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Antifungal Activity of Ethanolic and Aqueous Leaf Extracts of *Taraxicum officinale* and *Mentha arvensis* on the Growth of Some Selected Fungal Species under *In vitro* Conditions

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ABSTRACT

Antifungal activities of different solvent extracted samples of Taraxacum officinale Weber ex Wiggers and Mentha arvensis L. were carried out through agar well diffusion assay at three concentrations (25µl, 50µl and 75µl) against seven fungi, viz. Penicillium expansum, Aspergillus niger, Mucor plumbeus, Alternaria alternata, Penicillium chrysogenum, Trichothecium roseum and Rhizoctonia solani causing rot of tomato and brinjal. It was revealed from the present study that the ethanolic extract of Taraxicum officinale Weber ex Wiggers showed maximum antimycotic activity against Rhizoctonia solani and least activity against Mucor plumbeus. However, the aqueous extract of Taraxicum officinale Weber ex Wiggers showed maximum antimycotic activity against Rhizoctonia solani and least activity against Penicillium expansum and Aspergillus niger. It was observed from the present study that the ethanolic extract of Mentha arvensis L. showed maximum antimycotic activity against Alternaria alternata and least activity against Mucor plumbeus. While as the aqueous extract of Mentha arvensis L. showed maximum antifungal activity against Rhizoctonia solani and least activity against Trichothecium roseum.

Key words: *Antifungal assay, Taraxacum officinale, Mentha arvensis, Agar well diffusion.*

INTRODUCTION

Medicinal plants are the main and rich source of different activity especially antimicrobial agents. Plants have been used in many countries medicinally and as a source of many potent and powerful drugs[15]. *T. officinale* belongs to the family Asteraceae. It grows to a height of about 12 inches; traditionally *T. officinale* is commonly used as food. The leaves are used in salads and tea while the roots are often used as a coffee substitute. Leaf and root extracts of *T. officinale* have been used for different human ailments like treatment of liver, gall bladder, kidney, and joint problems, blood purifier, for treatment of poor digestion and other ailments[8,12].

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Likewise, *Mentha Arvensis* L belongs to the family Lamiaceae, has been found major source of antimicrobial compound and used in pharmaceutical, cosmetics and flavoring industries and different human ailments[6]. Many plants and their extracts have been evaluated for their antimycotic activity and are known to have good antifungal activity against plant pathogenic fungi causing diseases in plants[28,29]. Plant metabolites and plant based pesticides appear to be one of the better alternatives as they are known to have minimal environmental impact and danger to consumers in contrast to synthetic pesticides[27]. Extracts of many higher plants have been reported to exhibit antifungal properties under laboratory trails[3,5,6,10,16,19].Therefore, the aim of present study was to evaluate antifungal activities of ethanolic and aqueous extracts of leaves of two important medicinal plants, *T. officinale* (dandelion) and *Mentha arvensis* L. against some pathogenic fungi

MATERIALS AND METHODS

Plant collection and identification

The fresh plant material of *Taraxicum officinale* Weber ex Wiggers and *Mentha arvensis* L. were collected from Pulwama, Jammu and Kashmir, India. The authenticity of the plant was confirmed in Plant Taxonomy Department of Botany University of Kashmir.

Preparation of plant extracts

Fresh plant material of leaves of two test plants were washed under running tap water, shade dried or sun dried for 24 hours and then homogenized to fine powder and stored in airtight bottles. About 20g of coarsely powdered leaves (20g/100mL) were extracted separately in a soxhlet extractor for 8 to 10 hours (30-50°C) sequentially with ethanol and water separately in order to extract non-polar and polar compounds[9].

Preparation of inoculums

Seven fungal cultures such as *Penicillium expansum* Link ex Thorn., *Aspergillus niger* Van Tiegh, *Alternaria alternata* (Fr) Keissler, *Mucor plumbeus* Fisher, *Penicillium chrysogenum* Thorn, *Trichothecium roseum* (Pers.) Link and *Rhizoctonia solani* Kuhn were grown at 27°C on potato dextrose agar (PDA) medium. Spores of the each fungal species were collected from cultures on agar plates after 7 days[4]. PDA broth prepared by transferring a loop full of cells from the stock cultures was diluted with fresh potato dextrose broth. The sporangial suspension concentration was adjusted to $2x10^5$ (CFU/mL) spores as per the method by Abril, *et al*.[1]

Antifungal activity

Antifungal assay for ethanolic and aqueous extracts was performed by agar well diffusion method as described by Ahmad *et al*.[2] with some modification. 100 µl of standardized inoculum of each test fungi were inoculated on sterile molten Sabouraud Dextrose Agar, homogenised and poured onto a sterile Petri plate to yield a uniform depth of 4 mm. The petriplates were allowed to solidify inside the laminar hood. Sterile cork borers of 5 mm in diameter were used to make three wells at periphery of each Petriplate. Different concentrations (25µl, 50µl and 75µl) of each plant extract, prepared in respective solvents were loaded into four different peripheral wells. FLU-25 {(standard antifungal disc (20mcg/disc)} was loaded into the sterile molten Sabouraud Dextrose Agar in the same Petri plate at periphery. The plates were then incubated at 26 ± 2 ºC for 24 to 36 hours. After incubation period, the plates were observed for the zones of inhibition. Antifungal potential was evaluated by measuring inhibition zone diameters in millimeters (mm) with the help of standard measuring scale.

Statistical analysis

The antifungal activity of *Taraxicum officinale* and *Mentha arvensis* leaf extracts was indicated by zones of growth inhibition. All experiments were performed in triplicates and the results are presented as mean ± SD (Standard Deviation) according to New Duncan's Multiple Range Test[11].

RESULTS

It was revealed from the present study that the ethanolic extract of *Taraxicum officinale*

Weber ex Wiggers showed maximum activity at 25 µl, 50 µl and 75 µl with zone of inhibition of 18.66 ±0.57, 20.00 ± 1.00 and 22.00 ± 1.00 mm against *Rhizoctonia solani* respectively. While as *Mucor plumbeus* showed least activity with zone of inhibition of 9.00±1.00, 10.00±1.00 and 10.66±1.15 mm at 25 µl, 50 µl and 75 µl concentrations respectively. The moderate activity of ethanolic extract was shown against *Alternaria alternata* with zone of inhibition of 16.33± 0.57, 18.33± 0.57 and 20.33± 0.57 mm and *Trichothecium roseum* with zone of inhibition of 15.00± 1.00, 17.00 ± 1.00 and 19.00 ± 1.00 mm at 25 µl, 50 µl and 75 µl respectively. Antimycotic activity was also observed by the ethanolic extracts of *Taraxicum officinale* against *Penicillium chrysogenum* and *Aspergillus niger* with zone of inhibition of 13.66± 0.57, 15.66 ± 0.57, 17.66 ± 0.57 mm and 12.66± 0.57, 14.66 ± 0.57, 15.66 ± 0.57 mm at 25 µl, 50 µl and 75 µl concentrations respectively. Likewise antimycotic activity of leaf extracts was found against *Penicillium expansum* with zone of inhibition of 11.33 ±1.15, 12.33 ± 0.57 and 13.33 ± 0.57 mm at 25 µl, 50 µl and 75 µl concentrations (Table 1).

The aqueous extract of *Taraxicum officinale* Weber ex Wiggers showed maximum antifungal activity at 25 µl, 50 µl and 75 µl with zone of inhibition of 20.66 ± 0.57, 22.66 ± 0.57 and 24.66 ± 0.57 mm against *Rhizoctonia solani*. Moderate antifungal activity was recorded against *Trichothecium roseum*, *Mucor plumbeus* and *Alternaria alternata* with zone of inhibition of 18.33 ± 0.57, 20.00 ± 1.00, 21.00 ± 1.00., 18.33 ± 1.52, 19.33 ± 1.52, 19.66 ± 0.57 and 18.00 ± 1.00, 20.00 ± 1.00, 22.00 ± 1.00 mm at 25 µl, 50 µl and 75 µl respectively. The aqueous extract of *Taraxicum officinale* also showed active antifungal activity against *Penicillium expansum* and *Aspergillus niger* with zone of inhibition of 11.00 ± 1.00, 11.66 ± 0.57, 13.00 ± 1.00 mm and 11.00 ± 1.00, 11.33 ± 1.52, 13.33 ± 1.52 mm at 25 µl, 50 µl and 75 µl respectively. Similarly, aqueous extract of *T. officinale* against *Penicillium chrysogenum* was observed with zone of inhibition of 12.33 ± 0.57, 14.00 ± 1.00 and 15.66 ± 0.57 at 25 µl, 50 µl and 75 µl respectively (Table 2).

Table 1: Antifungal activity of ethanolic leaf extracts of *Taraxicum officinale* Weber ex Wiggers

Fungal Pathogen	Zone of Inhibition (mm), Concentration 100 mg/ml			
	25µl	50 µl	75 µl	20mcg/disc FLU 25
Penicillium expansum	11.33 ±1.15	12.33 ± 0.57	13.33 ± 0.57	14.66 ± 0.57
Aspergillus niger	12.66± 0.57	14.66 ± 0.57	15.66 ± 0.57	18.33 ± 1.52
Alternaria alternate	16.33± 0.57	18.33± 0.57	20.33± 0.57	21.33 ± 0.57
Mucor plumbeus	9.00±1.00	10.00±1.00	10.66±1.15	12.00 ± 1.00
Penicillium chrysogenum	13.66± 0.57	15.66 ± 0.57	17.66 ± 0.57	18.66 ± 0.57
Trichothecium roseum	15.00± 1.00	17.00 ± 1.00	19.00 ± 1.00	20.00 ± 1.00
Rhizoctonia solani	18.66 ±0.57	20.00 ± 1.00	22.00 ± 1.00	23.00 ± 1.00

Values were performed in triplicates and represented as mean ± SD

Mean values followed by different superscript in a column are significantly different ($p \leq 0.05$)

Table 2: Antifungal activity of aqueous leaf extracts of *Taraxicum officinale* Weber ex Wiggers

Fungal Pathogen	Zone of Inhibition (mm)., Concentration 100 mg/ml			
	25µl	50 µl	75 µl	20mcg/disc FLU 25
Penicillium expansum	11.00 ± 1.00	11.66 ± 0.57	13.00 ± 1.00	14.00 ± 1.00
Aspergillus niger	11.00 ± 1.00	11.33 ± 1.52	13.33 ± 1.52	13.66 ± 0.57
Alternaria alternate	18.00 ± 1.00	20.00 ± 1.00	22.00 ± 1.00	23.00 ± 1.00
Mucor plumbeus	18.33 ± 1.52	19.33 ± 1.52	19.66 ± 0.57	21.33 ± 0.57
Penicillium Chrysogenum	12.33 ± 0.57	14.00 ± 1.00	15.66 ± 0.57	17.00 ± 1.00
Trichothecium roseum	18.33 ± 0.57	20.00 ± 1.00	21.00 ± 1.00	22.00 ± 1.00
Rhizoctonia solani	20.66 ± 0.57	22.66 ± 0.57	24.66 ± 0.57	25.66 ± 0.57

Values were performed in triplicates and represented as mean ± SD, Mean values followed by different superscript in a column are significantly different ($p \leq 0.05$)

It was observed from the present study that the ethanolic extract of *Mentha arvensis* L. showed maximum antifungal activity at 25 µl, 50 µl and 75 µl with zone of inhibition of 26.00± 1.00, 27.00 ± 1.00 and 31.33 ± 0.57 mm against *Alternaria alternata* respectively. Likewise ethanolic extract of *Mentha arvensis* L. showed maximum antifungal activity *Penicillium chrysogenum* and *Aspergillus niger* with zone of inhibition of 25.33± 0.57, 27.00 ± 1.00, 29.00 ± 1.00 mm and 24.33± 1.52, 32.33 ± 1.52, 34.66 ± 1.52 mm at 25 µl, 50 µl and 75 µl respectively. The moderate antifungal activity was observe due to ethanolic extract on *Mentha arvensis* against *Penicillium expansum* with zone of inhibition of 23.33 ±0.57, 26.33 ± 1.52, 28.00 ± 2.00 mm, against *Rhizoctonia solani* with zone of inhibition of 23.33± 0.57, 24.33 ± 0.57, 26.00 ± 2.64 mm at 25 µl, 50 µl and 75 µl respectively and against *Trichothecium roseum* with zone of inhibition of 21.00 ±1.00, 22.33 ± 0.57, 25.33 ± 0.57 at 25 µl, 50 µl and 75 µl. While least fungal activity was observed against *Mucor plumbeus* with zone of inhibition of 16.00 ±1.00, 17.00 ±1.00 and 19.00 ± 1.00 mm at 25 µl, 50 µl and 75 µl concentration of ethanolic extracts of *M.* (Table 3).

The aqueous extract of *Mentha arvensis* L. showed maximum antifungal activity at 25 µl, 50 µl and 75 µl with zone of inhibition of 21.00±1.00, 22.00 ± 1.00 and 23.00 ± 1.00 mm against *Rhizoctonia solani*. Moderate antifungal activity was recorded due aqueous extract of *Mentha arvensis* against *Alternaria alternata* with zone of inhibition of 20.00 ±1.00, 21.33 ± 1.52, 23.66 ± 1.52., against *Penicillium expansum* with zone of inhibition of 19.66 ±1.52 , 21.00 ± 1.00 , 22.66± 1.52., and against *Penicillium chrysogenum* with one of inhibition of 18.66 ±0.57 , 21.00 ± 1.00 , 23.00 ± 1.00 at 25 µl, 50 µl and 75 µl concentrations respectively. Likewise, aqueous extract of *Mentha arvensis* showed antifungal activity against *Aspergillus niger* and *Mucor plumbeus* with zone of inhibition of 15.00 ± 1.00, 17.66 ± 1.52, 19.66 ± 1.52 and 15.00± 1.00, 18.00 ± 1.00, 20.00 ± 1.00 mm at 25 µl, 50 µl and 75 µl respectively and against *Trichothecium roseum* with zone of inhibition of 14.66±0.57, 16.66 ± 0.57 and 18.66 ± 0.57 mm at 25 µl, 50 µl and 75 µl respectively (Table 4). However, least effect in zone of inhibition was observed against *Trichothecium roseum.*

It was revealed from the present study that the aqueous extract of *Taraxicum officinale* Weber ex Wiggers are more effective in showing antifungal activity against selected fungi except *Alternaria alternata*, *Mucor plumbeus* and *Rhizoctonia solani* in which ethanolic extract shows more effectiveness. Likewise, the aqueous extract of *Mentha arvensis* L. is more effective than ethanolic extract against the isolated fungi except *Mucor plumbeus* respectively.

Table 3: Antifungal activity of ethanolic leaf extracts of *Mentha arvensis* L.

Fungal Pathogen	Zone of Inhibition (mm)., Concentration 100 mg/ml			
	25µl	**50 µl**	**75 µl**	**20mcg/disc FLU 25**
Penicillium expansum	23.33 ±0.57	26.33 ± 1.52	28.00 ± 2.00	28.00 ± 1.00
Aspergillus niger	24.33± 1.52	32.33 ± 1.52	34.66 ± 1.52	35.00 ± 1.00
Alternaria alternate	26.00± 1.00	27.00 ± 1.00	31.33 ± 0.57	32.33 ± 0.57
Mucor plumbeus	16.00 ±1.00	17.00 ±1.00	19.00 ± 1.00	20.00 ± 1.00
Penicillium chrysogenum	25.33± 0.57	27.00 ± 1.00	29.00 ± 1.00	29.33 ± 0.57
Trichothecium roseum	21.00 ±1.00	22.33 ± 0.57	25.33 ± 0.57	26.33 ± 0.57
Rhizoctonia solani	23.33± 0.57	24.33 ± 0.57	26.00 ± 2.64	27.00 ± 1.00

Values were performed in triplicates and represented as mean ± SD
Mean values followed by different superscript in a column are significantly different ($p \leq 0.05$)

Table 4: Antifungal activity of aqueous leaf extracts of *Mentha arvensis* L.

Fungal Pathogen	Zone of Inhibition (mm)., Concentration 100 mg/ml			
	25µl	50 µl	75 µl	20mcg/disc FLU 25
Penicillium expansum	19.66 ±1.52	21.00 ± 1.00	22.66± 1.52	24.00 ± 1.00
Aspergillus niger	15.00 ±1.00	17.66 ± 1.52	19.66 ± 1.52	22.00 ± 1.00
Alternaria alternata	20.00 ±1.00	21.33 ± 1.52	23.66 ± 1.52	25.00 ± 1.00
Mucor plumbeus	15.00± 1.00	18.00 ± 1.00	20.00 ± 1.00	21.00 ± 0.57
Penicillium chrysogenum	18.66 ±0.57	21.00 ± 1.00	23.00 ± 1.00	24.00 ± 1.00
Trichothecium roseum	14.66±0.57	16.66 ± 0.57	18.66 ± 0.57	19.66 ± 0.57
Rhizoctonia solani	21.00±1.00	22.00 ± 1.00	23.00 ± 1.00	24.33 ± 0.57

Values were performed in triplicates and represented as mean ± SD

Mean values followed by different superscript in a column are significantly different ($p \leq 0.05$)

DISCUSSION

In the present study some plant extracts *Taraxicum officinale* Weber ex Wiggers and *Mentha arvensis* L. were evaluated for their antifungal activity against the some fungi causing fungal rot of tomato and brinjal. It was clear from the results that all the tested plant extracts proved highly effective in reducing the mycelial growth of fungi under in-vitro conditions indicating their antifungal activity. In a similar study, Pawar[24] reported the antifungal activity of nine plant extracts against five phytopathogenic fungi, viz. *Alternaria alternata*, *Aspergillus niger*, *Curvularia lunata*, *Fusarium moniliforme* and *Trichoderma viride*. Hassanein *et al.*[13] studied the effectiveness of leaf extracts of neem and china berry against two tomato pathogenic fungi *Alternaria solani* and *Fusasuim oxysporum*, the causal agent of early blight and wilt disease of tomato plants respectively. Mondall *et al.*[17] reported the antifungal activity of aqueous and alcoholic neem leaf extracts on some fungi, viz. *Aspergillus*, *Rhizopus* and reported that the alcoholic extracts of neem leaf was most effective in comparison to aqueous extract for reducing the growth of *Rhizopus a*nd *Aspergillus*. The crude aqueous and alcoholic leaf extracts of neem was more effective in inhibitions of growth of *Aspergillus* in comparison to inhibitory effects on *Rhizopus* growth in culture medium. Taskeen-Un-Nisa *et al*.[26] tested plant extracts of three plants, onion (*Allium cepa*), garlic (*Allium sativum)* and mint (*Mentha arvensis*) for their antifungal activity against *Alternaria alternata* and *R. stolonifer*. They observed that the extract of *A. sativum* at highest concentration proved highly effective in reducing spore germination of *R. stolonifer* and *Alternaria alternata* followed by extract of *A. cepa* and *M. arvensis*. Jeyaseelan *et al*.[14] reported antifungal activity of organic extracts of leaf, flower and fruit of *Lawsonia inermis* L. against *Aspergillus niger, Penicillium notatum, Fusarium oxysporum*, *Colletotrichum gloeosporioides* and *Rhizopus stolonifer*. Parveen *et al*.[20] reported the inhibitory activity of five plant extracts, viz. *Artemisia absinthium*, *Rumex obtusifolius*, *Taraxacum officinale*, *Plantago lanceolata* and *Malva sylvestris* against the mycelial growth of three rot fungi, *Alternaria alternata*, *Penicillium expansum* and *Mucor piriformis*, and observed that all concentrations brought about significant reduction in the mycelial growth of these pathogenic fungi. Our results also showed the inhibition of mycelial growth due to plant extracts of *Taraxicum officinale* and *Mentha arvensis* against some pathogenic fungi**.** Dababneh and Khalil[7] studied the effect of five different medicinal plant extracts, viz. *Crupina crupinastrum*, *Teucrium polium*, *Achillea santolina*, *Micromeria nervosa* and *Ballota philistaea*, against four pathogenic fungi, viz. *Fusarium oxysporum*, *Rhizoctonia solani*, *Penicillium* sp. and *Verticillium s*p. It is concluded from this study that these plant extracts of *Taraxicum officinale and Mentha arvensis* can possibly be exploited in the management of pathogenic fungi to prevent biodeteriorations in an eco-friendly way and

to develop alternative and safe biopesticde. However, further investigation is needed to observe antimycotic activity of these plants.

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