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In Vitro Antioxidant Efficacy of the Flowers and Leaves of *Pogostemon quadrifolius* (Benth.) F. Muell. (Lamiaceae)

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ABSTRACT

In this study, the antioxidant efficacy of various extracts of an ethnobotanically important plant, Pogostemon quadrifolius (Benth.) belonging to Lamiaceae was examined by various antioxidant assays, including total antioxidant (Phosphomolybdenum assay), 1,1 diphenyl–2–picrylhydrazyl (DPPH) and Ferric Reducing Antioxidant Power (FRAP) assay. The various antioxidant activities were compared to standard antioxidant ascorbic acid. Most of the extracts showed strong antioxidant activities in all the tested methods. Methanolic extracts of leaves and flowers of P. quadrifolius showed high DPPH antioxidant activity followed by acetone extracts. Moreover floral extracts are more efficient DPPH radical scavengers than leaf extracts. Among the leaf extracts acetone extract showed maximum reducing power ability (FRAP) and among flower extracts petroleum ether extract showed maximum reducing power. The total antioxidant capacity (Phosphomolybdenum assay) results are also provided. The study indicates that P. quadrifolius may be a potential source of natural antioxidants and can be used for therapeutic purposes.

Keywords: *Pogostemon quadrifolius*, Lamiaceae, Antioxidant activity, Leaves, Flowers

INTRODUCTION

Free radicals are mainly different Reactive Oxygen Species (ROS). ROS include the hydroxyl radical, the superoxide anion radical, hydrogen peroxide, singlet oxygen, nitric oxide radical, hypochlorite radical and lipid peroxides [1]. Various metabolic processes and environmental stresses result in the generation of reactive species in living systems. Increased level of ROS increase oxidative stress in living systems which finally results in a variety of health problems such as cancer, age related disease and cardiovascular diseases [2,3]. Many antioxidant compounds, naturally occurring from plant sources, have been identified as a free radical or active oxygen scavengers [4]. Antioxidants inhibit or delay the oxidation of other molecules by inhibiting oxidizing chain reactions. Recently interest in natural antioxidants has increased considerably as they have no side effects. Hence development and utilization of effective natural antioxidants is essential as they can protect the human body from free radicals [5].

Pogostemon quadrifolius (Benth.) is a common shrub in India, Myanmar and Bangladesh and is used as folk medicine in India and Bangladesh for the treatment against chicken pox worms and also as a blood purifier [6-12]. (Z)-ethylidene-4,6-dimethoxycoumaran-3-one, a new compound from the leaf methanol extracts of *P. quadrifolius* (Benth.), exhibited antiproliferative property and it induces apoptosis in cancer cell line [13-15]. The plant also exhibited antioxidant, mosquito larvicidal and antimicrobial properties [16-20]. The GC MS analysis and preliminary phytochemical screening of various extracts of flowers and leaves of *P. quadrifolius* (Benth.) has shown the presence of various compounds like alkaloids, phenols, flavonoids, terpenes, tannin, saponins etc., in various extracts [21]. Quantitative estimation of flavonoids, phenolics, alkaloids, tannin and saponins had also been carried out [22,23]. The present study was aimed to investigate the *in vitro* antioxidant efficacy of various extracts of the flowers and leaves of *P. quadrifolius* (Benth.) by DPPH free radical scavenging assay, Ferric Reducing Antioxidant Power (FRAP) and total antioxidant capacity (Phosphomolybdenum assay).

MATERIALS AND METHODS

Chemicals

The major chemicals used in the study were DPPH-Sigma Chemicals (USA), Potassium Ferricyanide [$K_3Fe(CN)_6$]-Loba Chemie Pvt. Ltd. (India) and Ascorbic acid-SD Fine Chem. Ltd. (India). Other chemicals and solvents used for extraction were of analytical grade.

Collection of plant materials and extraction

Pogostemon quadrifolius (Benth.) collected from Panakkad, Karimbam, Kannur, Kerala was authenticated by Dr. A.K. Pradeep, Department of Botany, University of Calicut.

The fresh flowers and leaves which were washed and shade dried were then powdered using a mixer grinder and the powder was kept in small airtight bottles with proper labelling. Sequential extraction of powdered flowers and leaves using petroleum ether, acetone, methanol and water in increasing order of polarity using soxhlet apparatus were carried out until all the constituents were completely eluted. The extracts were then filtered and evaporated to dry, which were used for further studies.

Tests for antioxidant activity

DPPH free radical scavenging activity

The DPPH free radical scavenging activity has been carried out by the method described by Braca et al. [24]. 100-600 µg/ml of plant extract was added to 3 ml of a 0.004% ethanol solution of DPPH. After 30 min, absorbance at 517 nm was determined and the percentage inhibition activity was calculated by formula $[(A_0–A_1)/A_0] \times 100$, where A_0 is the absorbance of the control (DPPH solution) and A_1 is the absorbance of the extract/standard. The inhibition curves were prepared and IC_{50} values were calculated. Ascorbic acid was used as standard.

Determination of FRAP

The ferric reducing antioxidant power was determined as described by Oyaizu, [25]. By measuring the absorbance of Perl's Prussian blue complex, the reduction of Fe^{3+}-Fe^{2+} is determined. Extracts with concentrations 5-200 µg in 1 ml of distilled water were mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and $[K_3Fe(CN)_6]$ (2.5 ml, 1%). The incubated mixture (50°C for 20 min) was mixed with 2.5 ml trichloroacetic acid (10%), which was then centrifuged at 3000 rpm (10 min). To the supernatant (2.5 ml), distilled water (2.5 ml) and $FeCl_3$ (0.5 ml, 0.1%) was added and the absorbance was measured at 700 nm. Reducing power increases with increase in absorbance of the reaction mixture. The standard used was ascorbic acid.

Total antioxidant capacity (Phosphomolybdenum assay)

The total antioxidant capacity by phosphomolybdenum method was carried out as described by Prieto et al. [26]. 3 ml of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) and 0.3 ml extract was combined. After incubation of the reaction solution (95°C for 90 min), the absorbance of the solution was measured at 695 nm using a UV–visible spectrophotometer (Shimadzu, 1601) against blank (Methanol-0.3 ml) after cooling at room temperature. The antioxidant activity is given as the number of ascorbic acid equivalents.

Statistical analysis

The results are given as mean ± standard deviation (SD) (triplicate experiments) and evaluated by student's t-test. Differences were considered significant at a level of $P < 0.05$. IC_{50} was calculated using Graph Pad Prism 7.02 software.

RESULTS AND DISCUSSION

DPPH free radical scavenging activity

As per Figures 1 and 2, in the DPPH scavenging assay, various extracts of flowers and leaves exhibited free radical scavenging activity by inhibiting $DPPH^{\cdot}$ radical, which was dependent on concentrations of the extracts. The well-known antioxidant, ascorbic acid, showed high degree of free radical-scavenging activity than that of the plant extracts at each concentration points.

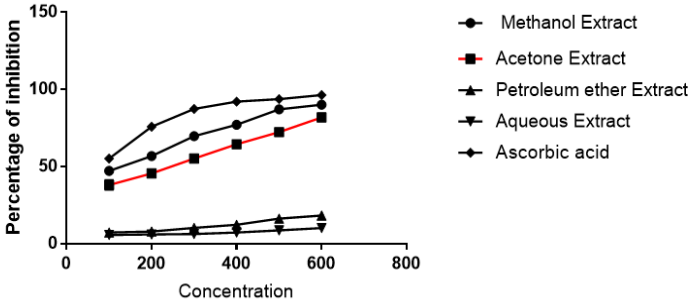

Figure 1: DPPH radical scavenging activity of various extracts of leaves of *Pogostemon quadrifolius* along with the standard ascorbic acid (Mean ± SD, n= 3). Concentration in µg/ml

The IC_{50} of the crude methanol extract of leaf was 133.97 ± 0.69 µg/ml and acetone extract was 245.47 ± 1.30 µg/ ml, respectively, whereas IC_{50} value for the standard ascorbic acid was 74.13 ± 0.84 µg/ml. The IC_{50} of the crude methanol extract of flower was 99.08 ± 0.84 µg/ml and acetone extract was 141.25 ± 0.88 µg/ml respectively, whereas IC_{50} of ascorbic acid was 74.13 ± 0.84 µg/ml. In the DPPH antioxidant assay, DPPH decolourise in the presence of antioxidants [27]. The method is based on the reduction of ethanolic DPPH solution in the presence of a hydrogen donating antioxidant, due to the formation of the non-radical form DPPH- H by reaction. The extract was able to reduce DPPH radical (visible deep purple colour) to the yellow colored diphenyl picrylhydrazine. It has been found that cystine, glutathione, ascorbic acid, tocopherol, polyhydroxy aromatic compounds (e.g. p-phenylenediamine, p-aminophenol) reduces and decolorizes DPPH by their hydrogen donating ability [28]. Methanolic extracts of flowers and leaves of *P. quadrifolius* (Benth.) showed high antioxidant activity followed by acetone extracts. Moreover floral extracts are more efficient DPPH radical scavengers than leaf extracts. Among the various natural antioxidants, phenolic compounds are reported to have the character of oxygen-derived free radicals by donating a hydrogen atom or an electron to the free radical [29,30]. Also, phenolic compounds of plant materials have been shown to neutralize free radicals in various model systems [31].

DPPH radical scavenging property of methanolic extracts of leaves of *P. quadrifolius* (Benth.) has been studied and it has been identified that the phenolic compounds in the extract is responsible for the activity [16]. It has also been identified that high phenolic content is present in the methanolic extracts and acetone extracts of the leaves and flowers of *P. quadrifolius* (Benth.) respectively [22]. So it can be concluded that phenolic compounds are responsible for antioxidant activity. Further studies regarding isolation and structural elucidation of major phenolic compounds in the methanolic and acetone extracts of flowers and leaves of *P. quadrifolius* (Benth.) are to be carried out.

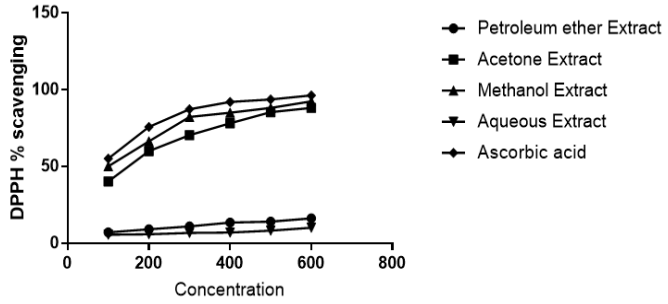

Figure 2: DPPH radical scavenging activity of various extracts of flowers of *Pogostemon quadrifolius* along with the standard ascorbic acid. (Mean ± SD, n=3). Concentration in µg/ml

Determination of FRAP assay

Figures 3 and 4 shows the reducing power capabilities of the plant extracts compared to ascorbic acid. The various extracts of leaves and flowers displayed good reducing power which was found to rise with increasing concentrations of the extract. In reducing power assays, the presence of antioxidants in the extracts can reduce the oxidised form of iron (Fe^{3+}) to its reduced form (Fe^{2+}) by donating an electron. Thus, it can be assumed that the presence of reductants (i.e., antioxidants) in the leaf and flower extracts of *P. quadrifolius* (Benth.) cause the reduction of the Fe^{3+}/ferricyanide complex to the ferrous form. Therefore, the Fe^{2+} complex can be monitored by measuring the formation of Perl's Prussian blue at 700 nm. A higher absorbance indicates greater reducing power ability [32]. Among the leaf extracts acetone extract showed maximum reducing power ability and among flower extracts petroleum ether extract showed maximum reducing power.

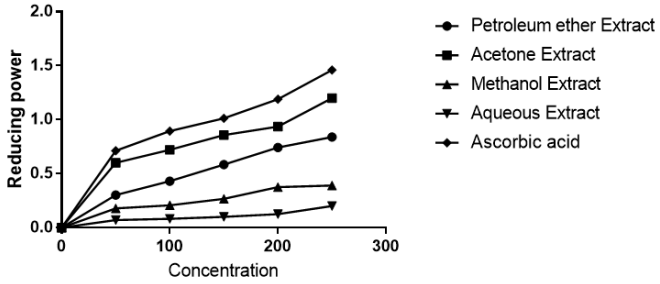

Figure 3: Reducing power of various extracts of leaves of *Pogostemon quadrifolius* along with the standard ascorbic acid (Mean ± SD, n=3)

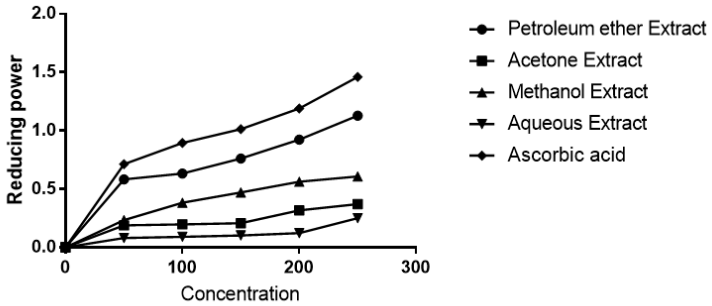

Figure 4: Reducing power of various extracts of flowers of *Pogostemon quadrifolius* along with the standard ascorbic acid (Mean ± SD, n=3)

Determination of total antioxidant capacity (Phosphomolybdenum assay)

The total antioxidant capacity of the leaf and flower extracts of *P. quadrifolius* (Benth.) is given in Tables 1 and 2 respectively. Significant amount of total antioxidant activity was obtained from petroleum ether, acetone and methanol extracts of leaves and flowers of *P. quadrifolius* at 200 µg/ml extract concentration. The phosphomolybdenum method was based on the reduction of Mo(VI)-Mo(V) by the antioxidant compound and the formation of a green phosphate/Mo(V) complex with a maximum absorption at 695 nm.

This assay was successfully used to quantify vitamin E in seeds. It was decided to extend its application to plant extracts because it was simple and independent of other commonly employed antioxidant measurements [26]. Moreover, it is a quantitative one, since the antioxidant activity is expressed as the number of equivalents of ascorbic acid.

Table 1: Total antioxidant capacity of leaf extracts of *Pogostemon quadrifolius*

S. No.	Name of extract	Total antioxidant capacity equivalent to ascorbic acid mg/g plant extract
1.	Petroleum ether extract	689.00 ± 1.00
2.	Acetone extract	404. 67 ± 3.51
3.	Methanol extract	146.33 ± 1.53
4.	Aqueous extract	101.00 ± 1.00

Table 2: Total antioxidant capacity of flower extracts of *Pogostemon quadrifolius*

S. No.	Name of extract	Total antioxidant capacity equivalent to ascorbic acid mg/g plant extract
1.	Petroleum ether extract	683.33 ± 2.08
2.	Acetone extract	272.00 ± 2.65
3.	Methanol extract	282.67 ± 1.53
4.	Aqueous extract	16.33 ± 1.53

Values are the average of triplicate experiments and represented as mean ± SD

CONCLUSION

The work has for first time determined the antioxidant activity of the flowers and leaves of *P. quadrifolius* (Benth.) by FRAP assay and total antioxidant capacity (Phosphomolybdenum assay). The results from various assays reveal that *P. quadrifolius* (Benth.) have significant antioxidant activity. The extracts are found to have different levels of antioxidant activity in all the systems tested. The study indicates that *P. quadrifolius* (Benth.) may be a potential source of antioxidants and can be used for therapeutic purposes. Further studies are being carried out for the isolation and identification of individual phenolic compounds responsible for antioxidant activities.

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