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

Sneha D. Borkar, Raghavendra Naik[1], Vinay J. Shukla[2], Rabinarayan Acharya[1]

Department of Agada Tantra, Mahatma Jyotiba Fule Medical College of Ayurveda, Chomu, Rajasthan, [1]Department of Dravyaguna, [2]Pharmaceutical 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 kcals/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.[3-5] 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

Address for correspondence: Prof. Rabinarayan Acharya, Head, Department of Dravyaguna, IPGT and RA, Gujarat Ayurved University, Jamnagar - 361 008, Gujarat, India.
E-mail: dr.sneha.borkar@gmail.com

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antioxidant potential because of the good stability, credible sensitivity, inexpensive, simplicity, and feasibility.[6,7]

The total antioxidant capacity was determined by phosphomolybdenum 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[19] and Keller–Killiani test;[18] detection of saponin by Foam test;[20] 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]

Figure 1: *Rivea hypocrateriformis* in its natural habitat

Figure 2: *Rivea hypocrateriformis* with its white-scented flowers

Figure 3: Herbarium specimen of *Rivea hypocrateriformis* with its flower and fruit

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.[29]

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.[30]

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.[30] The other remedial values of tannins include application on burns to heal the injury and cuts to stop bleeding. Tannins are proved hemostatics. 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	–
	Keller–Killiani test	–
Saponin	Foam test	–
Alkaloid	Mayer's test	+
	Dragendorff's test	+
Flavonoid	Alkaline reagent test	+
Phenol and tannins	Ferric chloride test	–
	Test for tannins	+
Phytosterols and Triterpenoids	Liebermann–Burchard test	–
	Salkowski test	–
Fixed oils and fats	Oily spot test	–

+: Present, –: Absent

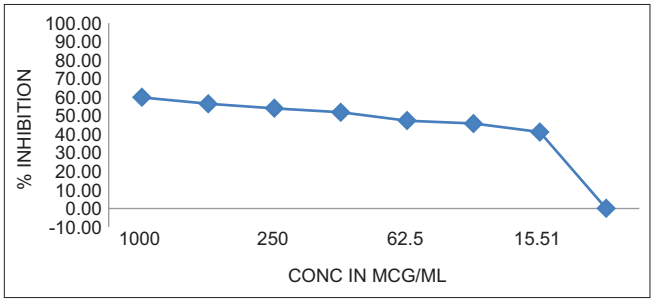
Table 2: Nutritional values of leaf of *Rivea hypocrateriformis*

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-picrylhydrazyl*	Total antioxidant activity*[a]
Rivea hypocrateriformis leaf	254±5.29	111.30±0.003
Standard (Ascorbic acid)	11.67±0.58	

*IC_{50} values µg/ml by methods. *[a]The 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 radicals 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.[34,35] 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.[36]

Phytochemical analysis of aqueous extract of leaf of *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.[5] 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.[37,38] Antioxidant supplementation is also reported for protective effect on the oxidant-mediated cough depression which may be of significance in respiratory infections.[39] 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 photoaging, ultraviolet-induced cancer, and certain skin diseases.[40] This proves its usefulness in ethnopharmacological claims to be useful in the management of cough, asthma, and skin disease in which it is used.

Conclusion

R. hypocrateriformis is considered as the botanical source of *Phanji*, a leafy vegetable mentioned in the Ayurvedic text which is said to be useful in *Shvas* and *Kasa*. The leaves of *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.

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Conflicts of interest

There are no conflicts of interest.

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हिन्दी सारांश

फंज़ी – रिविया हायपोक्रेटेरिफोरमिस की पादप रसायन सामग्री, पोषण तत्त्व एवं एंटीऑक्सिडेंट गतिविधी का मूल्यांकन

स्नेहा डी. बोरकर, राघवेंद्र नाईक, विनय जे. शुक्ला, रबीनारायण आचार्य

फंज़ी (रिविया हायपोक्रेटेरिफोरमिस (डेसर.) चोयसी), आयुर्वेद शास्त्र में वर्णित एक पत्तेदार सब्जी का स्रोत है, आज तक ओडीशा के पहाडी इलाकों में रहनेवाले कंध जाति के लोगों द्वारा एक सब्जी के रूप में इसका इस्तेमाल किया जाता है। हालांकि यह लंबे समय से उपयोग में है फिर भी अभी तक इसके पोषक तत्वों का मूल्यांकन नही किया गया है, अतः फंज़ी के पत्तों का पोषक मूल्य एवं एंटीऑक्सिडेंट गतिविधी के मूल्यांकन के उद्देश्य से फंज़ी के पत्तों की इन विट्रो में डी.पी.पी.एच. (१,१–डायफिनायल–२–पिक्रायलहायड्राझझायल) और टोटल एंटीऑक्सिडेंट केपेसिटी द्वारा एंटीऑक्सिडेंट क्षमता का मूल्यांकन किया गया। पौधे के पादपरसायन, फ्रूड प्रोटीन, वसा, कार्बो हायड्रेट, ऊर्जा और खनिज सामग्री का मानक प्रक्रियाओं से मूल्यांकन किया गया। फंज़ी पत्तों के चूर्ण के पादपरसायन विश्लेषण में अलकलॉइड, फ्लेवोनॉइड और कार्बोहायड्रेट पाये गये। ऊर्जा उच्चतम मात्रा में (३३१.५४ कि. कॅलरी/१०० ग्राम) पायी गयी। कार्बोहायड्रेट, वसा, प्रोटिन, कैल्शियम, मैग्नीशियम, फॉस्फोरस एवं झिंक क्रमशः ५७.६३%, २.६६%, १९.२७%, ०.९९%, ०.३४%, ०.३२% एवं ०.०११% मात्रा में पाये गये । सत्व और एस्कॉर्बिक एसिड के आइ सि.५० मूल्य क्रमशः २५४+५.२९ मा.ग्राम/मिली और ११।६७+०.५८ मा/ग्राम/मिली थे/डी.पी.पी.एच. रेडीकल का स्केवेन्जिग प्रतिशत क्रूड सत्व के कॉन्सेन्ट्रेशन के वृद्धि के साथ बढ़ रहा था। सत्व की कुल एंटीऑक्सिडेंट क्षमता १११.३०+०.००३ मा.ग्राम पायी गयी। इस अध्ययन के परिणामों से यह पता लगता है कि फंज़ी के पत्तों में सेकन्डरी मेटाबोलायट जैसे कि टेनिन तथा कम मात्रा में एंटीऑक्सिडेंट गुण पाये जाते है। पोषण विश्लेषण में ऊर्जा उच्चतम मात्रा में, कार्बोहायड्रेट, वसा, प्रोटीन, कैल्शियम, मैग्नीशियम, फॉस्फोरस और झिंक उपस्थित है।