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PHYTOCHEMICAL ANALYSIS AND ANTIOXIDANT POTENTIALITY OF *PREMNA SERRATIFOLIA* L. - AN AROMATIC MEDICINAL PLANT

[1]Lubaina A.S., [2]Brijithlal N.D. and [3]Murugan K.*

[1]Department of Botany, Christian College, Kattakada, Thiruvananthapuram, Kerala.
[2]Department of Botany, Bishop Moore College, Mavelikkara, Kerala – 690110.
[3]Department of Botany, University College, Thiruvananthapuram.

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***Corresponding Author**
Dr. Murugan K.
Department of Botany,
University College,
Thiruvananthapuram.

ABSTRACT

Documenting and validating traditional and indigenous system of medicine in India has been continuously increasing from the last few decades. The current study was made to evaluate the phytochemical constituents and antioxidant potentiality of *Premna serratifolia* L. an aromatic medicinal plant known as 'Agnimantha' in Ayurvedic system of medicine. It has immense ethno pharmacological importance in folk medicine for curing various human ailments. The qualitative phytochemical screening of the aqueous and ethanolic leaf extracts of *P. Serratifolia* revealed the presence of glycosides, flavonoids, alkaloids, tannins, terpenoids and steroids. Subsequently, the quantification of major secondary metabolites revealed remarkable level of phenols followed by terpenoids and flavonoids. The *in vitro* antioxidant activity was determined by 2,2-diphenyl-2-picrylhydrazyl (DPPH), ferric reducing antioxidant potentiality (FRAP), hydroxyl radical scavenging activity, hydrogen peroxide scavenging, superoxide anion radicals quenching effect and metal chelating assay. The aqueous extracts showed positive correlation between concentrations of the extracts with DPPH radical scavenging capacity. Similarly the other antioxidant potentials also displayed remarkable activities when comparable with synthetic antioxidants such as ascorbate and BHA. The results suggest that the aqueous extract of *P.serratifolia* are potential source for natural antioxidants due to the presence of polyphenols and that could have a protective role as well as prevention from life style diseases.

KEYWORDS: Antioxidant potentiality, *Premna serratifolia*, phenols, flavonoids, terpenoids, FRAP, DPPH.

INTRODUCTION

Herbals are treasure islands of potential drugs and in the recent years there has been an increasing awareness about the importance of medicinal plants. They are the richest bio-resource of drugs in traditional systems of medicine, allopathy, nutraceuticals food supplements, folk medicines, pharmaceutical intermediates and chemical templates for synthetic drugs.[1] Plant derived bioactive compounds have been the focus of recent research due to their health promoting effects. Drugs from the plants are easily available, less expensive, safe, efficient and having fewer side effects. The plants which have been selected for medicinal use contains several bioactive compounds constitute the most obvious choice of examining the current search for therapeutically effective new drugs. They are used for curing of various human diseases and also play an important role in healing because of the presence of phytochemical constituents such as alkaloids, steroids, tannins, glycosides, volatile oils, fixed oils, resins, phenols and flavonoids deposited in leaves, flowers, bark, seeds, fruits and root. The beneficial medicinal effect of plant materials wasdue to the synergistic action of these secondary metabolites.[2] Among them flavonoids are proven antioxidants constitute a wide range of molecules that play important role in protecting biological systems against the harmful effects of oxidative burst on macromolecules such as proteins, lipids and DNA.[3] In this juncture, the present study aims to analyse the major phytochemicals and their antioxidant potentiality from *P. serratifolia* of Verbenaceae to understand its medicinal potential and propose strategies for future utilization.

MATERIALS AND METHODS

Plant material

Plant material of *P. Serratifolia* was collected from Malayinkeezhu, Trivandrum, Kerala was used for the present study. The specimen was taxonomically identified and confirmed by comparing with authenticated herbarium specimen at JNTBGRI, Palode, Trivandrum, Kerala. A voucher specimen of the plant is kept in the herbarium of Department of Botany (CCB 046).

Preparation of extracts

Fresh leaves (50 g) was chopped, air dried at room temperature, finely powdered and successively extracted with 100 ml of ethanol and water for 6 h using soxhlet hot continuous

extraction method. The extracts were filtered and concentrated using rotary evaporator at 40°C.

Phytochemical screening

Alcoholic and aqueous extracts were subjected to various tests in order to identify the presence of different phytochemicals such as glycosides, tannins, coumarins, alkaloids, saponins, flavonoids, phenols, steroids, reducing sugar and terpenoids.[4]

Quantification of phenol, flavonoids and terpenoids

Total phenols of ethanolic and aqueous extracts were isolated and quantified by the method ofMayer.[5] the total flavonoid content determined by $AlCl_3$ method[6] and total terpenoids by the method of Van Beek et al.[7]

Antioxidant potentiality

Evaluation of DPPH radical scavenging activity

The free-radical scavenging activity of the different concentration of the extracts were measured with the stable radical 2,2-diphenyl-1-picrylhydrazyl in terms of hydrogen-donating or radical-scavenging activity following the method of Rajesh and Natvar.[8]

Ferric reducing antioxidant power (FRAP) assay

Antioxidant activity by FRAP assay estimated by the method of Benzie and Strain.[9]

Estimation of superoxide radical scavenging activity

Superoxide scavenging was determined by the nitrobluetetrazolium (NBT) reduction method.[10]

Hydroxyl radical scavenging activity

The effect of hydroxyl radicals of the extracts were assayed by using the deoxy ribose method.[11]

Hydrogen peroxide scavenging assay

The free radical scavenging activity of different concentrations of both the extracts was determined by Hydrogen peroxide assay of Gulcin*et al.*[12]

Chelating activity of Fe^{2+}

The different concentration of three extract were assessed for their ability to compete with ferrozine for iron (II) ions in free solution. The chelating ability of ferrous ions was estimated by the method of Koncic*et al.*[13]

Statistical analysis

The data was statistically analysed by one way analysis of variance (ANOVA) and t-test ($p < 0.05$). The results are mean of five replications and were represented as mean ± SD.

RESULTS AND DISCUSSION

Phytochemical analysis

The preliminary phytochemical tests are helpful in finding therapeutic phytochemical constituents in the plant material and also in pinpointing the source of pharmacologically active chemical compounds. The therapeutic value of herbals lies in its principle component which play potential physiological role on humans. Phytochemical screening of aqueous and ethanolic extracts of *P.serratifolia* showed the presence of glycosides, flavonoids, alkaloids, tannins, terpenoids and steroids (Table 1). These bioactive compounds present in *P. Serratifolia* are proven antioxidants.

The yields of the ethanol and water extracts of *P. serratifolia* were 0.58% and 0.92% respectively. In the present study the significant yield of water extract revealed the higher efficiency of water as extracting solvent and that might be attributed to the high polarity of the solvent system. The present result was in close agreement to the findings of other researchers regarding the yield of extract prepared in different solventsystem.[14] Increasing trend in the yield of antioxidant extracts has been observed with increasing polarity of solvent suggesting the polar nature of antioxidants compounds.

The results of quantitative estimation of total phenols, flavonoids and terpenoids from aqueous and ethanolic extracts were tabulated in Table 2. The maximum amount of phenols was detected in the water extract of *P. serratifolia*. The phenolic content in water extract was 76.4 ± 0.59 mg/g tissue and that of ethanol was 65.3 ± 0.38mg/g tissue. Plant polyphenols are phytochemicals that protects plants from oxidative stress i.e., they are having redox properties. So they can act as reducing agents, hydrogen donors and singlet oxygen quenchers.[15] Polyphenols also play an important role in giving protection to the plant against deleterious effects of UV rays[16] and also against phytopathogenic organisms. The high

phenolic concentrations observed in plants may contribute to its defence mechanism. Biosynthetic potentialities of plants are better illustrated by a group of natural products called terpenoids. Similar to phenols, terpenoids also showed significant amount in water extract (54.6 ± 0.24 mg/g tissue) followed by ethanolic extract (39.3 ± 0.68 mg/g tissue) (Table 2). The fragrance of plants is mainly due to the presence of terpenoid compounds present in them. Quantified data of terpenoids in many herbals revealed by Goto *et al.*[17] were at par with the present results. Terpenoids are generally lipid soluble compounds located in the cytoplasm which includes essential oils, carotenoids, derived sterols and saponins. Monoterpenes can also function as antigerminative phytotoxic allelopathic molecules.[18]

Like that of phenols and terpenoids *P. serratifolia* showed significant amount of flavonoid content in the water (46.5 ±0.14 mg/g tissue) andethanol extracts (31.2 ± 0.07 mg/g tissue). Thirumalai *et al.*,[19] estimated total flavonoids in *P. herbaceae*and revealed similar results. Flavonoids possess multiple biological and pharmacological potentialities like radical scavengers, cellular signalling, anti-inflammatory, antiallergic, antiviral, antimetastatic and antimutagenic.[20] In addition flavonoids can act as free radical scavengers and terminates radical chain reaction that occurs during oxidation of triglycerides in the food system. The quantitative estimation of phenols, terpenoids and flavonoids in *P. serratifolia* gives an insight into their phytochemical repository which can provide a rich data in understanding the basic pattern of growth and metabolism. At the same time phenols, terpenoids and flavonoids can be used as chemical markers in taxonomic studies.[21]

Antioxidant potentiality

Quantitative analysis of total phenols and flavanoids in the extract does not necessarily represent the total antioxidant potentiality of the constituents present in the extract. Therefore various antioxidant protocols were used for the determination of antioxidant potentiality of the extract. Besides, well known and traditionally used natural antioxidants from tea, fruits, vegetables and spices, some natural antioxidants are already exploited commercially either as antioxidant additives or as nutritional supplements.[22] Also, many other plant species have been investigated in the search for novel antioxidants.[23] But, still there are demands to find more information concerning the antioxidants from plant sources. It has been reported that antioxidant properties of plants might be due to their constituents including the phenolic and flavanoid compounds. Some evidence suggests that the biological actions of these compounds are related to their antioxidant activity.[24]

DPPH scavenging activity

DPPH method has been used to analyse the radical scavenging potentiality of the extracts of *P. Serratifolia* due to its simplicity, sensitivity and effectiveness. Aqueous and ethanolic plant extracts showed varying level of scavenging activities over DPPH free radicals (43.76 ± 0.17 and 86.58 ± 0.26 at 1000 µg/ml respectively) (Table 3). DPPH is one of the stable organic nitrogen free lipophilic radical which has been used to test the radical scavenging ability.[25] Antioxidants, on interacting with DPPH either transfer an electron or hydrogen atom to DPPH, thus neutralizing its free radical character.[26] There scavenging activities might be due to the presence of phenolic contents. Generally, the antioxidant activity of medicinal plants was not significant compared with synthetic antioxidants such as BHT and ascorbic acid but recommendable due to their natural source. The bleaching of DPPH absorption is representative of the capacity of tested compounds to scavenge the free radicals independently from any enzymatic activity. DPPH method provides a method to evaluate antioxidant activity in a relatively short time, compared to other methods. In the present study *P.serratifolia* ethanol extract showed high scavenging activity of DPPH radicals compared to water extract. Which may attributable to its significant hydrogen donating ability (Table 3). It could be inferred from the results that increasing the concentration of extract reduce the level of free radical in the reaction mixture, that would ultimately raise the potential to scavenge or chelate free radicals by the plant extract hence an increase in the antioxidant properties. Alothman*et al.*,[27] studied the antioxidant capacity and phenolic content of selected tropical fruits from Malaysia; using different solvent system the fruits studied were viz. *Ananas squamosus, Musa paradisiaca* and *Psidium guajava*. The DPPH results obtained by them (12.7- 93.7%) were similar to the results obtained in the present study.

Ferric reducing antioxidant power assay(FRAP)

FRAP assay measures the reducing potential of an antioxidant with ferric tripyridyltriazine (Fe^{3+}-TPTZ) complex to its ferrous coloured form tripyridyltriazine (Fe^{2+}-TPTZ) [9].Generally the reducing properties are associated with the presence of compounds, which exert their action by breaking the free radical chain by donating a hydrogen atom. Aqueous extract of *P.serratifolia* showed the highest ferric reducing power (442.3 ± 0.93 µM/g at 1000 µg/ml), followed by ethanolic (366.53 ± 0.98 µM/g at 1000 µg/ml) (Table 4). The values are comparable with antioxidant potentialities of many plant products.[28]

Superoxide radical scavenging activity

The superoxide anion scavenging ability of the extracts was determined using SOD assay kit-WST. Superoxide dismutase (SOD) is an enzymatic antioxidant that can scavenge superoxide anion radical ($O_2^{\cdot-}$) by catalyzing the dismutation of the superoxide anion into hydrogen peroxide and molecular oxygen. Superoxide ($O_2^{\cdot-}$) radical is known to be harmful to cellular components as a precursor of the more reactive oxygen species, contributing to the tissue damage and various diseases. This assay is based on the measurement of superoxide dismutase inhibition activity. In this assay the superoxide anion reduce WST-1 (2-(4-iodophenyl)-3-(4-nitrophenyl)-5-(2,4-disufophenyl)-2H-tetrazolium) to generate the water soluble formazan dye in the testing solution, which is measured using spectrophotometer at 450nm. In the presence of an enzymatic antioxidant, the reduction of WST-1 can be inhibited by neutralizing $O_2^{\cdot-}$. Thus the SOD neutralizing activity can be quantified by measuring the decrease in the colour development at 450nm. *P.serratifolia* aqueous extract exhibited the highest superoxide anion scavenging activity with inhibition rate of 90.32 ± 0.23% at 1000 µg/ml followed by ethanoicfraction, which is 81.54 ± 0.18% at 1000 µg/ml (Table 5).

Hydroxyl radical scavenging activity

Antioxidant activity of ethanol and aqueous extracts of *P.serratifolia* by hydroxyl radical scavenging activity is presented in table 6. Both the extracts exhibited strong hydroxyl radical scavenging activity which was dose dependent. The inhibition observed was found to be statistically significant with respect to the inhibition observed at all the dose levels tested. Ethanol and water extracts have moderate but statistically significant hydroxyl radical scavenging effects up to the doses in comparison to controls. The activity was found to increase at higher dose levels. *P.serratifolia* aqueous extract exhibited the highest hydroxyl radical scavenging activity with inhibition rate of 69.532 ± 0.78% at 1000 µg/ml followed by ethanolic fraction i.e., 42.023 ± 0.42% at 1000 µg/ml (Table 6).

Hydrogen peroxide scavenging assay

Hydrogen peroxide is a weak oxidizing agent and can inactivate a few enzymes directly, usually by oxidation of essential thiol (-SH) groups. Hydrogen peroxide can cross cell membrane rapidly. Once inside the cell, H_2O_2 can probably react with Fe^{2+} and possibly Cu^{2+} to form hydroxyl radical and this may be the origin of many of its toxic effects. It is therefore biologically advantageous for cells to control the amounts of hydrogen peroxide synthesis. The decomposition of H_2O_2 by aqueous and ethanolic extracts of *P. serratifolia* is presented

in table 7. Both the extracts exhibited strong ROS scavenging activity which was dose dependent. The inhibition observed was found to be statistically significant with respect to inhibition observed at all the dose levels tested. Aqueous extract have statistically significant hydrogen peroxide scavenging effects (84.005 ± 0.73% at 1000 µg/ml), followed by ethanol (59.002 ± 0.49% at 1000 µg/ml) (Table 7).

Metal chelating assay

Plant extracts have the ability to chelate or deactivate transition metals, which possess the ability to catalyse hydroperoxide decomposition and block Fenton-type reactions due to its antioxidant nature. Chelating agents serve as secondary antioxidants since they reduce redox potential, thereby stabilizing the oxidized forms of metal species. Reduction of the iron ion is an indicator of electron donating activity, which is an important mechanism of phenolic antioxidant action. Yellow colour of test solution changes in to various shades of green and blue depending upon the reducing power of the extract. The presence of antioxidants in *P.serratfolia* extracts causes the reduction of Fe^{3+}/Ferric cyanide complex to ferrous form. Therefore, the Fe^{2+} complex can be monitored by measuring the formation of Perl's Prussian blue. Results in Table 8 displayed that the aqueous extract showed highest iron reduction ability (55.61 ± 0.47 µg/ml) followed by ethanol (25.70 ± 0.19 µg/ml).

Table 1: Preliminary phytochemical analysis using aqueous and ethanol extracts of *P. serratifolia* L.

Phytochemicals	Ethanol	Water
Phenol	++	+++
Glycosides	-	+++
Flavonoids	-	++
Alkaloids	++	-
Tannins	+++	++
Terpenoids	++	+++
Steroids	+++	+
Saponins	-	+
Coumarins	-	+

Strong positive: + + +; moderately positive: + +; Low positive: +; negative test: -

Table 2: Total phenol, terpenoids and flavonoids of ethanol and water extracts of *P. serratifolia*. Values are mean ± SD of three independent replications.

Solvent	Total phenol (mg/g tissue)	Terpenoids (mg/g tissue)	Flavonoids (mg/g tissue)
Ethanol	65.3 ± 0.38	39.3 ± 0.68	31.2 ± 0.07
Water	76.4 ± 0.59	54.6 ± 0.24	46.5 ±0.14

Table 3: DPPH radical scavenging activity of water (AE) and ethanolic (EE) extracts of *P. serratifolia*. Values are mean ± SD of three independent replications.

	AE	EE
Concentration(µg/ml)	**% Inhibition ± SD**	**% Inhibition ± SD**
100	38.40±0.29	77.78±0.41
500	38.70±0.31	84.92±0.29
1000	43.76±0.17	86.58±0.26
Ascorbate (500µg)	96± 2.77%	
BHT(500µg)	90±0.98%	

Table 4: FRAP-potential of water (AE) and ethanol (EE) extracts of *P. serratifolia.* Values are mean ± SD of three independent replications.

	AE	EE
Concentration(µg/ml)	**µM/g**	**µM/g**
100	150.7 ±0.52	68.76 ± 0.18
500	229.4 ±0.58	170.50 ± 0.29
1000	442.3 ±0.93	366.53 ± 0.98
Ascorbate (500µg)	494.2 ±0.54%	
BHT(500µg)	483.2 ±1.6%	

Table 5: Superoxide anion ($O_2^{\cdot-}$) radicals quenching effects of water (AE) and ethanol (EE) extracts of *P. serratifolia.* Values are mean ± SD of three independent replication

	AE	EE
Concentration(µg/ml)	**% Inhibition ± SD**	**% Inhibition ± SD**
100	73.97 ± 1.78	48.35 ± 0.84
500	85.27 ± 1.63	70.02 ± 0.31
1000	90.32 ± 0.23	81.54 ± 0.18
Ascorbate(500µg)	94 ± 0.54%	
BHT(500µg)	83 ± 1.6%	

Table 6: Hydroxyl radical scavenging activity of water (AE) and ethanol (EE) extracts of *P. serratifolia.* Values are mean ± SD of three independent replications.

	AE	EE
Concentration(µg/ml)	**% Inhibition ± SD**	**% Inhibition ± SD**
100	36.531 ± 0.41	23.243 ± 0.21
500	58.289 ± 0.39	30.319 ± 0.20
1000	69.532 ± 0.78	42.023 ± 0.42
Ascorbate(500µg)	71 ±0.98	
BHT(500µg)	82 ±0.79	

Table 7: Hydrogen peroxide scavenging activities of water (AE) and ethanol (EE) extracts of *P. serratifolia*. Values are mean ± SD of three independent replications.

	AE	EE
Concentration(µg/ml)	**% Inhibition ± SD**	**% Inhibition ± SD**
100	42.384± 0.48	16.348 ± 0.18
500	68.182 ± 0.51	28.263 ± 0.29
1000	84.005 ± 0.73	59.002 ± 0.49
Ascorbate(500µg)	90 ± 5.1%	

Table 8: Metal chelating activities of water (AE) and ethanol (EE) extracts of *P. serratifolia*. Values are mean ± SD of three independent replications.

	AE	EE
Concentration(µg/ml)	**µg/ml**	**µg/ml**
100	26.18 ± 0.17	7.57 ± 0.05
500	43.95 ± 0.33	14.40 ± 0.09
1000	55.61 ± 0.47	25.70 ± 0.19
Ascorbate(500µg)	71 ± 0.98%	
BHT(500µg)	82 ± 0.79%	

CONCLUSION

Natural product antioxidants significantly contribute in preventions of pathological consequences caused by free radicals. Furthermore plant derived antioxidant are safer and cheaper than their synthetic counterparts. Total polyphenolic compounds were higher in the aqueous extract than alcoholic extract. Therefore, the plant *P.serratfolia* possesses strong antioxidant potential and it would be advantageous to use the plant antioxidant in therapeutic drugs for the implications of human health. Further studies are designed to isolate the lead molecule and to purify, characterize them in terms of their biological potentialities.

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