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Antioxidant Activity of *Premna serratifolia* Linn. Leaf Extracts: A Comprehensive Analysis Using Various Testing Methods

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***Abstract*— *Premna serratifolia* Linn leaf possesses medicinal properties, useful in the treatment of cardiovascular diseases, skin diseases, inflammatory diseases, arthritis, rheumatism, anorexia, jaundice, intestinal worms, bad breath, lung infections, febrifuge, hypolipidemic, antioxidants, antidiabetic, CNS depressant and thalasemia treatment, among other things. The purpose of this study was to investigate the antioxidant effect of *P. serratifolia* Linn leaf from Indonesia. The leaves of *P. serratifolia* Linn were extracted with 95% ethanol and fractionated with n-hexane and ethyl acetate. The antioxidant activity was evaluated by various antioxidant assays, including 1-diphenyl-2-picrylhydrazyl (DPPH), 2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS), nitric oxide scavenging and reducing power ability method. The extracts and fractions contained phytoconstituents such as alkaloid, flavonoid, phenolic, tanin, saponin, and steroid compounds, according to phytochemical analysis. The antioxidant activities were compared to standard antioxidant ascorbic acid. *P. serratifolia* Linn ethanol leaves extract showed a significant antioxidant activity in DPPH, ABTS, reducing power and nitric oxide scavenging methods compared others. The findings of the present study suggest that *P. serratifolia Linn* could be a potential source of natural antioxidant that could have greater importance as therapeutic agent in preventing or slowing oxidative stress related degenerative diseases.**

***Keywords*— *Premna serratifolia* Linn.; antioxidant; DPPH radical; ABTS; reducing power; nitric oxide scavenging**

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I. INTRODUCTION

Premna serratifolia Linn is an important plant belonging to the family Verbenaceae, and is one of the most widespread large shrubs in the forests of Indonesia. The whole plant possesses medicinal properties. *P. serratifolia* Linn. (bebuas) is a tropical and subtropical medicinal herb that is widely spread and frequently utilized in the traditional system of medicine. *P. serratifolia* is a thorny, erect shrub or small tree with a scandent stem and big branches [1-4]. The leaves are either opposite or whorled and they are either whole or serrate. *P. serratifolia* has a lot of therapeutic usefulness and it's been studied a lot [5-8]. The *P. serratifolia* leaf possesses medicinal properties, useful in the treatment of cardiovascular diseases, skin diseases, inflammatory diseases, arthritis, gonorrhea, rheumatism, anorexia, jaundice, colds, intestinal worms, bad breath, lung infections, diarrhea, rheumatism, headaches, febrifuge, hypolipidemic, antioxidants, anti-inflammatory, antidiabetic, CNS depressant, antitumor activity and can help restore women's health after childbirth have all been reported in previous pharmacological studies [6, 9-12]. Food stability, human

health, and nutrition all benefit from antioxidants [13-15]. ROS and free radicals are involved in the development of diseases like atherosclerosis, cancer, aging, rheumatoid arthritis, and irritation [16-20]. Antioxidants such as tocopherols, vitamin C, carotenoids and phenolic chemicals are abundant in plants [21-24]. Other antioxidants demonstrated to have health advantages include olive oil phenolics, tocotrienols, oryzanol, squalene, sesame lignans, pycnogenol, flavonoids, isoflavones, resverator and alpha lipoic acid [25-28]. However, the most often used synthetic antioxidants are BHA (tert-butyl-4-hydroxy-anisol) and BHT (tert-butyl-4-hydroxytoluene), both of which are suspected of causing liver damage [10,12,29,30]. As a result, it's critical to use various screening procedures to look for natural antioxidants that are both safe and efficient [10-12,29-31].

Many medicinal plants have become key research targets for discovering chemical compounds with high antioxidant potential that can protect cells from damage caused by free radicals and reactive oxygen species (ROS) [8-10,32-35]. Total antioxidant tests which employ absorption spectroscopy are used to compare the antioxidant activity of various compounds [9,10,36-38]. Many of these assays have been used to characterize antioxidants. The loss of absorbance at a specific wavelength can be used to track antioxidant activity. Antioxidants are substances that prevent or slow the oxidation of other molecules by preventing the onset or propagation of an oxidizing chain reaction [1,4,9,39].

P. serratifolia Linn. is one of the most common plants in the forest of Jambi in Indonesia. The whole part of plant has medicinal characteristics that can be used several diseases. *Premna serratifolia* Linn is an essential medicinal herb in Indonesia, where it's known as Bebuas. The leaf is used in a well-known jamu mixture for a number of medicinal purposes. *Bacillus subtilis*, *Staphylococcus aureus* and *Escherichia coli* were all resistant to ethanolic compounds found in the leaves of *Premna serratifolia* Linn. In both immunological and non immunological experimental models, decoction of the pant revealed significant anti-inflammatory and anti-arthritic efficacy against acute, subacute and chronic inflammation.

P. serratifolia leaves have previously been found to contain a variety of glycosides, including iridoid glycosides and phenylethanoids like premnethanoside A and B, some xanthones, steroids and saponins, flavonoids, triterpenoids, and diterpenoids (including premnones A and C), flavonoids, triterpenoids, and diterpenoids (including premnones A Volatile oil was discovered in *P. serratifolia* flower buds, primarily consisting of 1-octen-3-ol, (Z)-n-hexanol, 2-phenyl ethyl alcohol, (E,Z)-2,4-nonadienal, and linalool [3,4,9,]. Premcoryoside, a Verbascoside iridoid glycoside conjugate, was isolated from the leaves of *P. corymbosa* var. obtusifolia together with Verbascoside and three monoacyl 6-O-alpha-L-rhamnopyranosylcatalpols. Flavonoid and alkaloid are the main compounds found in this plant [7]. The volatile elements of *P. serratifolia* flower buds were extracted from a hexane concrete by vacuum distillation and the principal components are 1-octen-3-ol (16.9%), (Z)-3-hexenol (10.2%), 2-phenylethyl alcohol (8.9%), (E,Z)-2,4-nonadienal (6.2%), (E,Z)-2,6-nonadienal (5.0%), and linalool (4.4%) [1-4,8,9,19]. However, there hasn't been any extensive pharmacological testing of the *Premna serratifolia* Linn. leaf extract. Literature survey revealed that there was no work has been done on the antioxidant activity of *Premna serratifolia* Linn, against radical scavenging. This herb has been used to treat cardiovascular illness, arthritis and inflammation for centuries. This piqued our interest, so we decided to test it for antioxidant efficacy in vitro. The free radical scavenging activity of *P. serratifolia* Linn. leaves ethanol, n-hexane and ethyl acetate extracts was investigated in this work.

II. MATERIAL AND METHODS

Study Area

The bebuas (*P. serratifolia*) leaves were collected from Kuala Dendang village, Dendang district, Tanjung Jabung Timur Regency, Jambi Province, Indonesia. The specimen was taxonomically identified and confirmed by a taxonomist from the Research Center for Plant Conservation and Botanic Gardens, Indonesian Institute of Sciences. A voucher specimen (No. B-338/IPH.3/KS/II/2020) was deposited. This study was conducted at the Department of Pharmaceutical Biology, Faculty of Pharmacy, Universitas Padjadjaran, Indonesia.

Chemicals

DPPH [1,1-Diphenyl, 2-picryl-hydrazyl] (Sigma-Aldrich, St. Louis, MO, USA), Naphthylethylenediamine dihydrochloride (PanReac AppliChem, Darmstadt, Germany), 2'-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) (Sigma-Aldrich, St. Louis, MO, USA). Thiobarbituric acid (TBA) and Trichloro acetic acid (Sigma-Aldrich, St. Louis, MO, USA). All other reagents used were of analytical grade and were purchased from Sigma-Aldrich (Saint Louis, MO, USA) and PanReac AppliChem (Darmstadt, Germany).

Extraction of P. serratifolia leaves

The extract was made using 2 kg of dried *P. serratifolia* leaves in a sequential order by ethanol, which were then fractionated using n-hexane and ethyl acetate. *P. serratifolia* leaves were ground and extracted three times with 25 L of ethanol using maceration procedures at room temperature (24 h). Using a rotary evaporator, the macerate was concentrated, evaporated, and dried in a vacuum at 60°C (buchi rotavapor R-205). The percentage yields for ethanol, n-hexane, and ethyl acetate were 22.7%, 16.3% and 9.5% w/w, respectively. The dry extract was kept at 4°C in the refrigerator until it was needed. These extracts were used for the assessment of in vitro antioxidant screening and preliminary phytochemical screening.

Phytochemical screening

In accordance with qualitative phytochemical screening tests, the researchers carried out phytochemical screening of the ethanol, n-hexane and ethyl acetate extracts. The extracts were then subjected to preliminary phytochemical screening [8-12] to detect the presence of polyphenolic compounds and other chemical constituents such as alkaloids, flavonoids, saponins, triterpenoids, steroids, tannins, glycosides and phenolics [8-11]. The qualitative chemical test performed were Shinado test, Ammonia fuming test, lead acetate test, boric acid test for flavonoids and ferric chloride test, nitric acid test, ammonia hydroxidepotassium ferricyanide test, lead acetate test for tannins. All the tests confirmed the presence of flavonoids and tannins in all extracts.

Antioxidant Activity Evaluation

Scavenging Of DPPH Radical

This assay [9,10,11] based on the measurement of the scavenging ability of antioxidant test extracts towards the stable radical. The free radical scavenging activity of the ethanol, n- hexane and ethyl acetate extracts of *Premna serratifolia* Linn leaves were examined in-vitro using DPPH [1,1-Diphenyl, 2-picryl-hydrazyl] radical. The test extracts were treated with different concentrations from a minimum of 4 µg/mL to maximum of 250 µg/mL. The reaction mixture consisted of 1 ml of 0.1 mM DPPH in ethanol, 0.95 ml of 0.05 M Tris-HCl buffer (pH - 7.4), 1 ml of ethanol and 0.05 ml of the herbal extract. Thereafter, the absorbances of the mixture were determined in a spectrophotometer (Shimadzu 1601 UV-Visible pectrophotometer) at 517 nm exactly 30 sec after adding extracts. The experiment was performed in triplicate and percentage of scavenging activity was calculated using following equation,

DPPH radical scavenging (%) = [(control absorbance - sample absorbance) / control absorbance] x 100.

The blank was also carried out in similar manner, using distilled water in the place of extracts. The activity was compared with ascorbic acid, which was used as a standard antioxidant.

ABTS Radical Scavenging Assay

The ABTS assay was performed according to the methodology adapted from Miller et al. [35]. ABTS was prepared using 7 mM ABTS and 140 mM of potassium persulfate incubated at room temperature without light for 16 h. The solution was then diluted with phosphate-buffered saline until it reached an absorbance of 0.700 ± 0.02 at 734 nm. To measure the antioxidant capacity, 2.97 mL of the ABTS solution was transferred to the cuvette, and the absorbance at 734 nm was determined using a Shimadzu 1601 UV-Visible spectrophotometer. Then, 0.03 mL of the extracts was added to the cuvette containing the ABTS radical, and after 5 min, the second reading was performed. The data were expressed as percent scavenging.

semisolid form or (after removal of the solvent) in dry powder form, and are intended for oral or topical application.

Scavenging of Nitric Oxide

The tubes contain sodium nitroprusside (5 µM) (Sigma-Aldrich, St. Louis, MO, USA) in standard phosphate buffer solution and the different concentration of the ethanol, n-hexane and ethyl acetate extracts dissolved in standard 0.025 M phosphate buffer (pH 7.4) were incubated at 25°C for 5 hours [9,11,14]. 0.5 ml of the incubation solution was withdrawn after 5 hours and diluted with 0.5 ml of Griess reagent (prepared by mixing equal volume of 1% sulphanilamide in 2% phosphoric acid and 0.1% naphthylethylene diamine dihydrochloride in water). At 546 nm, the absorbance of the chromophore produced was measured in a spectrophotometer (Shimadzu 1601 UV-Visible spectrophotometer) at 546 nm.

Reducing Power Determination

Different amounts of the extracts in methanol [9,11,13] were mixed with 2.5 ml of (pH 6.6) 0.2 M phosphate buffer and 2.5 ml of 1% potassium ferricyanide [$K_3Fe(CN)_6$] (Sigma-Aldrich, St. Louis, MO, USA). The mixture was incubated at 50 ºC for 20 minutes. 2.5 ml of 10% trichloroacetic acid was added to the mixture, which was then centrifuged for 10 minutes at 1000 rpm. 2.5 ml upper layer of solution was mixed with 2.5 ml of distilled water and 0.5 ml of 0.1% ferric chloride. The absorbance was measured at 700 nm in a spectrophotometer (Shimadzu 1601 UV-Visible spectrophotometer). The blank was also carried out in similar manner, using distilled water in the place of extracts. Increase in the absorbance of the reaction mixture indicated the increase in the reducing power. The activity was compared with ascorbic acid, which was used as a standard antioxidant.

Data analysis

The experimental results included three replications, and the data were expressed as mean ± standard deviation (SD). The data were analyzed by an ANOVA ($p < 0.05$) using SPSS, and $p < 0.05$ was considered to be statistically significant.

III. RESULT AND DISCUSSION

Results

Recent interest in plant-derived compounds has increased due to their diverse applications. Medicinal plants are the richest bioresource of traditional medicines, modern medications, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates, and chemical entities for synthetic drugs. Extraction (as the term is used in the pharmaceutical industry) is the separation of medicinally active plant tissues using selective solvents and standard processes. The products so derived from plants are relatively complicated combinations of metabolites, in liquid or

Employing selected solvents, pharmaceutical extraction procedures separate the medicinally active sections of plant

tissues from the inactive/inert components. During extraction, solvents diffuse into solid plant material and solubilize similar polarity compounds.

Properties such as cardiotonic, anti-coagulant, anti-inflammatory, anti-hyperglycemic, anti-parasitic, antioxidant, stomachic, carminative, hepatoprotective, antibacterial, and anticancer are all possessed by the plant known as *Premna serratifolia* Linn. In order to treat a wide variety of infectious disorders, the majority of the plant parts of the *Premna serratifolia* Linn. species have been utilized in the traditional medical practice of Indonesia. During the course of this research, phytochemical screening was performed on three dry extracts of *Premna serratifolia* Linn. leaves. The results of the phytochemical screening (Table 1) gave the results.

Table 1. Phytochemical screening of *Premna serratifolia* Linn. leaf extract

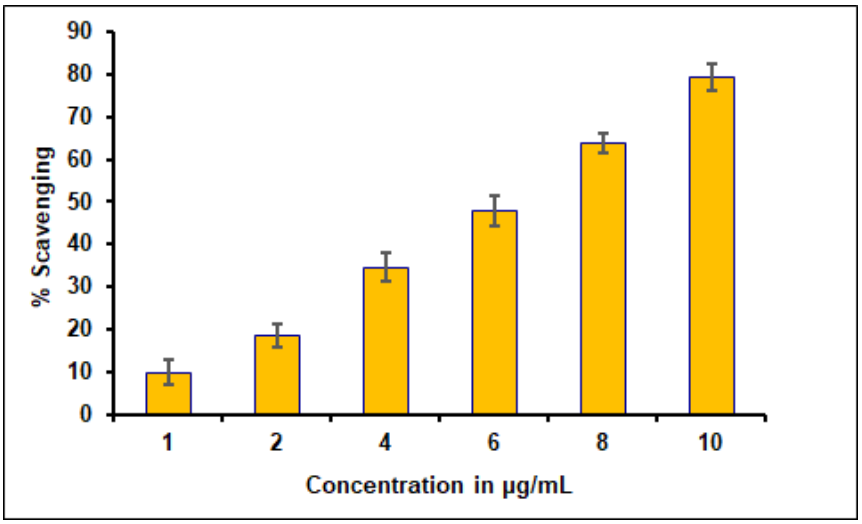
	Ethanol Extract	Hexane Extract	Ethyl acetate Extract
Alkaloids			
Meyer	-	-	+
Dragendorff	+	-	+
Flavonoids	+	-	+
Phenolics/Tanins	+	-	+
Saponins	+	-	-
Steroids	+	+	-
Terpenoids	-	-	-

Note: - : negative, + : positive

Based on the results of phytochemical screening performed on the extracts, the extracts contained a variety of phytoconstituents, including alkaloids, flavonoids, phenolics, tanins, saponins, and steroids. These results were positive. An in-depth examination into the connection between phytoconstituents and the antioxidant capabilities of the extracts may shed information on the specific chemical constituents of the extracts that are responsible for the antioxidant activity.

DPPH Scavenging

In a concentration-dependent manner, the ethanol, n-hexane, and ethyl acetate extracts of the leaves of *P. serratifolia* Linn. demonstrated promising free radical scavenging properties of DPPH up to 250 µg/mL. In terms of the ability to scavenge free radicals, the ethanol extract was superior to both the n-hexane and ethyl acetate extracts. Ascorbic acid, the reference standard, had significant radical scavenging potential when it was present in a concentration of 10 µg/mL. Ascorbic acid had a suppressive effect on the DPPH radical that was 79.24% [Figure 1a], whereas the ethanol, n-hexane, and ethyl acetate extracts had suppressive effects that were 75.21%, 52.02%, and 68.65% respectively [Figure 1b]. Table 2 shows the IC_{50} values obtained using the DPPH scavenging method for extracts of ethanol, n-hexane, and ethyl acetate, as well as ascorbic acid.



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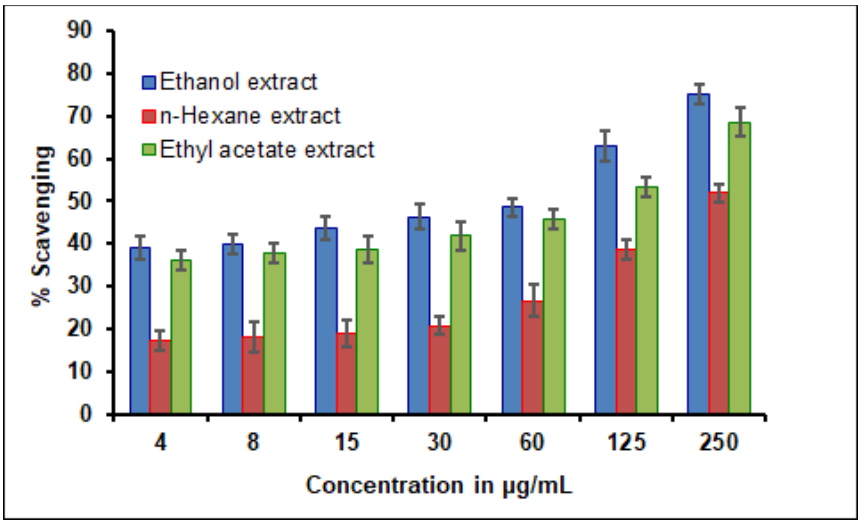

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Figure 1. (a) In vitro free radical scavenging effect of Ascorbic acid by DPPH method; and (b) In vitro free radical scavenging effect of *P. serratifolia* Linn. leave extracts, by DPPH method.

DPPH radical scavenging activity of *P. serratifolia* Linn. leaf extracts, each value is expressed as mean ± SD (n = 3), means within each graph are significantly different, $p < 0.05$.

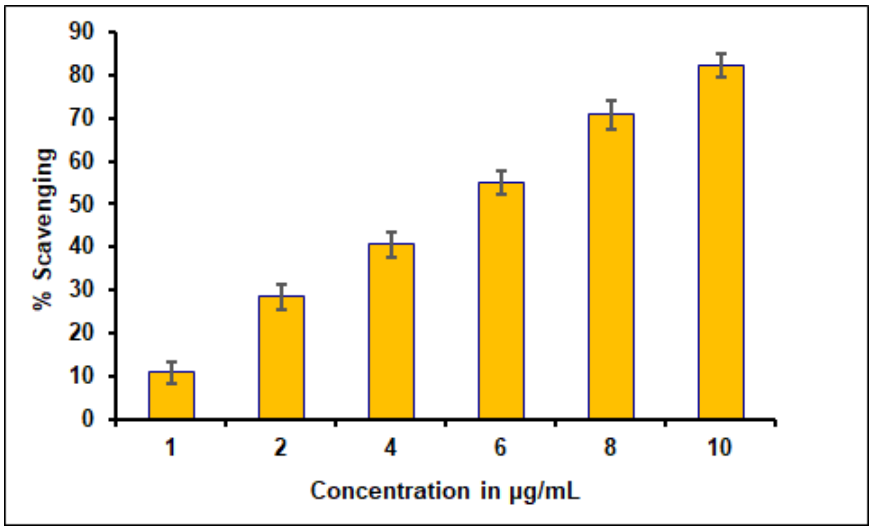
Table 2. Antioxidant activity of *P. serratifolia* Lin. leave extracts by DPPH Method

Extracts	IC_{50} (µg/mL)
Ethanol	64.41 ± 2.23
n-Hexane	224.80 ± 2.26
Ethyl acetate	101.46 ± 2.41
Ascorbit acid	6.20 ± 2.11

Data are means ± SD (n = 3).

ABTS Scavenging

The ability of the ethanol, n-hexane, and ethyl acetate extracts of *Premna serratifolia* Linn. Leaf to scavenge stable free ABTS (2,20-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) radicals was used to test the antioxidant potential of these extracts; the findings are given in Figure 2. As a result of ABTS radical scavenging, the antioxidant capacity of ascorbic acid was 82.24% [Figure 2a], whereas the antioxidant capacity of ethanol, n-hexane, and ethyl acetate extracts were 79.01%, 57.21%, and 82.65%, respectively [Figure 2b]. According to the results of the ABTS test, the ethyl acetate extract of *Premna serratifolia* leaves exhibited the highest antioxidant capacity. It's possible that the presence of flavonoids and phenolics is to blame for this. Table 3 show IC_{50} value from ethanol, n-hexane, ethyl acetate extracts and ascorbic acid by ABTS scavenging method.



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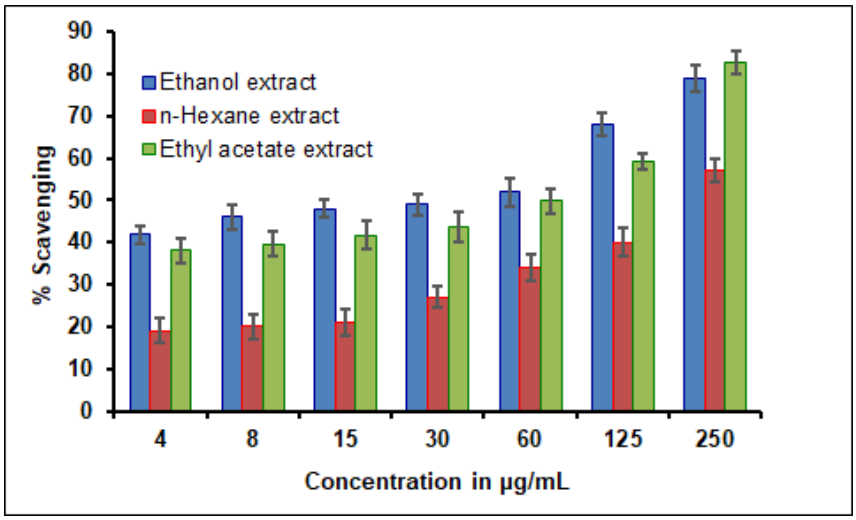

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Figure 2. (a) In vitro free radical scavenging effect of Ascorbic acid by ABTS method; and (b) In vitro free radical scavenging effect of *P. serratifolia* Linn. leave extracts, by ABTS method.

ABTS radical scavenging activity of *P. serratifolia* Linn. leaf extracts, each value is expressed as mean ± SD (n = 3), means within each graph are significantly different, $p < 0.05$.

Table 3. Antioxidant activity of *P. serratifolia* Lin. leave extracts by ABTS Method

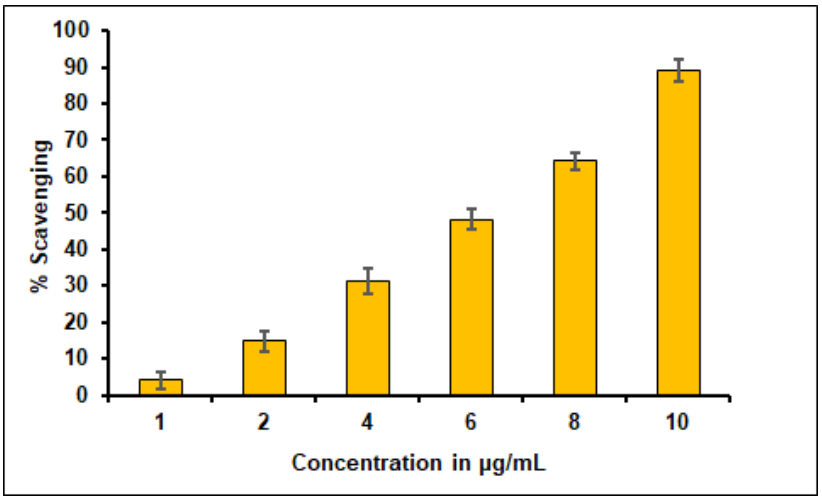
Extracts	IC_{50} (µg/mL)
Ethanol	37.23 ± 3.14
n-Hexane	194.65 ± 3.25
Ethyl acetate	66.23 ± 2.86
Ascorbit acid	5.43 ± 2.64

Data are means ± SD (n = 3).

Nitric oxide scavenging

An ethanol extract of the leaves of *P. serratifolia* Linn. showed significant free radical scavenging action against the nitric oxide (NO) induced release of free radicals at a concentration of 250 µg/mL. The extract had a percentage of scavenging that was 58.12%, which was higher than the percentages of scavenging that were achieved by n-hexane and ethyl acetate extracts, 41.35% and 52.92%, respectively

[Figure 3b], which Ascorbic acid served as the reference standard, and its percentage of inhibition was found to be 89.16% [Figure 3a]. Table 4 shows the IC_{50} value calculated using the nitric oxide scavenging method for extracts of ethanol, n-hexane, and ethyl acetate, as well as ascorbic acid.



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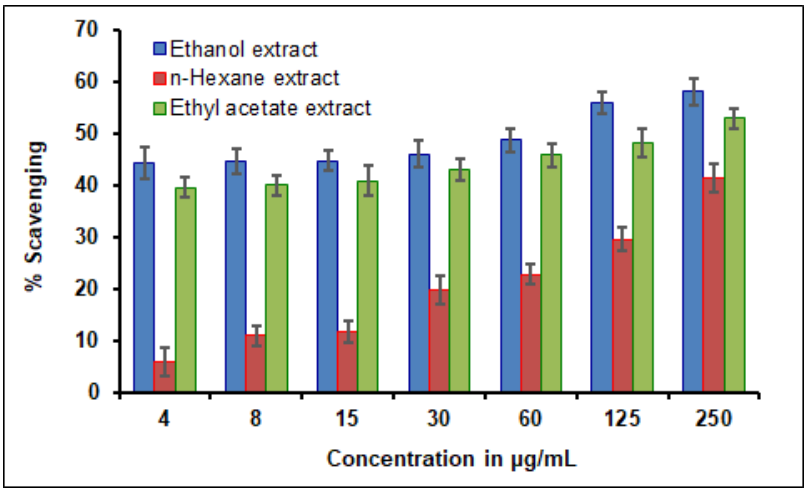

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Figure 3. (a) In vitro free radical scavenging effect of Ascorbic acid by Nitric Oxide Scavenging Method; (b) In vitro free radical scavenging effect of *P. serratifolia* Linn. leave extracts, by Nitric Oxide Scavenging Method.

Nitric oxide scavenging activity of *P. serratifolia* Linn. leaf extracts, each value is expressed as mean ± SD (n = 3), means within each graph are significantly different, $p < 0.05$.

Table 4. Antioxidant activity of *P. serratifolia* Linn. leave extracts by Nitric Oxide Scavenging Method

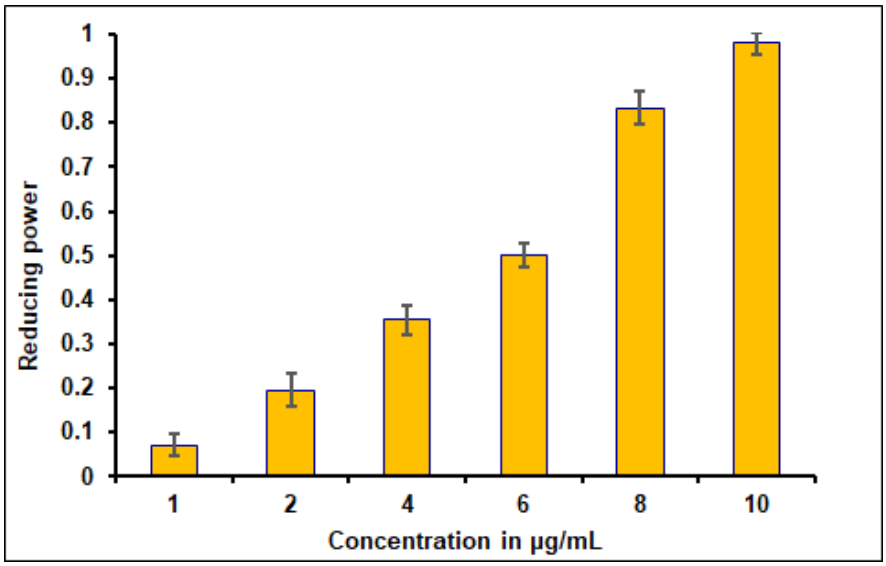
Extracts	IC_{50} (µg/mL)
Ethanol	87.57 ± 2.18
n-Hexane	298.19 ± 1.85
Ethyl acetate	176.61 ± 2.22
Ascorbit acid	6.05 ± 1.96

Data are means ± SD (n = 3).

Reducing power determination

The fact that the absorbance increased as the concentration of the extracts increased demonstrates that the extracts of ethanol, n-hexane, and ethyl acetate have high antioxidant activity because they have a lower power ability. When evaluated against the standard antioxidant ascorbic acid, the reducing capacity of the ethanol extract was found to be

higher than of the n-hexane and ethyl acetate extracts [Figure 4a and Figure 4b].



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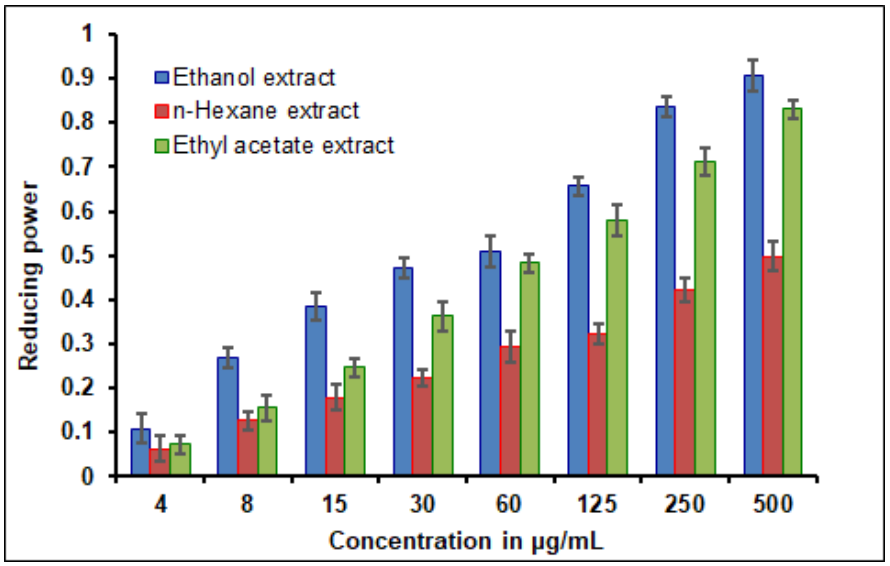

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Figure 4. (a) In vitro free radical scavenging effect of Ascorbic acid by Reducing Power Determination; (b) In vitro free radical scavenging effect of *P. serratifolia* Linn. leave extracts, by Reducing Power Determination

Reducing power of *P. serratifolia* Linn. leaf extracts, each value is expressed as mean ± SD (n = 3), means within each graph are significantly different, $p < 0.05$.

Discussion

P. serratifolia leaves extracted with ethanol, hexane, and ethyl acetate were found to contain secondary metabolites such as alkaloids, flavonoids, phenolics, tanins, saponins, and steroids. These compounds were shown to be present in the extracts (Table 1). According to the findings of this research, the secondary metabolites that may be discovered in the leaves of *P. serratifolia* have been shown to possess therapeutic properties, including an antioxidant function. The following antioxidant activity assay will have its basis laid by the screening for antioxidant properties of phytochemicals that were discovered in the ethanolic extract of *P. serratifolia*. Because hexane has a tendency to selectively extract nonpolar component groups like steroids, phytochemical screening revealed that the chemicals extracted in ethanol and ethyl acetate solvent were more diverse than those extracted

in hexane [1-4,6]. This was the case because hexane has a tendency to selectively extract nonpolar component groups like steroids.

Based on the phytochemical screening, the positive ethanol extract contains phenolic and flavonoid components. This conclusion was consistent with the typical compound groups of the premna genus. The most common secondary metabolites in premna plants are diterpenoids, iridoid glycosides, and flavonoids, followed by sesquiterpenes, lignans, phenylethanoids, megastigmanes, glyceroglycolipids, and ceramides [1-4,9,17,20,37-39]. The phytochemical analysis of the extract revealed the presence of antioxidant alkaloids, flavonoids, and steroids. This scientific evidence supports the use of *P. serratifolia* leaves as an anti-inflammatory medication, which is frequently employed in several country in Asian to treat a variety of inflammatory disorders [1-4,34].

In traditional medicine, it is believed that specific plants can treat a variety of pathological conditions. The claimed therapeutic reputation needs to be backed up by solid scientific data. The leaves of *P. serratifolia* Linn., one example of such a medicine, were utilized in the present experiment. Extracts of the leaves of *P. serratifolia* Linn. have a notable capacity for scavenging free radicals. Reactive oxygen species, both endogenous and exogenous, have been shown to have a role in the pathophysiology of a variety of diseases, including atherosclerosis, diabetes, cancer, arthritis, and the aging process [1-4,9,20-23,31,39]. Antioxidants that are able to "scavenge" reactive oxygen species are likely to be of help in the treatment of various illnesses as a result.

The free radical scavenging activities of the extracts were evaluated based on their capacity to remove man-made DPPH. This assay was very helpful in providing information on the compounds' reactivity with stable free radicals. This was made possible by the odd number of electrons in the compounds. One of the most prominent absorption bands of DPPH can be found in the visible spectrum at a wavelength of 517 nm. Because of the presence of free radical scavenger, the absorption disappears when the electron is paired off, and the resulting discoloration is stoichiometrically equal to the amount of electrons taken up. The bleaching of DPPH uptake is a representation of the ability of the ethanol, n-hexane, and ethyl acetate extracts to scavenge free radicals independently [1-4,9-11].

In the ABTS radical scavenging assay, the ethyl acetate extract of *P. serratifolia* leaves demonstrated the highest antioxidant capacity. This was determined by the ABTS assay. It's possible that the presence of flavonoid and phenolic compounds is responsible for this. The fact that the combination of the numerous organic chemical constituents present in ethyl acetate extract have a synergistic effect, increasing the biological activity, or, alternatively, an antagonistic effect may be related to the fact that this sample has a high free radical scavenging effect. This may be due to the fact that the combination of these constituents has a synergistic effect. In addition, bioactive flavonoid and phenolic compounds exhibit antioxidant activity. These compounds belong to the class of phenolics.

Nitric oxide is a gas that is required for a variety of physiological processes, such as the transmission of signals in the brain, the reaction of the immune system, and the regulation of blood pressure [9,10]. Nitric oxide levels, on the other hand, were shown to be significantly increased in patients suffering from a wide range of pathological conditions, such as cardiovascular disease, diabetes, and others [9-11]. Sodium nitroprusside is the most important contributor to the production of free radicals. As a marker for nitric oxide scavenging capacity, the chromophore that is formed following nitrite diazotization with sulphanilamide and subsequent coupling with napthylethylene diamine [28,32,36-38]. In the presence of leaf extracts (ethanol, n-hexane, and ethyl acetate extracts) of *P. serratifolia* Linn., the production of the chromophore was not completed. Since the leaf extracts scavenge the NO that is produced as a result of the sodium nitroprusside, the absorbance decreases as the concentration of the extracts increases in a dosage-dependent way.

The presence of reducing properties is frequently connected with the presence of reductones, which have been shown to have antioxidant properties by breaking the chain of free radicals by donating an atom of hydrogen [9,11]. The existence of reductones is often connected with the presence of reducing properties. It has also been discovered that reductones can react with the various peroxide precursors, hence preventing the production of peroxide. The increase in absorbance of the extracts is indicative of the presence of antioxidant action [9-12].

During early phytochemical screening, the presence of flavonoids, phenolics, and tannins in ethanol and ethyl acetate was detected, and these flavonoids have been proven to exhibit a variety of biological activities related to antioxidant mechanisms [36-38]. The ethanol extracted from *P. serratifolia* Linn. leaves suppresses DPPH radical scavenging, nitric oxide radical generation, and reducing power more efficiently than n-hexane and ethyl acetate extracts. The ethanol extract has a cardiotonic impact [14,18-21], and the in vitro antioxidant activity supports the antioxidant principles responsible for its cardiotonic and antioxidant activity.
The findings of this research with *P. serratifolia* Linn. leaf extracts suggested the presence of possible antioxidant activity. This activity has strong contacts with therapeutic application in the treatment of cardiovascular disorders within the community of traditional medicine. Flavonoids are found in high concentrations in plants belonging to the

Verbenaceae family, and bioflavanoids are well-known for the cardiotonic and antioxidant qualities they possess. The investigation for the biomolecules that are necessary for the antioxidant capacity is still active at this time. In conclusion, the findings of this study support the view, that the ethanol extract of plants are promising sources of potential antioxidant and may be efficient as preventive agents in some diseases. It can be concluded that the ethanol and ethyl acetate extracts of *P. serratifolia* Linn. leaves contain several metabolite secundery with antioxidant activity. Antioxidant activity be attributable to the presence of compounds such as flavonoids and phenolic. The ethanol extract of *P. serratifolia* Linn. leaves inhibits DPPH radical scavenging, ABTS radical scavenging, nitric oxide radical production and reducing power more effectively than n-hexane and ethyl acetate extracts.

IV. CONCLUSION

The findings of this study support the view, that the ethanol extract of plants are promising sources of potential antioxidant and may be efficient as preventive agents in some diseases. It can be concluded that the ethanol and ethyl acetate extracts of *P. serratifolia* Linn. leaves contain several metabolite secundery with antioxidant activity. Antioxidant activity be attributable to the presence of compounds such as flavonoids and phenolic. The ethanol extract of *P. serratifolia* Linn. leaves inhibits DPPH radical scavenging, ABTS radical scavenging, nitric oxide radical production and reducing power more effectively than n-hexane and ethyl acetate extracts.

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