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Antimycotic activity of ethanolic and aqueous leaf extracts of *Ajuga bracteosa* Wall. *ex* Benth. (Lamiale: Lamiaceae) and *Iris kashmiriana* Baker (Asparagales: Iridaceae) against some vegetable rot fungi

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Abstract. Antifungal activities of different solvent extracts of *Ajuga bracteosa* Wall. *ex* Benth. (Lamiale: Lamiaceae) and *Iris kashmiriana* Baker (Asparagales: Iridaceae) were carried out through agar well diffusion assay at three concentrations (25 µL, 50 µL and 75 µL) against seven rot causing fungi, viz. *Penicillium expansum*, *Aspergillus niger*, *Mucor plumbeus*, *Alternaria alternata*, *Penicillium chrysogenum*, *Trichothecium roseum* and *Rhizoctonia solani.* All the concentration of plant extracts showed antimycotic activity against tested pathogenic fungi. Antimycotic activity increased with the increased concentrations of plant extracts. However, higher concentrations proved more effective than lower concentrations. It was revealed from the present study that the ethanolic extract of *Ajuga bracteosa* showed maximum antimycotic activity against *Mucor plumbeus* and *Rhizoctonia solani* and least activity against *Penicillium chrysogenum*. However, the aqueous extract of *Ajuga bracteosa* showed maximum antifungal activity against *Rhizoctonia solani* and *Penicillium expansum* and least activity against *Trichothecium roseum.* It was further revealed from the present study that the ethanolic extract of *Iris kashmiriana* showed maximum antimycotic activity against *Aspergillus niger* and least activity against *Rhizoctonia solani*. Whereas the aqueous extract of *Iris kashmiriana* showed maximum antimycotic activity against *Penicillium expansum* and *Rhizoctonia solani* and least activity against *Aspergillus niger*.

Keywords: Antimycotic assay; Pathogenic fungi; Concentration; Plant extracts; Agar well diffusion.

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Introduction

Plant pathogenic fungi are known to cause huge losses to the crop plants and their produce in storage. Several control strategies have been employed by agricultural scientists to minimize the losses caused by pathogenic fungi. In this study, the important medicinal plants *Ajuga bracteosa* Wall. *ex* Benth. (Lamiale: Lamiaceae) and *Iris kashmiriana* Baker (Asparagales: Iridaceae) have been evaluated for their antimycotic activity.

Medicinal plants constitute the basis of primary health care for many people in Asia and are also a source of income for rural populations. Bioactive compounds currently extracted from these plants are used as pharmaceuticals, agrochemicals, flavour and fragrance ingredients, food additives and pesticides. *Ajuga bracteosa* is an important medicinal plant commonly known as "bungle" in English and "Jan-i-adam" in Kashmiri. *Ajuga bracteosa* has great medicinal and economic importance (Kayani et al., 2016). In North area of India, *Ajuga bracteosa* is given in the treatment of fevers, neuro diseases (Nisar et al., 2014). The leaves are diuretic, stimulant and used as a substitute for cinchona (Chopra et al*.,* 1956) the plant is also reported to possess cardio stimulant action in animals and anticancer activity in rats and mice (Dhar et al., 1968).

Iris kashmiriana is one of an important member of this Family Iridaceae, locally known as "Mazarmund" in Kashmir. The plant has been widely used in traditional medicine and modern clinical preparations to treat cold, flu, malaria, toothache, cancer, bacterial and viral infections and bruise (Hanawa et al., 1991). The phytochemical analyses of the different extracts of *Iris kashmiriana* have revealed the presence of different compounds including flavonoids, isoflavonoids, glycosides and tannins (Wani et al., 2012). The medicinal importance of the plant prompted isolation of a variety of pharmacologically active compounds including quinones, triterpenoids, flavonoids, isoflavonoids and stilbene glycosides (Amin et al*.*, 2013).

Keeping in view the medicinal importance of plants, study was carried out to evaluate the antifungal activities of ethanolic and aqueous extracts of leaves of *Ajuga bracteosa* and *Iris kashmiriana* against some selected fungi causing decaying of vegetables.

Materials and methods

Plant collection and identification

The fresh plant material of *Ajuga bracteosa* and *Iris kashmiriana* was collected from District Baramulla and Pulwama of Kashmir Valley. The authenticity of the plant was confirmed in Plant Taxonomy Department of Botany University of Kashmir. Adequate amount of the leaves of these plants were collected in polythene bags, brought to laboratory for evaluating their antimycotic activity under *in vitro* conditions.

Preparation of plant extracts

These plant leaves in a required quantity were sundried for 24 h and then milled into powder using morter and pestle. About 20 g of coarsely powdered leaves (20 g/100 mL) were extracted separately in a soxhlet extractor for 8 to 10 h (30 °C-50 °C) sequentially with ethanol and water separately in order to extract non-polar and polar compounds (Elgorashi et al., 2004).

Preparation of inoculums of fungi

Pure fungal cultures of *Penicillium expansum*, *Aspergillus niger*, *Alternaria alternata*, *Mucor plumbeus*, *Penicillium chrysogenum*, *Trichothecium roseum* and *Rhizoctonia solani* were obtained from Plant Pathology and Mycology Laboratory, Department of

Botany University of Kashmir. These pure cultures were grown on Potato dextrose agar (PDA) medium at 27 °C ± 1 °C in Petri plates. Spores of the each fungal species were collected from these cultures after 7 days (Broekaert et al., 1990). The density of spore suspension was measured and was adjusted to $2x10^5$ CFU/mL spores (Abril et al., 2008).

Antifungal activity

Antifungal activity of ethanolic and aqueous extracts was performed by agar well diffusion method (Alzoreky et al., 2003; Ahmad et al., 2012). 100 µL of standardized inoculum of each test fungi were inoculated onto sterile molten Sabouraud Dextrose Agar homogenised and poured into a sterile Petri plate to yield a uniform depth of 5 mm. The Petri plates were allowed to solidify inside the laminar airflow chamber. Sterile cork borers of 5 mm in diameter were used to make three wells at periphery of each Petri plate. Different concentrations (25 µL, 50 µL and 75 µL) of each plant extract, prepared in respective solvents were loaded into three different peripheral wells. Flucanazole solution (20 µL/well) was used as control in the separate well in the same Petri plate. The plates were then incubated at 26 °C ± 2 °C for 24 to 36 h. 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.

Results

It was observed from the results (Table 1, Figure 1) that the ethanolic extract of *Ajuga bracteosa* showed maximum inhibition in the fungal growth of *Mucor plumbeus* and *Rhizoctonia solani* with zone of inhibition as 20.33 mm ± 0.57 mm, 22.00 mm ± 1.00 mm, 24.66 mm ±0.57 mm and 20.33 mm ± 0.57 mm, 22.33 mm ± 0.57 mm, 24.00 mm ± 1.00 mm at 25 µL, 50 µL and 75µL, respectively. Whereas as moderate inhibitory activity of *Ajuga bracteosa* was shown against *Aspergillus niger*, and *Trichothecium roseum* with the zone of inhibition of 19.00 mm ± 1.00 mm, 21.66 mm ± 0.57 mm, 23.00 mm ± 1.00 mm and 17.66 mm ± 0.57 mm, 20.00 mm ± 1.00 mm, 21.66 mm ± 1.52 mm at 25 µL, 50 µL, and 75 µL concentration, respectively. The inhibition in zone of fungal growth due to ethanolic extract of *Ajuga bracteosa* against *Penicillium expansum* and *Alternaria alternata* was 16.00 mm ± 1.00 mm, 18.66 mm ± 0.57 mm, 21.00 mm ± 1.00 mm, and 16.00 mm ± 1.00 mm, 18.00 mm ± 1.00 mm, 20.66 mm ± 0.57 mm at 25 µL, 50 µL, and 75 µL concentration, respectively. The results were compared with solution of flucanazole as a positive control. Ethanolic extract of *Ajuga bracteosa* showed least inhibition in fungal growth of *Penicillium chrysogenum* which was found as 15.66 mm ± 0.57 mm,

Table 1. Antifungal activity of ethanolic leaf extracts of *Ajuga bracteosa*.

	Zone of Inhibition (mm)			
	25 µL	**50 µL**	**75 µL**	**Control**
Penicillium expansum	16.00 ± 1.00	18.66 ± 0.57	21.00 ± 1.00	22.00 ± 1.00
Aspergillus niger	19.00 ± 1.00	21.66 ± 0.57	23.00 ± 1.00	24.00 ± 1.00
Alternaria alternata	16.00 ± 1.00	18.00 ± 1.00	20.66 ± 0.57	21.66 ± 0.57
Mucor plumbeus	20.33 ± 0.57	22.00 ± 1.00	24.66 ± 0.57	25.00 ± 1.00
Penicillium chrysogenum	15.66 ± 0.57	18.00 ± 1.00	20.66 ± 0.57	21.00 ± 1.00
Trichothecium roseum	17.66 ± 0.57	20.00 ± 1.00	21.66 ± 1.52	23.33 ± 1.52
Rhizoctonia solani	20.33 ± 0.57	22.33 ± 0.57	24.00 ± 1.00	25.00 ± 1.00

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

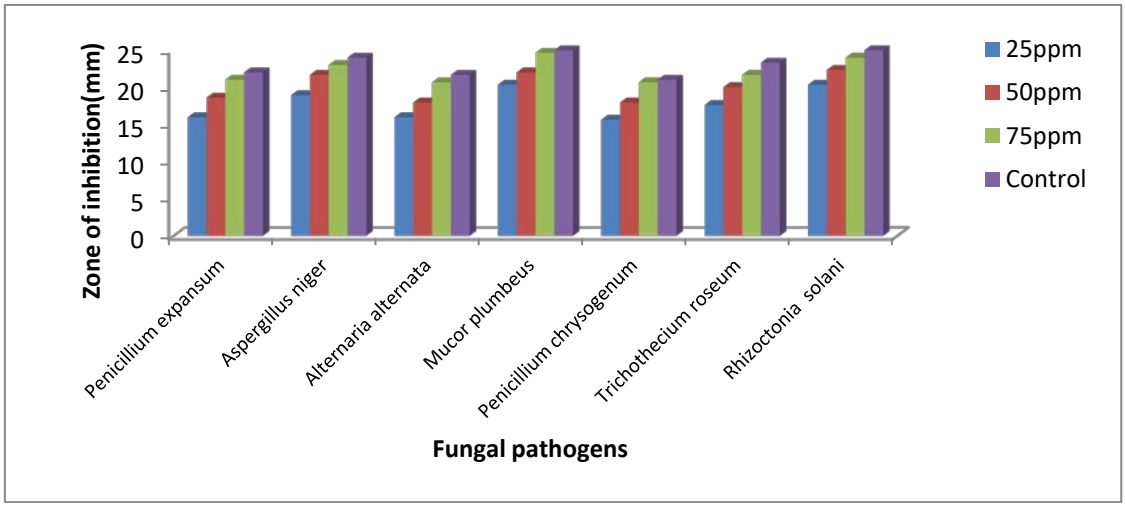

Figure 1. Antifungal activity of ethanolic leaf extracts of *Ajuga bracteosa*.

18.00 mm ± 1.00 mm and 20.66 mm ± 0.57 mm at 25 µL, 50 µL and 75 µL concentrations, respectively.

The aqueous extract of *Ajuga bracteosa* caused maximum inhibition in fungal growth at 25 µL, 50 µL and 75 µL with zone of inhibition as 20.66 mm ± 0.57 mm, 21.33 mm ± 1.52 mm, 23.66 mm ± 1.52 mm and 20.33 mm ± 0.57 mm, 23.33 mm ±1.52 mm, 26.00 mm ±1.00 mm against *Rhizoctonia solani* and *Penicillium expansum*, respectively. Moderate antifungal activity was recorded against *Aspergillus niger* and *Mucor plumbeus* with zone of inhibition as 20.00 mm ± 1.00 mm, 21.66 mm ± 1.52 mm, 22.33 mm ± 1.52 mm and 19.00 mm ± 1.00 mm, 21.33 mm ± 0.57 mm, 22.66 mm ± 1.52 mm at 25 µL, 50 µL and 75 µL concentration of plant extracts, respectively. The inhibition in fungal growth of *Alternaria alternata* and *Penicillium chrysogenum* was 18.33 mm ± 0.57 mm, 20.00 mm ± 1.00 mm, 22.66 mm ± 1.52 mm and 17.00 mm ± 1.00 mm, 18.66 mm ±1.15 mm, 20.33 mm ± 0.57 mm at 25 µL, 50 µL and 75 µL concentrations of plant extracts, respectively. The zone of inhibition in mycelia of *Trichothecium roseum* was 15.00 mm ± 1.00 mm, 16.66 mm ± 1.52 mm and 20.00 mm ± 1.00 mm, respectively at 25 µL, 50 µL and 75 µL concentration of plant extract of *Ajuga bracteosa* (Table 2, Figure 2).

Table 2. Antifungal activity of aqueous leaf extracts of *Ajuga bracteosa*.

	Zone of Inhibition (mm)			
	25 µL	**50 µL**	**75 µL**	**Control**
Penicillium expansum	20.33 ± 0.57	23.33 ± 1.52	26.00 ± 1.00	27.00 ± 1.00
Aspergillus niger	20.00 ± 1.00	21.66 ± 1.52	22.33 ± 1.52	23.33 ± 1.52
Alternaria alternata	18.33 ± 0.57	20.00 ± 1.00	22.66 ± 1.52	23.66 ± 1.52
Mucor plumbeus	19.00 ± 1.00	21.33 ± 0.57	22.66 ± 1.52	24.00 ± 1.00
Penicillium chrysogenum	17.00 ± 1.00	18.66 ± 1.15	20.33 ± 0.57	22.00 ± 1.00
Trichothecium roseum	15.00 ± 1.00	16.66 ± 1.52	20.00 ± 1.00	22.00 ± 1.00
Rhizoctonia solani	20.66 ± 0.57	21.33 ± 1.52	23.66 ± 1.52	26.00 ± 1.00

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

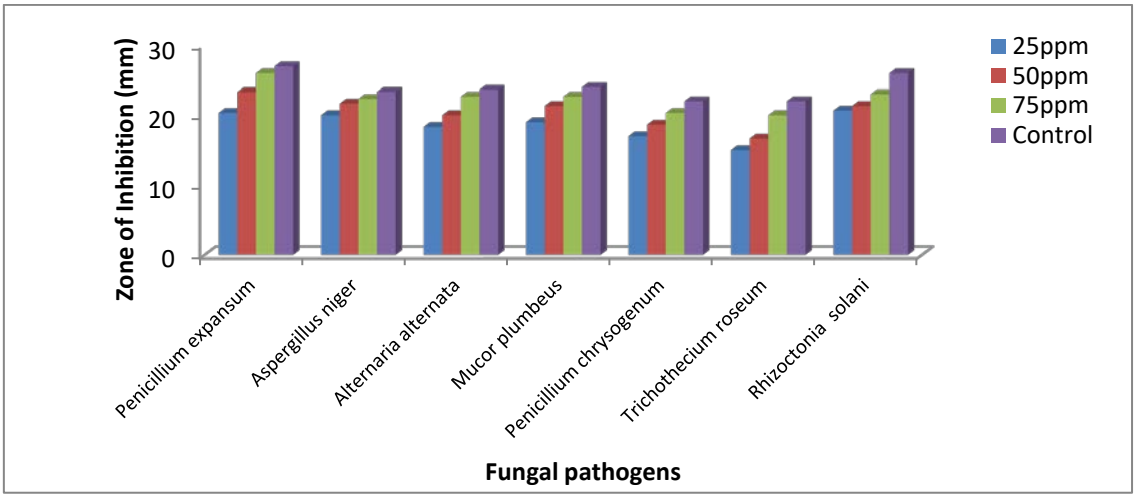

Figure 2. Antifungal activity of aqueous leaf extracts of *Ajuga bracteosa*.

It was revealed from the results (Table 3, Figure 3) that the ethanolic extract of *Iris kashmiriana* caused maximum inhibition in mycelial growth of *Aspergillus niger* with zone of inhibition of 20.33 mm ± 0.57 mm, 22.00 mm ± 1.00 mm and 24.33 mm ± 0.57 mm at 25 µL, 50 µL and 75 µL concentrations, respectively. The zone of inhibition in case of *Penicillium expansum* and *Mucor plumbeus* was 18.66 mm ± 0.57 mm, 20.00 mm ± 1.00 mm, 22.00 mm ± 1.00 mm, and 18.00 mm ± 1.00 mm, 20.00 mm ± 1.00 mm, 21.33 mm ± 0.57 mm at 25 µL, 50 µL and 75 µL concentrations of *Iris kashmiriana*, respectively. The zone of mycelial inhibition was 17.33 mm ± 1.15 mm, 18.66 mm ± 0.57 mm, 21.00 mm ± 1.00 mm at 25 µL, 50 µL and 75 µL concentrations of *Iris kashmiriana* against *Penicillium chrysogenum*. Moderate inhibitory effect of ethanolic extract was shown against *Trichothecium roseum* and *Alternaria alternata* with the zone of inhibition as 16.66 mm ± 1.52 mm, 18.00 mm ± 1.00 mm, 20.33 mm ± 0.57 mm and 16.00 mm ±1.00 mm, 17.66 mm ± 0.57 mm, 20.00 mm ± 1.00 mm at 25 µL, 50 µL and 75 µL concentrations of plant extract of *Iris kashmiriana*, respectively. Whereas, ethanolic extract of *Iris kashmiriana* caused least inhibition in fungal growth of *Rhizoctonia solani* as it varies from 13.33 mm ± 0.57 mm, 16.00 mm ± 1.00 mm, and 17.33 mm ± 0.57 mm at 25 µL, 50 µL and 75 µL concentrations.

Table 3. Antifungal activity of ethanolic leaf extracts of *Iris kashmiriana*.

	Zone of Inhibition (mm)			
	25 µL	**50 µL**	**75 µL**	**Control**
Penicillium expansum	18.66 ±0.57	20.00 ± 1.00	22.00 ± 1.00	23.00 ± 1.00
Aspergillus niger	20.33± 0.57	22.00 ± 1.00	24.33 ± 0.57	26.00 ± 1.00
Alternaria alternate	16.00±1.00	17.66 ± 0.57	20.00± 1.00	20.66 ± 0.57
Mucor plumbeus	18.00±1.00	20.00 ± 1.00	21.33 ± 0.57	23.33 ± 0.57
Penicillium chrysogenum	17.33±1.15	18.66± 0.57	21.00 ± 1.00	23.00 ± 1.00
Trichothecium roseum	16.66±1.52	18.00 ± 1.00	20.33 ± 0.57	21.00 ± 1.00
Rhizoctonia solani	13.33 ±0.57	16.00 ± 1.00	17.33 ± 0.57	18.33 ± 0.57

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

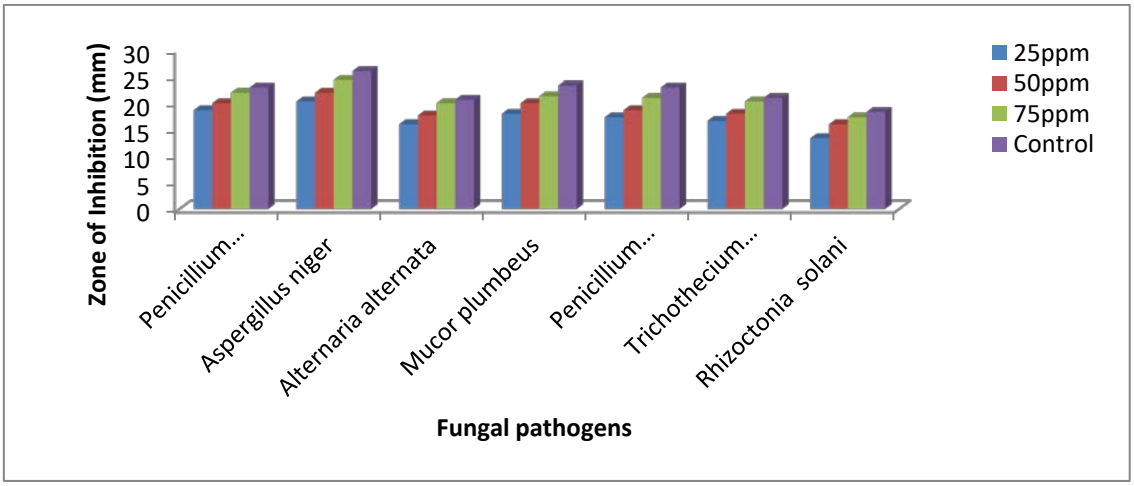

Figure 3. Antifungal activity of ethanolic leaf extracts of *Iris kashmiriana*.

The aqueous extract of *Iris kashmiriana* showed maximum antimycotic activity at 25 µL, 50 µL and 75 µL concentration against *Penicillium expansum* and *Rhizoctonia solani* with the zone of inhibition of 20.66 mm ± 1.15 mm, 22.00 mm ± 1.00 mm, 23.00 mm ± 1.00 mm and 20.00 mm ± 1.00 mm, 21.66 mm ± 1.52 mm, 22.66 mm ± 1.52 mm, respectively. The moderate antifungal activity was observed against *Mucor plumbeus* and *Trichothecium roseum* with the zone of inhibition of 19.66 mm ± 0.57 mm, 21.00 mm ± 1.00 mm, 23.33 mm ± 0.57 mm, and 19.33 mm ± 0.57 mm, 23.00 mm ± 1.00, 24.33 mm ± 0.57 mm at 25 µL, 50 µL and 75 µL concentrations of *Iris kashmiriana*, respectively. The inhibition in fungal growth of *Alternaria alternata* and *Penicillium chrysogenum* was 18.33 mm ± 0.57 mm, 20.00 mm ± 1.00 mm and 22.00 mm ±1.00 mm, and 18.00 mm ± 1.00 mm, 20.66 mm ± 0.57 mm, and 22.66 mm ± 0.57 mm at 25 µL, 50 µL and 75 µL concentrations of plant extracts, respectively. The fungal growth of *Aspergillus niger* showed least inhibitory activity with the zone of inhibition of 18.00 mm ± 1.00 mm, 19.33 mm ± 0.57 mm, 22.00 mm ± 1.00 mm at 25 µL, 50 µL and 75 µL concentrations of plant extract of *Iris kashmiriana*, respectively (Table 4, Figure 4).

Table 4. Antifungal activity of aqueous leaf extracts of *Iris kashmiriana*.

	Zone of Inhibition (mm)			
	25 µL	**50 µL**	**75 µL**	**Control**
Penicillium expansum	20.66 ± 1.15	22.00 ± 1.00	23.00 ± 1.00	24.66 ± 0.57
Aspergillus niger	18.00 ± 1.00	19.33 ± 0.57	22.00 ± 1.00	24.66 ± 0.57
Alternaria alternata	18.33 ± 0.57	20.00 ± 1.00	22.00 ± 1.00	24.00 ± 1.00
Mucor plumbeus	19.66 ± 0.57	21.00 ± 1.00	23.33 ± 0.57	25.00 ± 1.00
Penicillium chrysogenum	18.00 ± 1.00	20.66 ± 0.57	22.66 ± 0.57	25.00 ± 1.00
Trichothecium roseum	19.33 ± 0.57	23.00 ± 1.00	24.33 ± 0.57	26.00 ± 1.00
Rhizoctonia solani	20.00 ± 1.00	21.66 ± 1.52	22.66 ± 1.52	24.00 ± 1.00

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

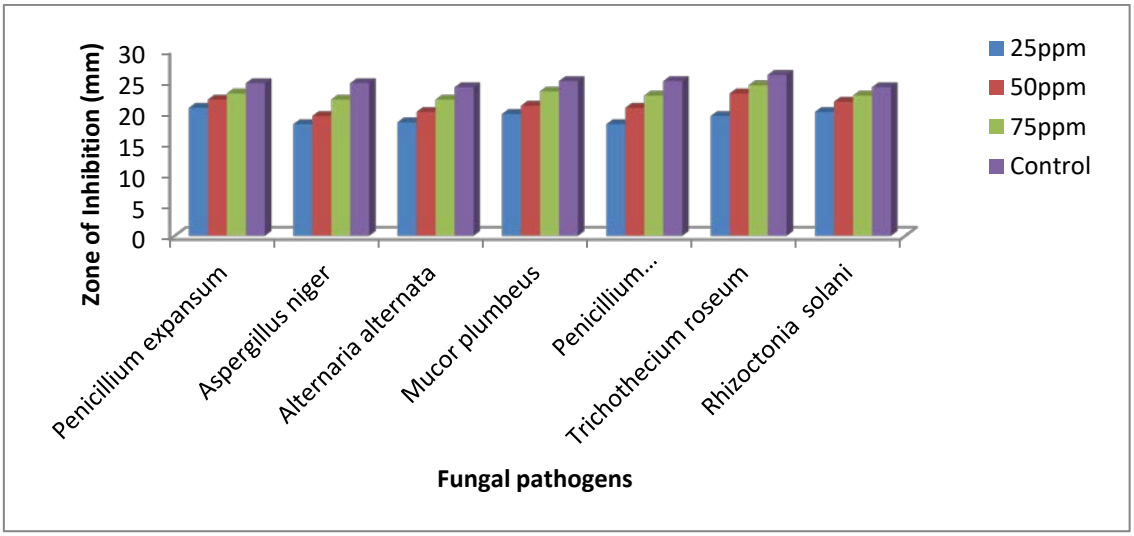

Figure 4. Antifungal activity of aqueous leaf extracts of *Iris kashmiriana*.

Discussion

The results clearly indicates that extracts of two medicinal plants *Ajuga bracteosa* and *Iris kashmiriana* brought about significant inhibition in the mycelial growth at their different concentration. Higher concentration proved effective than lower concentration. In the present study some plant extracts were evaluated for their antifungal activity against the fungus causing rot of tomato and brinjal. These two test plant species proved highly effective in reducing the mycelial growth of fungi causing rot diseases of tomato and brinjal fruits. Such study has been carried for the first time on the extracts of *Ajuga bracteosa* and *Iris kashmiriana*. However, extracts of other plants have been evaluated for their antimycotic activity in a similar way.

In a similar study, Singh et al. (1997) and Kumar et al. (2005) also revealed that fungicides and plant extracts inhibited the mycelial growth of *Aspergillus flavus* on chilli fruits. Webster et al. (2008) screened 14 plants for their antifungal activity against various pathogenic fungi and concluded that *Fragaria virginiana*, *Epilobium angustifolium* and *Potentilla simplex* show a promising antifungal potential. Gatto et al. (2011) studied the *in vitro* and *in vivo* activity of extracts from nine herbaceous species, viz. *Borago officinalis*, *Orobanche crenata*, *Plantago lanceolata*, *Plantago coronopus*, *Sanguisorba minor*, *Silene vulgaris*, *Sonchus asper*, *Sonchus oleraceus* and *Taraxacum officinale*, against some postharvest fungal rot causing pathogens *Monilinia laxa*, *Botrytis cinerea*, *Penicillium expansum*, *Penicillium digitatum*, *Penicillium italicum*, *Aspergillus carbonarius* and *Aspergillus niger*, and reported that the extract of *Sanguisorba minor* completely inhibited the spore germination of *Monilinia laxa*, *Penicillium digitatum*, *Penicillium italicum* and *Aspergillus niger.* Taskeen-Un-Nisa et al. (2010) tested plant extracts of three plants, onion (*Allium cepa*), garlic (*Allium sativum)* and mint (*Mentha arvensis*) for their antifungal activity against *Alternaria alternata* and *Rhizopus stolonifer*. They observed that the extract of *Allium sativum* at highest concentration proved highly effective in reducing spore germination of *Rhizopus stolonifer* and *Alternaria alternata* followed by extract of *Allium cepa* and *Mentha arvensis*. Saheb et al. (2011) reported the antifungal activity of

various extracts like aqueous, alcoholic and ethyl acetate extracts of leaves of five *Terminalia* species against five plant pathogenic fungi like *Aspergillus flavus*, *Aspergillus niger*, *Alternaria brassicicola*, *Alternaria alternata* and *Helminthosporium tetramera* and found that the ethyl acetate extract showed better inhibitory effect against all the fungi tested. Senguttuvan et al. (2013) reported the antifungal activity of chloroform and methanol extracts of leaf against seven fungi viz. *Mucor* sp., *Trichoderma viride*, *Verticillium lecanii*, *Candida albicans*, *Penicillium* sp., *A. fumigates* and *A. niger* and methanol extract showed maximum inhibition in *Fusarium* sp. Parveen and Wani (2015) 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. However, the highest concentration caused maximum inhibition in the mycelial growth. The extract of *Artemisia absinthium* leaves at highest concentration (S) proved highly effective in inhibiting the mycelial growth of all these pathogenic fungi followed by other plant extracts.

Similarly, other studies also confirmed the effect of different concentration of plant extracts against fungi causing rotting of tomato, brinjal and other fruits (Mangang et al., 2012; Parveen et al., 2014; Tijjani et al., 2014; Koka et al., 2017; Satpute et al., 2017).

Conflict of interest statement

Authors declare that they have no conflict of interests.

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