Bangladesh J. Bot. **50**(2): 441-443, 2021 (June) **- Short communication**

ANTIFUNGAL ACTIVITY OF SIX MEDICINAL PLANTS OF PAKISTAN AGAINST SELECTED FUNGI

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Keywords: Medicinal plants, Antifungal activity, Crude extract, Dichloromethane, *n*-hexane, Ethyl acetate.

Abstract

Antifungal properties of *Cucumis sativus*, *Portulaca oleracea, Malus baccata, Saxifraga flagillaris, Geranium wallichianum.* and *Monotheca buxifolia* were evaluated against *Alternaria, Acremonium, Verticillium, Pythium,* and *Trichoderma,* using agar well-diffusion method. The crude ethanolic extract of *G. wallichianum* showed the highest antifungal activity followed by *P. oleracae* and *S. flagillaris.* The dichloromethane fraction of *G. wallichianum* showed the highest antifungal activity against *Acremonium* (28 mm), *Alternaria* (20.50 mm) and *Trichoderma* (20 mm). The n-hexane fraction of *C. sativus* showed the maximum antifungal activity (20 mm) against *Pythium*. The present findings demonstrated that six selected plants contain precious natural products for treating infectious diseases and can be used to isolate chemical compounds for future drug sources.

Plants used for traditional medicines usually contain potentially active natural antimicrobial compounds that may serve as an alternative, effective, cheap and safe way for the treatment of common microbial infections (Mathur 2012). Well-known examples of these components are flavonoids, phenols and phenolic glycosides, unsaturated lactones, sulfur compounds, saponins, cyanogenic glycosides and glucosinolates (Ademe *et al.* 2013). Pathogenic fungi are mostly controlled by synthetic fungicides, which damage both humans and environment. Hence, an exploration of fresh antifungal medications is exceptionally needed. Therefore, the present study was conducted to identify the most potent medicinal plants that contain active ingredients against pathogenic fungi.

The collected plant powder was soaked in ethanol for 2 to 3 days, filtered and vacuum dried by rotary evaporator, and fractionation of crude ethanol extract (CEE) was performed in different organic solvents consisting of n-hexane, dichloromethane and ethyl acetate. So, one crude extract and three partitioned from the crude extract such as *n*-hexane, dichloromethane (DCM) and ethyl acetate were obtained. Each extract of 0.5 g was dissolved in 50 ml of dimethyl sulphoxide to get a final concentration of 20 mg/ml. Fungal species such as *Alternaria*, *Acremonium*, *Verticellium*, *Pythium*, and *Trichoderma* were used for antifungal assessment. Agar well diffusion method was followed as described by Samie *et al.* (2010). The 100 µl of different fungal cultures in distilled water was spread over an agar plate using a sterile inoculation loop. Holes were made in each culture plate using a sterile cork borer. The 75 µl of crude and fractions of the medicinal plant extracts were added. The culture plates were then incubated at 27°C, and the results were observed

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DOI: https://doi.org/10.3329/bjb.v50i2.54105

after 24 hrs depending on the fungal growth. The clear zones around each well were measured in millimeters. The experiment was repeated three times and statistical analysis was performed using Duncun's Multiple Range Test for the results.

Antifungal activity of crude ethanol extracts of six selected medicinal plants against the fungal strains is presented in Table 1. *Portulaca oleracae* showed the highest antifungal activities of 17.00, 16.41 and 16.17 mm against *Verticellium*, *Trichoderma* and *Pythium*, respectively. The crude extracts of remaining plants also showed considerable antifungal activities such as *C. sativus* against *Acremonium* (16.50 mm), *M. baccata* against *Trichoderma (*14.77 mm), *S. flagillaris* against *Acremonium* (15.33 mm), *G. wallichianum* against *Trichoderma* (16.06) and *M. buxifolia* against *Trichoderma*.

Table 1. Antifungal activity of crude extracts of six medicinal plants against fungal species.

Plant	*Alternaria* (mm)	*Acremonium* (mm)	*Verticellium* (mm)	*Pythium* (mm)	*Trichoderma* (mm)
Cucumis sativus	6.89 ± 0.11	16.50 ± 1.89	13.44 ± 0.53	10.67 ± 1.30	11.78 ± 1.75
Portulaca oleracae	10.33 ± 0.88	14.12 ± 0.19	17.00 ± 0.58	16.17 ± 0.93	16.41 ± 0.82
Malus baccata	10.90 ± 0.58	9.89 ± 0.63	13.62 ± 0.87	9.17 ± 1.01	14.77 ± 1.13
Saxifraga flagillaris	9.77 ± 1.65	15.33 ± 1.13	7.90 ± 0.66	10.23 ± 0.96	13.14 ± 1.03
Geranium wallichianum	14.50 ± 1.25	13.57 ± 0.87	12.83 ± 0.95	14.27 ± 1.22	16.06 ± 0.97
Monotheca buxifolia	11.00 ± 1.00	10.33 ± 0.88	12.33 ± 1.86	10.00 ± 3.60	12.00 ± 2.08

Table 2. Antifungal activity of fractions of six medicinal plants against fungal species.

Plant	Fraction	*Alternaria* (mm)	*Acremonium* (mm)	*Verticillium* (mm)	*Pythium* (mm)	*Trichoderma* (mm)
Cucumis sativus	*n*-hexane	07.00 ± 0.58	15.67 ± 1.20	14.67 ± 1.86	20.00 ± 0.58	16.33 ± 1.20
	DCM	10.33 ± 1.76	20.33 ± 0.88	15.83 ± 0.73	11.17 ± 0.44	15.83 ± 1.09
	Ethyl acetate	08.50 ± 1.50	15.00 ± 1.00	16.50 ± 3.50	09.75 ± 1.25	09.25 ± 0.75
Portulaca oleracae	n-hexane	16.50 ± 3.55	14.17 ± 1.92	16.83 ± 0.44	14.67 ± 0.33	17.33 ± 1.48
	Ethyl acetate	14.17 ± 2.52	16.50 ± 1.80	18.17 ± 2.17	18.67 ± 1.45	18.67 ± 1.45
Malus baccata	*n*-hexane	18.67 ± 1.45	13.33 ± 3.84	15.67 ± 0.76	10.67 ± 1.76	14.00 ± 1.73
	DCM	18.33 ± 1.76	11.00 ± 1.73	15.83 ± 1.30	09.67 ± 0.88	15.00 ± 1.58
	Ethyl acetate	12.83 ± 1.09	09.17 ± 0.44	12.17 ± 0.44	06.50 ± 0.29	17.67 ± 0.88
Saxifraga flagillaris	*n*-hexane	10.83 ± 1.88	15.83 ± 0.60	12.33 ± 3.53	11.67 ± 2.73	10.67 ± 0.67
	DCM	08.67 ± 1.76	13.50 ±0.29	09.67 ± 1.86	10.17 ± 0.44	13.50 ± 2.29
Geranium wallichianum	*n*-hexane	11.83 ± 1.30	11.33 ± 2.03	06.83 ± 0.60	09.00 ± 0.58	14.17 ± 1.48
	DCM	20.50 ± 1.04	28.83 ± 1.48	22.00 ± 1.73	19.67 ± 0.33	20.00 ± 0.58
	Ethyl acetate	14.83 ± 1.30	12.17 ± 1.92	10.17 ± 1.36	11.33 ± 1.87	12.17 ± 0.77
Monotheca buxifolia	*n*-hexane	10.50 ± 0.50	10.33 ± 2.40	07.17 ± 0.44	09.33 ± 0.67	14.50 ± 1.44
	DCM	16.67 ± 1.67	19.67 ± 0.88	17.67 ± 0.33	17.33 ± 0.33	18.67 ± 0.88
	Ethyl acetate	12.33 ± 1.45	10.33 ± 1.45	09.33 ± 1.20	09.67 ± 0.67	09.67 ± 0.33

The crude ethanolic extracts of medicinal plants were fractionated on polarity basis into n-hexane, dichloromethane and ethyl acetate fractions, and tested against the fungal strains as shown in Table 2. The DCM of *G. wallichianum* showed the highest antifungal activities such as 28.83, 22, 20.50, 20 and 19.67 mm were noted against *Acremonium*, *Verticillium*, *Alternaria*, *Trichoderma*, and *Pythium*, respectively. The DCM and n-hexane fractions of *C. sativus* revealed higher antifungal activity against *Acremonium* (20.33 mm) and *Pythium* (20.00 mm), respectively.

The crude ethanol extracts of all the medicinal plants were more or less active against all the fungal species and showed differences in their zones of inhibition. All the selected fungal strains showed a various degree of susceptibility to different fractions, evidently with previous findings that medicinal plants possess potential antifungal chemical constituents, which inhibit the growth of pathogenic fungi (Mahlo *et al.* 2016).

The present study indicated that the six medicinal plants exhibited antifungal activities against the selected fungal strains. Among the selected medicinal plants, *G. wallichianum* and *P. oleracae* showed high antifungal activity against all the selected strains. The fractions of medicinal plants were highly effective, particularly, DCM fractions were found highly active against the fungal strains.

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(Manuscript received on 2 January, 2020; revised on 21 May, 2020)