Phenolic acid analysis and biological activity of methanolic extracts of some medicinal plants against some phytopathogenic fungi

A Singh, S Singh, B Sarma, U Singh, R Srivastava, K Singh

Citation

A Singh, S Singh, B Sarma, U Singh, R Srivastava, K Singh. *Phenolic acid analysis and biological activity of methanolic extracts of some medicinal plants against some phytopathogenic fungi*. The Internet Journal of Alternative Medicine. 2008 Volume 6 Number 2.

Abstract

About three quarters of the world population rely mainly on plants and plant extracts for health care. The methanolic extracts of some potential medicinal plants such as Saraca indica, Withania somnifera and Bacopa monnieri were assayed against Alternaria cajani, Helminthosporium sp., Bipolaris sp., Curvularia lunata and Fusarium sp. at different concentrations (1000, 2000, 3000, 4000 and 5000 µg/ml). All the three extracts exhibited good inhibitory activity against A.
cajani while they were effective at lower concentrations against other fungi also. High performance liquid chromatography (HPLC) analysis of the crude extract of the above plants showed five different phenolic acids, viz., benzoic, gallic, ferulic, catechin and tannic acids. Seeing the antifungal efficacy of the above three plant extracts it can be suggested that they can be tried for the control of some plant diseases under field conditions also. The role of phenolic acids in human health has also been discussed.

INTRODUCTION

Extracts of several plants are highly effective against parasitic as well as saprophytic microbes. It is estimated that around 70,000 plant species, from lichens to trees, have been used at one or the other time for medicinal purposes ([1]). The demands of medicinal plants by the modern pharmaceutical industries has also increased manifold ([2]). The medicinal plants occupy a significant place in modern medicine for some important drugs, although synthetic drugs and antibiotics brought about a revolution in controlling different diseases ([3]). The anti-infectional compounds show broad-spectrum bioactivity against bacteria, fungi, protocists, protozoans, viruses, yeasts, etc. ([4]).

Saraca indica belongs to family Caesalpiniaceae and commonly called 'Ashoka' in Hindi in India. It is found in plenty all over India. The bark of Ashoka tree is used as antihypertensive in Dysmenorrhoea. It is Haemorrhoidic, Menorrhagic, Leucorrhoeic, Hemostatic, Anticonvulgant and Diuertic. It is also useful in menorrhagia due to uterine fibroids, in leucorrhea and in internal bleeding. Withania somnifera, also known as 'Ashwagandha', Indian ginseng, and winter cherry, is an important herb in the Ayurvedic medicine (ancient Indian medicine) being used for over 3000 years. Roots of W. somnifera reportedly exhibit anti-inflammatory, antitumour, antistress, antioxidant, immunomodulatory, haematopoietic and rejuvenating properties ([5]). Bacopa monnieri is immunostimulant, tranquilizing, mind pacifying, neuroleptic, psychotropic herb with great action on nervous system, anticonvulsant, antispasmodic, cholinesterase inhibition, CNS depressant or sedative, neuromuscular blocking, anesthetic and mild barbiturate potentiating effect. Besides it has antiviral, anti-bacterial, anti-tumor, antipyretic, antihistaminic or anti-allergic effects.

The objective of this research was to see whether methanolic extracts of these plants are antifungal. High performance liquid chromatographic (HPLC) analysis was also done to see the phenolic profile as some of the phenolic acids are antimicrobial and some others are potential agents for human health. The results are presented here.

MATERIALS AND METHODS

COLLECTION AND EXTRACTION OF MEDICINAL PLANT MATERIAL

The raw material (root and aerial parts) of Saraca indica, Withania somnifera and Bacopa monnieri were collected

from different fields. The dried plant parts were powdered and extracted separately with methanol:sterile water (1:1) using soxhlet apparatus for 48 h. The solvent was distilled at lower temperature under reduced pressure in rotary flash evaporator and concentrated on water bath to get the crude extract. The extracts were stored in desiccators for further experiments.

ANTIFUNGAL ACTIVITY

The crude extracts of S. indica, W. somnifera and B. monnieri were used in the present experiment. Alternaria cajani, Helminthosporium sp., Bipolaris sp., Curvularia lunata and Fusarium sp. were isolated from respective infected plant parts on potato dextrose agar (PDA) (peeled potato 250 g, dextrose 20 g, agar 15 g, distilled water 1 L) medium. The cultures were further purified by single spore isolation technique and maintained at 25 ± 2 ° C on PDA slants. Seven to ten-day-old cultures were used in the experiment.

Stock solutions (5000 µg/ml) of the crude extracts were prepared by dissolving 5 mg of the extract in 1 ml of distilled water. Required concentrations (1000, 2000, 3000, and 4000 µg/ml) were prepared from each stock solution by diluting with distilled water. One drop (30-35 µl) from each concentration was placed on grease-free glass slides. Fungal spores (200-300) were picked up from 7-10-day-old cultures with sterilized inoculation needle and mixed in solutions of different concentrations of the three extracts separately. The slides were placed in moist chambers made by placing two sterile filter papers each on the lid and base of the petri plates. They were incubated at 25 ± 2 ° C for 24 h. Germination was observed after mixing a drop of cotton blue prepared in lactophenol on every slide containing fungal spores under binocular microscope (Nikon, Japan Type 102). Spores mixed in only sterile distilled water served as control. All the experiments were conducted in triplicate.

SAMPLE PREPARATION FOR THE ANALYSIS OF PHENOLIC COMPOUNDS

The phenolic acids were analysed through High Performance Liquid Chromatography (HPLC) as per the method of Singh et al. ($_6$). The samples of each plant were prepared separately. One gram of each extract was macerated and suspended in 5 ml ethanol:water (80:20; v/v). The collected samples were subjected to ultrasonication (Branson Sonifier, Danbury, CT, USA) for 15 min at 4°C followed by centrifugation at 12500 x g for 15 min. The clear supernatant was subjected to charcoal treatment for the removal of pigments. The residue was re-extracted twice with the same extracting solution and the supernatant was pooled prior to evaporation under vacuum (Buchi Rotavapor Re Type, Labco, India; Ambala Cantt. India). Dried extracts were resuspended in 1.0 ml HPLC grade methanol by vortexing and filtered through ultra membrane filter (pore size 0.45 µm: Millipore) before HPLC analysis.

HPLC ANALYSIS

Quantitative analysis of the sample was performed according to the method of Singh et al. ($_6$). The HPLC system (Shimadzu Corporation, Kyoto, Japan) was equipped with two Shimadzu LC-10 ATVP reciprocating pumps, a variable Shimadzu SPD-10 AVP UV-VIS detector and a Rheodyne Model 7725 injector with a loop size of 20 µl. The peak area was calculated with a Winchrom integrator. Reverse-phase chromatographic analysis was carried out in isocratic conditions using a C-18 reverse phase column (250 x 4.6 mm i.d., particle size 5 µm, Luna 5µ C-18 ($_2$); phenomenex, Torrance, CA, USA) at 25°C. Running conditions included: injection volume 5µl; mobile phase, methanol: 0.4% acetic acid (80: 20 v/v); flow rate 1 ml/min; and detection at 290 nm. Samples were filtered through an ultra membrane filter (pore size 0.45 µm; E-Merck, Darmstadt, Germany) prior to injection in the sample loop. Benzoic, gallic, ferulic, catechin and tannic acids were used as internal and external standards. Phenolic acids present in each sample were identified by comparing chromatographic peaks with the retention time (R_t) of individual standards and further confirmed by co-injection with isolated standards. The amount of each phenolic acid is expressed as micrograms per gram of fresh weight unless otherwise stated.

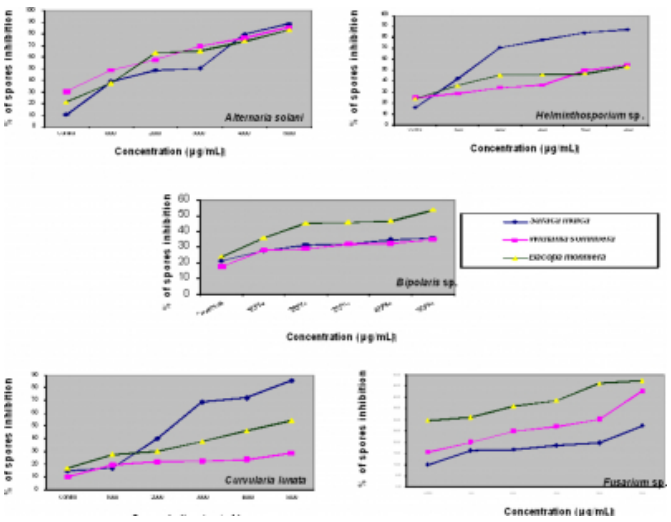
RESULTS AND DISCUSSION

COMPARATIVE ANALYSIS OF ANTIFUNGAL ACTIVITY

Crude extracts of S. indica, W. somnifera and B. monnieri were tested against Alternaria cajani, Helminthosporium sp., Bipolaris sp., Curvularia lunata and Fusarium sp. at concentrations of 1000, 2000, 3000, 4000 and 5000 µg/ml (Fig. 1). The methanolic extract inhibited growth of the test fungi to varying degrees. All the three extracts, i.e. of S. indica, W. somnifera and B. monnieri, were highly effective at 5000 µg/ml. S. indica was highly inhibitory against Fusarium sp. (90%), A. cajani (89.47%), Helminthosporium sp. (83.87%) and C. lunata (85.37%). Similarly, W. somnifera extract was highly inhibitory against C. lunata

(90.32%), Fusarium sp. (84.37%), and Bipolaris sp. (82.35%). Antifungal activity of B. monnieri was the least inhibitory compared to the other two plant extracts but highly inhibitory against C. lunata (83.33%) and A. cajani (79%) at 5000 µg/ml. The inhibitory effect was less at lower concentrations of the extracts. According to Joshi et al. ([7]) methanolic extract of S. indica showed good fungicidal activity against Aspergillus fumigatus, A. niger, Penicillium frequentence, P. notatum and Botrytis cinerea. It also showed antimicrobial activity against Aspergillus flavus, A. niger and Candida albicans in a range of 75-1200 µg/ml ([8]). S. indica also exhibited complete toxicity against Pythium debaryanum, Fusarium oxysporum, Rhizoctonia solani and Sclerotium rolfsii ([9]). W. somnifera has also potential antibacterial activity against Staphyloccus aureus and Pseudomonas aeruginosa ([10]). Its antifungal activity was demonstrated against A. flavus, F. oxysporum, F. verticilloides and antibacterial activity against Clvibacter michiganensis subsp. michiganensis ([11]). The phytochemicals betulinic acid, wogonin and oroxindin from aerial part of B. monnieri were found effective against Altenaria alternata and Fusarium fusiformis ([12]).

Figure 1



Recent researches indicate that phytophenols, being chief secondary metabolites, are present in rich amount in several plants. The HPLC fingerprints (Fig. 2 a, b and c) of the crude extracts of S. indica, W. somnifera and B. monnieri showed presence of several phenolic acids of which five were identified, i.e., benzoic, gallic, ferulic, catechin and tannic acids present in varying amounts (Table 1).

Figure 2

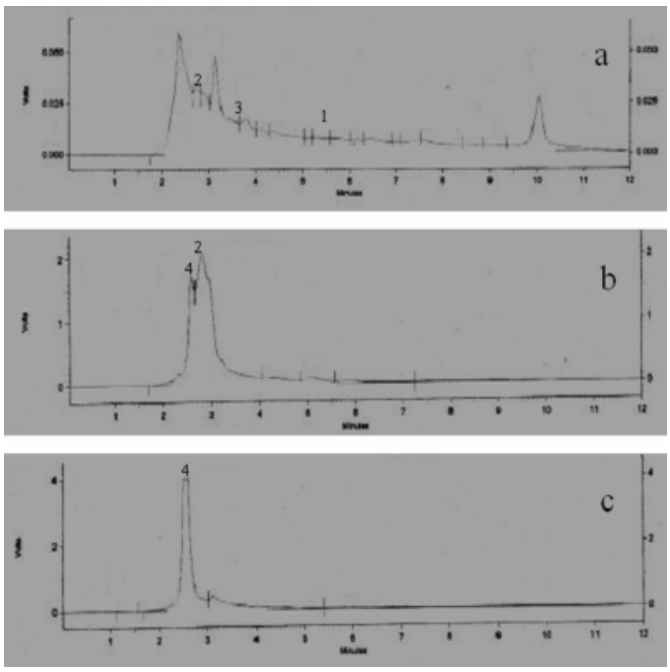
Table 1: Amount of phenolic acids in the crude extract of and

Crude Extract	Phenolic acid (µg/g dry wt.)				
	Catechin	Benzoic acid	Gallic acid	Ferulic acid	Tannic acid
Saraca indica	0.663	987.44	16.02	5.931	ND
Withania somnifera	111.85	ND	ND	ND	1467.24
Bacopa monnieri	ND	ND	892.17	ND	5380.8

ND = Not detectable

The retention times (min.) of the five phanolic acids were 5.67, 2.80, 3.77, 2.73 and 2.55, respectively, at a wavelength of 290 nm. Out of the three extracts, B. monnieri showed maximum amount of tannic acid (5380.89 µg/g) followed by W. somnifera (1467.24 µg/g). The amount of gallic acid was also maximum in B. monnieri (892.17 µg/g) compared to S. indica (16.016 µg/g). Out of the five phenolic acids, only catechin (0.663 µg/g), gallic (16.016 µg/g), ferulic (5.931 µg/g) and benzoic acids (987.44 µg/g) were detected in S. indica. Highest amount of catechin was recorded in W. somnifera (111.85 µg/g) followed by S. indica (0.663 µg/g). HPLC analysis of the samples revealed wide variability in their phenolic acid content (Fig. 2).

Figure 3



S. indica contains about 6% tannins, catechol, haematoxylin, ketosterol, saponin and organic calcium and iron compounds ([13]). Saracin, a seed integument lectin from S. indica is highly specific for binding N-acetyl-neuraminyl-N-acetyllactosamine ([14]). The enzymatic activity of S. indica

was determined by HPLC and the bark of this plant extracts showed inhibitory effect on human immunodeficiency virus type 1 (HIV-1) protease ([15]). In W. somnifera, the biological activities of withanolides, especially of the dominant withanolide A and withaferin A, were detected using HPLC analysis which were found to have anti-cancerous activity ([16], [17]). Similarly, Bandyopadhyay et al. ([18]) detected the accumulation of withaferin-A and withanolide-D in roots of W. somnifera. HPTLC method has been developed for the estimation of withaferin-A and withanolide-A in different plant parts such as, leaf, root, stem and fruit of two morphotypes of Withania somnifera ([19]). The pharmacological properties of B. monnieri were studied extensively which were attributed mainly to the presence of saponins called "bacosides" ([20]). In B. monnieri, an HPLC method was developed for the quantitative determination of Bacoside A, the putative bioactive component, was found to be a mixture of saponins with bacoside A_3 (1), bacopaside II (2), jujubogenin isomer of bacopasaponin C (3) and bacopasaponin C (4) as major constituents ([21]). Glycosides of the 20-deoxy derivatives of jujubogenin and pseudojujubogenin were selected from B. monnieri. The compounds were tested for their cytotoxicity, antileishmanial, antimalarial, antioxidant, and anti-inflammatory activities ([22]). Looking into the previous reports as well as evidence from the present investigation it can be concluded that the methanolic extracts of B. monnieri, W. somnifera and S. indica are antifungal effective against a diverse group of fungi and therefore could be exploited for their use in agricultural crop protection.

References

1. Purohit SS, Vyas SP. Medicinal plants cultivation a scientific approach including processing and financial guidelines. 1st edition. Publishers Agrobios, Jodhpur, India, 2004, pp. 1-3.
2. Ashraf M, Ali Q, Iqbal Z. Changes in chemical composition of fixed and essential oil of black cumin (Nigella sativa L.) seeds collected from plants grown at different soil nitrogen habitats. J. Sci. Food Agri. 2006, 86: 871-876.
3. Huynh QK, Borgmeyer JR, Smith CE, Bell LD, Shah DM. Isolation and characterization of a 30 kDa protein with antifungal activity from leaves of Engelmannia pinnatifida. J. Biol. Chem. 2001, 316: 723-727.
4. Conlon JM, Sonnevend A, Patel M, Daviuudson C, Npelsen PF, Pasl T, Smith LAR. Isolation of peptides of the brevinin-1 family with potent candidacidal activity from the skin secreations of the frog Rana boylii. J. Pep. Res. 2003, 62: 207.
5. Mishra LC, Singh BB, Dagenais S. Scientific basis for the therapeutic use of Withania somnifera (Ashwagandha): a review. Altern. Med. Rev. 2000, 5: 334-346.
6. Singh UP, Sarma BK, Singh DP, Bahadur A. Plant growth promotingrhizobacteria mediated induction of phenolics in pea (Pisum sativum) after infection with Erysiphe pisi. Curr. Microbiol. 2002, 44: 396-400.
7. Joshi BC, Pandey A, Chaurasia L, Pal M, Sharma RP, Khare A. Antifungal activity of stem bark of Ailanthus excelsa. Fitoterapia 2003, 74: 689-691.
8. Dabur R, Gupta G, Mandal TK, Singh DD, Vivek B, Lavekar GS. African Journal of Traditional, Complementary and Alternative Medicines. African Ethnomedicines Network 2007, 4: 313-318.
9. Kumar, Tripathi SC. Evaluation of the leaf juice of some higher plants for their toxicity against soil borne pathogens. Plant Soil 2006, 132: 297-301.
10. Ali NA, Julicch WD, Kusnick C, Lindequist U. Screening of Yemeni medicinal plants for antibacterial and cytotoxic activities. J. Ethnopharmacol. 2001, 74: 173-179.
11. Girish KS, Machiah KD, Ushanandini S, Kumar KH, Nagaraju S, Govindappa M, Vedacathi M, Kemparaju K. Antimicrobial properties of a non-toxic glycoprotein (WSG) from Withania somnifera (Ashwagandha). Basic Microbiol. 2006, 46: 365-374.
12. Chaudhuri PK, Srivastava R, Kumar S. Phytotoxic and antimicrobial constituents of Bacopa monnieri and Holmskioldia sanguinea. Phytother. Res. 2004, 18: 114-117.
13. Shaila HP, Udupa SL, Udupa AL. Hypolipidemic activity of three indigenous drugs in experimentally induced atherosclerosis. Int. J. Cardiol. 1998, 67: 119-24.
14. Ghosh S, Majumder M, Majumder S, Nirmal K, Bishnu G, Chatterjee P. Saracin: a Lectin from Saraca indica seed integement induces apoptosis in human T-lymphocytes. Arch. Biochem. Biophy. 1999, 371: 163-168.
15. Kusumoto IT, Nakabayashi T, Kida H, Miyashiro H, Hattori M, Nambat T, Shimotohno K. Screening of various plant extracts used in Ayurvedic medicine for inhibitory effects on human immunodeficiency virus type 1 (HIV-1) protease. Phytoth. Res. 1995, 9: 180-184.
16. Ichikawa H, Takada Y, Shishodia S, Jayaprakasam B, Nair MG, Aggarwal BB. Withanolides potentiate apoptosis, inhibit invasion and abolish osteoclastogenesis through suppression of nuclear factor-kappa B (NF-kappa B) activation and NF-kappa B regulated gene expression. Mol. Cancer Ther. 2006, 5: 1434-1445.
17. Dhar RS, Verma V, Suri KA, Sangwan RS, Satti NK, Kumar A, Tuli R, Qazi GN. Phytochemical and genetic analysis in selected chemotypes of Withania somnifera. Phytochem. 2006, 67: 2269-2276.
18. Bandyopadhyay M, Jha S, Tepfer D. Changes in morphological phenotypes and withanolide composition of Ri-transformed roots of Withania somnifera. Plant Cell Rep. 2007, 26: 599-609.
19. Sharma V, Gupta AP, Bhandari P, Raghbir Gupta C, Bikram Singh B. A validated and densitometric HPTLC method for the quantification of withanolide-A and withaferin-A in different plant parts of two morphotypes of Withania somnifera. Chromatographia 2007, 66: 801-804.
20. Deepak, Amit A. The need for establishing identities of bacoside A and B, the putative major bioactive saponins of Indian medicinal plant Bacopa monnieri. Phytomedicine 2004, 11: 264-268.
21. Deepak M, Sangli GK, Arun PC, Amit A. Quantitative determination of the major saponin mixture bacoside A in Bacopa monnieri by HPLC. Amit. Phytochem. Analy. 2005, 16: 24-29.
22. Pawar RS, Shabana I, Khan IA. Glycosides of 20-Deoxy Derivatives of Jujubogenin and Pseudojujubogenin from Bacopa monnieri. Planta Med. 2007, 73: 380-383.

Author Information

Amitabh Singh, M.Sc.(Ag.), Ph.D.
22, Ganesh Dham Colony, Newada, Sunderpur

Shalini Singh, M.Sc.
Institute of Bioengineering and Biological Sciences, Varuna Bridge

B.K. Sarma, M.Sc.(Ag.), Ph.D.
Department of Mycology and Plant Pathology, Institute of Agricultural Sciences, Banaras Hindu University

U.P. Singh, M.Sc.(Ag.), Ph.D.
22, Ganesh Dham Colony, Newada, Sunderpur

Rachna Srivastava, M.Sc., Ph.D.
Institute of Bioengineering and Biological Sciences, Varuna Bridge

K.P. Singh, M.Sc.(Ag.), Ph.D.
College of Forestry and Hill Agriculture, G. B. Pant University of Agriculture and Technology