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Research Article

IN VITRO ANTIOXIDANT ACTIVITY OF *PHYLLODIUM PULCHELLUM* L. DESV - AN THREATENED MEDICINAL PLANT

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ABSTRACT

Objectives: In this study, we determined the *in vitro* antioxidant capacity of *Phyllodium pulchellum* of aqueous, ethanol, and chloroform leaf extracts.

Methods: In this context, the *in vitro* antioxidant activity was demonstrated by 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2′-azinobis(3-ethylbenzothiazolone-6-sulfonic acid) (ABTS+) radical scavenging assay, the total antioxidant activity of phosphomolybdenum assay and hydroxyl radical scavenging activity in different leaf extracts of *P. pulchellum*. The antioxidant activity of the extracts was compared to standard ascorbic acid.

Results: All the four methods of antioxidant showed good reducing power and reducing capacity with increasing concentration again taking the ethanol leaf extract to the top position. Remarkable of antioxidant activity was observed in ethanol leaf extract on the hydrogen peroxide scavenging activity with the lowest inhibitory concentration 50 values of (155.40 µg/ml) followed by DPPH (432.90 µg/ml) and ABTS+ (524.40 µg/ml).

Conclusion: These results suggest that the leaf of *P. pulchellum* could be a valuable source of new antioxidant properties, from the above results it seen that this plant exhibits pharmaceutical activity.

Keywords: *Phyllodium pulchellum,* Fabaceae, Leaf extracts, 2,2-diphenyl-1-picrylhydrazyl, 2,2′-azinobis(3-ethylbenzothiazolone-6-sulfonic acid), Inhibitory concentration 50.

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INTRODUCTION

Phyllodium pulchellum L. Desv. (Vellalothi in Tamil) is a flowering plant species and widely distributed in South India as a threatened medicinal plant belonging to the family of Fabaceae. It is a Subshrub 1(3) m; branchlets downy pubescent to tomentose. *P. pulchellum* (Syn. *Desmodium pulchellum* (L.) Benth) is used to treat various diseases such as anti-inflammatory, analgesic, antioxidant, hemorrhage, diarrhea, poisoning, and eye diseases [1]. The genus relative plants are reported to have anthelmintic [2], anti-hepatic fibrotic [3], antidiarrheal [4], anti-inflammatory [5], and antidiabetic [6]. The international union for conservation of natural and national resource has a long time ago listed *P. pulchellum* (L.) Desv. as a threatened species [7]. The genus Desmodium, of the Fabaceae family, includes about 350 species distributed in tropical and subtropical zones of the worldwide. However, no studies to date have been able to reveal the antioxidant effect of any *Desmodium* species other than *D. gangeticum* and *D. triflorum*. Hence, for there is no scientific report on the antioxidant activities in the particular species. Therefore, the present study was undertaken to evaluate and compare the antioxidative activities of different solvent leaf extracts of *P. pulchellum* in different methods.

MATERIALS AND METHODS

Plant material

P. pulchellum was collected from the *Jambhudu hamlet* of Bodamalai at Namakkal District, Tamil Nadu, India, and identified following the Botanical Survey of India and the voucher specimen (BSI/SRC/5/23/2012-13/Tech-1795 & Serial No. 2) was deposited in the herbarium of the Department of Botany, National College (Autonomous), Tiruchirappalli - 620 001, Tamil Nadu, India.

Preparation of plant extracts

Fresh plant material was washed under running tap water, air dried and powdered. About 30 g of coarsely powdered plant materials (30 g/300 ml) were extracted in a Soxhlet extractor for 8-10 hrs, sequentially with aqueous, ethanol, and chloroform. All the solvent extracts were evaporated to remove the final traces of the respective solvents. Dried extracts were kept at 20°C until further test was carried out.

In vitro antioxidant activity

Free radical scavenging activity of the various solvent of leaf extracts in *P. pulchellum* was determined using various *in vitro* assays such as 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity, 2,2′-azinobis(3-ethylbenzothiazolone-6-sulfonic acid) (ABTS) radical scavenging assay, total antioxidant activity of phosphomolybdenum assay, and hydroxyl radical scavenging activity.

DPPH radical scavenging activity

Free radical scavenging activity was measured using DPPH method [8]. Different concentrations (1000, 800, 600, 400, and 200 µg/ml) of crude extracts and taken in test tubes in triplicates. Then, 5 ml of a 0.1 mM ethanol solution of DPPH (1,1,diphenyl-2-picrylhydrazyl) was added to each of the test tubes and was shaken vigorously. They were then allowed to stand at 37°C for 20 minutes. The control was prepared without any extracts. Ethanol was used for baseline corrections in absorbance (optical density [OD]) of the sample measured at 517 NM. Ascorbic acid is used as reference antioxidant compound. A radical scavenging activity was expressed as 1% scavenging activity and was calculated by the following formula:

$$\text{Radical scavenging activity}\,(\%) = \frac{\text{OD control} - \text{OD sample}}{\text{OD control}} \times 100$$

ABTS radical scavenging assay

The efficacy of plant extracts to scavenge free radicals was determined using ABTS radical scavenging assay with minor modification [9]. Freshly prepare the ABTS radical solution by adding 5 ml of 4.9 mM

ammonium persulfate solution to 5 ml of a 14 mM ABTS solution and keep for 16 hrs under dark condition. The solution is diluted with distilled water to yield an absorbance of 0.70±0.02 at 734 nm, and the same is used for the assay. To 900 µl of the ABTS radical solution, add 100 µl of the extract (1000, 800, 600, 400, and 200 µg/ml) and the reaction mixture is vortexed for 10 seconds and the assay was done. 6 minutes after record the absorbance at 734 nm against distilled water using (Beckman DU-530) ultraviolet-visible spectrophotometer. Ascorbic acid was used as reference standard. The radical scavenging activity was calculated using the formula:

$$\text{ABT scavenging activity} = \frac{\text{Absorbance of test sample}}{\text{Absorbance of blank sample}} \times 100$$

Total antioxidant activity of phosphomolybdenum assay

Determination of total antioxidant capacity developed by method [10]. 0.2 ml of plant extract (1000, 800, 600, 400, and 200 µg/ml) is mixed with 1.8 ml of distilled water, 2 ml of phosphomolybdenum reagent solution. Incubate it at 95°C for 90 minutes. The mixture is cooled to room temperature, and the absorbance is measured at 695 nm against a reagent blank. The test was performed in triplicate. The antioxidant capacity is expressed as ascorbic acid equivalent (AAE) and was calculated by the following theoretical formula:

$$A = \frac{c \times V}{M}$$

Where, A=Total content of antioxidant compounds, (mg/g) leaf extract, in AAE, c=The concentration of ascorbic acid established from the calibration curve, (mg/ml), V=The volume of extract (ml), and m=The weight of crude leaf extract (g).

Hydroxyl radical scavenging activity

The scavenging activity of the leaf extract on hydroxyl radical was measured according to the method [11]. Various concentrations (200, 400, 600, 800, and1000 µg/ml) of extracts were added to 1.0 ml of iron-ethylenediaminetetraacetate (EDTA) solution (0.13% ferrous ammonium sulfate and 0.26% EDTA), 0.5 ml of EDTA solution (0.018%), and 1.0 ml of dimethyl sulfoxide (0.85% v/v in 0.1 M phosphate buffer, pH 7.4). The reaction was initiated by adding 0.5 ml of ascorbic acid (0.22%) and incubated at 80-90°C for 15 minutes in a water bath. After incubation, the reaction was terminated by the addition of 1.0 ml of ice-cold trichloroacetic acid (17.5% w/v). 3 ml of Nash reagent (75.0 g of ammonium acetate, 3.0 ml of glacial acetic acid, and 2 ml of acetyl acetone were mixed and raised to 1 L with distilled water) were added and left at room temperature for 15 minutes, and the test was done 3 times. The reaction mixture without sample was used as a control. The intensity of the color formed was measured spectroscopically at 412 nm against the reagent blank. The percent hydroxyl radical scavenging activity is calculated by the following formula:

$$\text{Hydroxyl radical scavenging activity} = \frac{\text{Test sample absorbance}}{\text{Blank sample absorbance}} \times 100$$

RESULTS AND DISCUSSION

DPPH radical scavenging activity

The scavenging activity of all the extracts was found to be less when compared to that the standard ascorbic acid. Among the different extracts, the ethanol leaf extracts of showed high DPPH scavenging activity at 1000 µg/ml (81.5±0.02) followed by aqueous and chloroform (Table 1). From the results, it is known that the species, *P. pulchellum* possess hydrogen donating capabilities for ethanolic leaf extracts and execute scavenging free radicals.

ABTS radical scavenging assay

ABTS radical scavenging activity of different extracts of *P. pulchellum* is shown in Table 2. Ethanolic leaf extracts of *P. pulchellum* showed maximum free radical scavenging for ABTS assay at 1000 µg/ml concentration (75.13%) with a respective standard value of (82.13%) followed by aqueous and chloroform extracts. Among the all tested plant samples, ethanolic extracts of *P. pulchellum* exhibited the majority effective radical scavenging activity. All the extracts showed an increase in antioxidant capacity with an increase in the amount.

Total antioxidant activity of phosphomolybdenum assay

Phosphomolybdate method is also a quantitative assay, while the total antioxidant capacity is expressed as AAE. The total antioxidant activity of different concentrations in (200-1000 µg/ml) this method. The ethanolic leaf extract is higher than (2.069±0.002) with in respective standard value is 2.201±0.029. Although the antioxidant capacity of the extracts was found to the decrease arrange as followed by ethanol>aqueous>chloroform (Table 3).

Hydroxyl radical scavenging activity

The concentrations range from 200 to 1000 µg/ml of different extracts was assessed for their hydroxyl radical scavenging activity. From

Table 1: DPPH radical scavenging activity of leaf extract from *P. pulchellum*

Concentration (µg/ml)	Absorbance at 517 nm			
	Aqueous extract	Ethanol extract	Chloroform extract	Ascorbic acid (SD)
200	34.7±0.03	37.7±0.05	23.4±0.02	39.1±0.01
400	45.5±0.04	48.2±0.02	36.3±0.57	48.4±0.05
600	56.1±0.02	58.6±0.04	47.6±0.05	63.4±0.01
800	68.6±0.01	69.3±0.03	50.6±0.06	86.1±0.07
1000	76.8±0.03	81.5±0.02	67.2±0.10	91.1±0.08

Values were performed in triplicates and represented as mean±SD, DPPH: 2,2-diphenyl-1-picrylhydrazyl, *P. pulchellum: Phyllodium pulchellum*

Table 2: Activity of *P. pulchellum* leaf extracts in the ABTS cation radical assay

Concentration (µg/ml)	% inhibition of ABTS radical			
	Aqueous extract	Ethanol extract	Chloroform extract	Ascorbic acid (SD)
200	20.15	27.19	19.11	15.18
400	33.70	44.13	28.10	29.55
600	47.62	58.82	41.01	42.87
800	59.31	67.31	53.05	68.64
1000	72.41	75.13	68.82	82.13

ABTS: 2,2′-azinobis (3-ethylbenzothiazolone-6-sulfonic acid), *P. pulchellum: Phyllodium pulchellum*

Table 3: Total antioxidant activity of phosphomolybdenum assay

Concentration (µg/ml)	Absorbance at 695 nm			
	Aqueous extract	Ethanol extract	Chloroform extract	Ascorbic acid (SD)
200	1.258±0.008	1.311±0.021	1.125±0.012	1.121±0.018
400	1.422±0.005	1.572±0.011	1.322±0.010	1.346±0.024
600	1.629±0.006	1.692±0.013	1.492±0.023	1.657±0.072
800	1.811±0.007	1.881±0.005	1.662±0.043	1.824±0.043
1000	1.926±0.015	2.069±0.002	1.726±0.018	2.201±0.029

Values were performed in triplicates and represented as mean±SD

Table 4: Hydroxyl radical scavenging activity

Concentration (µg/ml)	% Inhibition of hydroxyl radical scavenging			
	Aqueous extract	Ethanol extract	Chloroform extract	Ascorbic acid (SD)
200	42.21	51.25	41.01	53.01
400	54.11	63.32	53.43	66.13
600	67.01	75.13	61.79	78.37
800	78.13	84.27	73.11	86.18
1000	84.51	93.70	81.15	96.67

Table 5: IC_{50} values of *P. pulchellum* in radical scavenging assay

Leaf extract	DPPH radical scavenging activity (µg/ml)	ABTS radical inhibition activity (µg/ml)	Hydroxyl radical scavenging activity (µg/ml)
Aqueous extract	481.37	651.32	320.29
Ethanol extract	432.90	524.40	155.40
Chloroform extract	698.42	728.18	357.80
Ascorbic acid (SD)	417.60	529.21	114.32

DPPH: 2,2-diphenyl-1-picrylhydrazyl, ABTS: 2,2′-azinobis (3-ethylbenzothiazolone-6-sulfonic acid), IC_{50}: Inhibitory concentration 50

Table 4, it is clear that the ethanolic leaf extracts of *P. pulchellum* showed greatest scavenging activity (93.70%) at 1000 µg/ml concentration followed by aqueous and chloroform with the standard values of 96.67%, respectively. The results showed the scavenging potential of *P. pulchellum* against hydroxyl radicals (Table 4).

The dosage of the extract is expressed in µg of the dry weight of the extract (compound)/mL of the assay mixture. Inhibitory concentration 50 (IC_{50}) value represents the concentration of test extract or compound where the inhibition of test activity reached 50%. Among the plant extracts, ethanolic extract showed the highest activity, with an IC_{50} value of 524.40 µg/ml. Moreover, the ABTs radical inhibition activity was in the order ethanol>chloroform>aqueous. The IC_{50} value of aqueous and chloroform extracts and ascorbic acid was 651.32 µg/ml, 728.18 µg/ml, and 529.21 µg/ml, respectively. The DPPH radical scavenging capacity in the order: Ethanol>chloroform>aqueous and then scavenging ability on hydroxyl radicals was in the order: Ethanol>chloroform>aqueous (Table 5).

Rising concern in the investigate for natural alternatives in favor of synthetic antioxidant has led to the evaluation of plant sources. In this study, leaf extract of *P. pulchellum* exhibited outstanding scavenging effects on DPPH radical scavenging activity, ABTS radical scavenging assay, total antioxidant activity of phosphomolybdenum assay, and hydroxyl radical scavenging activity. Along with the good number widely used procedures for measurement of antioxidant activity capacity, the DPPH radical scavenging analysis is one of the top known, correct, and regularly employed to measure the electron donating ability of the plant [12,13]. Dechayont *et al.,* reported that the moderate antioxidant activity from *Pogostemon cablin* and inhibited bacteria commonly responsible for community and hospital acquired infections [14]. Usmangani *et al.* reported that leaf and fruit extracts of *D. palmatus* were determined to have a certain level of radical scavenging effect, proportional to their level of phytochemicals [15]. Similar reported on antioxidant activity was Phyllodium genus relative synonymous species, *Desmodium gangeticum* (L.) DC., *Desmodium heterocarpon* (L.) DC., *Desmodium intortum* (DC.) Urb., *Desmodium microphyllum* (Thunb *ex* Murray) DC., *Desmodium renifolium* (L.) Schindl., *Desmodium scorpiurus* (Sw.) Desv., *Desmodium sequax* Wall. (DSE), *Desmodium tortuosum* (Sw.) DC., *D. triflorum* (L.) DC., and *D. uncinatum* DC.[16]. A similar observation was made in *Toddalia asiatica* [17], *Thevetia peruviana* [18], and *Leucas aspera* [19] reported that the amount of sensitivity increased with the regular increase in the concentration of extracts. There is no report that antioxidant activity of *P. pulchellum* leaf extracts. Hence, far we identify this is the first report that envisages the antioxidant activities of *P. pulchellum* leaf extracts. The extracts of *P. pulchellum* could be potential as a source of valuable phytochemical compounds used for the pharmaceutical industry and antioxidant mechanisms.

CONCLUSION

Based on the resulted find in this study, it is concluded that the ethanolic leaf extract of *P. pulchellum* exhibits considerable antioxidant radical scavenging activity on all tested assays. Therefore, further research is needed for the isolation and identification of the active components in the extracts.

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