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ANTIMICROBIAL SCREENING OF *IMPATIENS BICOLOR ROYLE*

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Abstract

Extracts of *Impatiens bicolor Royle* obtained from n-hexane (A); dichloromethane (B), ethyl acetate (C), n-butanol (D), aqueous (E) as well as crude (F) were tested *In vitro* for their antibacterial and antifungal activities. Antibacterial study performed against 6 bacteria viz., *Escherichia coli, Bacillus subtilis, Shigella flexenari, Staphylococcus aureus, Pseudomonas aeruginosa* and *Salmonella typhi* indicated that crude and its fractions had no activity at all against any microorganism. The antifungal activity of these extracts was performed against 6 fungi viz., *Trichophyton longifusus, Candida albicans, Aspergilus flavus, Microsporum canis, Fusarium solani and Candida glaberata*. The extracts showed moderate activity against different fungal strains.

Introduction

The genus *Impatiens* (*Balsaminaceae*) comprises of about 135 species of stove, greenhouse, or hardy, annual or biennial herbs, natives for the most part of the mountains of tropical Asia and Africa. *Impatiens bicolor Royle* (locally called bantil) is an annual herb, 45-60 cm tall. It has lateral small green sepals while its stem is purplish-green and woody at base but herbaceous above (Hara *et al*., 1978, 1979, 1982; Bernardi, 1963) .It is native of Indian subcontinent mainly India, Nepal and Pakistan. In Pakistan it is distributed in northern areas in Murree, Nathia Gali, Swat and Miran Jani and used as fodder. The plant is used locally as diuretic, tonic and has cooling effect. (Gilani *et al*., 2001).

A few flavonoids have been isolated form this plant (Hasan & Tahir, 2005) however antimicrobial screening was totally ignored. In this context as part of our continuous studies on exploring the hidden potential of indigenous flora of Pakistan (Nisar *et al*., 2007, 2008; 2009a,b; Zia-ul-Haq *et al*., 2007a,b; 2008; 2009), we have screened the extract of *I. bicolor Royle* for various *In vitro* biological activities to evaluate its phytomedicinal potential. To our knowledge, no data has been reported on the phytochemical screening of the *I. bicolor Royle* obtained from Pakistan. The present investigation will provide a broad base for the possibility of further detailed biological studies on *I. bicolor Royle* along with its biological standardization.

Material and Methods

Plant material, preparation of crude extract and fractionation: Whole plant of *Impatiens bicolor Royle* was collected from Khwazabhela, Swat, N.W.F.P. Pakistan, during September 2008. A taxonomist, Dr. Hassan Sher, Jahan Zeb Post Graduate College Saidu Sharif, Swat, Pakistan, identified the plant. A voucher, specimen No.18-NH-4-008 was deposited in the National Herbarium, Islamabad.

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Shade-dried *I. bicolor Royle* (10 kg) was grounded and extracted with MeOH and water at room temperature. The combined methanolic extract was filtered and evaporated under vacuum to obtain a thick greenish black gummy mass. It was fractionated into n-hexane (A); dichloromethane (B), ethyl acetate (C), n-butanol (D), aqueous (E) as well as crude (F) fractions. All these extracts (A-F) were tested for antibacterial and antifungal activities.

Antibacterial bioassay: The antibacterial activity was checked by the agar–well diffusion method (Kavanagh *et al*., 1963). In this method one loop full of 24 hours old culture containing approximately 104-106 CFU was spread on the surface of Mueller-Hinton Agar plates. Wells were dug in the medium with the help of sterile metallic cork borer. Stock solutions of the test samples (A-F) in the concentration of 1 mg/ml were prepared in dimethyle sulfoxide (DMSO) and 100 µl dilutions were added in their respective wells. The antibacterial activity of extracts (A-F) was compared with standard drug imepinem; the std. drug imepinem and DMSO were used as positive and negative control. The amount of growth in each well was determined visually by comparing with the growth in the control wells (Rashid *et al*., 2009).

Antifungal bioassay: The antifungal activity was determined by the Agar Well Diffusion Method (Atta-ur-Rahman *et al*., 1991). In this method Griseofulvin was used as the standard drug. The crude extract was dissolved in DMSO (50 mg / 5ml). Sterile Sabouraud's dextrose agar medium (5ml) was placed in a test tube and inoculated with the sample solution (400 µg /ml) kept in slanting position at room temperature overnight. The fungal culture was then inoculated on the slant. The samples were incubated for 7 days at 29^{o}C and growth inhibition was observed and percentage growth inhibition was calculated with reference to the negative control by applying the formula:

$$\% \text{ Inhibition of fungal growth} = 100 - \frac{\text{Linear growth and test (mm)}}{\text{Linear growth in control (mm)}} \text{ X } 100$$

Miconazole and amphotericin B were used as standard drugs, while miconazole, amphotericin B and DMSO were used as positive and negative controls (Rashid *et al*., 2009).

Results and Discussions

In recent years, there has been a resurgence of scientific interest in the use of medicinal plants for the development of new pharmacotherapeutic agents. Medicinal plants play an important role for the management of different microbial infections because overmedication and long-term side effects of synthetic drugs have assumed alarming range. Effective, safe and cheap medicinal agents from plants may appear as potential alternatives for controlling microbial infections particularly the resistant cases.

Different bacterial isolates comprising both Gram negative and Gram positive organisms were used for evaluation of antibacterial activity. The antibacterial study was performed against 6 bacteria viz., *Escherichia coli, Bacillus subtilis, Shigella flexenari, Staphylococcus aureus, Pseudomonas aeruginosa* and *Salmonella typh.* The dose was given in a single concentration (1mg/ml). Neither crude extract nor any of its fractions showed any activity against any microorganism.

Table 1. Antifungal bioassay.

Test organism	% Inhibition						Standard
	A	B	C	D	E	F	
T. longifusis	-	-	-	-	-	-	Miconazole70
C. albicans	-	-	-	-	-	-	Miconazole110.8
A. flavus	-	-	40	30	-	-	Amphotericin20
M. canis	30	30	50	40	20	10	Miconazole98.4
F. solani	-	-	-	-	10	10	Miconazole73
C. glabarata	-	-	-	-	-	-	Miconazole110.8

n-hexane (A); dichloromethane (B), ethyl acetate (C), n-butanol (D), aqueous (E), crude (F)

Antifungal activity of these extracts was performed against 6 fungi viz., *Trichophyton longifusus, Candida albicans, Aspergilus flavus, Microsporum canis, Fusarium solani and Candida glaberata*. The results indicated that these extracts were not active against the tested fungal strain *C. albicans, C.glabarata* and *T.longifusis* while all fractions as well as crude extract were most effective against *M. canis.* (Table 1). It was further observed that ethyl acetate fraction followed by n-butanol fraction were most active while crude extract was least active as it showed only 10% inhibition.

There does not appear to be any previous report on the antifungal activity of *Impatiens bicolor Royle*. The knowledge of extent and mode of inhibition of specific compounds which are present in plant extracts, may contribute to the successful application of such natural compounds for treatment of infection disorder like fungal and bacterial diseases. The present status of medicinal plants and their products provide opportunity for the developing countries to benefit from the emerging marks as the developing countries possess most biodiversity of medicinal plants. It is concluded that in co ordinance of the chemical literature finding resistant strains of organism plant biodiversity may lead to unexpected research findings (Mahmud *et al*., 2009).The present study will help the researchers as a basic data for future research in exploiting the hidden potential of this important plant which has not been explored so far.

References

Atta-ur-Rahman. 1991. *Studies in Natural Product Chemistry, Netherlands, Elsevier Science publishers*, 9: 383-384.

Bernardi, L. 1963. Revisio generis *Weinmanniae*. Pars I: Sectio *Weinmanniae*. *Candollea*, 18: 253.

Gilani, S.A., R.A. Qureshi and U. Farooq. 2001. Ethnobotanical studies of Ayubia National Park District Abbottabad, Pakistan.*On Line J. Bio. Sci*., 1(4): 284-286.

Hara, H., W.T. Stearn and L.H.J. Williams. 1978. An enumeration of the flowering plants of Nepal. Vol. I. Trustees of British Museum, London, UK.

Hara, H. and L.H.J. Williams. 1979. An enumeration of the flowering plants of Nepal. Vol. II. Trustees of British Museum, London, UK.

Hara, H., A.O. Chater and L.H.J. Williams. 1982. An enumeration of the Flowering Plants of Nepal. Vol. III. Trustees of British Museum, London, UK.

Hasan, A. and M.N. Tahir. 2005. Flavonoids from the leaves of *Impatiens bicolor. Turk. J. Chem*., 29: 65-70.

Kavanagh, F. 1963. *Analytical Microbiology.* Academic Press London, 125-141.

Mahmud, S., H. Shareef, U.F. Arrukh, A. Kamil and G.H. Rizwani.2009. Antifungal activities of *Vitex negundo* Linn. *Pak. J. Bot*., 41(4): 1941-1943.

Nair, N.C. 1977. *Flora of Bashahr Himalayas.* International Bioscience Publishers, Hissar, India.

Nasir, E. and S.I. Ali.1974. *Flora of West Pakistan*. Department of Botany, University of Karachi, Feroz Sons Press; Karachi.

Nisar, M., I. Khan, B.Ahmad, A. Ihsan, W. Ahmad and M.I. Choudhary..2008. Antifungal and antibacterial activities of *Taxus wallichiana* Zucc. *J. Enz. Inh. Med. Chem*., 23(2) :256-260.

Nisar, M, S.A.Tariq and Ihsanullah. 2009. Nutritional levels of *Indigofera gerardiana* wall and *Crataegus songrica* k. Koch. *Pak. J. Bot*., 41(3): 1359-1361.

Nisar, M, S.A. Tariq, I.K.Marwat, M.R. Shah and I.A. Khan. 2009. Antibacterial, antifungal, insecticidal, cytotoxicity and phytotoxicity studies on *Indigofera gerardiana*. *J. Enz. Inh. Med. Chem*., 24(1): 224-229.

Nisar, M, B. Adzu, K. Inamullah, A. Bashir, A. Ihsan and A.H. Gilani. 2007. Antinociceptive and antipyretic activities of the *Zizyphus oxyphylla* leaves. *Phyto. Res*., 21(7) :693-695.

Rashid, R., M. Farah and M.N. Mirza .2009.Biological screening of *Salvia cabulica. Pak. J. Bot*., 41(3): 1453-1462.

Sharma, B.D. and N.P. Balakrishnan. 1993. *Flora of India* vol. 1, Botanical Survey of India, Calcutta, India.

Zia-ul-Haq, M, S. Iqbal, S. Ahmad, M. Imran, A. Niaz and M.I. Bhanger..2007a. Nutritional & compositional study of desi chickpea (*Cicer arietinum* L.) cultivars grown in Punjab, Pakistan. *Food Chemistry*, 105: 1357-1363.

Zia-ul-Haq, M., M. Ahmad S. Iqbal, S. Ahmad and H. Ali .2007b. Characterization and compositional studies of oil from seeds of desi chickpea (*Cicer arietinum* L.) cultivars grown in Pakistan. *J. Am. Oil Chem. Soc*., 84:1143-1148.

Zia-ul-Haq, M, S. Iqbal and M. Ahmad.2008. Characteristics of oil from seeds of 4 mungbean (*Vigna radiata* (L.) Wilczek) cultivars grown in Pakistan. *J. Am. Oil Chem. Soc*., 85: 851-856.

Zia-ul-Haq, M., S. Ahmad, M. Ahmad, S. Iqbal and K.M. Khawar. 2009. Effects of cultivar and row spacing on tocopherol and sterol composition of chickpea (*Cicer arietinum* L) seed oil. *Tarim Bilimleri Dergisi*, 15: 25-30.

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