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Original Research Article

Phytochemical analysis and screening of antioxidant, antibacterial and anti-inflammatory activity of essential oil of *Premna mucronata* Roxb. leaves

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ABSTRACT

Premna mucronata Roxb., commonly known as Agyon, is a plant of family Lamiaceae. This study dealt with the phytochemical analysis of the essential oil from the leaves of *Premna mucronata* Roxb. (PMLO) using GC/MS technique and screening of its antioxidant activity using 2,2-diphenyl-1-picrylhydrazyl (DPPH), metal chelating and reducing power assays along with the corresponding anti-inflammatory activity using *in-vitro* albumin denaturation assay and antibacterial activity using well diffusion method against pathogenic bacterial strains, e.g. *Escherichia* coli and *Staphylococcus aureus*. The phytochemical analysis of PMLO revealed the presence of over 71 compounds. Accordingly, 3-octanone was found to be the major constituent component accounting for 23.6% of the total oil composition. The other major constituents identified were ethyl hexanol (13.9%), 1-octen-3-ol (9.6%), linalool (5.5%), methyl salicylate (2.9%) and (*E*)-caryophyllene (2.9%). Moreover, it was found that PMLO possessed satisfied antioxidant activity using DPPH (IC_{50} = 11.18 ± 0.03 µg/mL), metal chelating (IC_{50} = 18.82 ± 0.46 µg/mL), and reducing power activities (IC_{50} = 21.69 ± 0.02 µg/mL) assays. The assessment of *in-vitro* anti-inflammatory activity of PMLO also gave rise to an IC_{50} value of 30.27 ± 0.005 µg/mL. Antibacterial activity of PMLO showed zone of inhibitions of 14.33 ± 0.58 mm against *E. coli* and 14.66 ± 0.58 mm against *S. aureus* at higher concentrations (1000 ppm). This study revealed that PMLO contained high quantities of 3-octanone, 1-octen-3-ol, ethyl hexanol, linalool, and exhibited antioxidant, anti-inflammation and antibacterial activities.

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1. Introduction

Medicinal plants are scattered all over the world, having a large treasure of potential bioactive compounds hidden within them. It has been reported from time immemorial that plants possess great medicinal importance and the human race had a dependence on botanicals and herbs for their food and health care issues (Mohammadhosseini et al., 2017; Mohammadhosseini et al., 2019). Plants are major source of therapeutic agents since prehistoric time. As per WHO estimates, plant drugs, provide nearly 80% to the health needs of the entire world population. Medicinal herbs have been used in traditional medicine system for several herbal preparations since prehistoric times (Mohammadhosseini, 2017; Wansi et al., 2018). Many studies have revealed that plants promote health and well-being to human beings. The utility of herbal remedies is not only cost-effective but also safe and almost independent from any serious adverse effect. The rural elders, farmers and tribal cultures have tremendous knowledge about the plants being used for various purposes of health since thousands of years and are still a part of medical practices by folks of various regions of Indian sub-continents and China, Middle East and African countries, South American and other developing countries of the world (Venditti et al., 2018). India is a large hub of medicinal plants where more

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than 45,000 plant species have been documented and among these several thousands have been claimed to show medicinal properties (Mohammadhosseini, 2017). Ayurveda and other oriental medicinal systems describe how to use the plants in the treatment of a variety of ailments. To date, various phytochemicals possessing established or potential biological activities have been identified and discovered. In fact, the plant products are extensively used in various medicinal systems by different practitioners who require proper documentation and in some reports for further enhancement of their medicinal values (Mohammadhosseini, 2017). The interest in the area of natural product medicines is growing exponentially due to the increased awareness of people towards the adverse effects of synthetic drugs (Venditti et al., 2018). The genus *Premna* is a medicinally important member of Lamiaceae family. This herbal genus was formerly classified in the family Verbenaceae (Munir, 1984), but now is considered as a member of the family Lamiaceae, subfamily Viticodeae (Harley et al. 2004; Olmstead 2010, 2012). Plants of Lamiaceae are herbs, shrubs, trees, or rarely vines in habit and are widely distributed in tropical, subtropical and coastal areas of China to tropical Asia. According to the botanical nomenclature, *P. mucronata* and *Premna latifolia* are synonyms of *Premna mollissima*, Roth (The Plant List 2013; WCSP, 2018). *P. mucronata*, a low bushy tree with trunk up to 1.2 m height, commonly known as "Bari arani", "Ganiar", Agethu", "Agnimatha" and "Agyon" has great ethnobotanical value. Furthermore, P. mucronata is an aromatic and medicinal plant and one of the important ingredients of "Dashamula" herbal preparation. The barks have been recommended to cure boils (Rekha et al., 2015), while the leaves have been applied externally to treat dropsy as diuretic (*Suresh et al., 2011*). It has been reported that *P. mucronata* shows cardioprotective activity in myocardial infarction (Savasani et al., 2014), antioxidant (Subedi et al., 2014), larvicidal (Renjana and John, 2013), hypocholestremics, antimicrobial, wound healing power (Ram et al., 2004) and anti-inflammatory activities (Kumari et al., 2011, Patel et al., 2015, Dianita and Jantan, 2017; Jena et al., 2017). It has also been shown that its essential oil has remarkable antifeedant property (Patel et al., 2015; Rekha et al., 2015).

A simple literature search reveals that very little work has been done on *P. mucronata* from Uttarakhand state, India up to present. The present study aimed to establish a comprehensive characterization of the chemical profile of the essential oil of *Premna mucronata* Roxb. collected from Kumaun region of Uttarakhand and evaluate its in-vitro antioxidant, anti-inflammatory and antibacterial activity. To the best of our knowledge, this study is the first report dealing with the potential anti-inflammatory and antibacterial activities of this medicinal plant.

2. Experimental

2.1. Collection of plant material

The fresh leaves of *P. mucronata* Roxb. were collected from village Bhaurasa, Aadi Kailash region of district Nainital (29017'9.4"N 79037'19.8"E at1300 meters altitude), Kumaun region, Uttarakhand, India in June 2017 (Fig. 1). 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 and voucher specimen (Acc No. GBPUH-916/28.5.2018) was submitted to G.B. Pant University herbarium, Department of Biological Sciences, CBSH, Pantnagar.

Fig. 1. The photographs of *Premna mucronata* Roxb. tree.

2.2. Isolation of essential oils from fresh leaves

The essential oils from freshly collected leaves of *P. mucronata* Roxb. were obtained by hydrodistillation using Clevenger apparatus. The fresh leaves were weighed, chopped and hydrodistilled for 3-4 h and the subsequent oil was extracted with hexane and dried over Na_2SO_4. The essential oil, namely PMLO was stored at a low temperature (4 °C in refrigerator) for further use. The percent yield of the obtained oil was found to

be 0.093% (v/w).

2.3. GC-MS analysis

Chemical composition of PMLO was analyzed using GC-MS-QP 2010 Plus equipment. The column DB-5 (30 m × 0.25 mm, i.e. 0.25 µm of film thickness) was used for chromatographic-based analyses. Exactly, 1 µL of PMLO was injected and the injector temperature was set at 260 °C. The flow rate of the helium (carrier gas) was adjusted at 1.21 mL/min at a pressure of 69.0 kPa with the split ratio of 22.0. Initial temperature was 50 °C, and increased to 210 °C with a ramp of 3 °C/min up. The chemical profile of PMLO was tentatively identified by comparing the Kovats indices of the constituent components with those tabulated in the literature, the library and published data of NIST-MS and FFNSC Wiley libraries (Adams, R. P. 2007).

2.4. Antioxidant activity

2.4.1. DPPH radical scavenging activity

DPPH radical scavenging activity was evaluated based on some previous standard methods (Blois 1958; Liu et al., 2008; Lu et al., 2011) with slight modifications. In this regard, different concentrations of PMLO (5 µL-25 µL) were mixed with 5 mL of freshly prepared methanol solution of DPPH (0.004%) and then their absorbance 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 butylated hydroxytoluene (BHT) and catechin. Inhibition of DPPH radical by the sample, in percent inhibition (%) was determined through the following formula:

Percent inhibition(%) of radical scavenger = IC% = $(A_0 - A_t)/A_0 \times 100$
(Eqn. 1)

Where A_0 and A_t respectively account for absorbance value of control sample and absorbance value of test sample.
Percent inhibition was plotted against the corresponding concentrations and the standard curve was subsequently drawn using positive controls, BHT and catechin to calculate the IC_{50} values for standard and oil. A lower IC_{50} value indicated more radical scavenging activity.

2.4.2. Reducing power activity

The reducing power activity of PMLO was determined based on some reliable methods (Yen et al., 1993; Singh et al., 2005; Parki et al., 2017). Briefly, different concentrations of PMLO (5 µL-25 µL) were mixed with 2.5 mL of phosphate buffer (200 mM, pH = 6.6) and 2.5 mL of 1.0% potassium ferricyanide, $K_3[FeCN_6]$. After 20 minutes incubation at 50 ± 1 ºC, 2.5 mL of trichloroacetic acid was added to the mixtures, followed by centrifugation at 650 rpm for 10 min. The supernatant layer (1 mL) was mixed with 5 mL of distilled water and 1 mL of ferric chloride (0.1%) and the absorbance was finally measured at 700 nm. All the readings were taken in triplicate and ascorbic acid was used as positive control. The percent inhibition (%) of reducing power activity was calculated through the following formula (Eqn. 2).

Percent inhibition (%) of reducing power activity = $(A_0 - A_t)/A_0 \times 100$
(Eqn. 2)

Where A_0 and A_t are respectively the absorbance values of control and test samples. Percent inhibition was plotted against concentrations and the standard curve was drawn using standard antioxidant (ascorbic acid) to calculate the IC_{50} values for positive control and PMLO. The lower IC_{50} value indicates greater reducing power ability.

2.4.3. Metal chelating activity

The metal chelating activity of PMLO was examined spectrophotometrically based on the principle of the Fe^{2+} chelation by the antioxidant to form ferrous iron-ferrozine complex which was measured at 562 nm (Kunwar et al., 2013). 0.1 mL of $FeCl_2.4H_2O$ (2 mM), 0.2 mL of ferrozine (5 mM) and 4.7 mL of methanol were added to different concentrations of oil (5-25 µL/mL). The solutions were mixed and incubated for 10 min. The absorbance of test sample was measured at 562 nm using UV spectrophotometer. All the readings were taken in triplicate, EDTA (0.01 mM) was used as standard. The metal chelating activity of tested samples, was calculated using the formula:

Percent inhibition (%) of chelating ability = $(A_0 - A_t)/A_0 \times 100$
(Eqn. 3)

Where A_0 and A_t are absorbance values of control and test samples, respectively. The percent of chelating ability was plotted against the relevant concentrations and the standard curve was drawn using positive control, EDTA to calculate the IC_{50} values for standard and essential oil. A lower IC_{50} value indicates greater metal chelating ability.

2.5. Evaluation of anti-inflammatory activity

The *in-vitro* anti-inflammatory activity of PMLO was determined using protein denaturation assay (Williams et al., 2008; Chandra et al., 2012; Shunmugaperumal and Kaur, 2016). The reaction mixture (5 mL) comprised of 0.2 mL of egg albumin, 2.8 mL of phosphate buffer solution (pH = 6.4) and 2 mL of varying amounts, e.g. 25, 50, 75, 100, 125, 250 and 500 µL of oil. Double distilled water was used as control. The mixtures were incubated at 37 ± 2 °C in a biochemical oxygen demand (BOD) incubator for 15 min and then heated at 70 °C for 5 min in water bath. After cooling, their absorbance was measured at 660 nm using a UV/Vis. spectrophotometer. All the readings were taken in triplicate; diclofenac sodium was used as the positive

control.
The percentage inhibition of protein denaturation was calculated using the following formula:

Percent inhibition (%) of protein denaturation = $(A_0 - A_t)/A0 \times 100$ (Eqn. 4)

In this equation, A_0 and A_t are absorbance values of control and test samples, respectively. The concentration of PMLO to inhibit 50% protein denaturation was determined by plotting the percentage inhibition vs. its respective concentration.

2.6. Antibacterial activity

Screening of antibacterial activity of PMLO was performed against two pathogenic bacterial strains, *Escherichia coli* (MTCC No. 443) and *Staphylococcus aureus* (MTCC No. 737), using agar well diffusion method (Balouiri et al., 2016; Javed et al., 2016). 100 µL 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 (8 mm diameter). 30 µL-portions with varying concentrations of PMLO were poured into the well and plates were allowed to stand for 1 h for samples to get diffused into media and they were then incubated for 24 h at 37 °C. Gentamicine sulphate was used as positive control. The antibacterial activity was determined by measuring the mean of zone of inhibition (ZOI).

2.7. Statistical analysis

The data was analyzed by using ANOVA (Analysis of Variance). All the values were taken in triplicate and the respective 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. Results and Discussion

3.1. GC-MS analysis of essential oil of *Premna mucronata* leaves

The GC-MS analysis of PMLO revealed the presence of over 71 compounds comprising of 96.6% of the whole chemical profile (see Fig. 2). The major constituent characterized compound was 3-octanone that accounts for 23.6% of the total oil composition. The other major constituents were ethyl hexanol (13.9%), 1-octen-3-ol (9.6%), linalool (5.5%), methyl salicylate (2.9%) and (*E*)-caryophyllene (2.9%). The phytochemical composition of PMLO revealed the presence of a complex mixture of terpenoids and other identified constituents such as hydrogenated monoterpenoids (5.5%), oxygenated monoterpenoids (11.4%), hydrogenated sesquiterpenoids (13.9%), oxygenated sesquiterpenoids (7.1%), hydrogenated diterpenoids (0.4%) and others (61.0%).
Kumar et al. (2011) have reported the presence of 29 components in the essential oil of *Premna latifolia* from Kotdwar of Garhwal region, Uttarakhand constituting about 78.1% of the total oil. The most abundant constituents of the oil reported were 1-octen-3-ol (35.7%), terpendiol II (7.2%), δ-guaiene (7.5%), 2-undecanone (4.8%) and α-pinene (3.3%). Apart from the difference in yield and major constituents, PMLO from Kumaun region differed in its qualitative and quantitative compositions from the one from Garhwal region. Terpendiol II (7.19%), δ-guaiene (7.49%), 2-undecanone which were the major constituents of essential oil of Garhwal region were not found in PMLO of the present study. Compound 1-octen-3-ol (35.69%) which was major constituent of Garhwal region, is the third major constituent of PMLO. On the other hand, α-pinene with frequency of 3.27% in Garhwal collection, contributed only 0.1% to the total oil composition of PMLO. Similarly, ethyl hexanol and methyl salicylate were not present in Garhwal collection. Linalool was 2.4% in Garhwal collection, while in PMLO it accounted for 5.5%, and caryophyllene which was 2.9% in PMLO, was only 0.3% in Garhwal collection. These chemical variations may be due to cumulative effect of the climatic and soil variations. Chemical compositions of the essential oils from the leaves of *Premna mucronata* collected from Kumaun and Garhwal region of Uttarakhand have been compared with each other as shown in Table 1. Renjana and John (2013) reported the larvicidal activities of the leaf extracts and essential oil of *Premna latifolia* Roxb. against *Aedes albopictus* Skuse. In addition, hexane, ethyl acetate and ethanolic extract of *Premna latifolia* were investigated by Krishnamoorthi et al. (2015) for phytochemical analysis and antioxidant property.

3.2. Antioxidant activity

In the present study, the DPPH radical scavenging activity of PMLO was performed and the respective antioxidant activity was found to be IC_{50} = 11.18 ± 0.03 µg/mL comparable to positive controls, BHT and catechin with IC_{50} value of 9.48 ± 0.02 µg/mL and 6.2 ± 0.16 µg/mL respectively. PMLO was also found to have good antioxidant activity with IC_{50} = 18.82 ± 0.46 µg/mL comparable to the standard Na-EDTA with IC_{50} = 14.64 ± 0.03 µg/mL. The reducing power activity of PMLO was evaluated and showed good antioxidant activity with of IC_{50} = 21.69 ± 0.02 µg/mL comparable to ascorbic acid (13.97 ± 0.09 µg/mL, see Table 2). It has been previously reported that the antioxidant effectiveness of essential oil is due to the presence of 3-octanone (Jhang et al., 2008), 1-octen-3-ol (Zhang et al., 2008), linalool (Seol et al., 2016), methyl salicylate (Oloyede, 2016) and (*E*)-caryophyllene (Ali et al., 2017). The present study shows that all these compounds are present in PMLO as major constituents. Hence, these compounds might be responsible for lower IC_{50} value or higher antioxidant activity of PMLO.

Table 1
Comparison of leaves essential oil composition of *Premna mucronata* Roxb. collected from Kumaun and Garhwal region of Uttarakhand.

S.N.	Compound	KI	Composition (%)	
			Kumar et al. (2011): Garhwal region	Present study: Kumaun region
1	(*E*)-3-hexen-1-ol	851	-	0.1
2	3-hexen-1-ol	857	-	1.4
3	1-hexanol	858	-	2.8
4	3-hexanol	858	0.5	-
5	(*E*)-2-hexen-1-ol	874	-	0.8
6	α-pinene	936	3.3	0.1
7	pinane	947	0.6	-
8	3-octanone	970	-	23.6
9	sabinene	974	-	2.4
10	1-octen-3-ol	974	35.7	9.6
11	1-octen-3-one	978	-	0.3
12	β-pinene	993	0.7	t
13	3-octanol	1000	1.8	-
14	α-phellandrene	1003	0.3	-
15	2(10)-pinen(*E*)-3-one	1016	0.9	-
16	α-terpinene	1018	-	0.1
17	limonene	-	-	1.6
18	β-ocimene	1023	-	0.2
19	ethyl hexanol	1025	-	13.9
20	*p*-cymene	1026	-	0.2
21	β-phellandrene	1030	0.3	-
22	eucalyptol (1,8-cineole)	1031	-	0.3
23	2(3H)-furanone, 5-ethenyl dihydro-5-methyl	1036	1.6	-
24	*cis*-sabinene hydrate	1057	-	0.3
25	γ-terpinene	1062	-	0.3
26	1-octanol	1078	-	0.1
27	*cis*-linalool oxide	1078	0.2	-
28	pentyl iso propyl sulphide	-	-	0.7
29	*trans*-linalool oxide	1094	0.2	t
30	nonanal	1097	0.1	t
31	linalool	1102	2.4	5.5
32	1,5,7-octatrien-3-ol 3,7-dimethyl	1106	0.6	-
33	cyclohexanone, 3-(4-hydroxybutyl)-2methyl	1141	0.6	-
34	(-)-terpinen-4-ol	1182	-	0.8
35	α-terpineol	1189	-	0.3
36	terpendiol-I	1190	3.9	-
37	methyl salicylate	1193	-	2.9
38	β-cyclocitral	1204	-	0.4
39	3-t-pentyl cyclopentanone	1204	0.6	-
40	α-copaene	1221	-	0.1

Table 1 Continued

41	nerol	1228	-	0.3
42	geraniol	1255	-	0.5
43	ethyl-salicylate	1270	-	0.4
44	terpendiol-II	1272	7.2	-
45	2-undecanone	1294	4.9	-
46	propenylguaiacol	1324	0.3	-
47	(*E*)-2-hexenyl tiglate	-	-	0.1
48	α-terpinyl acetate	1350	-	0.1
49	eugenol	1356	-	1.9
50	β-damascenone	1391	-	1.4
51	β-elemene	1392	-	1.9
52	α-gurjunene	1410	-	0.4
53	2-cyclohexen-1-one,2-butyl-3methoxy-	1426	-	0.1
54	β-copaene	1428	-	0.1
55	(*E*)-caryophyllene	1434	0.7	-
56	*trans*-α-bergamotene	1436	-	0.2
57	α-humulene	1455	-	1.9
58	(*E*)-α-ionone	1456	-	0.1
59	geranyl acetone	1460	-	0.2
60	eudesma-4(14),11-diene	1476	-	0.7
61	germacrene D	1480	-	1.3
62	α-selinene	1494	-	0.6
63	(*Z*)-α-bisabolene	1504	-	0.1
64	(*E,E*)-α-farnesene	1509	-	1.9
65	β-sesquiphellandrene	1519	-	1.2
66	δ-cadinene	1524	-	0.1
67	(*E*)-nerolidol	1564	-	0.7
68	caryophyllene oxide	1583	-	0.8
69	*trans*-caryophyllene	1584	0.6	2.9
70	*n*-tetradecanal	1601	-	0.2
71	humulene epoxide II	1608	-	0.7
72	spathulenol	1619	-	0.2
73	cyclohexane butylidene	1621	0.3	-
74	γ-eudesmol	1630	-	0.8
75	guaiol	-	-	1.8
76	10,10-dimethyl-2,6-dimethylene bicycle [7.2.0] undecan-5 β-ol	1645	1.7	-
77	β-eudesmol	1651	-	0.1
78	pogostole	-	-	0.9
79	7-*epi*-α-eudesmol	1658	-	0.2
80	δ-guaiene	1667	7.5	-
81	α-bisabolol	1685	-	0.2
82	dodec-(7*Z*)-en-1-yl acetate	-	-	0.6
83	9-eicosene	-	-	0.4
84	patchulane	1685	0.5	-
85	1,1,1,3,5,7,7,7-octamethyl-3,5bis(tri 2-bromo dodecane methyl siloxy) tetrasiloxane	1702	0.4	-
86	α-agarofuran	1750	-	0.2

Table 1 Continued

87	2H-inden-2-one, 1,4,5,6,7,7a-hexahydro-7a-methyl-, (S)-	-	-	0.3
88	6,8-nonadien-2-one, 6-methyl-5-(1-methylethylidene)-	-	-	0.4
89	α-agarofuran	1750	-	0.2
90	2H-inden-2-one, 1,4,5,6,7,7a-hexahydro-7a-methyl-, (S)-	-	-	0.3
91	6,8-nonadien-2-one, 6-methyl-5-(1-methylethylidene)-	-	-	0.4
Total			78.1%	96.6%

KI: Kovats indices, t: trace (<0.1%)

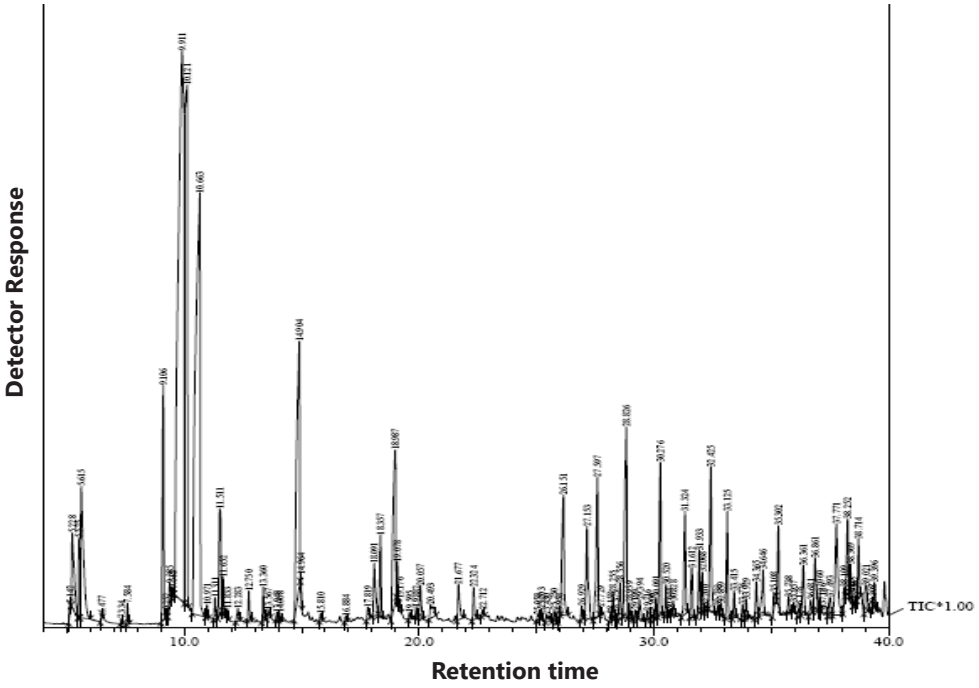


Fig. 2. Gas chromatogram of essential oil constituent components of *Premna mucronata* Roxb. (PMLO).

Table 2
Antioxidant activity of leaves essential oil of *P. mucronata*.

S.N.	Sample Name	Mean value ± SD		
		DPPH radical scavenging activity (IC_{50}: µg/mL)	Metal chelating activity (IC_{50}: µg/mL)	Reducing power activity (IC_{50}: µg/mL)
1	PMLO	11.18 ± 0.03[a]	18.82 ± 0.46[b]	21.69 ± 0.02[b]
2	BHT	9.48 ± 0.02[a]	-	-
3	Catechin	6.2 ± 0.16[c]	-	-
4	EDTA	-	14.64 ± 0.0[d]	-
5	Ascorbic acid	-	-	13.97 ± 0.09[d]

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 ($p<0.05$), PMLO: *Premna mucronata* leaves essential oil, BHT: Butylated hydroxytoluene, EDTA: Ethylene Diamine Tetra acetic acid (Sodium salt), SD: Standard Deviation, IC_{50}: Half minimal Inhibitory concentration.

3.3. Anti-inflammatory activity

The anti-inflammatory activity was performed using *in-vitro* albumin denaturation assay (Chandra et al., 2012). It was observed that PMLO showed good anti-inflammatory activity with IC_{50} = 30.27 ± 0.005 µg/mL comparable to Diclofenac sodium (IC_{50} = 13.42 ± 0.13 µg/mL) as positive control. The IC_{50} values for PMLO and positive control are given in Table 3. Previously, Kumari et al. (2011) reported the anti-inflammatory activity of *P. mucronta* leaves extract against carrageen induced rat hind paw edema. Furthermore, Mahire et al. (2009) reported anti-inflammatory effect of *P. latifolia* leaves using carrageenan-induced paw edema, cotton pellet-

induced granuloma, and acetic acid-induced vascular permeability models methods. The study revealed that some constituents like linalool (Peana et al., 2002), (*E*)-caryophyllene (Gertsch et al., 2008), methyl salicylate (Zhang et al., 2011), terpenin-4-ol (Hart, P.H., 2000), and α-terpineol (Held et al., 2007) were responsible for anti-inflammatory activity. The present study revealed that PMLO exhibits promising anti-inflammatory activity. Some valuable natural compounds, namely linalool, (*E*)-caryophyllene, methyl salicylate, terpenin-4-ol and α-terpineol were also characterized in PMLO. Hence, it might be possible that anti-inflammatory activity may be due to the cumulative effect of occurrence of these compounds in major, minor or trace amounts.

Table 3
Anti-inflammatory activity of leaves essential oil of *P. mucronata*.

S.N.	Sample Name	Mean IC_{50} values (µg/mL) with SD
1	PMLO	30.27 ± 0.005[a]
2	Diclofenac sodium	13.42 ± 0.13[b]

PMLO: *Premna mucronata* leaves Essential Oil, IC_{50}: Percent Inhibition of Protein Denaturaion

3.4. Antibacterial activity

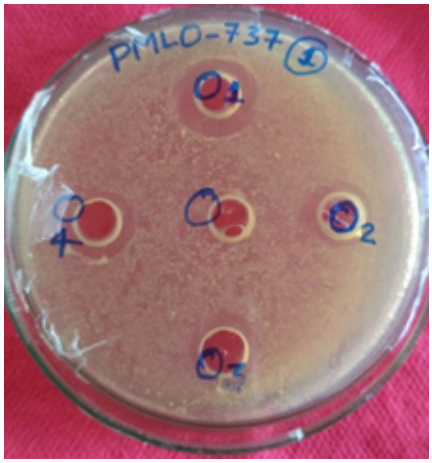
In the present study, antibacterial activity of PMLO was evaluated against the two bacterial strains, namely *E. coli* (MTCC No. 443) and *S. aureus* (MTCC No. 737) using agar well diffusion method. Gentamycin sulphate was used as positive control. Four concentrations involving 250 ppm, 500 ppm, 750 ppm and 1000 ppm of leaves essential oil were used. Regarding the obtained results, PMLO showed maximum zone of inhibition of 14.33 ± 0.58 mm against *E. coli* and 14.66 ± 0.58 mm against *S. aureus* both at concentrations ≥ 1000. The results of zone of inhibition (in mm) measurements by PMLO against *E. coli* and *S. aureus* are given in Table 4 and Fig. 3.

Natural compounds like linalool (Herman et al., 2016), (*E*)-caryophyllene (Montanari et al., 2011), methyl salicylate (Oloyede, 2016), sabinene (Arunkumar et al., 2014), terpenin-4-ol (Dorman and Deans, 2008) and α-terpineol (Dorman and Deans, 2008) were found to exhibit remarkable antibacterial activity. All these compounds were also found in the present study in different quantities. From these results and previous reports as mentioned above, it can be concluded that the antibacterial activity of PMLO might be due to the presence of these constituents or collective effect of various major, minor, and trace constituents.

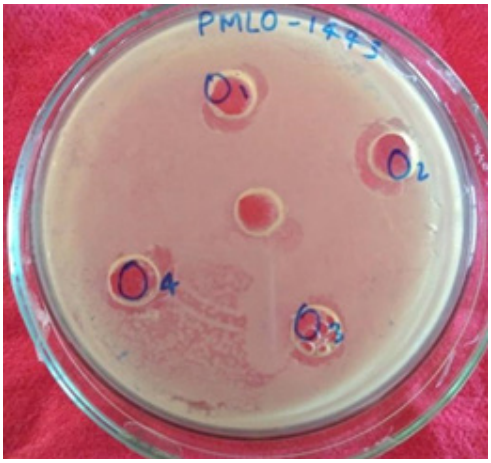
Table 4
Antibacterial activity of essential oil of *P. mucronata* against *E. coli* and *S. aureus*.

S.N.	Sample name	Concentration (ppm)	Zone of inhibition Mean (Mean value ± SD) mm	
			Against *E. coli*	Against *S. aureus*
1	PMLO	250	11.33 ± 0.58[b]	11.66 ± 0.58[b]
		500	10.33 ± 0.58[b]	8.66 ± 0.58[c]
		750	13.66 ± 0.58[a]	7.66 ± 0.58[c]
		1000	14.33 ± 0.58[a]	14.66 ± 0.58[a]
2	Gentamicin sulphate	250	20.33 ± 0.58[d]	18.33 ± 0.58[d]
		500	28.33 ± 0.58[c]	25.33 ± 0.58[c]
		750	34.66 ± 0.58[b]	32.33 ± 0.58[b]
		1000	40.33 ± 0.58[a]	38.33 ± 0.58[a]

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 ($p < 0.05$). PMLO = *Premna mucronata* Leaves Essential Oil.



Antibacterial activity of PMLO against *S. aureus* (MTCC No. 737)



Antibacterial activity of PMLO against *E. coli* (MTCC No. 443)

Fig 3. Representation of antibacterial activity of *Premna mucronata* Roxb. (PMLO).

4. Concluding remarks

In conclusion, the obtained results in the present study emphasized that the chemical composition of the essential oil separated from the leaves of *P. mucronata* was dominated by hydrogenated sesquiterpenoids (13.9%) and oxygenated monoterpenoids (11.4%). The present study revealed that *P. mucronata* can be a good source of compounds like 3-octanone (the major constitutent), ethyl hexanol, 1-octen-3-ol, linalool, methyl salicylate and (*E*)-caryophyllene which have found find wide applications in pharmacological activities as well as perfumery and flavoring industries (Zhang et al., 2008; Oloyede, 2016; Xiong et al., 2017). This study also revealed that the essential oil of *P. mucronata* possesses potential antibacterial, anti-inflammatory and antioxidant properties. The attributed activities are confirmed by the use of this plant as one of the component in dashmula and chawanprash. Thus, the separated oil of *P. mucronata* can be used as a resource of revenue for local communities as well as a potential herbal material for medicinal industries. However, further studies are still required to investigate new biological active compounds from the essential oil of *P. mucronata* or its organic extracts.

Conflict of interest

The authors declare that there is no conflict of interest.

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