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Phytochemical analysis and evaluation of antioxidant activity of *Premna latifolia* Roxb. A medicinal plant (Family: Lamiaceae)

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Abstract
Traditional medicine employing a plant-based system of treatment forms an important part of human medicine system. The most common reason for utilizing traditional medicine is its non- toxic nature, closely resembles the patient ideology and allays concerns about the adverse effects of chemical medicines, satisfies the desire for more personalized health care, and allows greater public access to health information. Plants and their bioactive principles have a long history of use in modern medicine and in certain systems of traditional medicine. Plant-derived substances have recently become of great interest owing to their versatile applications.

The bark and leaves extract of *Premna latifolia* Roxb. with different solvents were investigated for their phytochemical and antioxidant potential. The phytochemical composition was carried on the bark and leaves extracts of *Premna latifolia*, revealed the presence of active ingredients such as Glycosides, Steroids, Saponins, Phenols, Flavonoids, Terpenoids, and Tannins. The bark and leaves extracts were also evaluated for antioxidant activities by DPPH radical and ABTS scavenging assay. Among four different solvents used, the maximum antioxidant activity found in methanolic extract followed by hexane, ethanol, and chloroform. The present study reveals that this plant is of therapeutic potential due to their high free radical scavenging activity.

The methanolic extract of bark and leaves of *Premna latifolia* showed the presence of maximum phytochemicals (12&11) when compared to other solvent extracts *viz.* hexane (PLHEX) (10&9), ethanol (PLETH) (9&8), and chloroform (PLCHLO) (8&7) respectively. The amount of phenolics and flavonoids present in solvents were in the order of Methanol > Hexane > Ethanol > Chloroform. The best antioxidant potential was found in Methanolic extract of *Premna latifolia (*PLME). Methanolic extract of both bark and leaves gave an IC_{50} (135.53µg/ml &178.11µg/ml) for DPPH (2, 2-diphenyl-2-picryl hydrazyl) radical scavenging assay and (102.05µg/ml &111.26µg/ml) for ABTS (2, 2'-azinobis-3-ethyl-benzothiozoline-6-sulphonic acid) radical scavenging assay. This study shows that the bark and leaves of *Premna latifolia* extract could be used as a probable antioxidative agent. This study also revealed that *Premna latifolia* bark possesses greater antioxidant potential in comparison to leaves.

Keywords: Traditional medicine, *Premna latifolia*, bioactive principles, phenolics, flavonoids, antioxidant

Introduction
Before the advent of modern medicine and the pharmacopeia of these drugs, plants were judicially used by the ancient men to cure and treat a variety of ailments and diseases. This knowledge was passed on from one generation to the next and in today's context, these plants and their therapeutic actions are referred with manifold names- "herbal medicines", "herbal remedies" and "traditional medicines" to name a few.

The drawbacks and side effects of the allopathic medicines have led to a sudden increment in the development of herbal drugs globally [1]. Nowadays the scientific world has a great interest in the screening of medicinal plants for new therapies [2]. Medicinal plants have been identified to possess numerous phytochemicals with potential biological activity that play a restorative role in shielding humans from various diseases and complications, that is why they are used by a large proportion of the population. The use of medicinal plants is also well known among the indigenous population in the developing countries [3-5]. Today a substantial number of drugs are developed from plants which are effective against a number of diseases, which involves the isolation of the active compound from the particular specific medicinal plant and its further modification.

Medicinal plants in Uttarakhand

India can be regarded as the hub of medicinal plants with its forests being enriched with such useful plants. The old traditional system of Indian medicine (ISM), is one of the most ancient medicinal practice known to the world, which derives at most formulations from plants and their extracts that exist in its forests [6]. The Himalayan region has a great wealth of medicinal plants and an in-depth knowledge of their practical use. Out of 15,000 species of flowering plants found in India, about 17% of them possess medicinal importance [7] out of which several species (1,745) are from the Indian Himalayan region and a good number can also be located in Uttarakhand [8].

Oxidative stress and Antioxidants

The imbalance between the creation of the reactive oxygen species (ROS) and their detoxification by the biological system reflects the condition of oxidative stress which is one of the major factors implicated in cell stress and cell death. ROS are well identified to be one of the causes of diabetes, cancer, arthritis, atherosclerosis, etc [9]. Antioxidants act as a defense mechanism that prevents oxidative stress and removes the free radicals and repairs the damaged molecules [10] and hence is referred to as a scavenger of ROS.

Both natural and artificial antioxidants are being employed to fight off the oxidative stress. The phytochemicals present in the medicinal plants such as carotenoids, terpenoids, flavonoids, polyphenols, alkaloids, tannins and saponins possess various activities that help in scavenging the free radicals [11]. This antioxidant property is of huge importance as it has the potential to suppress the use of synthetic antioxidants [12].

***Premna latifolia Roxb*. – A Medicinal Plant**

Premna latifolia Roxb. popularly known as Agnimantha and Arani [13], belongs to the family Lamiaceae is a potent medicinal plant. It is a small tree with moderate to extensive branching.The genus contains around 200 species worldwide which are widely distributed in India in the coastal areas in plains and hills [14].

The plant is a rich source of a variety of antioxidants. Traditionally, the paste of *P. latifolia Roxb.* bark is applied to cure boils and the leaves show diuretic, anti-inflammatory, anti-cancerous property [15-16]. It is also employed for the treatment of liver complaints, diarrhea, beri-beri, and vaginal irritation and snake bites [17]. It is also believed to have anti-ulcer activity and the leaves are effective to cure hemorrhoids, dyspepsia, and cough [18].

The above-mentioned factors have thus generated research interest in carrying out this study, thus this work is design to evaluate the *in-vitro* antioxidant capacity effect and phytochemical analysis of different extracts of *Premna latifolia* leaves and bark.

2. Materials and Methods

Plant material

The samples were collected from Jeolikote, district Nainital, Uttarakhand (India) in the month of February 2018. Fresh leaves & bark of *Premna latifolia* Roxb. (Family: Lamiaceae) specimens were collected, at an altitude of 1219 m, strictly abiding by the standard precautions. The plant material was identified and authenticated by the Botany Department of DSB Campus, Kumaun University, Nainital. The leaves and bark samples have been indicated as *Premna latifolia* leaves (PLL) and *Premna latifolia* bark (PLB), respectively.

Processing of the plant material

Leaves and bark were washed thoroughly and then shade dried (for 3-4 days) to evaporate surface water, were chopped into small pieces. The completely dried samples were ground in a mixer to obtain a fine homogeneous powder. The powder was stored in a sealed plastic container in a dry place at room temperature until further use.

Extract preparation using various solvent

The powdered leaves and bark of *Premna latifolia* were extracted with different solvents like methanol, hexane, chloroform, and ethanol. The herb to solvent ratio was kept 1:10 to ensure complete extraction. The plant material was extracted by cold maceration for 72 hours with the respective solvent with intermittent agitation. After incubation, the extracts were filtered through Whatman@ filter paper and the extracts were collected and stored at 4°C in the refrigerator in an airtight container till further use [19].

Percentage yield calculation of various extracts

The percentage yield of various extracts of *Premna latifolia* was calculated from the product that was obtained after complete evaporation of the respective solvents as per the following formula [20].

Percentage Yield = $W_E/W_S \times 100$

Where (W_E = weight of the plant extract; W_S= Weight of the initial sample)

Qualitative phytochemicals screening of the extracts

The previously described method by Harborne, 1973 and Sofowara, 1982 were used with slight modification [21, 22]. The tests were performed to find out the presence of active chemical constituents such as carbohydrates, reducing sugars, amino acids, saponins, lactones, glycosides, flavonoids, tannins, alkaloids, sterols, triterpenes, and phenolics.

Determination of total Phenolics content

The total phenolic content (TPC) of the extracts was determined colorimetrically with some modifications Singleton and Rossi, 1965 [23]. The total phenolic content was determined by comparison with the standard calibration curve of Gallic acid. The calibration curve was prepared by mixing different solutions of Gallic acid (1ml; 20-120µg/ml) with 5 ml of Folin-Ciocalteu reagent (tenfold diluted) and 4 ml of Na_2CO_3 (7.5%). Results were presented as milligrams of Gallic acid equivalents (mg of GAE) per gram dry weight.

Determination of total flavonoids Content

The assay was performed by the aluminum chloride assay through colorimetric, following the procedure described by Zhishen *et al.* 1999 and Ruwali *et al.* 2015 with some modifications [24, 20]. Quercetin was used as standard (1mg/ml). All the tests were performed in triplicates. Flavonoid contents were determined from the standard curve (20-120µg/ml) and were expressed as quercetin equivalent.

Antioxidant assay: *in vitro*

DPPH Radical Scavenging Activity

The DPPH assay was performed according to the method described by Brand-Williams *et al.* 1995 with slight

modifications [25]. The sample concentration providing 50% inhibition (IC_{50}) was calculated from the graph of inhibition percentage against sample concentration. 1ml of 0.2mM DPPH solution in methanol was mixed with 1ml of plant extract solution of varying concentrations (25, 50, 100, 200 and 400µg/ml). The corresponding blank sample was prepared and Quercetin in different concentration was used as the reference standard. The reaction was carried out in triplicate and the decrease in absorbance was measured at 517nm after 30 minutes in dark using UV-Vis spectrophotometer. The inhibition % was calculated using the following formula.

Inhibition % = Ac-As/Ac×100

Where, Ac' is the absorbance of the control; 'As' is the absorbance of the sample

ABTS Radical Scavenging Activity

ABTS [2, 2′-azinobis (3-ethylbenzothiozoline-6-sulfonic acid) diammonium salt) assay measures the relative ability of an antioxidant to scavenge the ABTS generated in the aqueous phase, as compared with ascorbic acid standard. The ABTS is generated by reacting a strong oxidizing agent (e.g., potassium permanganate or potassium persulfate) with the ABTS salt. Reduction of blue-green ABTS radical coloured solution by hydrogen-donating antioxidant is measured by the suppression of its characteristic long wave (734 nm) absorption spectrum [26].

For ABTS assay, the procedure followed was the method of Re *et al.*, 1999 with some modifications [27]. ABTS radical cation ($ABTS^+$) was obtained by reacting $ABTS^+$ stock solution with 2.45 mM potassium persulfate (final concentration) (1/1, v/v) and allowing the mixture to stand in the dark for 12-16 hours (hrs) before use. The $ABTS^+$ solution was diluted with ethanol to an absorbance of 0.700 ± 0.05 at 734nm for measurements. The photometric assay was conducted on 0.9 ml of $ABTS^+$ solution and 0.1 ml of sample extract of various concentrations and mixed for 45 sec; measurement was taken immediately at 734nm after 15 min.

The scavenging activity was estimated based on the percentage of ABTS radicals scavenged by the following formula:

% scavenging = [(Ac− As) /As] × 100

Where, Ac' is absorption of control; 'As' is absorption of tested extract solution.

3. Statistical analysis

All the determinations were performed in triplicate (n=3) and the data were statistically analysed as mean±SD. All graphs were plotted using MS Excel software 2007.

4. Results and Discussion

Premna latifolia Roxb. healthy specimens were collected, clean plant leaves and bark were dried, powdered and extracted with various solvents viz. methanol, ethanol, hexane, and chloroform to yield the respective four different crude extracts. These extracts were subjected to qualitative and quantitative phytochemical analysis. All these extracts were also assessed for their *in vitro* antioxidative potential.

The percentage yield of extracts of *Premna latifolia*

The percentage yield of methanol, ethanol, hexane and chloroform extracts of *Premna latifolia* is presented in Table 1. Extraction is a process that aims to extract certain components present in the plant. it is a solid/liquid separation operation: a solid object is placed in contact with a fluid (the solvent). The extracts obtained with various solvents (methanol, ethanol, hexane, and chloroform) were weighed and their percentage yield was calculated [20].

The yields of soluble substances, expressed as in percentage in leaves and bark of *Premna latifolia* are closely dependent on the solvents, as shown in Table 1.

Table 1: Percentage yield of different extracts of *Premna latifolia*

Solvent used for extraction	Percentage yield of *Premna latifolia* (PLB)	Percentage yield of *Premna latifolia* (PLL)
Methanol	18.75	15
Ethanol	12.25	10.75
Hexane	11.5	9.37
Chloroform	8.62	7.25

As evident in the Table 1 the Methanolic extract of both bark and leaves gave the highest yield (18.75%), (15%) respectively, followed by Hexane (12.25%), (10.75%), Ethanolic extract (11.5%), (9.37%) and least in Chloroform extract (8.62%), (7.25) respectively. The plant contains structurally diverse different types of chemical constituents having solubility with solvents of varying polarity. Bark has a high yield percentage compare to leaves. Methanol extracts of bark and leaves have a high yield percentage.

Phytochemical analysis of various extracts of *Premna latifolia*

Qualitative phytochemical analysis

Phytochemicals are biologically active, naturally occurring chemical compounds found in plants, which provide health benefits for humans [28]. They protect plants from disease and damage and contribute to the plant's color, aroma and flavor. In general, the plant chemicals that protect plant cells from environmental hazards such as pollution, stress, drought, UV exposure, and pathogenic attack are called as phytochemicals [29, 30]. Recently, it is clearly known that they have roles in the protection of human health when their dietary intake is significant.

Phytochemical screening results (Table 2&3) of the bark and leaf extract in methanol, ethanol, hexane, and chloroform showed the presence of all major phytochemical constituents. The methanolic extracts of *Premna latifolia* bark (PLBM) showed the presence of maximum phytochemicals (12) when compared to other solvent extracts viz. PLBH (10,) PLBE (9), and PLBC (8), while in leaves extract PLLM showed (11), followed by PLLH (9), PLLE (8) and least in PLLC (7), thus inferring that methanol being a better solvent in this context.

Table 2: Phytochemical analysis of extracts of bark of *Premna latifolia*

Phytochemicals Group (Test for)	PLBM	PLBH	PLBE	PLBC
Carbohydrates	+	+	+	+
Reducing sugar	+	+	+	+
Tannins	+	-	+	+
Phenolic	+	+	+	-
Flavonoids	+	+	+	+
Lignin	+	+	-	+
Amino acid	+	+	-	-
Saponins	+	+	-	+
Glycosides	+	+	+	-
Sterols	+	+	+	-
Triterpenes	+	-	+	+
Alkaloids	+	+	+	+

(+) Presence of phytochemical compounds (-) absence of phytochemical compounds

Table 3: Phytochemical analysis of extracts of leaf of *Premna latifolia*

Phytochemicals Group (Test for)	PLLM	PLLH	PLLE	PLLC
Carbohydrates	+	+	+	+
Reducing sugar	+	+	-	+
Tannins	+	-	-	+
Phenolic	+	+	-	-
Flavonoids	+	+	+	+
Lignin	-	+	+	-
Amino acid	+	+	+	-
Saponins	+	-	+	+
Glycosides	+	+	+	-
Sterols	+	+	+	-
Triterpenes	+	-	-	+
Alkaloids	+	+	+	+

(+) Presence of phytochemical compounds (-) absence of phytochemical compounds

The phytochemical screening revealed the presence of carbohydrates, alkaloids and flavonoids in all four methanol, ethanol, hexane, and chloroform extracts of both bark and leaves. Sterols and Glycosides were present in Methanol, ethanol and hexane extracts of both bark and leaves but were absent in Chloroform extracts. Triterpenes and tannins were absent in hexane extract of bark, and also absent in hexane and ethanolic extracts of leaves. Amino acid absent in chloroform and ethanolic extracts of bark whereas amino acid is absent in chloroform extracts of leaves. Saponins are absent in ethanolic extracts of bark whereas saponins are absent in hexane extracts of leaves. In ethanolic extracts of leaves, the reducing sugar are absent whereas present in all extracts of bark. Plant extracts revealed the presence of phytochemicals such as phenols, tannins, flavonoids, saponins, glycosides, steroids, terpenoids, and alkaloids, which are known to exhibit medicinal as well as physiological activities.

Steroids have been reported to have antibacterial properties [31]. Terpenoids and tannins are attributed to analgesic and anti-inflammatory activities. Glycosides are known to lower the blood pressure according to many reports [32]. Glycosides also have vast therapeutic efficacy as they are found in almost every medicinal plant. Besides, they also are used as medications [33]. Saponins are used in medicine and pharmaceutical industries because of its foaming ability with the production of the frothy effect. Saponin is used in the medicinal uses for centuries and one of their common biological properties is their cytotoxicity [34].

Quantitave phytochemical analysis of various extracts of *Premna latifolia*

Total phenolic contents

Phenolic compounds possess a wide range of physiological properties such as anti-allergenic, anti-atherogenic, anti-inflammatory, anti-microbial, antioxidant, anti-thrombotic, cardioprotective and vasodilatory effects [35]. The phenolic compounds have been reported to be significantly associated with the antioxidant activity of plant and food extracts mainly because of their redox properties, allowing them to act as reducing agents, hydrogen donors, singlet oxygen quenchers, hydroxyl radical quenchers, and metal chelators [36].

The total phenolics of various extracts were assessed and expressed as mg GAE/gm of the dry weight of the extract. The content of phenolics varied among different extracting solvents used. The result of total phenolics of *Premna latifolia* leaves and bark in various extracts is summarized in Fig. 1 as (mg GAE/ gm of the dry weight of the extract).

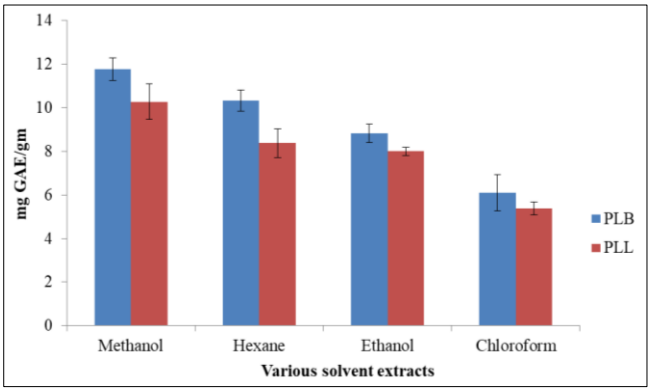

Fig 1: Total phenolic content of *Premna latifolia*. Values expressed are mean±standard deviation (n=3)

From this data the result of total phenolics of *Premna latifolia* bark and leaves in the various extracts is summarized as that

in Methanol extract has highest phenolic content in both bark and leaves (11.75mg GAE/gm & 10.27mg GAE/gm), followed by Hexane (10.32mg GAE/gm & 8.37mg GAE/gm), followed by Ethanol (8.82mg GAE/gm & 7.99mg GAE/gm) and least in Chloroform extract (6.1mg GAE/gm & 5.37mg GAE/gm). Thus the TPC of bark has high phenolic content as compared to leaves.

Total Flavonoid Contents

Flavonoids are ubiquitous in plants and are the most common type of polyphenolic compound found in the human diet. Flavonoids are a group of polyphenolic compounds, which exhibit several biological effects such as anti-inflammatory, anti-hepatotoxic, anti-ulcer, anti-allergic, anti-viral and anti-cancer activities [37]. They are capable of effectively scavenging the reactive O_2 species because of their phenolic hydroxyl groups and so they are potent antioxidants also [38]. Flavonoids are synthesized in all parts of the plant. Flavonoids are naturally occurring in plant and are thought to have positive effects on human health [39].

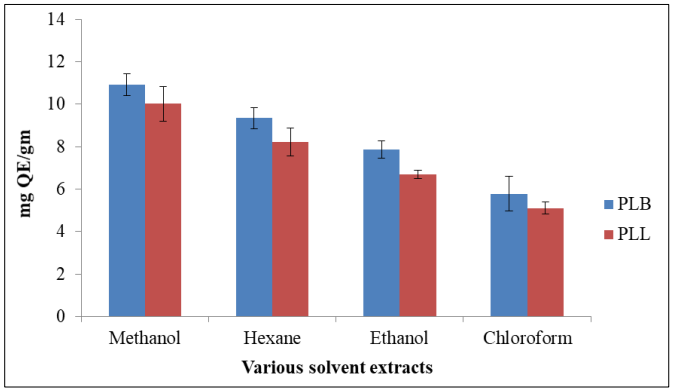

Fig 2: Total flavonoid content of *Premna latifolia*. Values expressed are mean±standard deviation (n=3)

From this data (Fig.2) the result of total phenolics of *Premna latifolia* bark and leaves in the various extracts is summarized as that in Methanol extract has highest flavonoid content in both bark and leaves (10.92mg QE/ gm & 10.02mg QE/gm), followed by Hexane (9.34 mg QE/gm & 8.21mg QE/gm), followed by Ethanol (7.86mg QE/gm & 6.69mg QE/gm) and least in Chloroform extract (5.78mg QE/gm & 5.11mg QE/gm). Thus, the TFC of bark has high flavonoid content as compared to leaves (Fig.2).

In vitro* Antioxidant activity of various extracts of *Premna latifolia

Antioxidant activity of various extracts of *Premna latifolia* was assessed by standard and currently most accepted methods *viz*. DPPH stable free radical scavenging assay and the ABTS radical scavenging assay.

The free radical scavenging ability of extracts on DPPH

DPPH is very convenient for the screening of the number of samples of different polarity. The measurement of the scavenging of DPPH radical allows determining the intrinsic ability of a substance to donate hydrogen atom or electrons to this reactive species in a homogenous system. Methanolic DPPH solution gets reduced because of the presence of antioxidant substances having hydrogen-donating groups such as phenols and flavonoid compounds due to the formation of non-radical DPPH-H form [40].

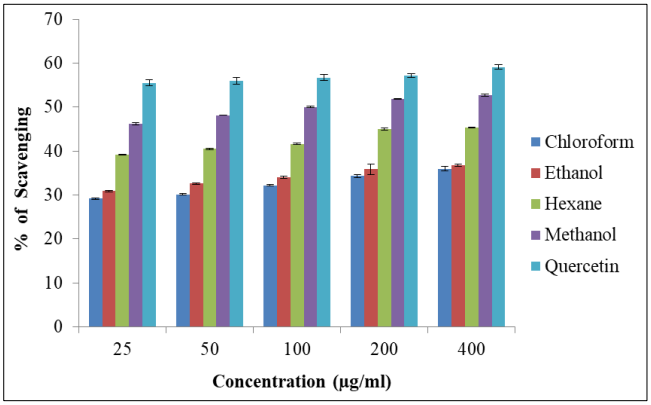

Fig 3: DPPH free radical scavenging activity of various extracts (PLL). values expressed are mean±standard deviation (n=3)

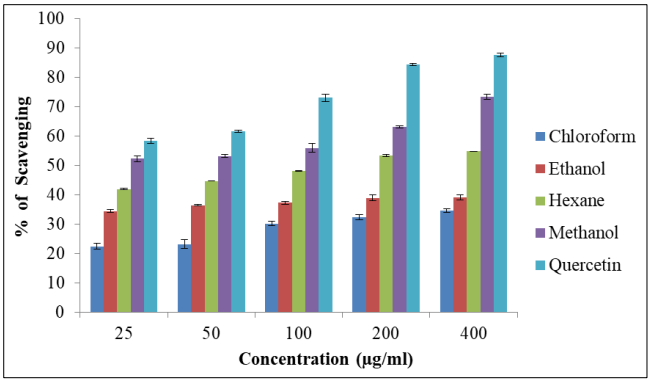

Fig 4: DPPH free radical scavenging activity of various extracts (PLB). values expressed are mean±standard deviation (n=3)

The effect of antioxidants on DPPH radical scavenging is thought to be due to their hydrogen donating ability [41]. The extent of decrease in the absorbance of DPPH in the presence of antioxidants correlates with the free radical scavenging potential of the antioxidant. Fig.3 & Fig.4 shows that both quercetin and various extracts scavenged DPPH radicals in a dose-dependent manner, though by different capabilities. These scavenging activities are most probably and mostly due to the presence of various phenolic compounds [42].

In case of Methanolic, Hexane, Ethanolic and Chloroform extract, 400µg/ml concentration showed maximum DPPH radical scavenging activity followed by 200µg/ml, 100µg/ml, 50µg/ml and 25µg/ml show the least activity.

The amount of antioxidant activity present in leaves and bark solvents are in the order of, Quercetin > Methanol > Hexane > Ethanol > Chloroform. These scavenging activities are most probably and mostly due to the presence of the various phenolic compound.

The antioxidant activity of the sample is evaluated from the determination of IC_{50} values corresponding to the amount of extracts required to scavenging 50% of DPPH radicals present in the reaction mixture. Lower IC_{50} values indicate higher radical scavenging ability. Thus, IC_{50} values are negatively related to the antioxidant activity, the lower the IC_{50} value, the higher the antioxidant activity of the tested sample and *vice-versa.*

Table 4: IC_{50} of Quercetin and various plant extracts (leaves & bark)

Sample	IC_{50} value (µg/ml) leaves	IC_{50} value (µg/ml) bark
Quercetin	126.14±0.45	124.09±0.21
Methanol	178.11±0.36	135.53±0.39
Hexane	260.05±0.16	171.68±0.11
Ethanol	279.84±0.21	204.75±1.52
Chloroform	290.77±0.32	240.88±0.03

The results (Table 4) of the present study showed that among various extracts, the lowest IC_{50} value was of methanolic extract and highest IC_{50} value was of the chloroform extract of *P. latifolia* of both the plant parts used (leaves and bark). The IC_{50} value of bark extracts is less than the IC_{50} value of leaf extracts. This indicates that bark of *Premna latifolia* can be a good source of natural antioxidants.

The free radical scavenging ability of extracts on ABTS

Free radical scavenging activity of plant samples (leaves and bark) was determined by ABTS radical cation decolorization assay. The peroxidise substrate 2, 2′-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^{+}$) radical is a nitrogen-centered synthetic free radical generated by oxidation of ABTS with ammonium persulphate, which is converted to non-radical form by reaction with antioxidants. ABTS radical scavenging method is a common spectrophotometric procedure for determining the antioxidant capacities of plants. The ABTS method is easy to use, has high sensitivity, and allows for rapid analyses of the antioxidant activity of a larger number of samples. The antioxidant can delay or diminish its absorbance [43-45].

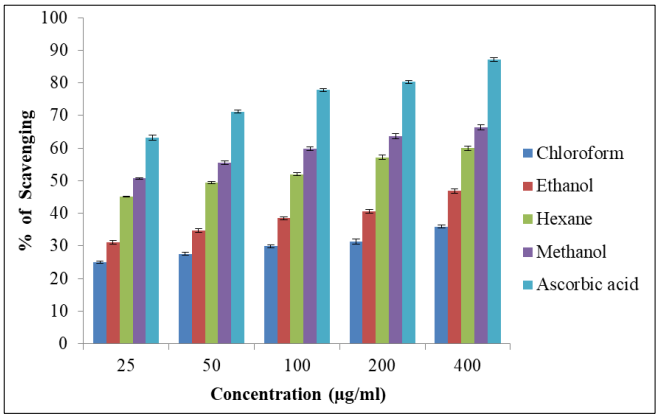

Fig 5: ABTS free radical scavenging activity of various extracts of (PLL). values expressed are mean±standard deviation (n=3)

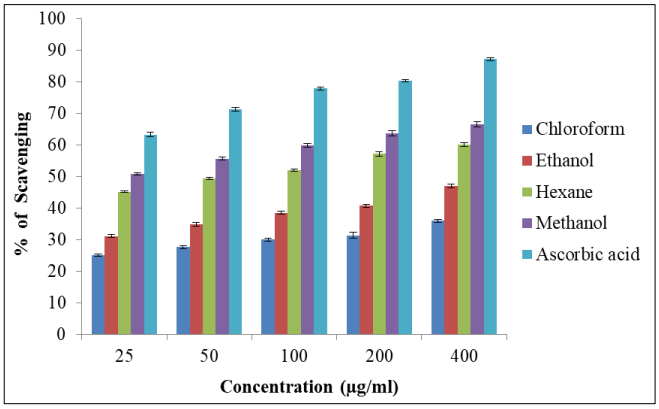

Fig 6: ABTS free radical scavenging activity of various extracts of (PLB). values expressed are mean±standard deviation (n=3)

In case of methanolic, ethanolic, hexane and chloroform extracts, 400µg/ml concentration of crude extract show maximum activity followed by 200µg/ml, 100µg/ml, 50µg/ml and 25µg/ml (Fig.5 & 6). The amount of ABTS free radical scavenging activity in were in the order of, Ascorbic acid > Methanol > Hexane > Ethanol > Chloroform. The results are indicative of that the methanolic, hexane, ethanolic and chloroform extracts of leaves and bark of *Premna latifolia* are efficient free radical scavenging. Among the four extracts of both leaves and bark tested, methanolic extracts possessed maximum radical scavenging activity, followed by hexane, ethanolic and chloroform extracts respectively.

Table 5: IC_{50} of Ascorbic acid and various plant extracts (leaves & bark)

Sample	IC_{50} value (µg/ml) leaves	IC_{50} value (µg/ml) bark
Ascorbic acid	93.43±0.80	87.09±1.6
Methanol	111.26±0.21	102.5±0.7
Hexane	156.72±1.21	132.17±1.3
Ethanol	219.24±0.31	187.92±0.5
Chloroform	264.76±0.29	226.59±1.2

Table 5. shows the significant decrease in the concentration of ABTS radical due to the scavenging ability of the *Premna latifolia*. The results of the present study showed that among various extracts, the lowest IC_{50} value was of methanolic extract and highest IC_{50} value was of the chloroform extract of *P. latifolia* of both the plant parts used (leaves and bark). The IC_{50} value of bark extracts is less than the IC_{50} value of leaf extracts. This indicates that bark of *Premna latifolia* can be a good source of natural antioxidant.

Flavonoids and phenolic acids make up one of the most pervasive groups of plant phenolics. Due to their importance in plants and human health, it would be useful to have a better understanding of flavonoid concentration and biological activities that could indicate their potentials as therapeutic agents, and also for predicting and controlling the quality of medicinal herbs. Plants and herbs consumed by humans may contain thousands of different phenolic acid and flavonoid components. The effect of dietary phenolics is currently of great interest due to their antioxidative and activities. Phenolic acids and flavonoids also function as reducing agents, free radical scavengers, and quenchers of singlet oxygen formation. In addition, flavonoids and phenolic acids components play important roles in the control of cancer and other human diseases.

5. Conclusion

The present research work concludes that qualitative phytochemical screening of crude extracts of *Premna latifolia*, supports the presence of bioactive compounds such as Flavonoids, steroids, terpenoids, tannins, glycosides, alkaloids reducing sugars and Phenols, Quinones, Lignin and fixed oils in the medicinal plant and thus responsible for the antioxidant activities. The estimation of phenolic and flavonoid content resulted in the highest content in the methanolic extract of leaves & bark, exhibit high antioxidant, and free radical scavenging activity. The present study results can be concluded that this plant showed the presence of significant amount of phenols, flavonoids, which directly influence the quality of secondary metabolites. Bark extracts possess potent antioxidant activity. Therefore, could be a potential source of natural antioxidant to combat the diseases

in which there is an increased free radical production. Further research is recommended for better characterization of important bioactive constituents responsible for antioxidant activity. The revealed antioxidant property of extracts may provide potential therapeutic intervention against oxidative threats and degenerative disorders. Further studies are needed for the isolation and identification of bioactive compounds responsible for antioxidant activity. Results for the assays for the antioxidative activity showed that *Premna latifolia* (leaves & bark) methanolic extracts has the ability of scavenging ABTS and DPPH radicals in dose-dependent manner, to much better extent than hexane, ethanol and chloroform extracts. Further studies are needed in this direction to explore more pharmacological actions of this plant. Further investigations on health-promoting aspects in animal models in the future will be made.

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Author's contributions
PR designed the study and wrote the manuscript. DN and PR performed the experiments. PR analyzed and verified the data.

Conflict of interests
We declare that there were no conflicts of interest.

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