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Research Article

ANTIOXIDATIVE AND FREE RADICAL SCAVENGING POTENTIALS OF *HABENARIA PECTINATA*

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ABSTRACT

Objective: This study was designed to evaluate the antioxidative potential of tubers of *Habenaria pectinata*.

Methods: The tubers of *H. pectinata* were extracted using hexane, ethyl acetate, methanol, and water as solvents. The anti-oxidant potential of extracts was evaluated using free radical scavenging and reducing power assays. The most active methanolic extract was then fractionated into four fractions using the above-mentioned solvents.

Results: The phenol and flavonoid content was found to be maximum in the methanol extracts. All the extracts and fractions showed significant levels of antioxidant activity except hexane extract.

Conclusion: The tubers of *H. pectinata* were found to possess a significant antioxidant potential and can be explored further for isolation and preclinical investigation for the ailment of various diseased states and disorders.

Keywords: *Habenaria*, Antioxidant, 1,1-diphenyl-2-picrylhydrazyl.

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INTRODUCTION

Natural resources have always been a good source of antioxidants. Several epidemiological studies suggest that a high intake of foods rich in natural antioxidants reduces the risk of several debilitating diseased states and disorders. Moreover, these antioxidants also contribute to the preservation of foods and food products. This necessitates the exploration of bioresources for newer and older antioxidants qualitatively as well as quantitatively to meet the demands of the food industry and health management systems [1,2]

Habenaria pectinata belongs to the family Orchidaceae; a terrestrial plant found in forests around 1800 m in India, Yunnan, and Nepal [3]. The crushed leaves are reported to treat snake bites and tubers for arthritis in India [4].

Preliminary investigation involving standard phytochemical tests on the prepared extracts revealed the presence of flavonoids and phenols in this plant. Moreover, the closely related species *Habenaria intermedia* has been reported to be a potential source of antioxidants [5]. Hence, we decided to check the total phenolic and flavonoid compounds quantitatively along with the evaluation of antioxidant properties of various extracts and fractions of *H. pectinata* connected to the presence of phenols and flavonoids in this plant.

METHODS

Plant material

The tubers of the plant *H. pectinata* were collected from Shimla and Dhanaulti Regions in August. The plant material was authenticated by Manager (Quality Control and Quality Assurance Department, Herbal Health Research Consortium Pvt. Ltd., established by National Medicinal Plants Board, MINISTRY OF AYUSH; Government of India).

Extraction and preliminary fractionation

Dried tubers were extracted with four different solvents: Hexane, ethyl acetate, methanol, and water and then methanolic extract (yield 12.5 g) was further fractionated using the above-mentioned solvents to obtain four different fractions (hexane, ethyl acetate, methanol and water).

Quantitative phytochemical analysis

Estimation of total phenolic compounds

The quantitative estimation of phenolic compounds was done using the Folin–Ciocalteu reagent method of Lister and Wilson [6]. A standard curve was prepared using different concentrations of Gallic acid (10–100 µg/ml). The absorbance of all the test samples (extracts and fractions) was measured at 760 nm. Total phenolic content was expressed as mg/g Gallic acid equivalent (GAE) [7].

Estimation of total flavonoid content

The flavonoid content in the extract was determined spectrophotometrically by the method of Quettier-Deleu *et al.* [8]. Rutin was used as the standard to make the calibration curve and the absorbance of the reaction mixture was measured at 430 nm. The flavonoid content was expressed as mg/g rutin equivalent (RE).

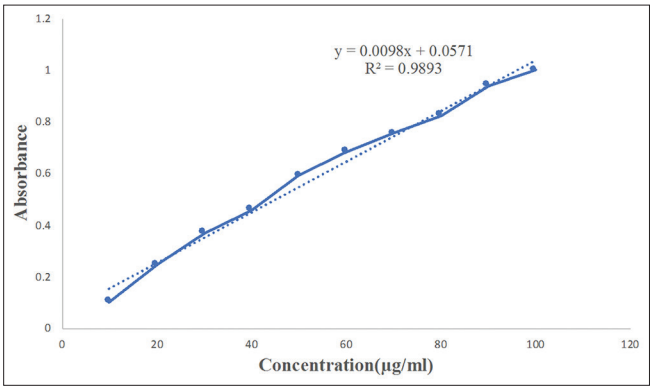

Fig. 1: Calibration curve of gallic acid

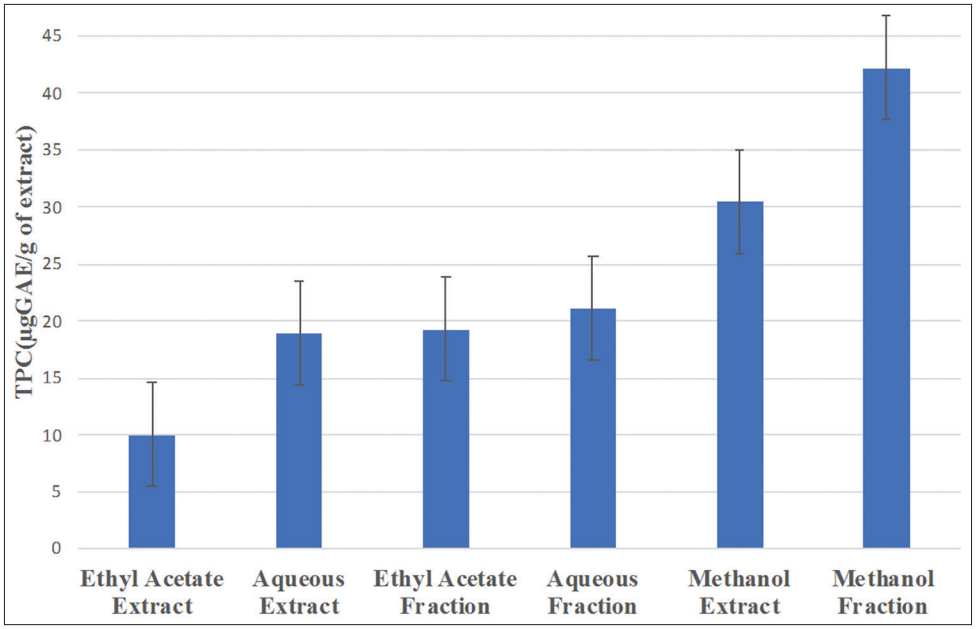

Fig. 2: Total phenolic content of different extracts and fractions of *Habenaria pectinata*. *All the data were reported as mean±standard error of three replicates (n=3)

Free radical scavenging ability (2,2-diphenyl-1-picrylhydrazyl [DPPH])

The scavenging ability of various extracts and fractions on DPPH free radicals was estimated according to the method of Shimada *et al*. [9] An equal amount of methanol and DPPH served as a control. The absorbance of the test solutions was measured at 517 nm against a blank. The DPPH radical scavenging activity was calculated by the following equation:

$$\% \text{ DPPH radical scavenging activity} = (A_o - A_1)/A_o \times 100\%.$$

Where A_o is the absorbance of the control reaction and A_1 is the absorbance of the sample of the tested extracts. IC_{50} values were also calculated for all the extracts and fractions.

Reducing power ability

The reducing power ability of methanol extract was determined by the method given by Oyaizu [10] using ascorbic acid as standard.

Statistical analysis

All the data were reported as mean±standard deviation of three replicates.

RESULTS

The total phenolic content of the extracts and fractions was determined using the calibration curve with different concentration of Gallic acid as y=0.0098x+0.0571, R^2=0.9893 (Fig. 1). The methanol extract of *H. pectinata* was found to possess phenolic content 30.51±4.87 mg/g GAE followed by ethyl acetate extract showed 10.020±4.96 mg/g GAE and aqueous extract revealed 18.938±5.23 mg/g GAE. The methanolic fraction obtained by fractionation from the methanolic extract was found to possess phenolic content 42.244±5.6 mg/g GAE; ethyl acetate fraction 19.285±4.76 mg/g GAE and aqueous fraction 21.122±5.21 mg/g GAE (Fig. 2).

The total flavonoid content of the extracts was determined as a rutin acid equivalent. The calibration curve with different concentrations of rutin acid was created as y=0.0096x+0.0065; R^2=0.9984 (Fig. 3). The methanol extract showed a significant amount of flavonoid content 16.608±1.28 mg/g RE followed by aqueous extract revealed 10.235±0.346 mg/g RE and ethyl acetate extract possess 5.208±0.49 mg/g RE (Fig. 4). The methanolic fraction showed flavonoid content 26.041±2.06 mg/g RE; aqueous fraction 12.625±1.23 mg/g RE; and ethyl acetate fraction 10.625±0.643 mg/g RE.

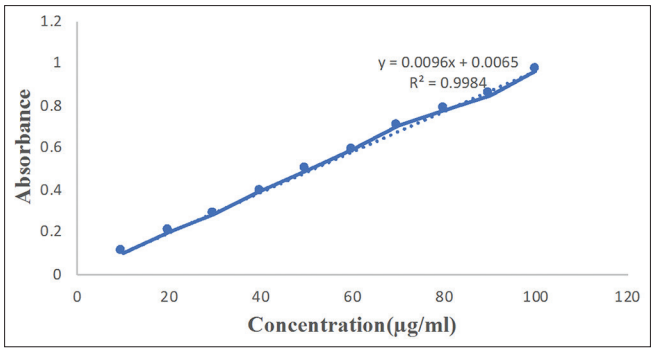

Fig. 3: Calibration curve of rutin

The DPPH radical scavenging activity of all extracts and fractions has been expressed in Fig. 5 in comparison with ascorbic acid as a standard. Ascorbic acid showed the lowest IC_{50} value of 0.586±0.014 and maximum scavenging activity. As compared to ascorbic acid, methanolic fraction and extract were found to possess significant DPPH radical scavenging activities with an IC_{50} value – 0.642±0.013 and 1.52±0.017, respectively. The aqueous fraction showed IC_{50} value 0.976±0.015 followed by ethyl acetate fraction and extract, as shown in Fig. 5.

Fig. 6 showed the reducing ability of ascorbic acid as a standard in comparison with different extracts and fractions of *H. pectinata*. Among them, methanol fraction and extract exhibited higher reducing activity followed by ethyl acetate extract dependent on concentration. At the highest concentration (1 mg/ml), the reducing potential of methanol extract and a fraction was found to be 0.975±0.032 and 0.987±0.014, respectively, followed by ethyl acetate extract (0.674±0.025) and fraction (0.773±0.034) as compared with ascorbic acid (0.687±0.013).

DISCUSSION

Flavonoids are globally known for free radical scavenging, antioxidative, anti-inflammatory, and anticancer activity [10]. In this study, the quantitative analysis of phenol and flavonoid

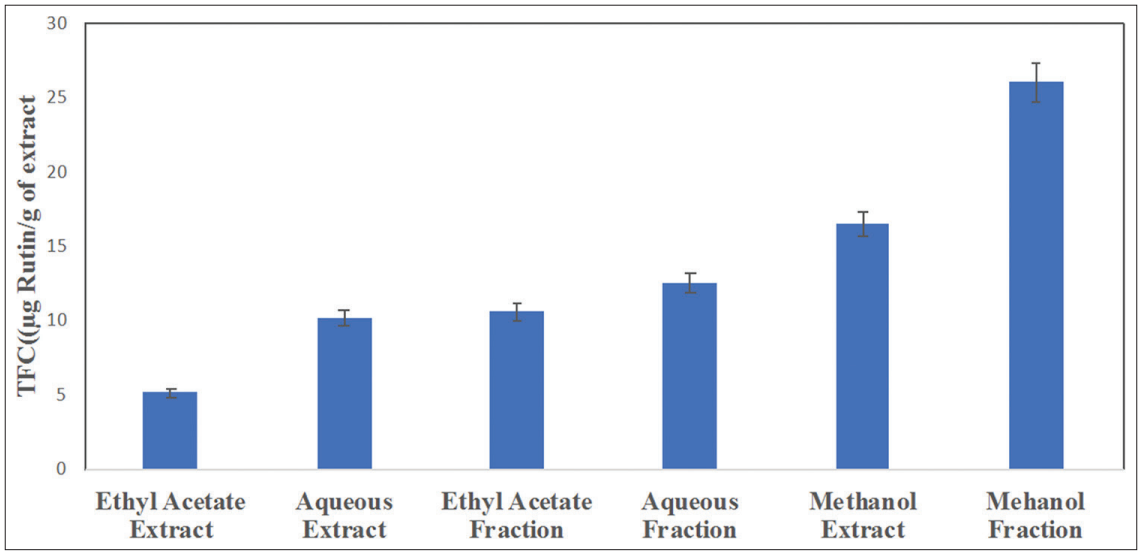

Fig. 4: Total flavonoid content of different extracts and fractions of tubers of *Habenaria pectinata.* *All the data were reported as mean±standard error of three replicates (n=3)

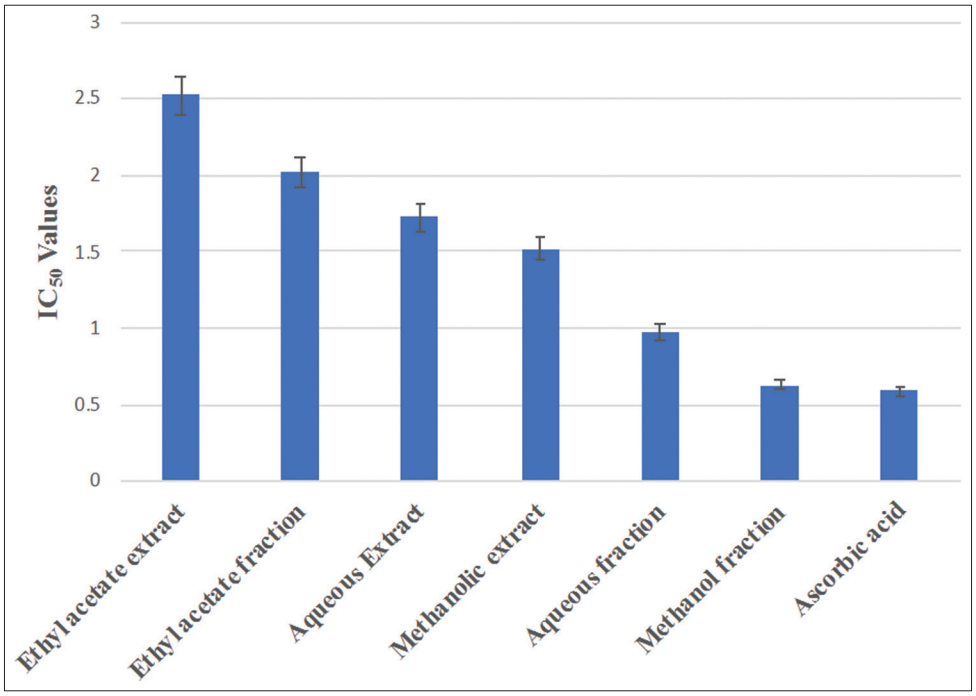

Fig. 5: 2,2-diphenyl-1-picrylhydrazyl radical scavenging activities of different extracts and fractions of tubers of *Habenaria pectinata* in comparison to standard ascorbic acid. *All the data were reported as mean±standard error of three replicates (n=3)

content (Figs. 2 and 4) of the extracts was performed based on the results of preliminary phytochemical investigations conducted to check the presence of phenols and flavonoids [11]. The methanol extract and fraction of *H. pectinata* were found to possess maximum phenolic and flavonoid content which signified its high antioxidant potential which depends on the concentration of phenols and flavonoids present in the plant [12]. This study is the first report of antioxidant potential of *H. pectinata.* The results showed the higher antioxidant potential in terms of DPPH free radical scavenging activity for methanol fraction followed by an aqueous fraction (Fig. 5). Reducing power signifies the reductive ability of antioxidants and the transformation of Fe^{+3} to Fe^{+2} in the presence of the extract [13]. Ascorbic acid showed the lowest IC_{50} value and maximum scavenging activity in comparison to the extracts and fractions. Several reports indicated that the phenolic content of the plant extracts had the reducing power of bioactive compounds and associated antioxidant activity [14-18]. The reducing power (Fig. 6) was found to increase with the increased concentration of extracts as well as fractions tested in this study and significant antioxidant activity was observed in the methanol extract and fraction in comparison to ascorbic acid. Our results revealed a substantial free radical scavenging as well as reducing potential of the different solvent extracts and fractions of tubers of *H. pectinata* when compared with ascorbic acid as standard.

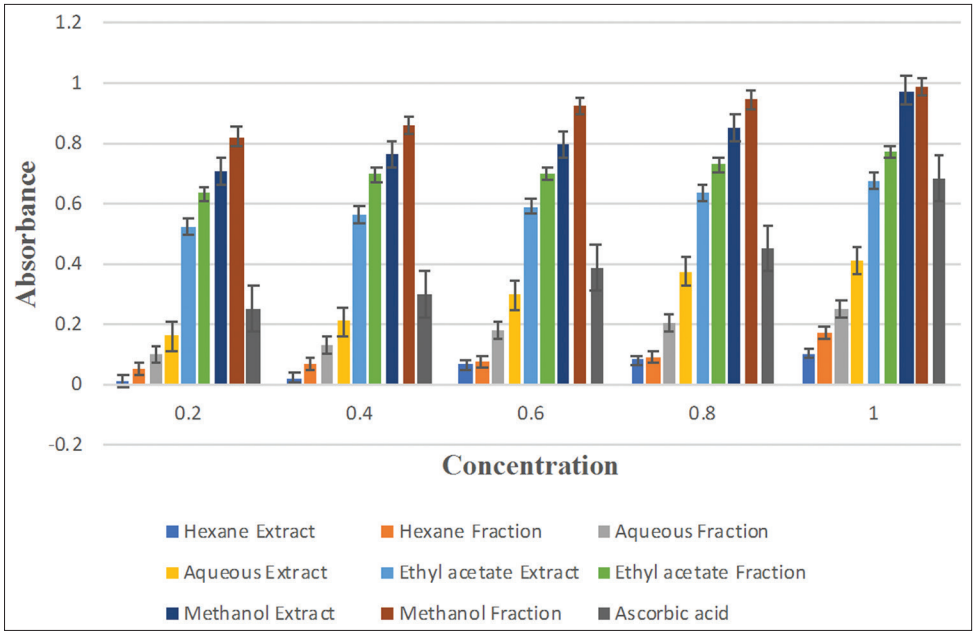

Fig. 6: Reducing powers of different extracts and fractions in comparison to ascorbic acid. *All the data were reported as mean±standard error of three replicates (n=3)

CONCLUSION

It can be assumed that the methanol extract and fraction of *H. pectinata* possess the potent antioxidant capacity in DPPH and ferric reducing antioxidant power assay methods. All other extracts and fractions also exhibited significant antioxidant potential except hexane extract in comparison to ascorbic acid standard. Thus, it may be considered as a potential biosource of antioxidants.

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AUTHORS' CONTRIBUTIONS

The authors declare that all the authors have contributed equally to this article.

CONFLICTS OF INTEREST

The authors have no conflicts of interest to disclose.

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