Asian Journal of Chemistry; Vol. 33, No. 4 (2021), 945-948

ASIAN JOURNAL OF CHEMISTRY

https://doi.org/10.14233/ajchem.2021.23155

An Indian Folkore Phytomedicine *Perilla frutescens* L.: Free Radical Scavenging Property along with Metal Detection by ICP-MS

AJAY SINGH[1], ARUNESH KUMAR DIXIT[1,*], S. FAROOQ[2], ZAFAR MEHMOOD[2] and SUMAN LATA CHHIMWAL[2]

[1]Department of Chemistry, School of Applied and Life Sciences, Uttaranchal University, Arcadia Grant, Premnagar, Dehradun-248007, India
[2]The Himalaya Drug Company, Saharanpur Road, Clement Town, Dehradun-248002 India

*Corresponding author: E-mail: dixit.arunesh@gmail.com

Received: 5 February 2021; *Accepted*: 27 February 2021; *Published online*: 20 March 2021; AJC-20308

In present study, free radical scavenging activity of methanolic extract of all parts (leaves, seeds, root, stem and flower) of *Perilla frutescens* L. has been estimated. The promising results, among the five plant parts leaves, seeds, root, stem, flower and the standard tested for the *in vitro* antioxidant activity using the DPPH method, the crude methanolic extracts of all parts showed antioxidant activity, with IC_{50} values of 5.95 ± 0.10, 8.28 ± 0.20, 66.27 ± 0.17, 80.03 ± 0.10 and 122.35 ± 0.17 µg/mL, respectively. The IC_{50} value for ascorbic acid was 5.19 ± 0.26 µg/mL. While butylated hydroxyl toluene (BHT) a synthetic commercial antioxidant has comparatively high IC_{50} value of 108.46 ± 0.57 µg/mL. Presence of zinc in all parts of plant in the range 17.20 ± 0.22 to 33.56 ± 0.32 ppm further supports the strong antioxidant activity of *Perilla frutescens* L. The phytochemical tests indicated that all parts of *Perilla frutescens* L. have considerable proportion of important phytochemicals and are in rich source of secondary metabolites like polyphenols, tannins, alkaloids and flavonoids. Several of such compounds are known to possess potent antioxidant activity.

Keywords: Oxidative stress, Antioxidant activity, Phytochemicals, Free radical scavengers, *Perilla frutescens* L.

INTRODUCTION

Because of the adverse health effects of artificial antioxidants, plant extract utilization as natural antioxidants has gained considerable attention [1-3]. Studies on antioxidants have focused on their utilization in preventing unsaturated fat from oxidation, which leads to rancidity [4]. Fruits, medicinal herbs, and vegetables are the richest sources of antioxidants, such as vitamins C, A, E and β-carotene as well as crucial minerals. Consumer preferences and strict legislation led manufacturers' attention to shift from synthetic to natural antioxidants because of the carcinogenic nature of some synthetic antioxidants and food additives. Synthetic antioxidants can be toxic [5,6]. Many phytophenolic compounds were used to reduce or inhibit lipid oxidation in lipid containing food products. The minerals like iron, zinc, calcium, potassium and magnesium, are the essential part of the dietary supplement [7]. The most common micronutrient elements like Fe and Zn lack in diets of young children and low age pregnant women especially in low and middle-income countries [8]. Free radicals are produced as metabolic byproducts by biological systems both endogenous and exogenous sources [9,10]. The production of endogenous free radical results from inflammation, immune cell activation, infection, ischemia, excessive exercise, cancer, aging and mental stress.

Exposure to heavy metals (Hg, Cd, Fe, Pb and As), environmental pollutants, certain drugs (tacrolimus, cyclosporine, bleomycin and gentamycin), cooking (used oil, smoked meat and fats) and chemical solvents leads to the production of exogenous free radicals. Presence of ROS inside the cells need to maintain at a low level because activation of several transcriptional factors (immunity and differentiation) and processes (like phosphorylation, protein and apoptosis) are all dependent on a proper production of ROS [11].

Perilla frutescens L. is an important herb of interior and remote villages of hilly region of Uttrakhand state of India, where peoples use seeds as a food as well as as folklore medicine for the remedy of various ailments cough, allergy, depression, anxiety, tumor, cough, intoxication and some intestinal problems, cancers, infectious diseases and cardiovascular ailments. In

northern regions of India, the stem part of the plant is used as pain reliever and anti-abortive agent while the leaves are said to helpful for relief in asthma, colds and flu [12]. To expel intestinal worms and healing the cuts & wounds, the leaf juice of this plant is used in Dekhatbhuli, Nepal [13]. The paste of *Perilla frutescens* L. root in combination with goat urine is used as a poultice to relief from rheumatoid arthritis [14,15].

Perilla frutescens L. is listed in the Chinese Pharmacopoeia as a traditional Chinese medicine (TCM) and has been used for centuries as a medicinal plant for asthma, influenza, cough, chronic bronchitis and vomiting [16]. *Perilla frutescens* L. belongs to the family Lamiaceae, generally called as Bhanjira. The plant is initially born to India and China and other ASEAN countries are also produce this plant in large scale. In this study, we investigated the phyto-chemicals, total phenolic content, total flavonoid content, presence of minerals in different parts of *Perilla frutescens* L. and conducted an *in vitro* evaluation of antioxidant activity in different parts of the plant.

EXPERIMENTAL

The plant *Perilla frutescens* L. was collected for this study from the local village of Chakrata, Dehradun, India. The plant was authenticated by Dr. Mayaram Uniyal, (Ex-Herbs Advisor, Government of Uttrakhand, India) and voucher specimen was deposited to Department of Pharmacogonosy, The Himalaya Drug Company, Dehradun, India. The root, stem, leaves were separated and washed with distilled water and sun dried. However, flowers and seeds were dried in shade. After drying foreign matters and dust and dirt were removed from all parts. The seeds were crushed in mortar and pestle while leaves, stem and flowers were converted into powder with help of a grinder. The method of Zurera *et al.* [17] was used with little modifications.

Sample preparation for phytochemical analysis: The powder of (root, leaves, stem and flower) and crushed seeds were extracted in aqueous and methanol and filtered using Whatmann filter paper No. 1. The phytochemicals screening procedure and the qualitative tests for alkaloids, flavonoids, carbohydrates, glycosides, saponnis, tannins, terpenoids, protein and anthraquinone were performed as per the procedure described by Harbone [18] with slight modifications.

Preparation of extract for total phenolic content, total flavonoid content and antioxidant studies: Powder of each plant parts (root, leaves, stem and flower) and crushed seeds were macerated in methanol for 4 h. The extracts were filtered and filtrates were used for assay of total phenolic, total flavonoid contents and antioxidant activity.

Determination of total phenolic content: Total phenolic content in different extracts of *Perilla frutescens* L. was determined by Folin-Ciocalteu's method. Aliquots of each extract (1 mL) and standard gallic acid (10, 20, 40, 60, 80, 100 µg/mL) was transferred into the 10 mL volumetric flask containing 5 mL of distilled water and 0.5 mL of Folin-Ciocalteu's reagent and shaken vigorously. After 5 min, 1.5 mL of 20% Na_2CO_3 solution was added and volume was made up to 10 mL with distilled water. All solutions were kept for incubation at room temperature for 2 h. After incubation, absorbance of dark blue colour was measured at 750 nm by using spectrophotometer. The extracts were performed in triplicates. The data for total phenolic contents of each plant part extract was expressed as mg of gallic acid equivalent weight (GAE)/100 g of dry mass [19,20].

Determination of total flavonoid content (TFC): The total flavonoid of each plant part extract was estimated by aluminum chloride colorimetric assay. Briefly, 0.5 mL aliquots of each of the extract and standard solution (0.01-1.0 mg/mL) of rutin were added into 5 mL volumetric flask containing 2 mL of distilled water and 0.15 mL of sodium nitrite (5% w/v) solution and mixed vigorously. After 6 min, 0.15 mL of (10% $AlCl_3$) solution was added and the solution was allowed to stand for further 6 min and then 2 mL of 4% NaOH solution was added to the mixture. The final volume was made up to 5 mL with distilled water, mixed thoroughly and allowed to stand for another 15 min. The absorbance was determined at 510 nm spectrophotometerically. The extracts were performed in triplicates. TFC was determined as mg rutin equivalent per gram of sample with the help of calibration curve of rutin [21].

Sample preparation for heavy metal analysis: 250 mg each of homogeneous material of different parts were weighed and mixed with 0.5 mL of gold solution (10 ppm) and 8 mL of nitric acid into the Teflon vessels of microwave digestion system. Vessels were closed and keep inside the multiwave PRO (Make-Anton Paar, Sr. No81321827) for digestion. The microwave digestion conditions are shown in Table-1.

TABLE-1
MICROWAVE DIGESTION CONDITIONS

Step	Temp. (°C)	Power in watts	Time (min)	Fan speed
Power ramp	–	650	6	1
Power hold	–	1000	20	1
Cooling	70	0	–	3

After digestion, the vessels were allowed to cool and solution was transferred into volumetric flask. The samples were transferred in polyethylene bottles and analyzed for metals contents by inductively coupled plasma mass spectrometry (ICP-MS), make Perkin-Elmer, Model-NexION 300X, Sr. No. 81XN3041501as per method described elsewehre used [22].

Antioxidant activity: The antioxidant activity of the prepared extracts was estimated using stable 2,2-diphenyl-1-picryl-hydrazyl (DPPH) free radical according to Brand-Willams *et al.* [23] method with some modifications. A methanol solution (1 mL of 0.05 mM) of DPPH was mixed with1 mL of sample solution with varying concentrations of plant extracts and standard solution separately. The reaction was carried out in triplicate and absorbance was measured at 517 nm after 30 min incubation in dark using UV-VIS spectrophotometer (Shimadzu, Model No. 1800). The inhibition % was calculated using the following formula given below [24]:

$$\text{Inhibition of DPPH activity (\%)} = \frac{A - B}{A} \times 100$$

where, A = absorbance of blank and B = absorbance of sample.

RESULTS AND DISCUSSION

Phytochemical screening: The phytochemical screening variations in the extracts of aqueous and methanol extracts are shown in Table-2. The polyphenols were found present in all aqueous and methanolic extracts. Tannins were observed in all parts of aqueous and methanolic extracts except the flower and methanolic extract of root. Only aqueous extract of leaves had shown the presence of flavonoids, which were present in methanolic extract of all parts. Alkaloids were present in all methanolic extract of all parts where as it was absent in the other aqueous extracts of stem, flower and root. It was revealed that seeds contained the maximum number of phytochemicals, while polyphenols were present in all part of the plant followed by tannins, alkaloids and flavonoids.

Quantification of total phenolic content and flavonoids: It is noted that total phenolic and flavonoids were found in all parts of the plant.

The free radical scavenging activity of all parts of *Perilla frutescens* L. was confirmed in the present investigation and it is obvious that the constituents like tannins, flavonoids, polyphenols and proteins present in all extracts may be responsible for such activity as illustrated in Table-2.

Heavy metal analysis: The concentration of various elements in different parts of *Perilla frutescens* L. is shown in Table-3. It is revealed that the plant have high content of iron followed by calcium, magnesium, manganese, zinc, copper, cobalt. The presence of zinc in all parts of the plant in the concentration range of 17.20 ± 0.22 to 33.56 ± 0.32 ppm further supports this study (Table-3).

Total phenolic content: The gallic acid solution of concentration (10-100 µg/mL) conformed to Beer's law at wavelength 750 nm with a regression coefficient (R^2 = 0. 997). It is cleared from the Table-4 that highest value of total phenolic content is present in flower followed by leaves, stem, root and seeds.

Total flavonoid content (TFC): The rutin solution of concentration (0.01-1.0 mg/mL) conformed to Beer's law at 510 nm with a regression co-efficient (R^2 = 0.994). It is cleared from Table-4 that stem shown the maximum flavonoids content among the all parts followed by leaves, flower, root and seeds.

Antioxidant activity by DPPH assay: HPTLC technique was performed to assess the presence of antioxidant phytochemicals in various parts of *Perilla frutescens* L. The yellow colour spots clearly indicate the antioxidant activity. Maximum number of yellow bands were appeared in leaves followed by seeds, stem and root. Ascorbic acid was taken as a positive

TABLE-2
PHYTOCHEMICAL SCREENING IN DIFFERENT PARTS OF *Perilla frutescens* L.

Name of phytochemical	Stem extract		Leaves extract		Seeds extract		Flower extract		Root extract	
	Aqueous	Methanol	Aqueous	Methanol	Aqueous	Methanol	Aqueous	Methanol	Aqueous	Methanol
Tannins	+	+	+	+	+	+	–	+	+	–
Flavonoids	–	+	+	+	–	+	–	+	–	+
Alkaloids	–	+	+	+	+	+	–	+	–	+
Terpenoids	–	–	–	–	–	–	–	–	+	–
Carbohydrate	–	–	+	+	–	–	–	–	–	–
Saponin	–	–	–	–	–	+	–	–	–	–
Steroids	–	–	–	–	–	–	–	–	–	+
Glycosides	–	+	–	–	–	+	–	+	–	+
Starch	+	+	–	–	+	+	–	–	–	–
Protein	–	+	+	–	–	+	–	+	+	–
Polyphenols	+	+	+	+	+	+	+	+	+	+

TABLE-3
HEAVY METAL CONTENT IN DIFFERENT PART OF *Perilla frutescens* L.

Elements	Leave (ppm)	Stem (ppm)	Root (ppm)	Flower (ppm)	Seed (ppm)
Copper	7.43 ± 0.320	7.50 ± 0.33	23.55 ± 0.36	10.05 ± 0.18	13.07 ± 0.170
Cobalt	0.17 ± 0.002	0.11 ± 0.03	1.65 ± 0.10	0.48 ± 0.04	0.217 ± 0.001
Iron	283.20 ± 0.330	118.38 ± 0.31	4163.24 ± 0.42	1243.35 ± 0.41	67.49 ± 0.390
Magnesium	2609.98 ± 0.810	1501.48 ± 0.44	3084.17 ± 0.25	2701.55 ± 0.33	2785.27 ± 0.360
Manganese	57.18 ± 0.350	20.21 ± 0.25	167.51 ± 0.71	69.80 ± 0.65	30.09 ± 0.200
Zinc	33.56 ± 0.320	17.20 ± 0.22	31.64 ± 0.10	20.71 ± 0.20	25.99 ± 0.140
Calcium	2948.87 ± 0.350	1104.74 ± 0.42	1575.46 ± 0.24	1528.14 ± 0.17	77.47 ± 0.440

Data expressed as mean ± standard deviation of three samples analyzed separately.

TABLE-4
TOTAL PHENOLIC CONTENT AND FLAVONOID CONTENT IN DIFFERENT PART OF *Perilla frutescens* L.

Total phenolic content					Flavonoid content				
Stem	Seeds	Leaves	Flower	Root	Stem	Seeds	Leaves	Flower	Root
0.980 ± 0.004	0.088 ± 0.001	1.256 ± 0.002	1.516 ± 0.005	0.965 ± 0.001	4.262 ± 0.011	0.501 ± 0.001	4.064 ± 0.005	3.776 ± 0.044	2.599 ± 0.003
Total phenolic content (mg of gallic acid equivalent/g dry material)					Flavonoid content (mg of rutin equivalent/g dry material)				

Data expressed as mean ± standard deviation of three samples analyzed separately.

control. The intensity of bands showed potential of scavenging activity, darker band indicate higher scavenging and lighter bands showed weak scavenging activity.

DPPH radical scavenging assay: In the present study, the antioxidant activities of all parts of *Perilla frutescens* L. plant were evaluated in a series of *in vitro* test using DPPH radical scavenging, total polyphenols and flavonoids. The values of percent decolorization of DPPH radicals are reported in Table-5, where the leaves displayed the maximum scavenging activity of 98.41 ± 0.24% followed by stem roots and seeds followed by flowers. The antioxidants were able to reduce the stable radical DPPH to the yellow coloured diphenylpicryl hydrazine.

TABLE-5
DPPH RADICAL SCAVENGING ACTIVITY IN DIFFERENT PARTS OF *Perilla frutescens* (L.)

Name of part/sample	Scavenging activity (%)
Roots	86.57 ± 0.40
Leaves	98.41 ± 0.24
Stem	86.65 ± 0.26
Flower	78.62 ± 0.30
Seeds	85.56 ± 0.24
Ascorbic acid	97.48 ± 0.26
BHT	51.35 ± 0.24

Among the five different parts of *Perilla frutescens* L. and standard tested for the *in vitro* antioxidant activity using the DPPH method, showed antioxidant activity, with IC_{50} values of 5.95 ± 0.10, 8.28 ± 0.20, 66.27 ± 0.17, 80.03 ± 0.03 and 122.35 ± 0.17 µg/mL, respectively. The IC_{50} value for ascorbic acid was 5.19 ± 0.26 µg/mL. The methanolic extract of leaves showed excellent DPPH radical scavenging activity that was enhanced with increasing concentration followed by stem, roots, seeds and flowers. The DPPH radical scavenging activities of stem, root and flowers were low may be due to the change in the contents of flavonoids and polyphenols at different growth stages.

The extract of leaves and flowers was found to have the highest phenolic content [1.256 µg and 1.516 µg (gallic acid equiv.)/mL (extract)] amongst all parts of plant extracts followed by 0.980, 0.965 and 0.088 µg/mL. Phenolic compounds played an important role in stabilizing lipid oxidation associated with its antioxidant activity [25,26]. On the other hand, total flavonoid contents in the leaves and stem of *Perilla frutescens* L. were also found to be superior [4.064 ± 0.005 µg and 4.262 ± 0.011 µg (rutin equiv.)/g (extract)]. The lowest flavonoid content was exhibited in the seed (0.501 ± 0.001 µg/mL).

Conclusion

Based on this study, it is proposed that the free radical scavenging and antioxidative activity of *Perilla frutescens* L. plant might result from its high contents of polyphenols and flavonoid type compounds. The findings indicated promising *in vitro* antioxidant activity of all parts of the *Perilla frutescens* L. more than that of synthetic and commercial antioxidant and can be seen as a potential source of useful new drugs but needs further exploration for their effective use after *in vivo* clinical studies to validate its therapeutic effects.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

REFERENCES

1. D.-P. Xu, Y. Li, X. Meng, T. Zhou, Y. Zhou, J. Zheng, J.-J. Zhang and H.-B. Li, *Int. J. Mol. Sci.*, **18**, 96 (2017); https://doi.org/10.3390/ijms18010096
2. S.C. Lourenço, M. Moldão-Martins and V.D. Alves, *Molecules*, **24**, 4132 (2019); https://doi.org/10.3390/molecules24224132
3. M. Selvamuthukumaran and J. Shi, *Food Qual. Saf.*, **1**, 61 (2017); https://doi.org/10.1093/fqsafe/fyx004
4. F. Shahidia and Y. Zhong, *Chem. Soc. Rev*., **39**, 4067 (2010); https://doi.org/10.1039/B922183M
5. R. Liu and R. Liu, *Environ. Sci. Technol*. 54, 11706 (2020); https://doi.org/10.1021/acs.est.0c05077
6. N. Nakatani, *Biofactors*, **13**, 141 (2000); https://doi.org/10.1002/biof.5520130123
7. J.T. Dwyer, M. Frances Picciano, J.M. Betz, K.D. Fisher, L.G. Saldanha, E.A. Yetley, P.M. Coates, K. Radimer, B. Bindewald, K.E. Sharpless, J. Holden, K. Andrews, C. Zhao, J. Harnly, W.R. Wolf and C.R. Perry, *J. Food Compos. Anal.*, **19**, S108 (2006); https://doi.org/10.1016/j.jfca.2005.09.001
8. M.R. La Frano, F.F. de Moura, E. Boy, B. Lönnerdal and B.J. Burri, *Nutr. Rev.*, **72**, 289 (2014); https://doi.org/10.1111/nure.12108
9. H. Sato, H. Shibata, T. Shimizu, S. Shibata, H. Toriumi, T. Ebine, T. Kuroi, T. Iwashita, M. Funakubo, Y. Kayama, C. Akazawa, K. Wajima, T. Nakagawa, H. Okano and N. Suzuki, *Neuroscience*, **248**, 345 (2013); https://doi.org/10.1016/j.neuroscience.2013.06.010
10. J. Navarro-Yepes, L. Zavala-Flores, A. Anandhan, F. Wang, M. Skotak, N. Chandra, M. Li, A. Pappa, D. Martinez-Fong, L.M. Del Razo, B. Quintanilla-Vega and R. Franco, *Pharmacol. Ther.*, **142**, 206 (2014); https://doi.org/10.1016/j.pharmthera.2013.12.007
11. P. Rajendran, N. Nandakumar, T. Rengarajan, R. Palaniswami, E.N. Gnanadhas, U. Lakshminarasaiah, J. Gopas and I. Nishigaki, *Clin. Chim. Acta*, **436**, 332 (2014); https://doi.org/10.1016/j.cca.2014.06.004
12. A. Dhyani, R. Chopra and M. Garg, *Biomed. Pharmacol. J.*, **12**, 649 (2019); https://dx.doi.org/10.13005/bpj/1685
13. N. Dhami, Ethnomedicinal uses of plants is Western Terai of Nepal: A Case Study of Dekhatbhuli VDC of Kanchanpur district; In: Medicinal Plants in Nepal: An Anthology of Contemporary Research, Ecological Society; Kathmandu, Nepal, pp. 165-177 (2008).
14. S.Y. Yang, C.O. Hong, H. Lee, S. Park, B. Park and K.-W. Lee, *Food Chem.*, **133**, 337 (2012); https://doi.org/10.1016/j.foodchem.2012.01.037
15. J. Suneetha and R.T.V.V. Seetharami, *J. Med. Plant Res*., **6**, 1 (2016).
16. T. Song and L. Liu, *Anal. Methods*, **8**, 295 (2016); https://doi.org/10.1039/C5AY01685A
17. G. Zurera, B. Estrada, F. Rincón and R. Pozo, *Bull. Environ. Contam. Toxicol*., **38**, 805 (1987); https://doi.org/10.1007/BF01616705
18. J.B. Harnorne. Phytochemical Methods, Chapman and Hall, Ltd.: London, p. 49 (1973)
19. N. Bhalodia, P. Nariya, R. Acharya and V. Shukla, *Int. J. PharmTech. Res.*, **3**, 589 (2011).
20. V.L. Singleton, R. Orthofer and R.M. Lamuela-Raventós, *Methods Enzymol*., **299**, 152 (1999); https://doi.org/10.1016/S0076-6879(99)99017-1
21. J. Zhishen, T. Mengcheng and W. Jianming, *Food Chem.*, **64**, 555 (1999); https://doi.org/10.1016/S0308-8146(98)00102-2
22. The United States pharmacopeia. National formulary (USP29/NF-24),Asian Edition. Rockville (MD): United States Pharmacopeial Convention; 2006. Plasma Spectrochemistry.pp 2700-2703.
23. W. Brand-Williams, M.E. Cuveliver and C. Berset, *LWT-Food Sci. Technol.*, **28**, 25 (1995); https://doi.org/10.1016/S0023-6438(95)80008-5
24. W. Bors, M. Saran and E.F. Elstner, eds.: H.F. Linskens and J.F. Jackson, Screening for Plant Antioxidants, In: Modern Methods of Plant Analysis Plant Toxin Analysis, New Series, Springer: Berlin, vol. 13, pp. 277-295 (1992).
25. N. Osakabe, A. Yasuda, M. Natsume, C. Sanbongi, Y. Kato, T. Osawa and T. Yoshikawa, *Free Radic. Biol. Med.*, **33**, 798 (2002); https://doi.org/10.1016/S0891-5849(02)00970-X
26. Y. Gulcin, M.E. Buyukokuroglu, M. Oktay and Ö.Ý. Küfrevioglu, *Holmboe. J. Ethnopharmacol.*, **86**, 51 (2003); https://doi.org/10.1016/S0378-8741(03)00036-9