Penicillium chrysogenum*, *Aspergillus niger*, *Cladosporium herbarum* a *Trichothecium roseum*.

Všechny rostlinné extrakty v různých koncentracích mají prokazatelný účinek na růst testovaných hub. Z jejich srovnání vychází, že extrakty *B. lyceum* vykazují vyšší účinnost oproti *R. obtusifolius*

a že etanolové extrakty obou rostlin inhibují růst mycelia účinneji než vodné. Nejsilnější antimykotickou aktivitu vykazuje etanolový extrakt *B. lyceum* proti *Penicillium chrysogenum* (průměr inhibiční zóny 41 mm), následovaný etanolovým extraktem *R. obtusifolius* proti téže houbě (průměr inhibiční zóny 39 mm).

Závěrem lze konstatovat, že tyto léčivé rostliny obsahují široké spektrum látek, které mohou potlačovat růst hub, a mohou tak představovat potenciální alternativu pro eliminaci houbových nákaz.

INTRODUCTION

Fruits are attacked by a number of microbial pathogens in storage and on plants causing many diseases. Fungal rot of fruits is a predominant postharvest disease causing huge losses of upto 30% (Janisiewicz & Korsten 2002). Various workers have isolated and identified a diverse range of fungal pathogens belonging to the genera *Alternaria*, *Aspergillus*, *Botryodiplodia*, *Botrytis*, *Cladosporium*, *Colletotrichum*, *Rhizopus*, *Monilinia*, *Mucor*, *Penicillium*, *Phacidiopycnis*, *Phytophthora*, *Sphaeropsis*, causing rot diseases of various fruits in storage (Xiao & Boal 2002, Mari et al. 2003, Parveen et al. 2014, Parveen & Wani 2015, Abata et al. 2016, Wenneker et al. 2017). Several management practices have been used to manage postharvest diseases of fruits, but these methods have some limitations (Griffiths 1981, Spotts & Cervantes 1986).

Medicinal plants and their products have been used as a source of medicines in the past. They are the richest source of drugs for traditional medicinal systems, pharmaceuticals, modern medicines, food supplements and intermediate chemicals for synthetic drugs (Clark 1996, Anwar et al. 2013, Shakya 2016). In India more than 70% of the population uses herbal drugs to meet their daily health care needs (Vaidya & Devasagayam 2007). Extracts obtained from plants have gained great popularity and scientific interest for their antibacterial and antifungal activity (Santas et al. 2010). Several extracts of medicinal plants have been used against fungal plant pathogens causing plant diseases (Bobbarala et al. 2009, Gatto et al. 2011, Murtaza et al. 2015, Parveen et al. 2017).

Berberis lyceum Royle is an evergreen shrub belonging to the family *Berberidaceae*. It is a well-known medicinal plant every part of which has some medicinal importance (Bhattacharjee 2000). The plant is used for the treatment of various skin diseases, abdominal disorders and cough, and it is known to exhibit antibacterial, febrifugal, antidiabetic, laxative, carminative, hepatoprotective and ophthalmic properties (Gupta et al. 2015). The plant contains berberine, an isoquinoline alkaloid, as the major active agent. Other chemical constituents present in this plant are berbamine, palmitine, ascorbic acid, chinabine, karakoramine, sindamine and malic acid (Sharma 2003).

Rumex obtusifolius L. belongs to the family *Polygonaceae*. This plant is also of great medicinal importance, being used as a laxative, antidote to nettle, astringent, antibacterial, depurative, contraceptive, as blood purifier, in cancer treatment and can also be used to treat constipation, skin diseases, jaundice, blisters and sores (Harshaw et al. 2010). The plant contains anthracene derivatives, flavonoids and oxalic acid (Wegiera et al. 2007).

Keeping in view the medicinal importance of these plants, the present study was undertaken to check the efficacy of *Berberis lyceum* and *Rumex obtusifolius* against *Penicillium chrysogenum* Thom, *Aspergillus niger* Van Tiegh., *Cladosporium herbarum* (Pers.) Link and *Trichothecium roseum* (Pers.) Link, fungi which have been found to be pathogenic to most fruits (Parveen et al. 2014). A comparative study was carried out to assess the antifungal activities of aqueous and ethanolic extracts of these two medicinal plants, and to screen these plants for the presence of different phytochemicals.

MATERIAL AND METHODS

Plant material. Fresh samples of the local medicinal plants *Berberis lyceum* and *Rumex obtusifolius* were collected from Kashmir Valley and identified at Kashmir University Herbarium (KASH), Center of Plant Taxonomy, Department of Botany, University of Kashmir, Srinagar.

Preparation of plant extracts. The aboveground parts of plants of *B. lyceum* and *R. obtusifolius* were thoroughly cleaned and dried in the shade for at least one week. After drying, the plant material was chopped and ground to powder. Dried plant powder was then packed into a Soxhlet apparatus and extracted with ethanol and water at 50–65 °C. The extract was then filtered through Whatmann's filter paper no. 1. The pallet was discarded and the supernatant was collected and concentrated under reduced pressure. The extract was then dried, labelled and stored at 4 °C in sterilised storage vials for experimental use.

Test organisms. The fungal test organisms, viz. *Penicillium chrysogenum*, *Aspergillus niger*, *Cladosporium herbarum* and *Trichothecium roseum*, used in this study were isolated and identified from rotten fruits. The tested strains are stored at the Section of Mycology and Plant Pathology, Department of Botany, University of Kashmir, Srinagar under codes PPRL – 1702, PPRL – 1703, PPRL – 1704, and PPRL – 1705, respectively.

Phytochemical screening. The ethanolic and aqueous extracts of the medicinal plants selected for antifungal activity were screened for the presence of various phytochemicals, using standard qualitative methods as described by

Rizk & Bashir (1980), Das et al. (2010), and Eleazu et al. (2012). The plant extracts were screened for the presence of various biologically active compounds like phenols, alkaloids, tannins, flavonoids, quinones and terpenoids.

Antifungal activity assay. The antifungal activity of the plant extracts was determined by the agar well diffusion method as adopted by Perez et al. (1990). Seven- to eight-day old fungal cultures grown on PDA medium were used to assess the antifungal activity of selected plant extracts. An aliquot of 0.02 ml of inocula (3×10^3 conidia/ml) from each fungal species was inoculated in 20 ml of molten SDA medium in culture tubes. The culture tubes were then homogenised manually and poured into 90 mm Petri plates. The culture plates were then allowed to solidify in a Laminar airflow chamber and then wells were made on the agar plate using a 5-mm standard cork borer. A 2 mg/ml stock solution was made from the plant extract and then different volumes (25 µl, 50 µl and 75 µl) from that stock solution were added to respective wells. Hexahit (0.1 mg/ml) (a fungicide, Hexaconozole 5% EC, HPM Chemicals and Fertilizers Ltd., Azadpur, Delhi, India) was used as a standard (positive control). The effect of plant extracts on the different rot fungi was evaluated and compared with the standard. The plates were then sealed and incubated at 25 ± 2 °C for 5 days. Three replications were made for each treatment. The antifungal activity was calculated by measuring the inhibition zone with the help of a standard scale (Norrel & Messley 1997).

Determination of minimum inhibitory concentration (MIC). The broth dilution method (Barsi & Fan 2005) was followed for determination of minimum inhibitory concentration (MIC) values for ethanolic plant extracts showing highest antimicrobial activity against the test fungi. Minimum inhibitory concentration is the lowest concentration of a test sample at which the fungi do not show any growth. To measure MIC values, various concentrations of the plant extract (3.000, 1.500, 0.750, 0.375, 0.188, 0.094, 0.047, 0.024, 0.012, 0.006 mg/ml) were assayed against different test species. For determination of the MIC value of the standard, various concentrations (2.000, 1.000, 0.500, 0.250, 0.125, 0.063, 0.032, 0.016, 0.008, 0.004, 0.002 mg/ml) were assayed against the test species. Ethanolic plant extracts were resuspended in ethanol to make a 3 mg/ml final concentration and then two-fold serially diluted. One ml of the extract was added to the test tubes containing 1 ml of the SDA broth. The tubes were then inoculated with standard-size fungal spore suspension of 3×10^3 CFU/ml, made from a 5-day old fungal culture. The tubes were then incubated at 25 °C for 48 h in a BOD incubator. Three replications were made for each treatment.

RESULTS

In vitro screening of plant extracts for their antifungal activity

The antimycotic effectiveness of *Berberis lyceum* and *Rumex obtusifolius* extracts was determined by measuring inhibition zone diameters using the agar well diffusion method and MIC values using broth dilution assays. The presence of an inhibition zone on culture media clearly indicates the antifungal activity of these plant extracts. The aqueous and ethanolic extracts of both plants significantly reduced the mycelial growth of all the selected fungal species (Tabs. 1–4). However, ethanolic plant extracts were found to be more effective than aqueous plant extracts.

The ethanolic extract of *B. lyceum* was found to be most effective against *Penicillium chrysogenum* with an inhibition zone of 41.00–31.67 mm followed by the aqueous extract against *P. chrysogenum* with an inhibition zone of 36.67–27.00 mm. Both the ethanolic and the aqueous extracts of *B. lyceum* were found to be least effective against *Cladosporium herbarum* with inhibition zones of 11.33–6.67 mm (aqueous extract) and 19.33–8.33 mm (ethanolic extract). The ethanolic extract of *R. obtusifolius* was most effective against *P. chrysogenum* with an inhibition zone of 39.00–31.33 mm followed by the ethanolic extract of the same plant against *Aspergillus niger* showing a zone of 35.33–25.67 mm. Both the aqueous and ethanolic extracts of *R. obtusifolius* were found to be least effective against *Trichothecium roseum*. The standard used was the same throughout the study, but it showed a slight variation of a few millimeters in the inhibition zone in different Petri plates for the same fungi. The standard used was found to be most effective against *A. niger*, followed by *P. chrysogenum*, *C. herbarum* and *T. roseum*.

Tab. 1. Effect of aqueous and ethanolic extracts of *Berberis lyceum* and *Rumex obtusifolius* on *Penicillium chrysogenum*.

Treatment	Inhibition zone (mm)			
	Berberis lyceum		*Rumex obtusifolius*	
	Aqueous extract	Ethanolic extract	Aqueous extract	Ethanolic extract
25 µl	27.00 ± 1.73	31.67 ± 1.53	25.67 ± 1.15	31.33 ± 3.21
50 µl	32.33 ± 2.08	37.00 ± 1.73	30.00 ± 1.00	35.33 ± 0.58
75 µl	36.67 ± 1.53	41.00 ± 1.73	34.67 ± 2.52	39.00 ± 1.00
Standard	38.33 ± 2.89	38.00 ± 2.65	37.33 ± 2.08	37.67 ± 2.52

Values are represented as mean ± SD of three replicates. The data was analysed by means of two-way ANOVA. The inhibition zone varies significantly according to plant extract concentration ($F = 53.794$, $P = 0.001$) and type of extract ($F = 15.038$, $P = 0.001$). No significant variation in the inhibition zone was caused by the interaction concentration * extract type ($F = 1.626$, $P = 0.150$).

Tab. 2. Effect of aqueous and ethanolic extracts of *Berberis lyceum* and *Rumex obtusifolius* on *Aspergillus niger*.

Treatment	Inhibition zone (mm)			
	Berberis lyceum		*Rumex obtusifolius*	
	Aqueous extract	Ethanolic extract	Aqueous extract	Ethanolic extract
25 µl	22.00 ± 3.00	25.33 ± 0.58	19.67 ± 2.08	25.67 ± 2.08
50 µl	25.00 ± 2.00	29.33 ± 2.52	25.00 ± 2.00	31.33 ± 1.15
75 µl	30.00 ± 1.53	34.00 ± 1.73	32.00 ± 2.00	35.33 ± 1.53
Standard	39.33 ± 0.58	39.33 ± 1.52	40.00 ± 2.00	39.00 ± 4.00

Values are represented as mean ± SD of three replicates. The data was analysed by means of two-way ANOVA. The inhibition zone varies significantly according to plant extract concentration (F = 139.534, P = 0.001) and type of extract (F = 10.018, P = 0.001). No significant variation in the inhibition zone was caused by the interaction concentration * extract type (F = 2.157, P = 0.053).

Tab. 3. Effect of aqueous and ethanolic extracts of *Berberis lyceum* and *Rumex obtusifolius* on *Trichothecium roseum*.

Treatment	Inhibition zone (mm)			
	Berberis lyceum		*Rumex obtusifolius*	
	Aqueous extract	Ethanolic extract	Aqueous extract	Ethanolic extract
25 µl	12.33 ± 1.53	15.33 ± 1.53	9.00 ± 1.00	13.00 ± 2.00
50 µl	14.00 ± 1.00	17.33 ± 1.15	12.33 ± 1.53	17.33 ± 2.51
75 µl	17.00 ± 1.73	22.33 ± 3.05	15.67 ± 1.15	22.00 ± 2.00
Standard	31.00 ± 1.73	31.33 ± 1.15	30.33 ± 3.21	30.00 ± 2.00

Values are represented as mean ± SD of three replicates. The data was analysed by means of two-way ANOVA. The inhibition zone varies significantly according to plant extract concentration (F = 216.645, P = 0.001) and type of extract (F = 15.070, P = 0.001). No significant variation in the inhibition zone was caused by the interaction concentration * extract type (F = 2.028, P = 0.069).

Tab. 4. Effect of aqueous and ethanolic extracts of *Berberis lyceum* and *Rumex obtusifolius* on *Cladosporium herbarum*.

Treatment	Inhibition zone (mm)			
	Berberis lyceum		*Rumex obtusifolius*	
	Aqueous extract	Ethanolic extract	Aqueous extract	Ethanolic extract
25 µl	6.67 ± 1.53	8.33 ± 0.58	11.67 ± 0.58	16.67 ± 0.58
50 µl	9.33 ± 0.58	14.00 ± 1.00	17.00 ± 1.00	23.33 ± 1.53
75 µl	11.33 ± 2.08	19.33 ± 0.58	22.33 ± 1.53	27.33 ± 0.58
Standard	30.67 ± 3.21	31.33 ± 3.06	33.00 ± 1.73	33.33 ± 2.89

Values are represented as mean ± SD of three replicates. The data was analysed by means of two-way ANOVA. The inhibition zone varies significantly according to plant extract concentration (F = 340.381, P = 0.001) and type of extract (F = 15.070, P = 0.001) as well as the interaction concentration * extract type (F = 6.722, P = 0.001).

Minimum inhibitory concentration for ethanolic extracts

The results revealed that the standard fungicide Hexahit showed a MIC value of 0.016 mg/ml for *Penicillium chrysogenum*, *Aspergillus niger* and 0.032 mg/ml for *Cladosporium herbarum* and *Trichothecium roseum* (Tab. 5). Of the two plant extracts, the lowest MIC was found in *Berberis lyceum* for *P. chrysogenum* (0.188 mg/ml), showing that the extracts are more effective in reducing the growth of *P. chrysogenum.* The highest MIC value was shown by *Rumex obtusifolius* for *T. roseum* (1.500 mg/ml). For *A. niger* (0.375) and *C. herbarum* (0.750) both tested plant extracts show the same MIC value. The MIC value of the *B. lyceum* plant extract varies between 0.188 and 0.750 mg/ml and that of the *R. obtusifolius* plant extract between 0.375 and 1.500 mg/ml for different fungi.

Tab. 5. Minimum inhibitory concentration of the ethanolic plant extracts against the selected fungi.

Treatment	MIC (mg/ml)			
	Penicillium chrysogenum	***Aspergillus niger***	***Trichothecium roseum***	***Cladosporium herbarum***
Berberis lyceum	0.188[b]	0.375[c]	0.750[d]	0.750[d]
Rumex obtusifolius	0.375[c]	0.375[c]	1.500[e]	0.750[d]
Standard	0.016[a]	0.016[a]	0.032[a]	0.032[a]

Mean values were compared using Tukey's multiple comparison test. Values followed by different letters are statistically different ($F = 6.375$, $P < 0.05$).

Phytochemical screening

The plant extracts of *Berberis lyceum* and *Rumex obtusifolius* were screened for the presence of different phytochemicals like phenols, alkaloids, tannins, quinones, terpenoids and flavonoids (Tab. 6). All the phytochemicals tested were found present in both aqueous and ethanolic extracts of *B. lyceum*. Alkaloids were found to be absent in the aqueous extract of *R. obtusifolius*, hence *B. lyceum* extracts are more effective against different fungal pathogens than those of *R. obtusifolius*.

Tab. 6. Qualitative screening of different phytochemicals present in *Berberis lyceum* and *Rumex obtusifolius*. Symbols: **++** = strong presence, **+** = moderate presence, **–** = absence.

Plant extracts		Phytochemicals tested					
		Phenols	**Alkaloids**	**Tannins**	**Flavonoids**	**Quinones**	**Terpenoids**
Berberis lyceum	Eth.	++	++	+	++	+	+
	Aq.	+	++	++	+	+	++
Rumex obtusifolius	Eth.	++	++	+	+	+	+
	Aq.	+	–	++	++	+	+

Discussion

The results revealed that ethanolic as well as aqueous plant extracts of *Berberis lyceum* and *Rumex obtusifolius* significantly reduced mycelial growth of all the tested rot fungi. Of the two plant extracts used, *B. lyceum* plant extracts were found more effective against *Penicillium chrysogenum* and *Trichothecium roseum*. Likewise, the *R. obtusifolius* plant extracts were more effective against *Aspergillus niger* and *Cladosporium herbarum*. Ethanolic plant extracts were more effective than aqueous extracts in inhibiting the mycelial growth of different fungal pathogens, thus showing a larger inhibition zone. The extent of inhibition depends on the concentration of the plant extract and also fungal spore concentration. Plant extracts with a larger inhibition zone show lower MIC values and are thus more effective.

Several reports of similar studies have demonstrated that extracts of medicinal plants are effective in controlling many pathogenic fungi (Raji & Raveendran 2013, Znini et al. 2013, Parveen & Wani 2015, Parveen et al. 2016, 2017). Parveen & Wani (2015) evaluated the efficacy of five plants, namely *Artemisia absinthium*, *Rumex obtusifolius*, *Plantago lanceolata*, *Taraxacum officinale* and *Malva sylvestris* against *Mucor piriformis*, and reported that all plants tested caused significant inhibition in spore germination and mycelial growth of the tested fungus. Jantasorn et al. (2016) evaluated the antifungal activity of five plant extracts, viz. *Hydnocarpus anthelminthicus*, *Crateva magna*, *Caesalpinia sappan*, *Xanthophyllum lanceatum* and *Carallia brachiata*, against five pathogenic fungi, *Pyricularia oryzae*, *Rhizoctonia solani*, *Phytophthora palmivora*, *Sclerotium rolfsii* and *Colletotrichum gloeosporiodes*, causing diseases of economic crops, and reported that *Hydnocarpus anthelminthicus*, *Caesalpinia sappan* and *Xanthophyllum lanceatum* have strong antimycotic activities and could be used as antifungal agents to control the growth of many pathogenic fungi and as an alternative method to reduce the application of fungicides. Parveen et al. (2017) determined the inhibitory effects of five phytoextracts, viz. *Artemisia absinthium*, *Malva sylvestris*, *Plantago lanceolata*, *Rumex obtusifolius* and *Taraxacum officinale*, on the mycelial growth and spore germination of *Rhizopus stolonifer*, *Drechslera* sp., *Penicillium expansum*, *Aspergillus niger* and *Aspergillus flavus*, revealing that all concentrations of plant extracts caused significant inhibition in the mycelial growth and spore germination of the test fungi as compared to the control. However, the maximum inhibition in mycelial growth and spore germination was found at the highest concentration of the plant extract, followed by lower concentrations.

The antifungal activities of these plant extracts are attributed to different chemical compounds like phenols, flavonoids, isoflavonoids, coumarins, pyrones and alkaloids present in these plants, which affect the growth of pathogenic fungi

(Jantasorn et al. 2016). The present study also revealed the presence of various phytochemicals, e.g. alkaloids, phenols, quinones, flavonoids, terpenoids and tannins, which may be responsible for their antifungal activity. Hence, these plant extracts may have a potential as antifungal agents for the control of different fungal pests.

Although the plant extracts showed lower efficacy than the fungicide used as a standard during the present study, the use of these fungicides has many limitations due to their harmful effects on the environment and living beings. Therefore the use of alternative control strategies like the use of plant extracts should be preferred, as they do not have any deletarious effect on environment or living beings. However, further study is needed to explore the possibility of using plant extracts against other pathogenic fungi responsible for causing different fungal plant diseases.

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