Pharmaceutical Standardization

Evaluation of phytochemical content, nutritional value and antioxidant activity of Phanji - Rivea hypocrateriformis (Desr.) Choisy leaf

Sneha D. Borkar, Raghavendra Naik¹, Vinay J. Shukla², Rabinarayan Acharya¹

Department of Agada Tantra, Mahatma Jyotiba Fule Medical College of Ayurveda, Chomu, Rajasthan, ¹Department of Dravyaguna, ²Pharmaceutical Chemistry Laboratory, Institute for Post Graduate Teaching and Research in Ayurveda, Gujarat Ayurved University, Jamnagar, Gujarat, India

Abstract

Background: Rivea hypocrateriformis (Desr.) Choisy is known to be the source plant of Phanji, a classically delineated leafy vegetable which is till date used by some hill dwelling Kandha tribes of Odisha. Though it is in use since a long time, it is not yet evaluated for its nutritive value. Aim: The leaves of R. hypocrateriformis were evaluated for its nutritive value and antioxidant potential. Materials and Methods: The in vitro antioxidant properties of the leaf of R. hypocrateriformis were screened through 1,1-diphenyl-2-picrylhydrazyl (DPPH) and total antioxidant capacity. Phytochemicals, crude protein, fat, carbohydrate, energy value, and mineral content of the leaves of the plant were evaluated with standard procedures. Results: In phytochemical analysis, tannin, alkaloids, flavonoids, and carbohydrates were present in leaf powder of R. hypocrateriformis. Energy content was found to be highest (331.54 kcal/100 g). Carbohydrate, fat, protein, calcium, magnesium, phosphorous, and zinc were present in 57.63%, 2.66%, 19.27%, 0.99%, 0.34%, 0.32%, and 0.011%, respectively. The IC50 values of the extract and ascorbic acid were found to be 254 ± 5.29 µg/ml and 11.67 ± 0.58 µg/ml, respectively. Percentage scavenging of DPPH radical was found to rise with increasing concentration of the crude extract. Total antioxidant capacity of the extract was found to be 111.30 ± 0.003 mcg. Conclusion: The results of this study indicate that the leaves of R. hypocrateriformis contain secondary metabolites such as tannin and possess mild antioxidant properties. Nutritional analysis indicates the presence of energy in highest amount, carbohydrates, proteins, fats, calcium, phosphorous, zinc, and magnesium. Key words: 1, 1‑diphenyl‑2‑picrylhydrazyl (DPPH), antioxidant, leafy vegetable, nutritional value, Phanji, Rivea hypocrateriformis, Shaka

Introduction

Plants especially fruits and vegetables are known to possess phytochemicals such as flavonoids and vitamins that exhibit significant amounts of antioxidant activity and that can be utilized to scavenge the excess free radicals from human body.¹,² Natural antioxidants exhibit many biologically important functions which include protection against oxidative stress, degenerative diseases and are reported to possess antibacterial, antiallergic, antiviral, anti-inflammatory, anticancer, antiaging activity, and hepatoprotective properties.¹⁻⁵ Therefore, the evaluation of antioxidant activity of various indigenous vegetables that were delineated by various texts of Ayurveda and used till date, is necessary for the identification of their capacity to scavenge the free radicals.

Though there are a number of methods to evaluate antioxidant activity, 1,1-diphenyl-2-picrylhydrazyl (DPPH), free radical scavenging method offers the first approach for evaluating the...
antioxidant potential because of the good stability, credible sensitivity, inexpensive, simplicity, and feasibility.[6,7]

The total antioxidant capacity was determined by phosphomolybdnem method and is based on the reduction by the antioxidant compounds and the formation of a green complex.[8]

*Rivea hypocrateriformis* (Desr.) Choisy of family convolvulaceae is considered botanical source of “Phanji” mentioned in Ayurvedic text *Raja Nighantu* under the group of vegetables with *Grahi, Deepana, Pachana, Ruchya, and Tridosha Shamaka* properties[9] and is used traditionally by hill dwelling *Kandha* tribes of Odisha as vegetables.[10] Besides known to be consumed as a leafy vegetable, it is also reported for its ethnomedicinal uses in cough, headache, skin disease, etc.[11,12] Hence, the objective of this study was to determine antioxidant properties of leaves of *R. hypocrateriformis* using a set of *in vitro* antioxidant assays including scavenging of DPPH and total antioxidant capacity along with the nutritional evaluation.

**Materials and Methods**

**Collection and preservation of the sample**

Leaves of *R. hypocrateriformis* were collected from its natural habitat [Figure 1], Rakha Khatia forest area, Jamnagar, Gujarat, during October 2012 on the basis of its morphological characters such as twining shrub with dark purple glands present at the base where lamina is attached to the petiole[13] and comparing them with the reported characters mentioned in Flora[14] [Figure 2]. A sample specimen was authenticated by an expert taxonomist and deposited to institutes pharmacognosy museum (Specimen No: PHM 6063/21/09/2012) for future references [Figure 3]. The leaves were washed, shade dried, powdered, sieved through 80 mesh, and preserved in an air-tight glass vessel.

**Preliminary qualitative tests**

The aqueous extraction was done with Soxhlet apparatus,[15,16] which was evaporated under reduced pressure to get dried extract, and was utilized for various qualitative tests such as detection of alkaloids by using Mayer’s test[17] and Dragendorff’s test,[18] detection of glycosides by modified Borntrager’s test,[18] and Keller–Killiani test;[18] detection of saponin by Foam test,[19] detection of phytosterols and triterpenoids by Liebermann’s test,[17] Liebermann–Burchard test,[17] and Salkowski test;[21] detection of fixed oils and fats by oily spot test;[18] detection of flavonoids by alkaline reagent test[22,23] and detection of phenols and tannins by ferric chloride test[20] and test for tannins.[20] The presence of carbohydrates was detected by Molisch’s test.[18]

**Nutritional evaluation**

Estimation of energy value - The sample calorific value was estimated (in kcal) by multiplying the percentage crude protein, crude lipid, and carbohydrate by the recommended factor (2.44, 8.37, and 3.57, respectively) used in analysis. The caloric value was determined based on the Atwater factor.[24] Carbohydrates was determined using cupric tartrate; the precipitate formed was compared with dextrose of known concentration.[25] Estimation of crude fat was performed using n-hexane as solvent by Soxhlet extraction method.[25] The crude protein was determined by the Kjeldahl method with slight modification and the absorbance at 470 nm.[25] Determination of moisture content was carried out by standard procedure mentioned in Ayurvedic Pharmacopeia of India.[26] All the minerals except phosphorus were analyzed from a triple acid-digested sample by an atomic absorption spectrophotometer.[27] The phosphorus content in the triple acid digested extract was determined colorimetrically.[28]
Antioxidant assay

1,1-diphenyl-2-picrylhydrazyl radical scavenging activity

Methanol was used to prepare 100 µM of DPPH solution. Dimethyl sulfoxide (DMSO) was used to obtain 10.5 mg/ml, 10.5 mg/ml, and 21 mg/ml concentrations of ascorbic acid, rutin, and extract, respectively which were serially diluted with DMSO to obtain lower concentrations. Various concentrations of sample were added to DPPH solution, and the absorbance of DPPH reagent was determined at 490 nm after 30 min of incubation, using a microplate reader.[20]

Total antioxidants assay

Accurately weighed 55 mg of the R. hypocrateriformis aqueous extract and standard ascorbic acid dissolved in 5 ml of DMSO. The lower dilutions were made serially with DMSO. An aliquot of 0.1 ml of the sample solution containing a reducing species in DMSO was combined with 1 ml of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). The tubes were capped and incubated in water bath at 95°C for 90 min, and the absorbance was measured at 695 nm. The total antioxidant capacity was expressed as mM equivalent of ascorbic acid.[19]

Results and Discussion

Preliminary qualitative tests

The observed results show the presence of tannin, alkaloids, flavonoids, carbohydrates and the absence of phenols, glycosides, saponin, phytosterols, and triterpenoids [Table 1]. Carbohydrates are rich source of energy. Alkaloids have pharmacological effects and are used as medications,[31] foods rich in tannins are considered to be of low nutritional value,[32] and also have got anti-inflammatory effect.[18] The other remedial values of tannins include application on burns to heal the injury and cuts to stop bleeding. Tannins are proved to be effective in reducing the healing time of wounds.[33] Flavonoids have a wide range of biological and pharmacological activities in in vitro studies such as antiallergic, anti-inflammatory, antioxidant, antimicrobial (antibacterial, antifungal, and antiviral), anticancer, and antidiarrheal activities.[33]

Nutritional evaluation

The results of nutritional analysis of R. hypocrateriformis leaf are presented in Table 2. Leaves of R. hypocrateriformis are a good source of energy and micronutrients. It possesses zinc, phosphorous, magnesium, and calcium along with protein, fat, and carbohydrate. It has the highest amount of energy content.

Antioxidant assay

1,1-diphenyl-2-picrylhydrazyl radical scavenging activity

The IC50 values of the extract and ascorbic acid were found to be 254 ± 5.29 µg/ml and 11.67 ± 0.58 µg/ml, respectively. Percentage scavenging of DPPH radical was found to rise with increasing concentration of the crude extract [Graph 1].

Total antioxidant capacity

Total antioxidant capacity of the extract is 111.30 ± 0.003 mcg [Table 3]. Total antioxidant capacity is expressed as the number of AAE.

Table 1: Phytochemical analysis of Rivea hypocrateriformis leaf aqueous extract

| Phytoconstituents | Test                          | Water extract |
|-------------------|-------------------------------|---------------|
| Carbohydrates     | Molisch’s test                | +             |
| Glycosides        | Modified Borntrager’s test    | –             |
| Saponin           | Foam test                     | –             |
| Alkaloid          | Mayer’s test                  | +             |
| Triterpenoids     | Dragendorff’s test            | +             |
| Flavonoid         | Alkaline reagent test         | +             |
| Phenol and tannins| Ferric chloride test          | –             |
| Tannins           | Test for tannins              | +             |
| Fixed oils and fats| Oily spot test                | –             |

+: Present, –: Absent

Table 2: Nutritional values of leaf of Rivea hypocrateriformis

| Parameters                | Results          |
|---------------------------|------------------|
| Energy                    | 331.54 kcals/100 g |
| Carbohydrate (%)          | 57.63            |
| Fat (%)                   | 2.66             |
| Protein (%)               | 19.27            |
| Moisture content (%)      | 6.25             |
| Calcium (%)               | 0.99             |
| Magnesium (%)             | 0.34             |
| Phosphorous (%)           | 0.32             |
| Zinc (%)                  | 0.011            |

Table 3: Antioxidant assay of Rivea hypocrateriformis leaf

| Samples                   | 1,1-diphenyl-2-piycrylhydrazyla | Total antioxidant activity a |
|---------------------------|---------------------------------|-------------------------------|
| Rivea hypocrateriformis    | 254±5.29                        | 111.30±0.003                  |
| Standard (Ascorbic acid)   | 11.67±0.58                      |                               |

aIC50 values µg/ml by methods. bThe total antioxidant capacity was expressed as mcg equivalent of ascorbic acid per gram of dry weight.

Graph 1: Graphical presentation of 1,1-diphenyl-2-picrylhydrazyl radical scavenging assay of Rivea hypocrateriformis

The drug possesses mild antioxidant potential as compared to the standard ascorbic acid by DPPH radical scavenging activity.
Reactive oxygen species (ROS) or free radicals cause damage to the cell and its organelles thus, resulting in various diseases. The naturally occurring antioxidants of the body are not able to protect the body from the excessive free radical damage, so there is need to supply antioxidants through food that we eat. The fruits and vegetables are known to possess natural antioxidants which are safer and possess various pharmacological properties.\textsuperscript{[17,38]} Epidemiological studies show that the consumption of vegetables and fruits can protect humans against oxidative damage by inhibiting or quenching free radicals and ROS. Flavonoids are powerful antioxidants against free radicals and are described as free-radical scavengers.\textsuperscript{[19]}

Phytochemical analysis of aqueous extract of leaf of \textit{R. hypocrateriformis} (Desr.) Choisy showed the presence of alkaloids, carbohydrates, flavonoids, and tannins. The drug is possessing phytoconstituents such as flavonoids and tannin, which have been proved as potent antioxidants occurring in different plants.\textsuperscript{[35]} Together, these compounds act as protective scavengers against oxygen-derived free radicals and ROS that play a healing role in aging and various disease processes.

Many recent studies demonstrate that antioxidants diminish oxidative damage by virtue of anti-inflammatory effects thus protect the lung in a model of oxidative lung injury.\textsuperscript{[17,38]} Antioxidant supplementation is also reported for protective effect on the oxidant-mediated cough depression which may be of significance in respiratory infections.\textsuperscript{[19]} Antioxidant protects cells against oxidative injury, which induce protein damage, apoptosis, or release of pro-inflammatory mediators, such as cytokines. Topical application or oral administration of antioxidants has been recently suggested as preventive therapy for skin photaging, ultraviolet-induced cancer, and certain skin diseases.\textsuperscript{[40]} This proves its usefulness in ethnomedical claims to be useful in the management of cough, asthma, and skin disease in which it is used.

**Conclusion**

\textit{R. hypocrateriformis} is considered as the botanical name of \textit{Phanji}, a leafy vegetable mentioned in the Ayurvedic text which is said to be useful in \textit{Shvas} and \textit{Kasa}. The leaves of \textit{R. hypocrateriformis} possess mild antioxidant potential that may be because of the constituents possessed by the plant such as flavonoids. The study revealed that the plant is good source of energy and micronutrient and can be used as nutritious leafy vegetable in daily life and specifically in conditions such as cough, skin disease, and asthma. Further study of the plant is essential to evaluate its usefulness in the management of asthma, cough, and skin disease.

**Acknowledgments**

The authors are thankful to Director IPGT & RA, Gujarat Ayurved University, Jamnagar, for providing facilities to carry out the research work. Authors extend special thanks to Radiant Research Services Pvt. Ltd., and Aristogene Biosciences Pvt. Ltd., Bengaluru, for their timely work and contribution.

**Financial support and sponsorship**

Institute for Post Graduate Teaching and Research in Ayurveda, Gujarat Ayurved University.

**Conflicts of interest**

There are no conflicts of interest.

**References**

1. Rout OP, Rabinarayan A, Mishra SK. In-vitro antioxidant potentials in leaves of \textit{Colesus aromatics} Benth and \textit{rhizomes of Zingiber zerumbet} (L.) SM. J Appl Pharm Sci 2011;1:194-8.
2. Atrooz OM. The antioxidant activity and polyphenolic contents of different plant seeds extracts. Pak J Biol Sci 2009;12:1063-8.
3. Wasson GR, McKelvey-Martin VJ, Downes CS. The use of the comet assay in the study of human nutrition and cancer. Mutagenesis 2008;23:153-62.
4. Cook NC, Samman S. Flavonoids: Chemistry, metabolism, cardioprotective effects and dietary sources. J Nutr Biochem 1996;7:66-76.
5. Gulcin I, Huyut Z, Elmasetas M, Aboul-Enein HY. Radical scavenging and antioxidant activity of tannic acid. Arabian J Chem 2010;3:43-53.
6. Jin J, Li Z, Zhang F. Scavenging function of mulberry vinegar extractives for 1,1-diphenyl-2-picrylhydrazyl (DPPH). J Northwest Sci-Tech University of Agri and Forestry 2006;34:135-7.
7. Aparadh VT, Naik VV, Karadge BA. Antioxidative properties (TPC, DPPH, FRAP, Metal Chelating Ability, Reducing Power and TAC) within some \textit{Gleome} species. Annali Di Botanica 2012;2:49-56.
8. Mojca S, Petra K, Majda H, Aandreja R, Marjana S, Knez Z. Phenols, proanthocyanidins, flavones and flavonoids in some plant materials and their antioxidant activities. Food Chem 2005;89:191-8.
9. Pandit N, Nigahantu R. Mullakadi varga, 7/157-159. Commentary by Dr. Indradev Tripathi. 2nd ed. Varanasi: Chaukhamba Krishnaasaa Academy; 1998. p. 217.
10. Panda T, Pandhy RN. Sustainable food habits of the hill dwelling Kandha tribe in Kalanadi district of Orissa. Indian J Tradit Med 2007;6:103-5.
11. Nadkarni KM. Indian Material Medica. 13th ed., Vol. I. Mumbai: Popular Prakashana; 1976. p. 1071.
12. Sarvalingam A. Curative climbers of Maruthanalai hills in Southern Western Ghats of Tamilnadu. Int J Med Aromat Plants 2011;1:326-32.
13. Sneha DB, Naik R, Harisha CR, Acharya RN. Development of random amplified polymorphic DNA markers for authentification of \textit{Rivea hypocrateriformis} (Desr.) Choisy. JRMRI 2013:5:278-91.
14. Cooke T. Flora of the Presidency of Bombay. Vol. 2. Dehradun: Bishen Singh Mahendra Pal Singh; 2006. p. 254.
15. Ahmad A, Alkarkhi AF, Hena S, Lim HK. Extraction, separation and identification of chemical ingredients of \textit{Elephantopus scaber} L. using factorial design of experiment. Int J Chem 2009;1:38.
16. Handa SS, Singh Khanauja SP, Genna L, Rakesh DD. Extraction Technologies for Medicinal and Aromatic Plants. Trieste: International Centre for Science and High Technology; 2008. p. 102.
17. Peach K, Tracey MV. Modern Methods of Plant Analysis. Berlin: Springer Verlag; 1955. p. 387.
18. Rosenthaler L. Chemical Investigations of Plants. London: G. Bell and Sons; 1930. p. 23-9, 119-32.
19. Middledone H. Systematic Qualitative Analysis. London: Edward Arnold Publishers Ltd.; 1956. p. 91-4.
20. Kokate CK, Purohit AP, Gokhale SB. Textbook of Pharmacognosy. 7th ed. Pune: Nirali Prakashan; 2001. p. 108-9.
21. Finar IJ. Organic Chemistry. 2nd ed. London: The English Language Book Society and Longman Green Co. Ltd.; 1959. p. 280-431.
22. Shellard EJ. Practical Plant Chemistry. London: Ritman Medical Publishing; 1957.
23. Baxi Aj, Shukla VJ, Bhatt UB. Methods of Qualitative Testing of Some Ayurvedic Formulations. Jamnagar: Gujarat Ayurvedic University; 2001. p. 5-10.
24. FAO Corporate Document Repository. Agriculture and Consumer Protection: Calculation of the Energy Content of Foods – Energy Conversion Factors; 2006a. Available from: http://www.fao.org/docrep/006/y5022e/y5022e04.htm. [Last cited on 2014 Apr 25].
25. Anonymous. Official Methods of Analysis. 15th ed. Washington, DC: Association of official analyst chemists; 1990. p. 375-9.
Borkar, et al.: Nutritional study on Phanji a leafy vegetable

302

AYU | Jul-Sep 2015 | Vol 36 | Issue 3

26. Anonymous. The Ayurvedic Pharmacopoeia of India, Part I, Vol. 1. 1st ed. New Delhi: Govt. of India; 1999. App. 2 (2.2.3), p. 213.

27. Issac RA, Johnson WC. Collaborative study of wet and dry techniques for the elemental analysis of plant tissue by atomic absorption spectrophotometer. J Assoc Off Anal Chem 1975;58:436-40.

28. Dickman SR, Bray RH. Colorimetric determination of phosphate. Ind Eng Chem Anal 1940;12:665-8.

29. Jinesh VK, Jaishree V, Shrishailappa B, Shyam W. Comparative evaluation of antioxidant properties of edible and non-edible leaves of Anethum graveolens Linn. Indian J Nat Prod Resour 2010;1:168-73.

30. Viana GS, Bandeira MA, Moura LC, Souza Filho MV, Matos FJ, Ribeiro RA. Analgesic and antiinflammatory effects of the tannin fraction from Myracrodruon urundeuva Fr. All. Phytother Res 1997;11:118-22.

31. Mahajan M, Kumar V, Yadav SK. Alkaloids: Properties, Applications and Pharmacological Effects. Palampur: CSIR; 2011. p. 1-36.

32. Chung KT, Wong TY, Wei CI, Huang YW, Lin Y. Tannins and human health: A review. Crit Rev Food Sci Nutr 1998;38:421-64.

33. Wang HK. The therapeutic potential of flavonoids. Expert Opin Investig Drugs 2000;9:2103-19.

34. Daffodil ED, Rajalakshmi K, Mohan VR. Estimates total phenolic, flavonoid content and in vitro antioxidant activity of root of Sueda monoica Forsak ex Gmel (Chenopodiaceae). Appl Bot 2012;53:11885-9.

35. Paulpriya K, Mohan VR. In vitro antioxidant potential of methanol extract of Dioscorea oppositifolia. Sci Res Rep 2012;2:239-45.

36. Sandhar HK, Kumar B, Prasher S, Tiwari P, Salhan M, Sharma P. A review of phytochemistry and pharmacology of flavonoids. Internationale Pharmaceutica Scientia 2011;1:25-41.

37. Pagano A, Barazzone-Agrofro C. Alveolar cell death in hypoxia-induced lung injury. Ann N Y Acad Sci 2003;1010:405-16.

38. Jyonouchi H, Sun S, Abiru T, Charancholvanch S, Ingbard DH. The effects of hyperoxic injury and antioxidant Vitamins on death and proliferation of human small airway epithelial cells. Am J Respir Cell Mol Biol 1998;19:426-36.

39. Brozmanova M, Plevkova J, Barton V, Plank L, Javorka M, Tatar M. The interaction of dietary antioxidant vitamins and oxidative stress on cough reflex in Guinea-pigs after long term oxygen therapy. J Physiol Pharmacol 2006;57 Suppl 4:45-54.

40. Briganti S, Picardo M. Antioxidant activity, lipid peroxidation and skin diseases. What’s new. J Eur Acad Dermatol Venereol 2003;17:663-9.