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Phytochemical analysis and biological activities of leaves and bark hexane extracts of *Premna mucronata* Roxb. collected from Kumaun hills of Uttarakhand

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Abstract
Premna mucronata Roxb. commonly known as agyon, is a high medicinal value plant belonging to the family Lamiaceae. The phytochemical, antioxidant, anti-inflammatory and antibacterial potential of leaves and bark hexane extracts of *Premna mucronata* (PMLH and PMBH) were investigated in this study. GC/MS analysis was done to determine the compounds present in the extracts. The extracts were subjected to biochemical assay for total phenolics, flavanoid and orthodihydric phenols; their biological activities was determined using developed protocols. GC-MS analysis revealed the occurrence of (*Z*)-7-tetradecenal and linoelaidic acid, TMS as a major compound in PMLH and PMBH respectively. PMLH and PMBH were found to contain potentially significant amount of total phenolics, total flavonoids and total ortho-dihydric phenol content. Antioxidant activity of extracts assessment using, DPPH radical scavenging, metal chelating and reducing power activity resulted significant antioxidant potential presumably due to qualitative and quantitative difference of their antioxidative components. Moderate anti-inflammatory activity was exhibited by both the extracts with IB_{50} value ranging from 63.77±0.04 µg/mL to 76.25±0.90µg/mL. Antibacterial activity was also performed using well diffusion method and it has been found that both the sample showed sustainable antibacterial activity against the bacterial strains *Escherichia coli* and *Staphylococcus aureus*.

Keywords: *Premna mucronata*, 7-tetradecenal, linoelaidic acid, antioxidant, antibacterial, anti-inflammatory

1. Introduction
Plants are major source of therapeutic compounds and are the essential foundation of medicine since prehistoric time. Medicinal plants are important source of new chemical compounds with potential therapeutic effects [1]. Plants synthesize thousands of chemical compounds possessing different properties like defense against insects, bacteria, fungi, diseases and herbivorous mammals. Herbal and natural products have been used in folk medicine for centuries throughout the world. Some Indian medicines like Ayurveda, Sindha and Unani entirely and homeopathy to some extent, depend on plant materials or their derivatives for treating human diseases [2]. Medicinal plants are widely used in non-industrialized societies, mainly because they are readily available and cheaper than modern medicines. Medicinal plants have been discovered and used in traditional medicine practices since prehistoric times. There has been renewed interest in screening higher plants for novel biologically active compounds, particularly those that effectively intervene in human ailments in the field of chronic diseases. Currently, research is focused on the isolation of pharmacologically active compounds from natural sources in the area of those diseases where presently available drugs are not so effective. Also herbal medicines are experiencing greater resurgence as many people are turning their attention from modern drugs toward parallel herbal systems which are also known as alternative medicine. Plants have been used for centuries as a remedy for human diseases because they posses phytochemicals of therapeutic values [3].
Lamiaceae is a family belonging to angiosperms which is characterized by plants with strong aroma and large therapeutic value. *Premna mucronata* Roxb. synonym *Premna latifolia* an aromatic medicinal plant belonging to Lamiaceae family, is widely distributed in tropical, subtropical and coastal areas. *Premna* is one of the important constitutent of the herbal preparation Dashmularisth and Chaywanprash. In Indian traditional systems of medicine, plant parts of some species of the genus *Premna* have been used for the treatment of several disorders such as hepatic disorder, cardiac, and for antioxidant, immunomodulatory effects [4] as well as myocardial salvaging effect [5]. In Ayurvedic systems of medicine it is used in the treatment of various ailments like liver disorders, piles, hyperlipidemia, dyspepsia,

constipation bronchitis, and fever [5,6]. Premna mucronata is also used for improving digestion, as blood purifier, cough expectorant, cardiac stimulant and also useful in skin disorders like ring worm [5, 6].

Taking into consideration the traditional use and medicinal value of *Premna mucronata* Roxb. the present study reveals the phytochemical analysis of leaves and bark hexane extracts (PMLH and PMBH) and their antioxidant activity, anti-inflammatory activity and antibacterial activity against the two bacterial strains *E. coli* and *S. aureus*.

2. Material and Methods

2.1 Collection of Plant Material

The Plant material of *Premna mucronata* Roxb. was collected from village Bhaurasa, Aadi Kailash region of district Nainital, Uttarakhand (29017'9.4"N 79037'19.8"E at 1300 meters altitude) in the month of June, 2017. The plant material was taxonomically identified by Dr. D. S. Rawat, Assistant Professor (Plant Taxonomist), Department of Biological Sciences, College of Basic Science and Humanities, Pantnagar. The voucher specimen **(Acc No. GBPUH-916/28.5.2018)** was submitted to G. B. Pant University Herbarium, Department of Biological Sciences, CBSH, Pantnagar, Uttarakhand.

2.2 Prepration of Extracts

The fresh leaves and bark of *Premna mucronata* were finely chopped and shade dried. The shade dried leaves and barks were finely grinded and the powdered material (250g) was subjected for extraction by cold percolation method in hexane. The solvent of extracts were evaporated by using rotatory vacuum evaporator and the final yield of the extracts was weighed. The yields in percentage obtained (w/w) for PMLH and PMBH were 0.2% and 0.08% respectively [7].

2.3 GC-MS Analysis

GC/MS analysis of PMLH and PMBH was performed using a GC MS-QP 2010. The GC capillary column DB-5 (30 m × 0.25 mm i.d.; 0.25µm film thickness; J&W Scientific, Agilent, Santa Clara, CA, USA) was used. Helium was used as a carrier gas with a flow rate of 1.21 mL/min, at a pressure of 73.3 kPa. The extracts were injected at temperature: 260 °C with oven temperature programme as: Initial temperature 60°C, RAMP 3°C/min upto 210°C (isotherm for 2 min), then RAMP 6°C/min upto 280°C (isotherm for 2 min), finally hold for 11 min. The compounds were identified with the help of NIST-MS, FFNSC Wiley Library, and comparing the data with literature reports and retention indices (RI) [8].

2.4 Biochemical assays:

2.4.1 Total phenolic assay

The total phenols were determined by the Folin-Ciocalteu reagent (FCR) method developed by Singleton and Rossi [9]. In brief, 1mL of the sample extract was transferred into a test tube and mixed with 1mL of 80% methanol and 8mL of distilled water now to each sample 0.5mL of 1 N Folin-Ciocalteu reagent was added and mixed. After 5 min., 1mL of saturated Na_2CO_3 was added to the reaction mixture and allowed to stand for 60min and then absorbance of test sample was measured at 650nm using a UV spectrophotometer (Thermo Scientific *EVOLUTION 201* series). The standard curve was drawn using various concentrations of gallic acid and results were expressed as mg of gallic acid per gram of sample in dried weight.

2.4.2 Estimation of flavanols

Aluminum chloride colorimetric assay was applied for estimation of flavanols [10]. 10 mg of extract were dissolved in 10mL of 80% methanol to prepare stock solution. 0.1 mL of stock solution was mixed with 1.25mL water and 0.75mL of 5% $NaNO_2$ in a test tube. The mixture was incubated for 5 min. After incubation, 0.15mL of 10% $AlCl_3$ was added to the mixtures. After 6 min. 0.5mL of 1N NaOH and 275µL of distilled water was added, after proper mixing of the solution the intensity of pink color was obtained at 510nm. The flavanol content standard curve was established using various concentrations of catechin and the concentrations were calculated with the help of calibration curve and expressed in mg/100gm of dry material material.

2.4.3 Estimation of orthodihydric phenols (ODP)

10 mg of extract was dissolved in 10mL of 80% methanol to prepare stock solution, 0.1mL of the extract solution was taken in a test tube and mixed with 0.4mL water and 1mL of 0.05N HCl, 1mL of Arnow's reagent (10g sodium nitrite and 10 g sodium molybdate made up to 100mL with distilled water), 10mL of water and 2mL of 1N NaOH. The solutions were mixed thoroughly (pink colour was appeared) and absorbance at 515nm was measured using a UV spectrophotometer (Thermo Scientific *EVOLUTION 201* series). To calculate the amount of ortho-dihydric phenols present in the sample standard curve was prepared with the help of working standard catechol solution at different concentrations. The concentration were calculated from the calibration curve and expressed in mg/100gm of material [11].

2.5 Evaluation of antioxidant activity

2.6.1 DPPH Radical scavenging activity

DPPH Radical scavenging activity was evaluated according to the method developed by Singh *et al.* [12], and Dhami *et al.* [13]. with slight modification. The tested samples of different concentrations were mixed with 5mL of a 0.004% methanol solution of freshly prepared DPPH. In brief, different concentration of extracts (50µg/mL-250µg/mL) was taken. The optical density was measured by using UV-visible spectrophotometer (Thermo Scientific *EVOLUTION-201* series*)* at 517 nm. All observations were taken in triplicate and the standard antioxidants used were BHT and catechin. Inhibition of free radical by DPPH in percent (IC %) was calculated by using the equation $IC\% = (A_0 - A_t)/A_0 \times 100$ where, A_0 = Absorbance value of control sample, A_t = Absorbance value of test sample and IC = Inhibitory concentration. Percent inhibition was plotted against concentrations and the standard curve was drawn using standard antioxidant (BHT and catechin) to calculate the IC_{50} values for standard and extracts.

2.6.2 Reducing power Activity

The reducing power of extracts was determined by the method developed earlier with slight modifications [14, 15]. Different concentrations of extracts (50µg/mL-250µg/mL) were mixed with 2.5mL of phosphate buffer (200mM, pH= 6.6) and 2.5mL of 1% potassium ferricyanide, $K_3[FeCN_6]$. After 20 minute incubation at 50±1 ºC, 2.5mL of trichloroacetic acid was added to the mixtures, followed by centrifugation at 650 rpm for 10 min. The supernatant layer (1mL) was mixed with 5mL distilled water and 1mL of 0.1% ferric chloride and absorbance of the resultant solution were measured spectrophotometrically at 700 nm. All the readings were taken in triplicate and ascorbic acid was used as the

standard. The reducing power of samples was calculated using the formula: RP% = $(A_0- A_t)/A_0\times100$ where, A_0 = Absorbance value of control sample, A_t = Absorbance value of test sample and RP= Reducing Power. Percent inhibition was plotted against concentrations and the standard curve was drawn using standard antioxidant (Ascorbic acid) to calculate the RP_{50} values for standard and extracts. The lower RP_{50} value indicates greater reducing power ability.

2.6.3 Metal chelating activity

The Fe^{2+} metal chelating activity of extracts was examined by spectrophotometric method. It is based on the principle of the Fe^{2+} chelating ability of the antioxidant by measuring the absorbance of ferrous iron-ferrozine complex formed at 562 nm [16, 17]. 0.1mL of 2mM $FeCl_2.4H_2O$, 0.2mL of 5mM ferrozine and 4.7mL of methanol was added to different concentrations of extracts (50-250µg/mL). The solutions were mixed and incubated for 10 min. The absorbance of test sample was measured at 562nm in a UV spectrophotometer (Thermo Scientific *EVOLUTION 201* series). All the readings were taken in triplicate; EDTA (0.01 mM) was used as the standard. The metal-chelating activity of tested samples, expressed as percentage was calculated using the formula: IC% = $(A_0- A_t)/A_0\times100$ where, A_0 = Absorbance value of control sample, A_t = Absorbance value of test sample and IC = Inhibitory concentration. The percent of chelating ability was plotted against concentrations and the standard curve was drawn using standard antioxidant (EDTA) to calculate the IC_{50} values of standard and sample extracts.

2.7 Evaluation of anti-inflammatory activity

The *in-vitro* anti-inflammatory activity of extracts was determined by using protein denaturation assay, by the standard protocols as reported in literature [18-20]. To study anti-inflammatory activity, the reaction mixture (5mL) comprised of 0.2mL of egg albumin, 2.8mL of phosphate buffer solution (pH= 6.4) and 2mL of varying amount of extracts (25-500µg/mL). Double distilled water filled as control. The mixtures were incubated at 37±2 °C in a BOD incubator for 15 min and then heated at 70 °C for 5 min in water bath. Subsequent to cooling, their absorbance was measured at 660nm by using a UV spectrophotometer (Thermo Scientific *EVOLUTION 201* series). All the readings were taken in triplicate, diclofenac sodium was used as the standard. The percentage inhibition of protein denaturation was calculated using the formula: IC% = $(A_0- A_t)/A_0\times100$ where, A_0 = Absorbance value of control sample, A_t = Absorbance value of test sample and IC = Inhibitory concentration. The drug concentration for 50% inhibition (IB_{50}) was determined by plotting percentage inhibition with respect to control against treatment concentration.

2.8 Antibacterial activity

Screening of antibacterial activity of leaves and bark hexane extract of *Premna mucronata* against two pathogenic bacterial strains namely *Escherichia coli* (MTCC No. 443) and *Staphylococcus aureus* (MTCC No. 737), was done by agar well diffusion method [21,22]. 100µL of bacterial strains were inoculated by spreading on the nutrient agar plates separately, after which well was made in the plates with the help of a sterile borer (8mm diameter). 30µL of varying concentrations of extracts were poured into the well and plates were allowed to stand for 1 hour for samples to get diffused in media then they were incubate for 24 hours at 370 °C. The same procedure was done for gentamicine sulphate which was taken as standard. When the bacteria have been grown completely on the surface of the media, then the results were determined by measuring mean of zone of inhibition (ZOI) in mm produced by the different concentrations of extracts.

2.9 Statistical Analysis

The data was analysed by using ANOVA (Analysis of Variance) using STPR. All the values were taken in triplicate and means were separated by the Tukey's test when analysis of variance was significant ($p<0.05$). IC_{50} was determined by linear regression analysis using Microsoft Excel 2007.

3. Result and Discussion

3.1 Phytochemical Analysis

In present study, fifty eight compounds comprising of 96.5 % of the total composition were identified in leaves hexane extract of *Premna mucronata* (PMLH) collected from Kumaun region of Uttarakhand, India. (*Z*)-7-tetradecenal (13.2%) identified as the major compound followed by palmitic acid (12.7%), 1-chloro-octadecane (9.8%), squalene (8.3%), phytol (8.1%), tetracontane (7.7%), palmitic acid TMS derivative (3.5%) and 24(*S*)-stigmast-5-ene-3β, TMS (3.2%). Previously leaves hexane extract analysis of *Premna mucronata* has been done by Kumar *et al*., [23] and plant was collected from Garhwal region of Uttarakhand, India. Fourty components comprising of 89.3% of the total components has been reported in Garhwal collection with hexadecanoic acid (25.04%), 8,11,14-docosatrienoic acid (13.62%), octadecanoic acid (6.82%) and 9,12-octadecadienoic acid (4.19%) as major fatty acid constituents. However present study results were completely different from the reported data both qualitatively and quantitatively. These differences in constituents might be due to some edaphic, climatic, genetic or altitudinal differences.

More than forty four compounds comprising of 95.9% were identified in the bark hexane extract *Premna mucronata* (PMBH) and linoelaidic acid, TMS (20%) was found as major compound followed by palmitic acid TMS (16.7%), oleic acid TMS derivative (13.1), stigmasterol TMS (9.9%), γ-sitosterol (6.8%), stearic acid TMS (5.3%), and squalene (4.5%). However comparison between the phytochemicals of leaves and bark hexane extracts (PMLH and PMBH) also showed difference in the quantity of major and minor compounds, besides this some compounds are found to be common in PMLH and PMBH like palmitic acid, tetratetracontane, stigmasta-5,22-diene-3-ol, stigmasterol and squalene. Literature search reveals no report on the phytochemical constituents of bark hexane extract of *P. mucronata,* however study on the whole plant part was done by Krishnamoorthi and Bai, [3] and plant hexane extract was found to show the presence of carbohydrates, flavanoids, cardiac glycosides, terpenoids, phenols and coumarins.

A comparison of leaves hexane extract composition (collected from Kumaun and Garhwal) and bark hexane extract composition of *Premna mucronata* is given in Table 1 and structures of major compounds found in PMBH and PMLH illustrated in Figure 1. The phytochemical composition of hexane extracts also revealed the presence of complex mixture of terpenoids and other identified constituents which varied in extracts in terms of hydrogenated monoterpenoids (HM), oxygenated monoterpenoids (OM), hydrogenated sesquiterpenoids (HS), oxygenated sesquiterpenoids (OS), hydrogenated diterpenoids (HD) and oxygenated diterpenoids (OD). The quantitative makeup of these classes of compounds has been given in Table 2.

3.2 Biochemical Assays
3.2.1 Total Phenolics
PMBH and PMLH found to contain 32±0.86 µg/g GAE and 30±0.5 µg/g GAE phenolic content respectively. Literature search showed that phenolics are responsible for scavenging free radicals and chelating metal ions i.e. antioxidant property of plants [24]. Besides this, Phenolics have been reported to show positive correlation with the anti-inflammatory activity [24].

3.2.2 Orthodihydric Phenols
The orthodihydric phenol content of extracts PMBH and PMLH were found to contain 16.7±0.06 mg/g CLE and 14.8±0.12 mg/g CLE respectively.

3.2.3 Total flavanoid
High amount of flavanoids in the plant show it's ethanobotanical importance as flavanoids have property to reduce risk of heart disease, cancer, stroke and asthma. They may also help in protecting the brain. It is found that certain flavanoids have antihistamine, antimicrobial, and even mood-enhancing properties [24, 25]. The flavanoid content reported in PMBH and PMLH were 54.2±0.31 µg/g CNE and 84.1±0.23 µg/g CNE respectively. Literature search reveals that there is no report on the quantification of phenols of hexane extract of *P. mucronata* however methanolic extracts of *Premna* has been analysed by Subedi *et al.* [26] and 28.04±0.10 mg GAE/g dry extract wt. phenols and 27.89±14.40 mg QE/g dry extract wt. flavanoids were quantified.

The quantitative make up of phenolics, orthodihydric phenols and total flavanoid in hexane extracts has been represented in Table 3.

3.3 Antioxidant Activity
3.3.1 DPPH Radical Scavenging Activity
The 2,2'-diphenyl-1-picrylhydrazyl radical has been widely used to evaluate free radical scavenging capacity of the antioxidants [13]. The scavenging activity evaluated for the hexane extracts was found to be less with respect to the standard antioxidant compounds, BHT and catechin which showed the activity in terms of mean of its IC_{50} value present as 17.55±0.44 µg/mL and IC_{50} =10.94±0.12 µg/mL respectively. The IC_{50} values for the extracts PMLH and PMBH was reported to be 67.7±0.65µg/mL and 57.27±0.69 µg/mL respectively (Table 4).

3.3.2 Metal Chelating Activity
To indicate the presence of chelator in the reaction system, a chelating reagent ferrozine is used. Ferrozine forms complexes only with free Fe^{+2} ions, in presence of chelating agents, the complex formation between Fe^{+2} ions and ferrozine is disturbed which results in change in colour of the complex. This change of colour is measured spectrophotometrically to determine the metal chelating activity of the chelator present in the reaction system. Chelating activity on Fe^{+2} of leaves and bark hexane extract of *Premna mucronata* was examined and it was found that plant possess a strong metal chelating power. The IC_{50} value for PMLH and PMBH reported to be 33.87±0.15 µg/mL and 28.57±0.82µg/mL respectively with respect to standard antioxidant EDTA (14.08±0.11 µg/mL) (Table 4).

3.3.3 Reducing Power Activity
Both the extracts PMLH (IC_{50}= 50.76±0.07 µg/mL) and PMBH (IC_{50}= 47.87±0.86 µg/mL) were reported to have moderate reducing power as compared to standard antioxidant (Table 4). The reducing power in the extracts may be due to the presence of significant amount of phenolic compounds in the extracts [27]. The literature search revealed that the constituents like squalene [28, 29], γ-sitosterol [30, 31], phytol [32, 33], stigmasterol [34] and palmitic acid [35] were reported to show antioxidant activity. Hence, these compounds namely squalene, γ-sitosterol, phytol, stigmasterol and palmitic acid found in hexane extracts (PMLH and PMBH) in present research, might be responsible for good antioxidant activity of plant.

Previously no work has done on leaves hexane extract and bark hexane extract separately however whole plant has been analyzed for antioxidant activity. DPPH radical scavenging activity of hexane, ethanol and ethyl acetate extracts has been reported by Krishnamoorthi and Bai,[3] and it was found that ethanol extract gives higher activity, hexane extract showed moderate activity and ethyl acetate extract showed least activity [3]. Besides this, antioxidant activity of methanol extract of *Premna mucronata* are reported by Subedi *et al.*, [26] and plant methanol extract was found to show weak radical scavenging activity and a good metal chelating activity [26].

3.4 Anti-inflammatory Activity
In-vitro anti-inflammatory activity of hexane extracts was performed using protein denaturation assay. Both the extracts exhibit moderate anti-inflammatory activity with IB_{50} values 76.25±0.90µg/mL and 63.77±0.04µg/mL respectively with respect to the standard anti-inflammatory drug diclofenac sodium (IB_{50}=19.63±0.06 µg/mL). IB_{50} values for extracts and standard are illustrated in Table 5. Previously, it has been reported that the anti-inflammatory effectiveness of plants is due to the presence of squalene [36], oleic acid [37], γ-sitosterol [38, 39], palmitic acid[37]and stigmasterol [40]. Present study shows that all these compounds are present in hexane extracts of *Premna* as major constituents. Hence, these compounds might be responsible for lower IB_{50} value or higher anti-inflammatory activity of PMLH and PMBH.

Anti-inflammatory activity of *Premna mucronata* has not been reported previously but anti-inflammatory activity of other species of genus *Premna* (*P. herbacea, P. integrifolia, P. corymbosa, P. tomentosa* etc.) has been evaluated and these were found to possesses strong anti-inflammatory activity [27] [41-45].

3.5 Antibacterial Activity
This investigation reveals that both hexane extracts of *P. mucronata* exhibits a good antibacterial activity against the two bacterial strains *E. coli* and *S. aureus* by using agar well diffusion method**.** Maximum zone of inhibition shown by PMLH and PMBH against *E. coli* were 14.33±0.58mm (at 500ppm) and 14.66±0.58mm (at 1000ppm) respectively. While against *S. aureus* it was observed to be 15.33±0.58mm (at 750ppm) and 16.33±0.58mm (at 1000ppm) respectively. PMBH was found to show concentration dependent antibacterial activity against the bacterial strain *S. aureus*. The zone of inhibition (in mm) by PMLH and PMBH against *E. coli* and *S. aureus* is given in Table 6. The compounds tetradecanal [46,47], γ-sitosterol [48], phytol [32,33] and oleic acid [49] were found to exhibit strong antibacterial activity. all these compounds were also found in present study in different quantities. From these results and previous reports as above it can be concluded that the antibacterial activity of PMLH and PMBH might be due to the presence of these constituents in hexane extracts of *Premna* or synergetic effect of various

major, minor, and trace constituents.

Previously hexane extract of *Premna* has not been examined for antimicrobial activities however ethanol extract of plant has been analyzed by Ram *et al.* [45] against the bacterial strains *Micrococcus luteus*, *Micrococcus roseus*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and fungal strain *Candida albicans* and it was found to show ZOI of 10 mm, 12 mm, 13 mm, 14 mm, 11mm respectively at 5000µg concentration [45].

Table 1: Comparative chemical composition of hexane extract of *Premna mucronate*

S. N.	Compound Name	% Contribution		
		PMLH		PMBH
		Present Study	Kumar *et al.* [23]	Present Study
1.	decanoic acid	-	t	-
2.	pentadecane	-	0.1	-
3.	dodecene	t	0.2	0.3
4.	1-dotriacontanol	-	t	-
5.	decane, 2-methyl	-	t	-
6.	dodecanoic acid	t	0.2	-
7.	octadecane	-	t	-
8.	heptadecane,2,6,10,15 tetramethyl	-	t	-
9.	tetradecanoic acid	0.3	1.0	-
10.	octadecane	t	0.2	-
11.	5-octadecenoic acid	-	t	-
12.	neophytadiene	0.3	1.0	-
13.	citronellyl propionate	-	1.3	-
14.	9-hexadecanoic acid	3.5	0.9	0.1
15.	methyl 3-acetyl hydroxyl palmitate	-	1.2	-
16.	hexadecanoic acid	-	25.0	-
17.	2-heptadec-5 ‘‘en’’ yloxy tetrahydrofuran	-	2.4	-
18.	heptadecanoic acid	-	0.3	-
19.	9,12-octadecadienoic acid	2.0	4.2	-
20.	8,11,14-docosatrienoic acid	-	13.6	-
21.	9-octadecenoic acid	-	1.2	-
22.	octadecanoic acid	0.9	6.8	-
23.	2-nonadecene	-	t	-
24.	octacosane	0.1	0.1	-
25.	octadecanoic acid, 11-methyl	-	t	-
26.	tricosane	-	0.2	-
27.	eicosanoic acid	0.7	2.7	-
28.	dodecane, 1-fluro	-	0.1	-
29.	tetradecanoic acid, 12-methyl	-	0.2	-
30.	tritetracontane	-	0.3	-
31.	docosanoic acid	0.1	2.6	-
32.	methyl 13-methyl tetradecanoate	-	0.1	-
33.	2[-1-(trimethylsily)-3-(tert-butyldimethy l)propyl]-1-hydroxy	-	t	-
34.	eicosane	-	3.5	-
35.	tetracosanoic acid	-	1.6	-
36.	nonacosane	-	0.5	-
37.	1-octen-3-ol	t	-	-
38.	psi-cumene	0.1	-	-
39.	acetophenone	0.4	-	-
40.	α,α- dimethyl benzene methanol	0.3	-	-
41.	n-tetradecane	t	-	-
42.	docosanoic acid, heptyl ester	t	-	-
43.	isoeugenol	0.1	-	-
44.	3-octenoic acid, TMS	1.8	-	-
45.	dodecamethyl-cyclohexasiloxane	0.1	-	-
46.	β- caryophyllene	t	-	-
47.	cyclomethicone 7	0.1	-	-
48.	β-asarone	t	-	-
49.	(*Z*) 7-tetradecenal	13.2	-	-
50.	9,12-octadecadienoic acid, methyl ester	0.1	-	-
51.	9, 12- octadecadienoic acid, TMS	2.0	-	-
52.	(*E,E,E*)- α-springene	0.6	-	-
53.	1,6,10,14,18,22-tetracosahexaen-3-ol, 2,6,10,15,19,23-hexa	0.4	-	-
54.	palmitic acid, TMS	12.7	-	16.7
55.	fitone	t	-	-
56.	phytol	2.8	-	0.2
57.	methyl palmitoleate	0.1	-	-
58.	myristoleic acid	0.3	-	-

59.	methyl stearate	0.3	-	-
60.	phytol, TMS	8.1	-	-
61.	oleic acid	0.2	-	13.1
62.	palmitoleic acid	0.1	-	-
63.	methyl linolenate	0.5	-	-
64.	linoelaidic acid, trimethylsilylester	0.3	-	20.0
65.	trimethylsilyl (9E)-9-octadecenoate	0.1	-	-
66.	1-chloro-octadecane	9.7	-	-
67.	stigmast-5 ene-3β, TMS 24(*S*)	3.2	-	-
68.	olean-12-en-3-one	1.4	-	-
69.	24-norursa-3,12-diene	0.5	-	-
70.	3β-ergost-5-en-3-ol	0.6	-	0.6
71.	stigmasta-5,22-diene-3-ol	2.5	-	1.0
72.	stigmasterol	1.3	-	9.9
73.	squalene	8.3	-	4.5
74.	oxirane,2,2-dimethyl -3-(3,7,12,16,20-pentamethyl-3,7,11,15,19-heneicosapentaenyl)	0.6	-	0.8
75.	squalene, TMS	0.4	-	-
76.	γ-tocopherol TMS derivative	0.3	-	-
77.	γ- tocopherol	3.4	-	-
78.	(+)-sesamin	0.2	-	-
79.	2-heptadecyloxirane	t	-	-
80.	triacontyl acetate	0.3	-	-
81.	tetracontane	7.7	-	-
82.	2-[1-hydroxy-2-(3-methylphenyl)ethyl]cholestan-3-one	1.3	-	-
83.	hexacosanal	0.3	-	-
84.	phytyl decanoate	2.5	-	-
85.	2-[1-hydroxy-2-(3-methylphenyl)ethyl]cholestan-3-one	-	-	-
86.	tetratetracontane	1.4	-	2.2
87.	propyl benzene	-	-	t
88.	meta ethyl toluene	-	-	0.4
89.	psicumene	-	-	0.3
90.	pseudocumene	-	-	1.3
91.	decane	-	-	0.1
92.	mesitylene	-	-	0.3
93.	dodecane	-	-	0.3
94.	2,6,10-trimethyl-4-trimethylsiloxy-deca-2,5,9,11-tetraene	-	-	0.2
95.	myrtensaeure, TMS	-	-	0.4
96.	myristic acid TMS derivative	-	-	0.3
97.	pentadecanoic acid	-	-	0.6
98.	9-hexadecenoic acid, (Z)-,	-	-	0.1
99.	margaric acid TMS	-	-	1.2
100.	methyl linoleate	-	-	0.3
101.	heneicosane	-	-	0.4
102.	linoleoyl chloride	-	-	0.1
103.	stearic acid, TMS	-	-	5.3
104.	oleic acid TMS derivative	-	-	13.1
105.	3,7,11-trimethyl-2,6,10-dodecatrienyl trimethylsilyl ether	-	-	0.4
106.	phthalic acid	-	-	0.2
107.	linoleic acid	-	-	0.2
108.	nonadecanoic acid TMS derivative	-	-	0.4
109.	ecosanoic acid TMS	-	-	1.1
110.	heneicosanoic acid	-	-	0.1
111.	behenic acid	-	-	0.5
112.	campesterol trimethylsilyl ether	-	-	1.0
113.	γ-sitosterol	-	-	6.8
114.	stigmasterol TMS	-	-	2.2
115.	docosa-8-,14-diyn-1,22-diol, (*Z*)	-	-	0.5
116.	trimethylsilyl tetracosanoate	-	-	0.7
117.	trimethylsilyltricosanoate	-	-	0.3
118.	melissyl alcohol	-	-	0.7
119.	sitostenone	-	-	0.6
120.	trimethylsilyltriacontanoate	-	-	0.6
	Total	98.5%	89.3%	95.9%

PMLH= *Premna mucronata* leaves hexane extract, PMBH= *Premna mucronata* bark hexane extract, t= trace (> than 0.1%)

Table 2: Comparative class composition of terpenoids in hexane extracts of *Premna mucronata* Roxb.\

Compounds	PMLH	PMBH
HM	0.10	0.12
OM	-	-
HS	0.08	-
OS	-	0.57
HD	0.88	-
OD	3.64	0.22
Others	83.12	93.3
Total	87.82%	94.2%

HM= hydrogenated monoterpenoids, **OM=** oxygenated monoterpenoids**,** **HS=** hydrogenated sesquiterpenoids, **OS=** oxygenated sesquiterpenoids, **HD=** hydrogenated diterpenoids, **OD=** oxygenated diterpenoids, - = not detected, **PMLH=** *Premna mucronata* leaves hexane extract, **PMBH=** *Premna mucronata* bark hexane extract.

Table 3: Orthodihydric Phenols, Flavanols and Phenolic content in hexane extracts of *Premna mucronate*

S. N.	Sample Name	Total Phenolic Content (µg/g GAE)	Orthodihydric Phenols (mg/g CLE)	Flavanols (µg/g CNE)
1.	PMLH	30±0.5^{a}	14.8±0.12^{b}	84.1±0.23^{a}
2.	PMBH	32±0.86^{b}	16.7±0.06^{e}	54.2±0.31^{b}

Values are means of three replicates ± SD. Within a column, mean values with the same letter are not significantly different according to Tukey's test (p<0.05), PMLH= *Premna mucronata* leaves hexane extract, PMBH= *Premna mucronata* bark hexane extract, GAE =Gallic acid equivalent, CNE =Catechin equivalent, CLE = Catechol Equivalent.

Table 4: Antioxidant activity of hexane extracts of *Premna mucronata* Roxb.

S.N.	Sample Name	Mean value ± SD (µg/mL)		
		DPPH radical scavenging activity (IC_{50})	Metal chelating activity (IC_{50})	Reducing power activity (RP_{50})
1.	PMLH	67.7±0.65^{a}	33.87±0.15^{b}	50.76±0.07^{a}
2.	PMBH	57.27±0.69^{b}	28.57±0.82^{c}	47.87±0.86^{b}
2.	BHT	17.55±0.44^{c}	-	-
3.	Catechin	10.94±0.12^{d}	-	-
4.	EDTA	-	14.08±0.11^{a}	-
5.	Ascorbic acid	-	-	22.03±0.30^{c}

Values are means of three replicates ± SD. Within a column, mean values with the same letter are not significantly different according to Tukey's test (p<0.05), PMLH = *Premna mucronata* leaves hexane extract, PMBH = *Premna mucronata* bark hexane extract, BHT = butylated hydroxyl toluene, EDTA=ethylene diamine tetra acetic acid (Sodium salt), SD= standard deviation, IC_{50}= half minimal Inhibitory concentration

Table 5: Anti-inflammatory activity of hexane extracts of *Premna mucronate*

S.N.	Sample Name	Mean IB_{50} values (µg/mL)
1.	PMLH	76.25±0.90^{a}
2.	PMBH	63.77±0.04^{b}
2.	Diclofenac Sodium	19.63±0.06^{c}

Values are means of three replicates ± SD. Within a column, mean values with the same letter are not significantly different according to Tukey's test (p<0.05). PMLH = *Premna mucronata* leaves hexane extract, PMBH = *Premna mucronata* bark hexane extract

Table 6: Antibacterial activity of hexane extracts of *Premna mucronata* against *E. coli and S. aureus*

S.N.	Sample name	Concentration(ppm)	Zone of inhibition Mean(R±SD) mm	
			Against *E. coli*	Against *S. aureus*
1.	PMLH	250	5.33±0.58^{f}	7.66±0.58^{l}
		500	14.33±0.58^{h}	12.33±0.58^{e}
		750	8.33±0.58^{k}	15.33±0.58^{m}
		1000	11.33±0.58^{g}	10.33±0.58^{g}
2.	PMBH	250	13.33±0.58^{e}	12.66±0.58^{f}
		500	13.66±0.58^{e}	12±0.00^{e}
		750	12.66±0.58^{d}	13.33±0.58^{d}
		1000	14.66±0.58^{c}	16.33±0.58^{c}
3.	Standard	250	20.33±0.58^{b}	20.33±0.58^{b}
		500	28.33±0.58^{a}	28.33±0.58^{a}
		750	34.66±0.58^{i}	34.66±0.58^{h}
		1000	40.33±0.58^{j}	40.33±0.58^{k}

Values are means of three replicates ± SD. Within a column, mean values with the same letter are not significantly different according to Tukey's test (p<0.05). PMLH = *Premna mucronata* leaves hexane extract; PMBH = *Premna mucronata* bark hexane extract.

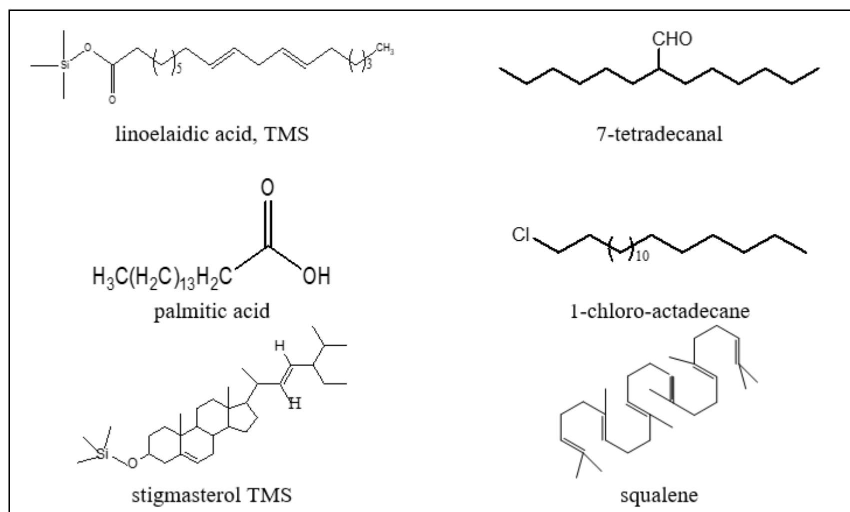

Fig 1: Structures of major compounds found in PMBH and PMLH

4. Conclusion

Present research reveals that the plant *Premna mucronata* Roxb. is a good source of compounds like 7-tetradecenal, linoelaidic acid, 1-chloro-actadecane, phytol, tetracontane, palmitic acid, oleic acid, stigmasterol, γ-sitosterol, stearic acid and squalene. On the other hand the plant is a potent antioxidant and anti-inflammatory agent, indicating its efficacy in the field of pharmaceutical, food and cosmetic industry. Substantial antibacterial activity has also been shown by the plant predicting its possible use as herbal antibacterial agent as a substitute to synthetic antibacterial drugs. Considerable amount of phenolics, orthodihydric phenols and flavanols are also quantified in plant. Further investigations are essential to transform the claims of the traditional uses of *Premna* by rurals and tribals into evidence based information for the future research purpose of this plant.

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