Pak. J. Bot., 43(1): 579-585, 2011.

ANTIBACTERIAL AND ANTIOXIDANT ACTIVITIES OF AN ETHNOBOTANICALLY IMPORTANT PLANT *SAUROMATUM VENOSUM* (Ait.) SCHOTT. OF DISTRICT KOTLI, AZAD JAMMU & KASHMIR

MUHAMMAD AJAIB[1], ZAHEER-UD-DIN KHAN[1], NASRULLAH KHAN[3] MUHAMMAD ATHAR ABBASI[2], DURRE SHAHWAR[2], MUHAMMAD WAHAB[3] AND MUHAMMAD FAHEEM SADDIQUI[3]

[1]Department of Botany, GC University Lahore, Pakistan
[2]Department of Chemistry, GC University Lahore, Pakistan
[3]Department of Botany, Federal Urdu University, Karachi Pakistan
E-mail: majaibchaudhry@yahoo.com

Abstract

In order to verify the ethnopharmacological effects of local plant, *Sauromatum venosum* (Ait.) Schott., on scientific lines the antibacterial activity including MIC and antioxidant activity of the crude extracts of its fruits were tested against Gram-positive and Gram-negative bacteria using well diffusion method and 2, 2́-dipheńyl-1-picrylhydrazyl (DPPH) radical scavenging activity test. The results indicated a reasonable antibacterial potential and significant total antioxidant activity, thus supporting its traditional medicinal practices.

Introduction

Plants play a vital role in our lives more than animals mainly due to their extraordinary array of diverse class of biochemicals with a variety of biological activities (Cotton, 1996; Buckingham, 1999). In Pakistan and Azad Kashmir the medicinal plants have immense potential but, unfortunately, very little are known about the actual production, size and potential of various species, their conservation status, actual trade and production areas.

Plant constituents have become an important source of active natural products which differ widely in terms of their structure and biological properties. In recent years, the prevention of many disorders such as cancer and cardiovascular diseases has been found associated with the ingestion of fresh fruits, vegetables, tea or plant beverages that are rich in natural antioxidants. The antioxidant and antimicrobial potential of plant products is due to the presence of several compounds in them which have distinct mechanisms of action; some are enzymes and proteins while others are low molecular weight compounds such as vitamins, carotenoids, flavonoids, anthocyanins and other phenolic compounds. The reason of investigations on medicinal plants is the ethnobotanic knowledge which still exists in remote areas (Khan *et al*., 2009).

Meurer-Grmes *et al*., (1996) tested the antimicrobial activity of 59 species of family Acanthaceae and Scrophulariaceae. All the plants were selected using ethnobotanical information and a significant antimicrobial activity was found. Tewary *et al*., (2005) studied the pesticidal activities of five plants collected from mid-hill of Western Himalayas and reported that the extracts of all the plants were active against the pests, a study useful in promoting research on the development of new agents for pest control from plants. Bisht *et*

al., (2006) tested the extracts from rhizomes of *Hedychium spicatum* for their antimicrobial and antifungal activity. Essential oil, petroleum ether and chloroform extracts showed inhibitory activity against Gram-positive and Gram-negative bacterial cultures, including a strain of methicillin and vancomycin resistant *Staphylococcus aureus* and fungal cultures. Buruk *et al*., (2006) studied the antimicrobial activity of 22 endemic plants growing in the Easter Black Region, Turkey. Among the 30 active crude extracts, water-insoluble crude extacts from *Betula medwediewii, Heracleum platytaenium, Primula longipes, Anthemis cretica and Centuarea helenioides* were having promising MIC values. Khan *et al*., (2006) tested various crude extracts of different parts of *Derris elliptica, Derris indica* and *Deris trifoliate.* A Good activity was exhibited by methanol fractions of the leaves and root heartwood, petroleum ether, butanol and methanol fractions of the root bark of *D. indica* and petroleum ether and ethyl acetate fractions of *D. trifoliata*. None of the plants showed antifungal activity. Usman (2008) investigated the ethnoparmacological effects of the crude extracts of Sohanjana (*Moringa oleifera*), locally used as an important medicinal plant. It was found having a reasonable antioxidant activity as well as potent against fungi and bacteria used in the study.

A somewhat similar investigation was carried out on the antimicrobial and antioxidant activities of the crude extracts of *Sauromatum venosum* (Ait.) Schott., to test and verify the local ethnomedicinal knowledge of this plant.

Material and Methods

Collection and preparation of samples: Fruits of *Sauromatum venosum* (Ait.) Schott. locally known as Sanp buti of the family Araceae was obtained from Khui-Ratta, District Kotli, Azad Jammu & Kashmir (Fig. 3). The plant specimen was preserved properly and deposited in Dr. Sultan Ahmad Herbarium, Department of Botany, GC University Lahore. It is used locally as a medicinal plant to treat diseases like cancer, etc.

Gram +ve, *Streptococcus faecelis* and *Staphylococcus aureus* and Gram –ve, *Escherichia coli* and *Pseudomonas aeruginosa,* obtained from Zeenat Laboratories and PCSIR Laboratories Lahore were used as test organisms.

About 250 gm of powdered fruit was extracted successively with polar and non-polar solvents, like petroleum ether, chloroform and methanol by maceration for 8 days in each of the solvents.

Antibacterial activity: All the crude extracts were studied for their antibacterial activity using well diffusion method according to Ortega & Julian (1996) and Ferreira *et al*., (1996). Two series of experiments were conducted in the present study. In the first, crude extracts were tested for their antibacterial activity against bacteria, while in second series of experiments, commercially available antibiotic discs, such as Erythromycin (15µg) and Amikacin (30µg) were used to compare their antimicrobial activity with that of crude extracts. All the experiments were performed in aseptic conditions. The zone of inhibitions produced by inhibitory action of different plant extracts and standard antibiotic discs were taken as the antibacterial activity. MIC of only methanolic extract was carried out according to Murray *et al.,* (1999) by modified Broth dilution assay with the help of Spectrophotometer at 595 nm in mg/ml.

DPPH free radical scavenging activity: The free radical scavenging activity of the crude extracts of the fruit of *Sauromatum venosum* (Ait.) Schott., was evaluated

according to Blois (1958), Brand *et al.,* (1995), Sanchez-Moreno *et al*., (1998) and Khan *et al.,* (2009). The final solutions were prepared by dissolving 0.5 mg/ml of each dried extract in the respective extraction solvent. 0.2 ml of each Petroleum ether extract (SV1) and Methanol extract (SV2) solution was added to 3.8ml of 0.025% (w/v) solution of DPPH (2,2/-diphenyl-1-picrylhydrazyl) in 95% ethanol. The reaction mixture was incubated at 28°C for 40 minutes. The scavenging activity on DPPH radical was determined by measuring the absorbance at 517nm. The antioxidant activity was expressed as %age of scavenging activity on DPPH radical: SC%= [1-(absorbance of sample)/(absorbance of control)] ×100. The control contained all reagents except the extract. The DPPH radical scavenging activity of standard antioxidant, BHT was also assayed for comparison. All tests were performed in triplicate and the means were calculated.

Total antioxidant capacity assay (TAOC): The total antioxidant capacity of SV1 and SV2 was assayed according to the method of Prieto *et al*., (1999) and Khan *et al*., (2009). 0.1 ml of each SV1 and SV2 solutions (0.5mg/ml) combined with 1.9ml of reagent solution (0.6 M H_2SO_4, 28 mM Sodium phosphate and 4mM Ammonium molybdate). The blank solution contained 2.0 ml reagent solution only. The mixtures were incubated at 95^oC for 150 minutes. After the mixture had cooled to room temperature, absorbance was measured at 695 nm. The antioxidant activity was expressed as the absorbance of the sample. The antioxidant activity of BHT was also assayed for comparison as standards.

Results

Antibacterial activity: The results indicated that fruit extracts had inhibitory effect against most of the bacteria tested especially *Escherichia coli.* The methanol and petroleum ether extracts were more potent against *E. coli* with zone of inhibition 22 and 21mm respectively (Fig. 1). The standard antimicrobial discs i.e., Cephalaxine 30µg and Amikacine 30µg both showed 25mm zone of inhibition against *Streptococcus faecelis* and *E. coli* and 22mm against *Staphylococcus aureus* and *Pseudomonas aeruginosa.* All the extracts showed inhibitory action against the bacteria as given in Table 1.

The MIC of methanolic extracts indicated more resistance against Gram-negative bacterium, *E. Coli* with a value 1.94mg/ml and least against Gram-positive bacterium, *Streptococcus faecelis* with a value 2.12mg/ml, which indicated that methanolic extract was more potent against Gram-negative bacteria.

Antioxidant activity: A flat concentration assay was carried out with two extracts SV1 & SV2 of the fruit of *Sauromatum venosum* and the results are presented in Fig. 1. These results provided a direct comparison of the antioxidant activity shown by the extracts and by the standard, BHT. Both the extracts (SV1 & SV2) possessed good scavenging activity on the DPPH radical which was 37.5%and 33% respectively, hence acting as antioxidants (Fig. 1).

Total antioxidant capacity assay: The assay was based on the reduction of Mo (VI) to Mo (V) by SVs and subsequent formation of a green phosphate/Mo (V) complex. The total antioxidant activity was measured and compared with that of BHT (standard). The high absorbance values indicated that the sample possessed significant antioxidant activity (Fig. 2).

Table 1. Zones of inhibition produced by extracts of *Sauromatum venosum* (Ait.) Schott., and Standard discs.

Bacteria	Zone of inhibition (mm)				
	Extracts			Standard Discs	
	Petroleum ether	Chloroform	Methanol	Cephalaxine	Amikacine
i. Gram-positive bacteria					
Streptococcus faecelis	10	15	10	25	25
Staphylococcus aureus	09	10	10	22	22
ii. Gram-negative bacteria					
Escherichia coli	21	18	22	25	25
Pseudomonas aeruginosa	19	15	20	22	22

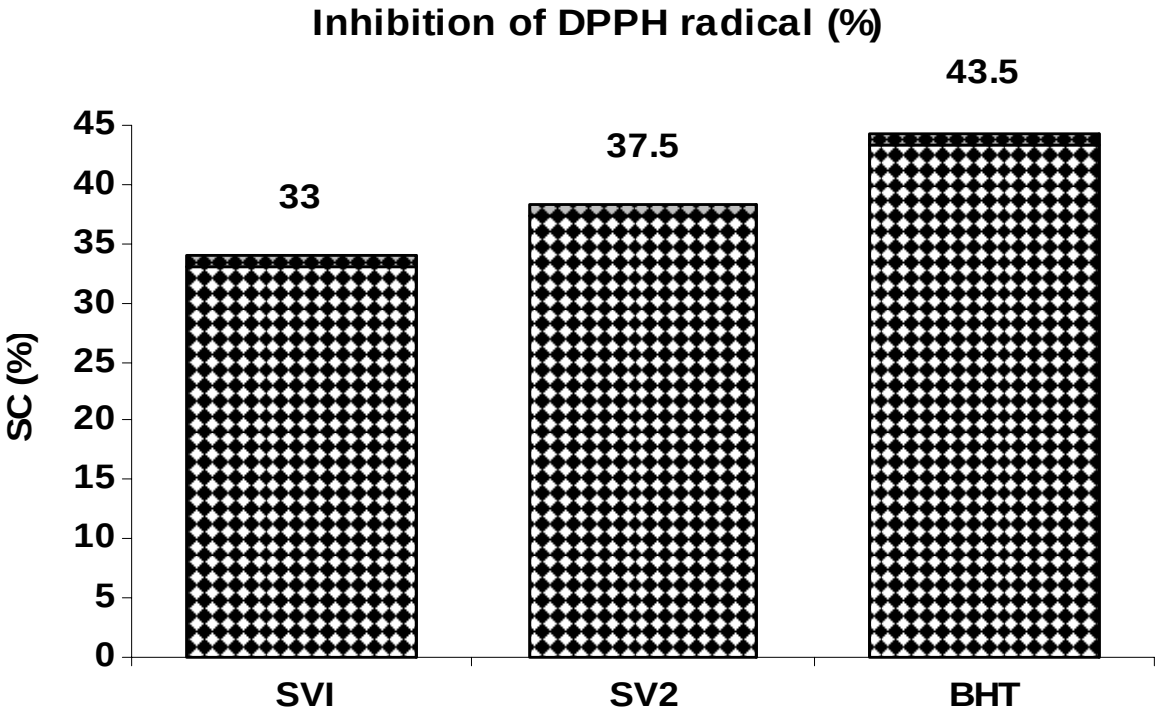


Fig. 1. DPPH free radical scavenging activity of Extracts and a standard antioxidant BHT.

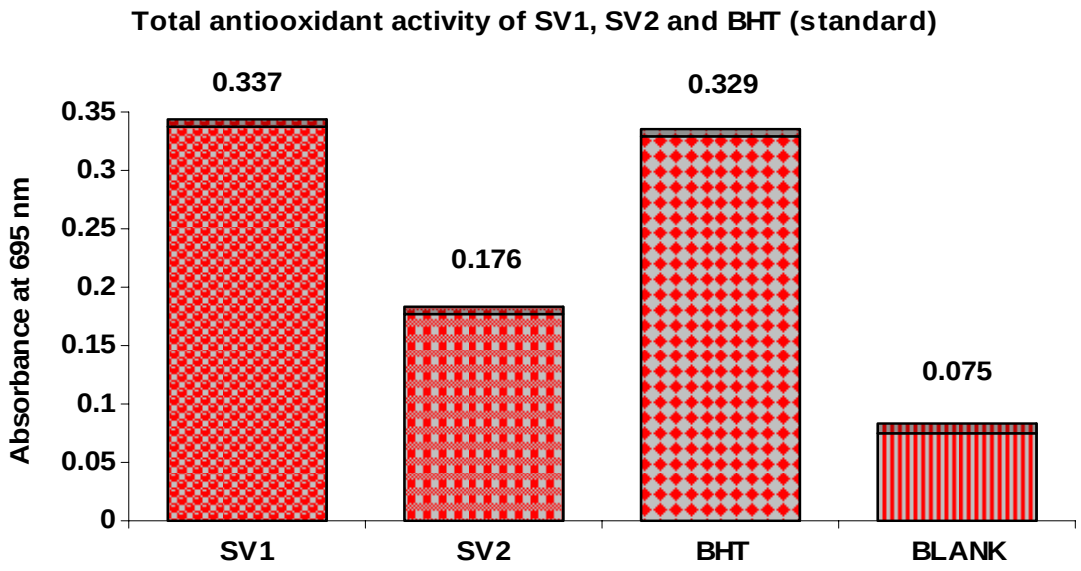
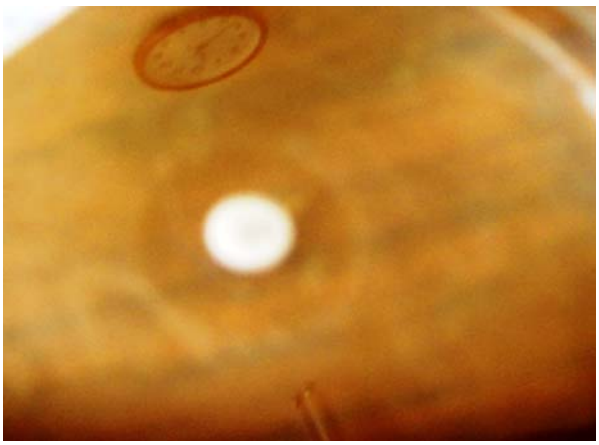


Fig. 2. Total antioxidant capacity assay.

a. *Sauromatum venosum* (Ait.)Schott.

b. Fruit of *Sauromatum venosum* (Ait.)Schott.



c. Standard Disc against *E. coli*

d. Chloroform extract against *E. coli*

e. Methanol extract against *E. coli*

f. Petroleum Ether against *E. coli*

Fig. 3. **a.** *Sauromatum venosum* (Ait.)Schott. in its natural habitat
b. Its fruits
c-f. Zone of inhibition

Discussion

In the present study, the antibacterial activity was found higher in all solvents against Gram-negative and Gram-positive bacteria. This may be due to the presence of different alkaloids or other antimicrobial chemicals in these extracts. It was observed that antimicrobial activity of the standard discs was higher than those of the crude extracts of the plant. Petroleum ether and chloroform extracts showed comparatively higher inhibitory action than the methonolic extracts, against the bacteria used (Table 1).

The results revealed that SV2 had significant free radical scavenging activities on DPPH radical. The total antioxidant activity SV1 was significant than SV2 extract against BHT (standard). The free radical scavenging property might be one of the mechanisms by which this herbal medicine exhibits higher antioxidant activity. Thus, this study gives a strong initiation for its use in food industry and medicine. It also provides useful information on pharmacological activities associated with this traditional folk remedy. Antioxidant activity of the compounds present in two extracts is strong; the overall antioxidant effect could be higher by the combined and synergistic effects of other compounds. Therefore, isolation and identification of individual active compounds, their *In vivo* antioxidant activities as well as different antioxidant mechanisms *In vitro* need to be studied.

References

Bisht, G.S., A.K. Awasthi and T.N. Dhole. 2006. Antimicrobial activity of *Hedychium spicatum*. *Fitoterapia,* 77(3): 240-242.

Blois, M.S. 1958. Antioxidant determinations by the use of a stable free radical. *Nature,* 26: 1199-1299.

Brand, W.W., M.E. Cuvelier and C. Berset. 1995. Use of a fee radical method to evaluate antioxidant activity. *Food Science Technology (London),* 28: 25-30.

Buckingham, J. 1999. *Dictionary of Natural Compounds*. Chapman and Hall, U.K: PP 14-20.

Buruk, K., A. Sokman, F. Aydin and M. Erturk. 2006. Antimicrobial activity of some endemic plants growing in the Eastern Black Sea Region, Turkey. *Fitoterapia.,* 77(5): 388-391

Cotton, C.M. 1996. *Ethnobotany: Principals and Applications*. John Wiley and Sons Ltd. Chichister, England.

Ferreira, M.J.U., A. Daurte and J.R. Ascenso. 1996. Antimicrobial and Phytochemical Studies of *Euphorbia tuckeyana. Fitoterapia,* 67: 85-86.

Khan, M.A., D. Shahwar, N. Ahmad, Z. Khan and M. Ajaib 2009. Chemical constituents of *Carissa opaca* extracts and their evaluation as antioxidant and preservative in Edible Oils. *Asian J. Chemistry,* 21(9): under press.

Khan, M.A., D. Shahwar, N. Ahmad, Z. Khan and M. Ajaib. 2009. Chemical Constituents of *Carissa opaca* Extracts and their evaluation as antioxidant and preservative in edible oils. *Asian J. Chemistry*, 22(1): 379-388.

Khan, M.R., A.D. Omoloso and Y. Barewai. 2006. Antimicrobial activity of the alkaloidal constituents of root bark of *Eupomatia laurina*. *Pharmaceut. Biol.,* 41: 277-280.

Meurer-Grmes, B., D.L. Mcbeth, B. Hllihan and S. Delph. 1996. Antimicrobial activity of ethnobotanically important plants of Acanthaceae and Scrophulariaceae. *Pharmaceutical Biology*, 34(4): 243-248.

Murray, P.R., E.J. Baron, M.A. Pfaller, F.C. Tenevor and R.H. Yolke. 1999. *Mannual of clinical Microbiology* (7^{th} ed). Washington, PP: 1527-1539.

Ortega, M.G. and H.R. Julian. 1996. Antimicrobial Agents in *Dalea elegant*, *Fitoterapia,* 67(1): 81.

Prieto, P., Pineda M. and M. Agular. 1999. Spectrophotometric quantitation antioxidant capacity through the formation of a phosphomolybdenum complex. *Analytical Biochemistry,* 269:337-341.

Scanchez-Moreno, J.A. Larrauri and S. Fulgencio. 1998. A procedure to measure the antiradical efficiency of polyphenols. *J. Food Sciences and Agriculture,* 76: 270-276.

Tewary, D.K., A. Bhardwaj and A. Shanker. 2005. Pesticidal activities in five plants collected from mid hills of Himalayas. *Industrial Crops and Products,* 22: 241-247.

Usman, M. 2008. The ethnopharmacological effects of the crude extracts of Sohanjana (*Moringa oleifera*). An unpublished M.Phil. Thesis submitted in Sustainable Development Study Centre, GC University Lahore, Pakistan.

(Received for publication 13 July 2009)