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Original Article

Phytochemical Screening and In Vitro Antioxidant Activity of Premna Serratifolia L. Leaf Extracts

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Abstract

The current work is based on the study of the antioxidant properties of *Premna serratifolia* L. *P. serratifolia* is a small shrub that belongs to the family Lamiaceae which has a high degree of medicinal properties and hence they are used for the treatment of several diseases. Phytochemical screening of leaf extracts showed the existence of various phytochemicals like tannins, alkaloids, resins, phenols, and coumarins. Antioxidant properties of *P. serratifolia* were assessed by DPPH as well as ABTS radical scavenging activity.

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Introduction

Medicinal plants have been a vital source of medical therapy preparation for human beings. The discovery and use of herbs with medicinal properties were closely linked with human civilization from ancient times (1). Due to the approximately 500,000 plants that exist worldwide, medicinal herbs have a bright future; most of which have not yet been investigated in the past, present, or even in future medical research. Almost all cultures turn to medicinal plants for treatment (2). Medicinal herbs are used for treating leprosy, intestinal worms, anorexia, skin, urinary disorder, diabetes, wounds, asthma, piles, blood morbidity, itching, and burning sensation and as curative of fever.

Various kinds of medicinal plants/herbs are used in certain countries to keep ants, flies, and fleeing away from offices and homes. Medicinal plant activities are mainly owing to the presence of certain phytochemicals. These phytochemicals are divided into primary metabolites and secondary metabolites. Carbohydrates, proteins, amino acids, and chlorophylls are primary metabolites. Tannins, flavonoids, steroids, saponins, and alkaloids, are secondary metabolites (3).

The medicinal plant *Premna serratifolia L.* is broadly distributed in subtropical and tropical areas and is often utilized in conventional drugs (4). *P. serratifolia* is a potent source for phytomedicine development in the future. This genus *Premna* originally categorized in the family Verbenaceae but now moved to the family Lamiaceae. It now consists of 200 species that are mostly found in tropical and subtropical Pacific Islands, Africa, Asia, and Australia (5). It is used by conventional practitioners as an antitumor, hepatoprotective, carminative, stomachic, anticoagulant, antibacterial, cardiotonic, etc. (6).

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In this current study, antioxidant activity, and phytochemical screening of *P. serratifolia* leaf extract were analyzed.

Materials & Methods

Plant materials

P. serratifolia was gathered locally during the January-February period.

Preparation of extract

The rinsed leaves were hot air-dried. The dried leaves of each plant were powdered using a mixer grinder. Then stored in airtight glass containers until used. The weighed sample was separately extracted with ethyl acetate, petroleum ether, and water using various solvents. The extracts were filtered and the residue of each extract was stored in a glass beaker and covered with aluminum foil.

Extractive Value

The extraction yield is represented as a proportion of the total mass of extracts (Mext) w.r.t. the mass of material used (7).

Qualitative Phytochemical Screening

Qualitative phytochemical analysis of different leaf extracts was done by standard procedure (7).

Antioxidant activity measurement

The antioxidant activity of *P. serratifolia* leaf extracts were assessed using DPPH as well as ABTS radical scavenging activity.

DPPH radical scavenging activity

It was found by mixing 1.25mL of the extracts and standard (gallic acid) at different concentration levels together with 1ml of 0.135mM DPPH radical in methanol. After being vortexed, the solution was preserved in the dark at 25°C for 30 minutes. The spectrophotometric absorbance at 517nm was assessed with methanol as a blank. The formula for calculating radical scavenging activity: Absorbance of (control-sample)/absorbance of control multiplied by 100 to get the DPPH inhibition % (8).

ABTS radical scavenging activity

A 7µM concentration of ABTS was prepared by dissolving it in water. A reaction between ABTS stock solution and 2.45µM potassium per sulfate was used to create $ABTS^+$, which was then stored in the dark for 12 to 16h at room temperature before usage. When maintained in the dark, the radical remained stable in this condition for over 2 days. The $ABTS^+$ solution-containing samples were diluted with "redistilled" water to an absorbance of 734nm and equilibrated at 30°C for the infusion research. A0 was recorded as the reagent blank. The absorbance measurement was assessed precisely 6min after the first mixing (A_t) of 3.0ml of diluted $ABTS^+$ with 30 l of polyphenolic extracts. All analyses were carried out in triplicate (9). The formula used to determine the ratio of ABTS inhibition was [absorbance of (control-sample)/absorbance of control]x100.

Results

Preliminary phytochemical analysis of different *P. serratifolia* extracts

Preliminary phytochemical screening of distilled water, ethyl acetate, and petroleum ether extract of *P. serratifolia* exhibited the existence of flavonoids, alkaloids, steroids/triterpenoids, tannins, coumarins, phenols, and resins (Table 1).

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Table 1. Phytochemical analysis of different *P. serratifolia* extracts

Phytochemical constituents	Leaf extracts of *P. serratifolia*		
	Distilled water	Ethyl acetate	Petroleum ether
Alkaloids	+	+	+
Flavonoids	+	+	+
Steroids/triterpenoids	+	+	+
Tannins	+	+	+
Coumarins	+	+	+
Phenols	+	+	+
Resins	+	+	+

***In vitro* antioxidant activity of different *P. serratifolia* extracts**

The antioxidant activity of *P. serratifolia* was examined by the DPPH as well as ABTS radical scavenging activity.

DPPH radical scavenging activity in diverse *P. serratifolia* extracts

In vitro, antioxidant test by DPPH radical scavenging activity revealed the presence of antioxidant potential in the various extracts of *P. serratifolia* (Figure 1). DPPH ("1,1-diphenyl-2-picrylhydrazyl radical") is a stable free radical that may receive hydrogen or electron from antioxidants to form a stable molecule. In DPPH radical scavenging activity case, in *P. serratifolia*, ethyl acetate, distilled water, & petroleum ether extract showed the percentage of inhibition of 48.61%, 68.02%, and 23.25% respectively. In the case of ethyl acetate extract, *Premna* shows the highest percentage of inhibition. The proportion of inhibition showed that the plant extract scavenged free radicals in a concentration-dependent style in all of the extracts. IC_{50} values of different extracts were computed (Figure 2).

Figure 1.

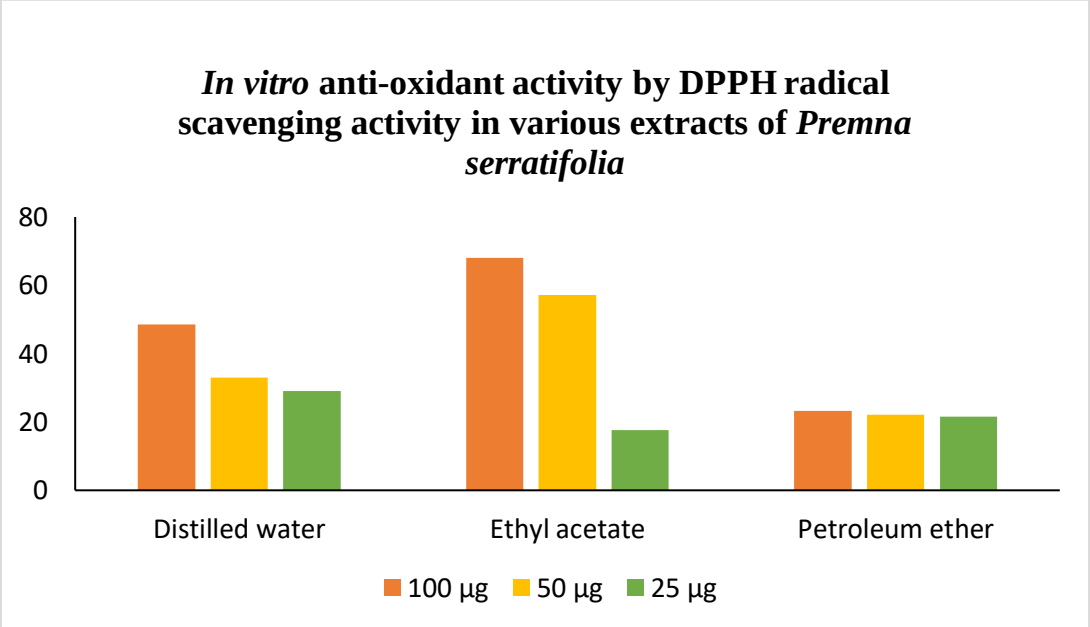

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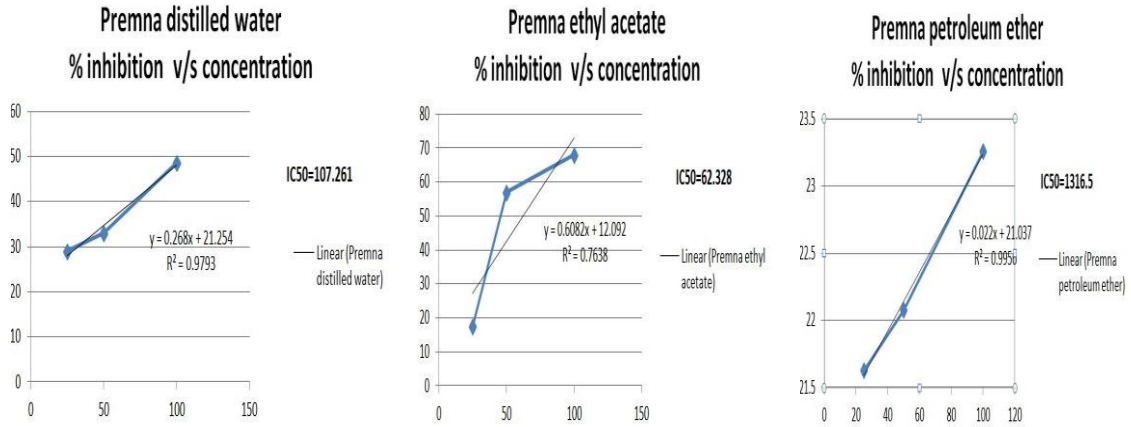
Figure 2: IC_{50} for DPPH radical scavenging activity



***In vitro* ABTS radical scavenging activity in diverse *P. serratifolia* extracts**

In the case of *P. serratifolia*, distilled water extract was found to possess 52.85% of inhibition, while ethyl acetate and petroleum ether extract has 45.71%, and 6.57% respectively (Table 2). The maximum ABTS•+ radical cation scavenging activity in distilled water extract *of P. serratifolia* at 30 µg/mL concentration was compared with standard Rutin.

Table 2. *In vitro* ABTS radical scavenging activity in different *P. serratifolia* extracts

Extract	OD	Percentage of inhibition	mg equivalents of Rutin
Distilled water	0.330	52.85	270
Ethyl acetate	0.380	45.71	300
Petroleum ether	0.654	6.57	470

Discussion

The leaves, bark, roots, and fruit of *P. serratifolia* were used in traditional medicine to cure a variety of ailments, like cardiovascular disorders, inflammation, rheumatism, cough, diabetes, and stomach disorders. Pharmacological analyses of *P.serratifolia* was conducted by antioxidant activity.

Phytochemical analysis of different leaf extracts of *P. serratifolia* showed the existence of steroids, alkaloids, flavonoids, triterpenoids, phenols, coumarins, tannins, and resins. DPPH and ABTS radical scavenging assay revealed that *P. serratifolia* possesses antioxidant activity. In the ABTS radical scavenging activity, distilled water extract of *P. serratifolia* showed a high percentage of inhibition 52.85%. Ethyl acetate extract of *P. serratifolia* has shown the highest percentage of DPPH inhibition.

Numerous studies have shown that the presence of phytochemicals gives the plants under study pharmacological and physiological properties that may be used to help treat several illnesses. The existence of the detected phytochemicals could be responsible for the extracts' antioxidant activity. Tannins and flavonoids are plant phenolics and phenolic compounds are a significant class of chemicals that function as free radical scavengers or main antioxidants (10). Terpenoids function similarly to vitamins

in that they control metabolism and serve as antioxidants to provide protection. The leaf's pharmacological activity is caused by the presence of recognized phytochemicals (11).

The uses of plants in the treatment and management of various ailments may be highly impacted by the antioxidant activity of plant extracts. Antioxidants lessen oxidative stress, which is caused by dangerous free radicals that damage vital biomolecules and cause injury to cells. They stop free radical chain reactions by eliminating free radical intermediates and limiting additional oxidation activities (12). From the literature, it may be inferred that the existence of phytochemical constituents indicates the medicinal behavior such as the anti-oxidant behavior of the plants.

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