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Chemical Composition of Leaves, Stem bark and Fruit Essential Oil from *Premna foetida* Linn

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Abstract

Premna foetida is a woody plant with short and twisted trunk. *P. foetida* is a scandent, erect shrub or small tree, thorny on the trunk and large branches. Leaves are opposite or whorled and entire or serrate. *Premna foetida* is a wild plant locally known as "*Daun Sebuas*". *P. foetida* is used for it nutritive and as traditional treatment. The fruit and leaves of *P. foetida* are prepared for salad. The study aimed at the hydrodistillation and antioxidant activity of leaves, stem-bark and fruits essential oil from Premna foetida Linn, they were analysed by capillary GC and GC-MS. Ninety eight compounds representing 81.68±0.02, 37.31±0.05and 93.45±0.03 of the isolates of leaves, stem-bark and fruits respectively were identified, the most abundant were α –Duprezianene (77.27±0.03, leaves, α-Gurjunene (36.06 ±0.05) fruits and Hinesol acetate (77.19±0.03) stem-bark. Components among which sesquiterpenoids dominated. The total volatiles were assayed for antioxidant potentials using 2, 2-diphenyl-1-picrylhydrazyl (DPPH). The total volatiles showed strong activity with IC_{50} of 11.74±0.82µg/ml, 9.63±0.34 µg/ml and 49.73±1.12 µg/ml for leaves fruits and stem-bark respectively.

Keywords: Chemical leaves, stem bark, fruit, Essential Oil, Premna foetida Linn

Major classifications: Food Science, Health Science

1. Introduction

The Lamiaceae are largest family of Lamiales, flowering and aromatic plants with 236 genera and 7,000 species. The genus *Premna* is consist of 200 species and distributed throughout Asia region. Premna species found in Nigeria is generally

characterized by trees, shrubs, and climbers (Mali, 2016). *Premna foetida* is a woody plant with short and twisted trunk. *P. foetida* is a scandent, erect shrub or small tree, thorny on the trunk and large branches (Minh, 2019). Leaves are opposite or whorled and entire or serrate. *Premna foetida* is a wild plant locally known as "*Daun Sebuas*". *P. foetida* is used for it nutritive and as traditional treatment. The fruit and leaves of *P. foetida* are prepared for salad. It is reported that the leaves of *P. foetida* were used to prepare traditional medicine to treat skin diseases. Essential oils (EOs) are aromatic oils that extracted from plants parts including leaves, flowers, fruits, seeds, barks, and roots (Padalia *et al.*, 2016; Tian *et al.*, 2020). EOs mainly consist of terpenoids, aromatic and aliphatic compounds with low-molecular-weight compounds (Chanegriha *et al.*, 1993; Bulatovic *et al.*, 2006; Abdel-Lateif *et al.*, 2016; Padalia *et al.*, 2016). The significant of natural products in phamarceutical, food, agricultural, and cosmetic industries has shown innumerable studies on the biological responds of those compounds (Abdel-Lateif *et al.*, 2016). Over the years, natural products have been claimed to play a key role in scavenging free radicals in an attempt to inhibit lipid peroxidation and cells damage (Umaru et al., 2019a). They act as natural antioxidants to reduce the risk of various diseases as well as to delay food deterioration. Hence, the demand of antioxidant from natural sources is increased over the years compared to synthetic antioxidants (Falowo et al., 2014; Lourenco et al., 2019). From the literature study, chemical constituents, and biological activity of EOs from the genus *Premna* has been reported. However, limited studies conducted on the *P. foetida* especially on its volatile constituents of essential oil, neither on its biological responds. As well as its impact on this departments; Food Science (Restaurant Management, Customer Eating-out behavior, Restaurant Marketing, Food Nutrition, Healthy Food), Health Science (Environmental Safety and Engineering, Medical Imaging and Detector, Nursing and Mental Health, Public Health, Health Policy and Economy, Dental Health), as well as other Hospitality Related Areas. Thus, this study aimed to isolate the essential oil from leaves, stem bark, and fruits of *P. foetida* along with their antioxidative potential.

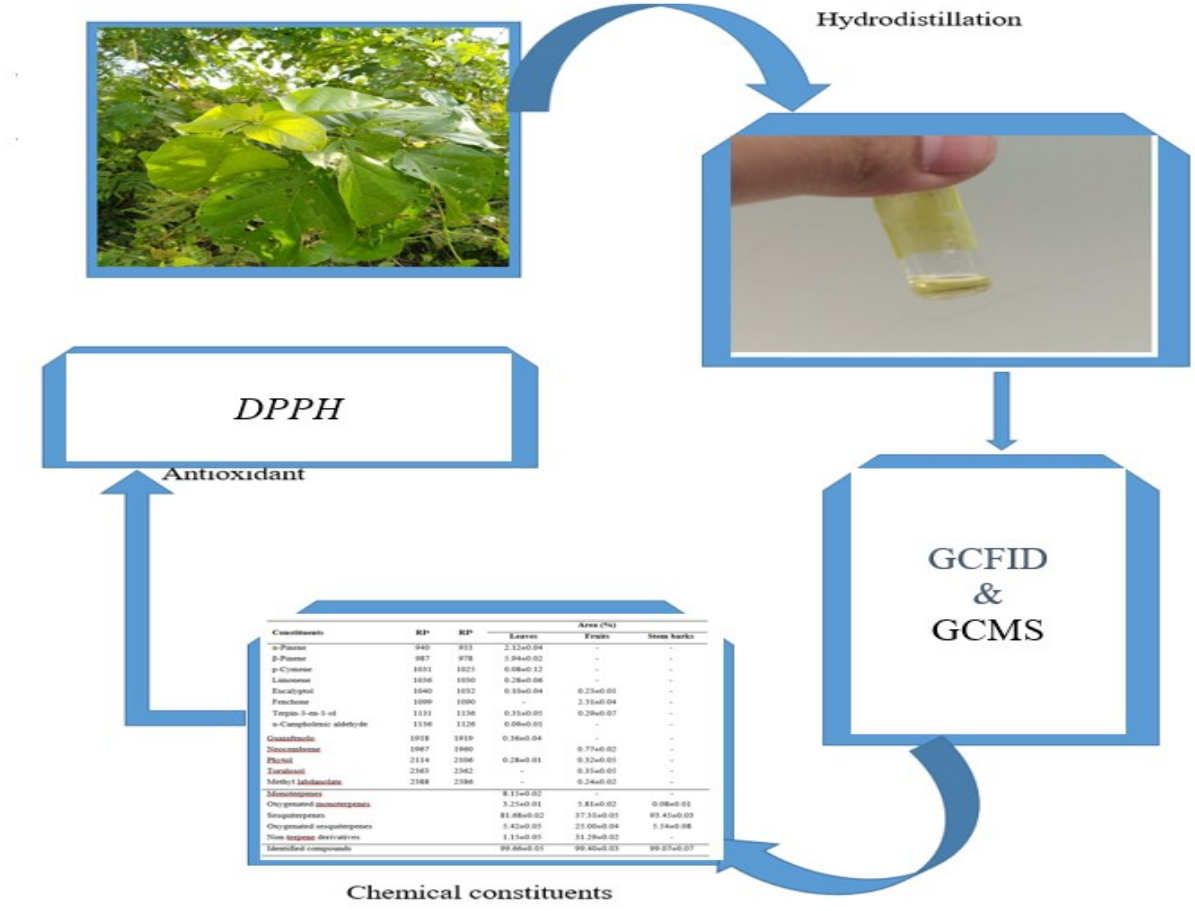

Figure 1: Structural abstract

2. Materials and method

2.1 Plant material and essential oil isolation

The plant materials were collected in February 2020. The plant was identified by a botanist from Department of Plant Resource Science and Management, Faculty of Resource Science and Technology, Universiti Malaysia Sarawak. A voucher specimen (BEN002) was deposited in the herbarium of Universiti Malaysia Sarawak. After harvesting, the leaves, roots, and stem bark of *P. foetida* were washed using distilled water prior to oil extraction. Roots and stem bark were kept in room temperature and ground into powder.

2.2. Essential oil Extraction and isolation

200 g of leaves, fruits, and stem bark of *P. foetida* were subjected to 6 hrs. hydrodistillation using Clevenger-type apparatus and distilled n-pentane as the collecting solvent. Distilled n-pentane contain essential oil was concentrated using nitrogen gas to obtain yellowish oil. EOs collected were preserved in 4^{o}C until analysis. The extraction of EOs for every plant part was carried out in triplicate and the percentage yield was calculated.

2.3. Essential oil analysis

2.3.1. Gas Chromatography

Gas Chromatography analysis were performed using a Perkin Elmer Clarus 680 (Perkin Elmer, United States). A BPX-5 fused-silica capillary column (Trajan Scientific and Medical, Australia) with 5% phenyl polysilphenylene-siloxane, 30 m x 0.25 mm i.e., film thickness, 0.25 µm was used. The temperature set as follow: Initial; 50 ºC for 1 min then ramped to final temperature 250 ºC at a rate of 6.5 ºC/min and held for 10 min, injector and detector temperatures were set at 260 ºC, interface temperature was set at 250 ºC. Helium was used as a carrier gas with a flow rate of 1.0 mL/min. Injection volume was 1 µL while splitting ratio of 20:1 was applied. The relative percentage amount of each constituent calculated was based on the average peak areas of three injections from three independent extractions. Experimental retention indices (RIs) of the constituents were calculated relative to those of *n*-alkanes (C_9-C_{24}) as reported by Umaru et al. (2018).

2.3.2. Gas Chromatography Mass Spectrometry

GC-MS analysis was performed using a Shimadzu GCMS-QP2010 Plus (Shimadzu, Japan) with the same capillary GC conditions as described by Umaru et al. (2020). Interface temperature was set at 250 ºC while 70 eV of electron impact ionization energy was used with a scan mass range 28-400 *m/z*. The constituents were identified by matching their mass spectra with NIST-17 MS library and by comparing their experimental RIs with those published in literature (Adams, 2007).

2.3.3. DPPH radical scavenging activity

The 2, 2-diphenyl-1-picrylhydrazyl (DPPH) scavenging effects of EOs from the leaves, twigs and fruits of *P. foetida* were assessed according to Umaru et al. (2019b) with slight modifications. Briefly, samples of EOs were diluted using methanol at different concentrations. Each sample solution was then mixed well with DPPH solution (0.2 mM). The mixture was incubated in the dark at room temperature for 30 min. Then, the absorbance was recorded at 517 nm using a UV/Vis spectrophotometer (PerkinElmer, Massachusetts, USA). Each sample solution was tested in triplicate and results were expressed as mean ± SD. Ascorbic acid was used as a positive control in this study. IC_{50} obtained was based on the concentration of EOs that required to give 50% inhibition using the graph of inhibition percentage versus EOs concentration.

3. Results

Table 1. Chemical constituents of the essential oil from *Premna serratifolia.*

	Constituents	RI[a]	RI[b]	Area (%)		
				Leaves	Fruits	Stem barks
1	α-Pinene	940	933	2.12±0.04	-	-
2	β-Pinene	987	978	5.94±0.02	-	-
3	p-Cymene	1031	1025	0.08±0.12	-	-
4	Limonene	1036	1030	0.28±0.06	-	-
5	Eucalyptol	1040	1032	0.10±0.04	0.23±0.01	-
6	Fenchone	1099	1090	-	2.31±0.04	-
7	Terpin-3-en-1-ol	1131	1136	0.31±0.05	0.29±0.07	-
8	α-Campholenic aldehyde	1136	1126	0.09±0.01	-	-
9	Pinocampheol	1155	1165	0.20±0.06	0.99±0.05	-
10	trans-Pinocamphone	1160	1160	-	0.51±0.14	-
11	Umbellulone	1175	1170	-	0.36±0.11	-
12	Borneol	1178	1173	0.32±0.05	-	-
13	trans-Mayol	1186	1181	0.78±0.11	0.36±0.07	0.08±0.05
14	4-Terpineol	1192	1184	0.29±0.15	-	-
15	p-Cymen-8-ol	1198	1188	0.13±0.04	-	-
16	Bergamal	1199	1194	-	0.33±0.01	-
17	γ-Terpineol	1207	1200	1.53±0.02	-	-
18	E-Violettyne	1210	1213	-	3.79±0.06	-
19	Alpha-E-Bisabolene	1221	1216	0.08±0.01	-	-
20	Aphermate	1230	1228	-	0.24±0.02	-
21	Geraniol	1255	1255	-	0.26±0.01	-
22	Nopol	1269	1279	-	-	0.17±0.03
23	Decyl alcohol	1275	1278	-	0.85±0.06	-
24	Undecan-2-one	1294	1294	-	0.27±0.02	-
25	n-Undecanal	1311	1309	-	0.51±0.19	-
26	isobutyl-Cinnamate	1313	1311	-	1.18±0.25	-
27	Undecanol	1380	1379	-	0.48±0.11	-
28	α-Duprezianene	1389	1385	77.27±0.03	-	-
29	β-Cubebene	1395	1392	0.64±0.04	-	-
30	α-chamipinene	1400	1400	0.13±0.08	-	-
31	α-Gurjunene	1404	1406	0.93±0.05	-	-
32	α -Gurjunene	1409	1406	-	36.06±0.05	-
33	Myrtenyl tiglate	1410	1407	0.38±0.01	-	-
34	4,8-α-Caryophyllene	1416	1418	-	6.98±0.08	-
35	α-Sinensal	1425	1425	0.14±0.05	-	-

36	α-Himachalene	1443	1449	0.13±0.06	-	-
37	α-Humulene	1452	1454	-	0.22±0.03	-
38	ethyl-, (Z)-Cinnamate	1482	1473	-	-	15.97±0.09
39	γ-Himachalene	1483	1482	0.28±0.08	-	-
40	Citronellyl isobutyrate	1484	1483	-	21.62±0.06	-
41	cis-β-Guaiene	1492	1498	0.17±0.10	-	-
42	Eremophilene	1492	1491	-	0.27±0.05	-
43	α-Bulnesene	1509	1505	0.12±0.11	0.49±0.01	-
44	α-Cuprenene	1512	1508	0.53±0.21	-	-
45	7-epi-α-Selinene	1519	1518	0.63±0.04	-	-
46	Z-Nerolidol	1533	1531	-	0.44±0.03	-
47	trans-Cadina-1,4-diene	1535	1536	0.86±0.02	-	-
48	E-α-bisabolene	1539	1540	-	0.26±0.04	-
49	Occidentalol	1541	1548	0.17±0.03	-	-
50	cis-Muurol-5-en-4-beta-ol	1550	1548	-	-	0.06±0.09
51	β-Calacorene	1562	1564	0.20±0.02	-	-
52	E-Nerolidol	1566	1561	-	-	0.08±0.11
53	Palustrol	1569	1568	1.07±0.11	-	-
54	Pentadecanolide	1573	1571	-	2.01±0.02	-
55	Globulol	1592	1592	0.08±0.21	-	-
56	Copaborneol	1603	1613	0.28±0.02	-	-
57	Humulol	1608	1604		2.02±0.06	0.08±0.18
58	cis-Timberol	1610	1618	0.28±0.06	-	-
59	cis-Timberol	1618	1618	-	0.24±0.05	-
60	epi-gamma-Eudesmol	1622	1624	-	-	1.50±0.06
61	epi-Cedrol	1624	1621	-	0.39±0.03	-
62	Eremoligenol	1632	1627	-	-	0.13±0.15
63	γ-Eudesmol	1633	1632	0.11±0.14	-	-
64	Epicubenol	1638	1631	0.30±0.03	0.67±0.02	0.06±0.11
65	Hinesol	1649	1645	0.18±0.07	0.23±0.01	-
66	Himachalol	1650	1650	-	-	0.06±0.04
67	α-Muurolol	1651	1651	0.20±0.05	-	-
68	Allohimachalol	1660	1664	-	0.25±0.14	-
69	Epi-β-Bisabolol	1667	1675	0.36±0.06	0.72±0.11	-
70	Bulnesol	1669	1673	-	-	2.60±0.01
71	β-Bisabolol	1672	1677	0.42±0.14	1.74±0.06	-
72	Cis-Carvyl tiglate	1677	1679	-	-	0.36±0.07
73	Occidenol	1682	1681	-	-	0.31±0.05
74	Shyobunol	1683	1686	-	4.39±0.01	-

75	5-neo-Cedranol	1686	1862	0.12±0.21	-	-
76	8-Cedren-13-ol	1690	1986	-	-	0.27±0.05
77	Junicedranol	1695	1690	-	0.32±0.02	-
78	11-αH-Himachal-4-en-1-beta-ol	1702	1700	0.34±0.07	-	-
79	(2Z,6E)-Farnesol	1725	1720	0.36±0.07	-	-
80	β-Acoradienol	1763	1760	-	4.29±0.03	-
81	Cedrenyl acetate	1769	1763	-	0.31±0.05	-
82	Hinesol acetate	1785	1786	-		77.19±0.03
83	Phytone	1844	1841	-	0.27±0.09	-
84	(E)-beta-Santalol acetate	1873	1870	-	-	0.05±0.09
85	Nootkatone	1879	1877	-	-	0.03±0.22
86	methyl-Linolenate	1885	1893	-	-	0.07±0.12
87	(E)-2-tridecenyl tiglate	1917	1914	-	0.23±0.18	-
88	Guaiafenolo	1918	1919	0.36±0.04	-	-
89	Neocembrene	1967	1960		0.77±0.02	-
90	Phytol	2114	2106	0.28±0.01	0.32±0.05	-
91	Torulosol	2363	2362	-	0.35±0.05	-
92	Methyl labdanolate	2388	2386	-	0.24±0.02	-
	Monoterpenes			8.15±0.02	-	-
	Oxygenated monoterpenes			3.25±0.01	5.81±0.02	0.08±0.01
	Sesquiterpenes			81.68±0.02	37.31±0.05	93.45±0.03
	Oxygenated sesquiterpenes			5.42±0.05	25.00±0.04	5.54±0.08
	Non-terpene derivatives			1.15±0.05	31.29±0.02	-
	Identified compounds			99.66±0.05	99.40±0.03	99.07±0.07

4. Discussion

The extraction yield of the EOs obtained from the leaves, twigs and fruits were recorded as 0.25 ± 0.05%, 0.24 ± 0.02% and 0.43 ± 0.02% (w/w), respectively. In the EOs of the leaves, fourty-five constituents were identified with the dominant constituents being α-Duprezianene (77.27%), β-Pinene (5.94%) and α-Pinene (2.12%). The leaf oil gave an earthy scent upon hydrodistillation. Meanwhile, thirty-two constituents were obtained in the EOs of the stem bark. Hinesol aceate (77.19%), a non-terpenes derivatives, was found as the major constituent in the twig oil. Interestingly, woody smell was detected from the stem bark oil. On the other hand, sesquitepenes were predominated in the fruit oil, accounting for 93.45% of the total oil. This was largely due to the presence of α-Gurjunene (37.31%). It is worth to note that the fruit oil resembled the floral scent.

Generally, terpenes family represent the main class of compounds in plants' EOs. They consist of low molecular weight compounds at atmospheric pressure and temperature (Lejonklev *et al.*, 2013; Cho *et al.*, 2018). According to the results, sesquiterpenes and its oxygenated hydrocarbons constituted 87.10% of the total leaf oil. This explain the odor of the leaves since the sesquiterpenes is typically responsible for the aroma characteristics of essential oil. This gives the communities the advantage to continue to discover new techniques that can transform the demands in the field of Food Science (Restaurant Management, Customer Eating-out behavior, Restaurant Marketing, Food Nutrition, Healthy Food), Heahth Science of the benefit of essential oil.

Table 2: Antioxidant of *P. foetida* leaf, Fruits and Stem-bark

Antioxidant potential of P. foetida in µg/ml				
	Ascorbic acid	Leaf	Fruits	Stem-bark
IC_{50}	26.34±0.52	11.74±0.82	9.63±0.34	49.73±1.12

The antioxidant activity of the leaf, fruit and stem bark EOs of *P. foetida* was evaluated using DPPH radical scavenging activity (Table 2). The assay is focused on the assessment of antioxidants' scavenging ability against it. By acquiring a hydrogen atom from antioxidants to the subsequent hydrazine, the odd electron of the nitrogen atom in DPPH is reduced (Pękal & Pyrzynska, 2015). In the present study, the leaf and fruit oil showed better scavenging activity than the stem bark oils, with IC_{50} of 11.74±0.82, 9.63±0.34 and 49.73±1.12 µg/ml, respectively. Meanwhile, ascorbic acid which act as a positive control displayed an IC_{50} of 26.34±0.52 µg/ml. A high radical scavenging activity of the leaf and ffruit oil could be attributed to its high content of α-Duprezianene in leaf oil and α-Gurjunene in fruit oil. A sesquiterpene compounds had been reported inhibit good antioxidant activity (Sghaier *et al.*, 2011; Proshkina *et al.*, 2020). Meanwhile, other minor sequiterpenes compounds, such as α-Cuprenene and *trans*-Cadina-1,4-diene may also have contributed to the antioxidant activity in interdependent behaviors. Meanwhile, weak antioxidant activity was shown by the stem bark oil. It was documented that non-terpenoids, were largely contributed to the antioxidant activity of plant Eos. However, our findings indicated that major composition of Hinesol acetate (77.19%) did not play a significant role in scavenging DPPH radicals. It recommended the enmity between the various constituents which diminished its cell reinforcement movement.

5. Conclusion

The EOs from the leaf, twig and fruit of *P. foetida* have been obtained using conventional hydrodistillation method. Chemical divergence among the EOs composition from different plant parts revealed their varied antioxidant strength. Sesquiterpenoids rich EOs from the leaf and fruit of *P. foetida* exhibited a significant antioxidant potential. These oils is predominantly constituted by α-Duprezianene and α -Gurjunene, respectively. The use of leaf and fruit oil is promising as natural antioxidant. To the best of our knowledge, this is the first study on the chemical composition and biological activities of *P. foetida* EOs. The findings could serve as an important reference for future investigations on other *Premna* species, which are rare and limited scientific information.

Recommendation

In this modern world healthy food and efficient medicine is highly recognized for a better health as recognized by the government and nongovernmental organization, hence increasing the discovery and influence of essential for the integration to be possible. It is however recommended that essential oil be recognized in providing healthy food and health care for the people. There should be a regular communication and collaboration of research among Food Science (Restaurant Management, Customer Eating-out behavior, Restaurant Marketing, Food Nutrition, Healthy Food), Health Science (Environmental Safety and Engineering, Medical Imaging and Detector, Nursing and Mental Health, Public Health, Health Policy and Economy, Dental Health), Other Hospitality Related Areas, to help get a better understanding of each methods, composition and chemical constituents and hence bridge the gap between them. Further research and studies should be of collaborative to know the knowledge, use and integration of food and its medicinal value of the essential oil.

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