International Journal of Basic and Applied Biology (IJBAB)
Print ISSN: 2349 – 5820; Online ISSN: 2349 – 5839 ; Volume 2, Number 1; October, 2014 pp. 94 – 97

http://www.krishisanskriti.org/ijbab.html

Bioactive Compounds and Antioxidant Activity of Polygonum odoratum Lour

Somananda K. Ahongshangbam[1], G. A. Shantibala Devi [1], S. Chattopadhyay[2]

[1]*Department of Life Sciences Manipur University, Canchipur-795003, Manipur*
[2] *Bio-Organic Division, Bhabha Atomic Research Centre, Trombay, Mumbai-400085, India*

Abstract: **Plants with rich phenolic compounds are of interest as they constitute one of the major targets to search for natural antioxidants. The ethanolic extract of Polygonum odoratum Lour., a perennial herb which is used as spices in the state of Manipur, India, was analysed for its bioactive compounds, total phenols, flavonoids and antioxidant activities. The plant has a total phenolic content of 13.03±0.61 GAE/mg/g dry weight and flavonoid content of 4.92±0.629 mg/g of dry weight. Antioxidant activity of the extract was evaluated by using DPPH (1, 1-Diphenyl- 2-picryl hydrazyl) assay. IC_{50} value of the plant has been determined as 190.19±0.424 µg/ml. In high-performance liquid chromatography, Gallic acid, apigenin, ferulic acid, quercetin, ellagic acid and p-coumaric acid has been identified and quantified as the main bioactive compounds. The plant extract showed good radical scavenging activity which may be, due to high phenolic and flavonoid contents. The results from this study indicates that the plant has prominent antioxidant activity and showed the presence various phenolic compounds that can be harnessed as a potent source of natural antioxidants to treat free radical mediated diseases.**

Key words: natural antioxidants, HPLC, bioactive compounds, flavonoids, free radical scavenging.

1. INTRODUCTION

Polygonum odoratum Lour., has been reclassified as Persicaria odorata (Lour.) Soja´k [1]. The plant is a perennial herb that belongs to the family Polygonaceae and grows to a height of 60-90cm tall with reddish stems divided into sections. The plant has pointed leaves 6-15 cm with a distinctive dark purple marking in the centre of the leaves. The plant has a strong coriander like scent and clear lemony note. The aerial part of the plant is used in garnishing salads and other local cuisines in the state of Manipur, India, due to its distinct aroma and flavor. The state of Manipur has rich flora comprising of various aromatic plants which forms a part of human diet but are yet to be fully investigated in terms of their potentials.

Spices and herbs have been used since the dawn of civilisation by many cultures to add flavour and aroma of food. Spices not only form a major part of human diet to boost flavour but are also known for their role as preservatives and for their medicinal values, which forms one of the oldest sciences [2, 3]. Many spices and herbs are known to exert antioxidant activity and constitute one of the most important targets to search for natural antioxidants. Natural antioxidants commonly exist in plants which contain polyphenolic compounds [4, 5].

Typical phenolics that possess antioxidant activity are known to be mainly phenolic acids and flavonoids [6]. Polyphenolic compounds are commonly found in both edible and inedible plants and have multiple applications in food, cosmetic and pharmaceutical industries [7]. This study aimed to investigate the possible antioxidant activity and to determine bioactive compounds, total phenols and flavonoids of the plant.

Antioxidants play a major role in protecting human body against free radical mediated diseases [8]. However the use of synthetic antioxidants have been restricted due to their carcinogenic effects [9, 10]. As such the focus have been shifted to plant phytochemicals. Spices and aromatic plants besides being the vital source of natural antioxidants, are also used in food industry to prolong shelf life of food which are rich in polyunsaturated fat [11].

2. MATERIALS AND METHODS

2.1 Chemicals

DPPH (1, 1- diphenyl-2-picryl-hydrazyl), Gallic acid, Quercetin, Apigenin, Ferulic acid, Ellagic acid, p-coumaric acid, Methanol HPLC grade and Aluminium trichloride were obtained from Sigma Co. (St Louis, MO, USA). Folin-Ciocalteu reagent, Sodium carbonate and Ascorbic acid were from Merck (Darmstadt, Germany). All other chemicals and solvents used in this study were of analytical grade.

2.2 Plant material

The aerial part of *Polygonum odoratum* Lour., was collected in June 2013 and authenticated. The voucher specimen of the plant was deposited in the Department of Life

Sciences, Manipur University, Canchipur, Manipur, India under registry no. ASK000876 (Man. Univ. Mus. Pl.)

2.3 Extraction of samples

The dried plant sample was ground to fine powder and 5g of the sample was soaked with 100ml of ethanol. The mixture was allowed to stand at room temperature for 48 hours with occasional agitation. The aqueous extract was filtered through Whatman no.1 filter paper while the residue was further extracted under the same conditions three times. The filtrates collected from these separate extractions were pooled together and evaporated to dryness under reduced pressure at temperature not exceeding 40^{o}C using Buchi rotary vacuum evaporator. This concentrated extract was designated as whole concentrate (WC).

Known weight of the sample from whole extract was extracted three times with ethyl acetate. The ethyl acetate extracts were combined passed over anhydrous sodium sulphate for 30 min. and filtered through Whatman filter paper no. 42. The Combined ethyl acetate extracts were then evaporated to dryness under reduced pressure and stored in a desiccator at low temperature prior to analysis by HPLC.

2.4 Total flavonoids estimation

Aluminium chloride colorimetric technique was used for flavonoids estimation with slight modification [12]. Each extract (0.2ml) in methanol was separately mixed with 1.8ml of methanol, 0.1ml of 10% aluminium chloride, 0.1ml of 1M potassium acetate and 2.8 ml of distilled water. It was left at room temperature for 30 min. after which the absorbance of the reaction mixture was measured at 415 nm with a UV-visible spectrophotometer. The calibration curve was plotted by preparing quercetin solutions at concentrations 12.5 to 100µg/mL in methanol. All the determinations were performed in triplicates (n=3).

2.5 Total phenols estimation

The amount of total phenolic content in extract was determined according to Folin-Ciocalteu method of Slingkard and Singleton [13]. 0.1 ml of sample solution (1mg/ml) was introduced into test tube containing 1 ml of Folin-Ciocalteu's reagent and 2 ml of Na_2CO_3 (7.5%). The final volume was brought up to 7 ml with deionized water. After 2 h incubation at room temperature, the absorbance was measured at 765 nm with spectrophotometer. All the determinations were performed in triplicates. The total phenolic content was expressed as gallic acid equivalents (GAE) in milligram per gram of dry weight.

2.6 DPPH radical scavenging activity

DPPH radical scavenging activity of extracts was measured according to the method of Blois with some modifications [14]. Different concentrations of the extract (100, 200, 400, 600 µg/ml) was mixed with 2.5 ml of stock solution of 100 µM DPPH in ethanol. The mixture was shaken vigorously and kept in the dark at room temperature for 30 min. Special care was taken to minimize the loss of free radical activity of the DPPH radical stock solution [14]. Absorbance of the mixture was measured at 517 nm in a UV–visible spectrophotometer. Ethanol was used to zero the spectrophotometer. The scavenging activity of the extracts was expressed as the percentage of inhibition of the DPPH radical.

Inhibition percentage (IP) = $[(A_{control} - A_{sample}) - 100]/A_{control}$, where $A_{control}$ is the absorbance of the control (containing all reagent except the sample), A_{sample} is the absorbance of the sample, both measured at 517 nm. The IC_{50} (DPPH) value, which represents the concentration of extract that gives 50% reduction in DPPH absorbance, was determined by linear regression analysis of absorbance versus concentration.

2.7 HPLC Analysis

For quantification and identification purposes of phenolic compounds in the plant samples, a 1.0% solution (w/v) in HPLC grade methanol was made for ethanol extracts. The samples were then filtered first through Whatman filter paper no. 42 and then through 0.22 mm membrane filters (Millipore). Separation of phenolic compounds was carried out by HPLC. Aliquot of 20 µL sample was injected for estimation purposes in a C-18 column. Prior to analysis, the analytical column was thoroughly washed with methanol followed by mobile phase for 1 h. The mobile phase consisted of formic acid system with 0.1% formic acid as solvent A and HPLC grade methanol as solvent B. Flow rate of mobile phase was 1 mL/min.

Following gradient was used during the run; A = 90%, T = 0 min; A = 85%, T = 35 min; A = 60%, T = 50 min; A = 40%, T = 55 min; A = 25%, T = 60 min; A=5%, T=65min. Detector wavelength was set at 280 nm. Identification of the phenolic compounds present in the plant sample was done on comparison of the retention times (Rt) with standard samples.

Further verification of most of the identified compounds was done by comparison of the UV–visible spectra with the standard compound. For this purpose, the fraction of a particular compound coming out of the column at the respective Rt was collected till a measurable amount was obtained. The sample was again dried under vacuum and re-dissolved in methanol followed by recording the spectra by scanning the sample at wavelength from 200 to 780 nm and

comparison with the known standard. The quantification was done with the help of standard curve obtained by plotting percentage peak area versus concentration.

3. RESULT AND DISCUSSION

The phytochemical study showed high amount of total phenolics and flavonoids present in the ethanolic extract of *P. odoratum*. The total phenolic and flavonoid contents were determined as 13.03±0.61mg GAE/g and 4.92±0.42mg QE/g respectively (Table 1). Plant phenolics have received enthusiastic attention due to their potential antioxidant activity [15, 16]. The antioxidant capacity of the plant was determined by DPPH (1, 1-diphehyl-2-picrylhydrazyl) assay and showed prominent IC_{50} value of 190.19±0.42 µg/ml as compared with ascorbic acid which is a well known antioxidant that showed an IC_{50} value of 29.98±0.24 µg/ml (Table 1).

Table 1: Total phenolic content, Flavonoid content and Antioxidant Activity

Sample	GAE mg/g	QE mg/g	IC_{50} (µg/ml)
P. odoratum	13.03±0.61*	4.92±0.63*	190.19±0.42*

*All the values are mean of triplicates ± SD

Table 2: Phenolic compounds identified and quantified through HPLC

Phenolic compounds	Content in µg/g (Mean ± S.D.)
Gallic acid	2172.32±0.330
G-resorcyclic acid	45.22±0.235
Chlorogenic	1193.27±0.395
p-coumaric acid	875.18±0.297
Ferulic acid	1292.14±0.238
Ellagic acid	2956.25±0.530
Quercetin	2672.18±0.241
Luteolin	198.13±0.136
Kaempferol	460.38±0.217
Apigenin	955.90±0.710

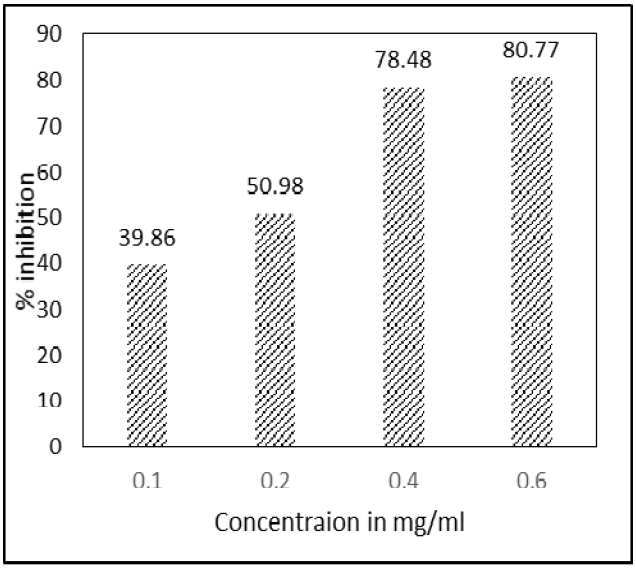

Figure 1: Inhibition of DPPH in presence of different concentration of *P. odoratum* extract.

DPPH being a stable free radical, is frequently used to determine radical scavenging activity of natural compounds. In its radical form, DPPH absorbs at 517 nm, but upon reduction with an antioxidant, its absorption decreases due to the formation of its non-radical form, DPPH–H [14]. Thus, the decrease in the absorbance of the DPPH solution can be monitored as the radical scavenging activity. The radical scavenging activity of the plant extract increases with increasing concentration [Figure 1].

The result indicates that the ethanolic extract of the *P. odoratum* exhibited the ability to quench DPPH radical that serve as a good antioxidant with free radical scavenging activity. The high potential of phenolics to scavenge free radicals may be attributed the hydroxyl groups they possess [17]. It has also been acknowledged that compounds such as flavonoids are responsible for the radical scavenging capacity in plants [18]. Phenolic acid have been largely studied in relation to their antioxidant activity but flavonoids have other health promoting activities including anti-allergic, anti-inflammatory, antimicrobial and anticancer properties [19].

The phenolic compounds identified and quantified through HPLC consists mainly of Ellagic acid, Quercetin, Gallic acid, Ferulic acid, Apigenin and p-coumaric acid with corresponding values of 2956.25±0.53 µg/g, 2672.18±0.241 µg/g, 2172.32±0.33 µg/g, 1292.14±0.238 µg/g, 955.90±0.710 µg/g and 875.18±0.297 µg/g respectively. Kaempferol, Luteolin and G-resorcyclic acid are detected in traces [Table 1]. The prominent antioxidant activity of the plant may be due to the presence of polyphenols like gallic acid, quercetin, ferulic acid and apigenin. Gallic acid is naturally occurring polyphenol with antioxidant capacity which is essential for the antioxidant capacity of black tea [20].

The study indicates that *P. odoratum* has prominent antioxidant activity and in addition to its use as a spice to add flavour and aroma, may also be harnessed as a preservative ingredients in food as well as a source of natural antioxidants. Bioactive compounds in *P. odoratum*, especially polyphenols can provide a boost in dietary phytochemicals and also play an important role in human health to counter oxidative stress mediated diseases caused by free radicals.

4. REFERENCES

[1] Wilson, K. L., "Polygonum sensu lato (Polygonaceae) in Australia", Telopea, 1988, pp.177-182.

[2] Nielson, P. V. and Rios, R., "Inhibition of fungal growth on bread by volatile compounds from spices and herbs and mustard essential oil", International journal of food microbiology, 2000, pp. 219-229.

[3] Fisher, C., "Phenolic compounds in food and their effects on health", ACS Symposium, 1992, pp. 506.

[4] Stoilova,I., Krastanov,A., Stoyanova, A., Denev, P. and Gargova, S., " Antioxidant activity of a ginger extract (*Zingiber officinale*)", Food Chemistry, 2007, pp. 764-770.

[5] Gülçin, I., Oktay, M., Küfreviolu, Ö.I and Aslan, A., "Determinations of antioxidant activity of lichen *Cetraria islandica* (L)", Ach. J. Ethnopharmacol., 2002, pp. 325-329.

[6] Wojdylo, A., Oszmianski, J., Czemerys, R., "Antioxidant activity and phenolic compounds in 32 selected herbs", Food Chemistry, 2007, pp. 940-949.

[7] Kahkonen, M.P., Hopia, A.I., Vuorela, H.J. Rauha, J.P. Pihlaja, K. and Kujala, T.S., "Antioxidant activity of plant extracts containing phenolic compounds", Journal of Agriculture and Food Chemistry, 1999, pp. 3954-3962.

[8] Halliwell, B. and Gutteridge, J.C.M., "Free radicals in Biology and Medicine", 1989, Clarendon Press, Oxford.

[9] Guner, A., Ozhatay, N., Ekim, T., and Baser, K.H.C., "Flora of Turkey and the East Aegean Islands", 1988, Edinburgh, Edinburgh University Press.

[10] Kargioglu, M., Cenkei, M., Serteser, A., Evliyaoglu, N., Konuk, M., Kok, M.S., and Bager, Y., "An ethnobotanical survey of inner West Anatolia, Turkey", Hum. Ecol., 2008, pp. 763-777.

[11] Shih Peng Wong, S.P., Leong, L.P. and Koh, J.H.W., "Antioxidant activities of aqueous extracts of selected plants", Food chemistry, 2006, pp. 775-783.

[12] Chang, C., Yang, M., Wen, H., Chern, J., "Estimation of total flavonoid content in Propolis by two complementary colorimetric methods", J. Food Drug Anal., 2002, pp. 178-182.

[13] Slinkard, K. and Singleton, V.L., "Total phenol analysis: automation and comparison with manual methods", American Journal of Enology and Viticulture, 1977, pp. 49-55.
.

[14] Blois, M.S., "Antioxidant determinations by the use of a stable free radical', Nature, 1958, pp. 1199-1200.

[15] Dziedzic, S.Z. and Hudson, B.F.J, "Polyhydroxy chalcones and flavonones as antioxidants for edible oils", Food Chemistry, 1983, pp. 205-212.

[16] Lopez-Velez, M., Martinez- Martinez, F. and Del Valle-Ribes, C., "The study of phenolic compounds as natural antioxidant in wines", Crit. Rev. Food Sci., 2003, pp. 233-244.

[17] Sawa, T., Nako, M., Akaike, T., Ono, K. and Maeda, H., "Alkylperoxyl radical scavenging activity of various flavonoids and other phenolics compounds: Implementations for the antitumour promoter effect of vegetables", J. Agric. Food Chem., 1999, pp. 397-492.

[18] Younes, M., "Inhibitory action of some flavonoids on enhanced spontaneous lipid peroxidation following glutathione depletion", Plant medica, 1981, pp. 240-245.

[19] Ho, C.T., Chen, Q., Shi, H., Zhang, K.Q. and Rosen, R.T, "Antioxidative effect of polyphenol extract prepared from various Chinese teas", Prev. Med., 1992, pp. 520-525.

[20] Cody V., Middleton E. J., and Harborne J. B., "Plant Flavonoids in Biology and Medicine: Biochemical, Pharmacological and Structure Activity Relationships", 1986, Alan R. Liss, New York.