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Research Article

Variation in chemical constituents of essential oils of the fresh, dried and fermented leaves of *Premna serratifolia*

Khin Su Yee[1,2], Penpun Wetwitayaklung[1], Worrakanya Narakornwit[1], Tasamaporn Sukwattanasinit[1], Bunyapa Wangwattana[1], Uthai Sotanaphun[1*]

[1] Department of Pharmacognosy, Faculty of Pharmacy, Silpakorn University, Nakhon Pathom, Thailand
[2] Department of Pharmacognosy, University of Pharmacy, Mandalay, Myanmar

ABSTRACT

This study aimed to identify the composition of the essential oils of the fresh, dried and fermented leaves of *Premna serratifolia* by gas chromatography-mass spectrometry (GC-MS) using DB-5 and Carbowax 20M columns. A total of 77, 82 and 90 compounds were detected, which involved the main compound categories of hydrocarbons, terpenoids, and phenolics. Amyl vinyl carbinol (15.8-32.6%), linalool (11.1-15.1%), phytol (7.7-12.5%), salicylic acid methyl ester (3.9-7.2%) and (*E*)-caryophyllene (3.1-6.6%) were predominant components of the fresh leaf oils. After drying and fermentation, the chemical compositions were changed by various reactions. The amounts of amyl vinyl carbinol were decreased to 6.3-13.8% and 6.9-11.5% after drying and fermentation, respectively. Likewise, linalool and phytol were decreased to 6.3-7.5% and 7.3-9.0% after drying, and decreased to 5.3-7.9% and 2.0-3.4% after fermentation, respectively. In both the dried and the fermented leaf oils, alpha-humulene was disappeared and beta-myrcene was detected as a new compound. The noticeable changes in chemical composition after drying process were much increasing in the amount of (*E*)-caryophyllene (6.6-12.2%) to become the most abundant compound, and the hydrolysis of palmitic acid ethyl ester to palmitic acid (5.0%). The fermentation method could dramatically increase the amounts of phenolic compounds especially *p*-vinylanisole (2.4-41.1%) which became the major compound, marked decrease the phytol and found acorenone B (4.4%) as a new compound. The present study demonstrated that drying and fermentation processes affected the volatile composition of the leaves of *P. serratifolia*.

Keywords:
Premna serratifolia, Essential oil, GC-MS, Drying, Fermentation

1. INTRODUCTION

Premna serratifolia L. (family Lamiaceae) is a shrub or tree in tropical and subtropical regions. Its leaves have a characteristic fetid smell and native people in Celebes use it as a food additive to reduce the fishy smell[1]. In Peninsula, Malaysia, and Indonesia, young leaves of this plant are used as vegetables[2]. In Myanmar, the leaves were used for the treatments of cancer and liver diseases. Some people take the preparation of the fresh leaves and some others use the dried leaves. If the fresh leaves of this plant were kept at room temperature or dried in the shade under inappropriate condition, some leaves will be fermented and turned to dark brown color. The fresh, dried and fermented leaves have different smells which suggested that their volatile chemical constituents should be in variation. Many medicinal plants have been reported for chemical variation between their fresh and dried leaves, such as *Tapinanthus bangwensis*[3], *Artemisia afra*[4], *Ocimum sanctum*[5], and *Cymbopogon citratus*[6]. One of the most well-known fermented example was the hydrolysis of aroma precursors by endogenous glycosidase during the manufacturing processes of Oolong tea and black tea, and many aroma compounds occurred[7].

This study was a keen interest to investigate the difference among volatile phytochemical constituents of the fresh, dried and fermented leaves of *P. serratifolia*

***Corresponding author:**
*Uthai Sotanaphun sotanaphun_u@su.ac.th

using gas chromatography coupled with mass spectrometry (GC-MS) technique. The result would be declared as basic scientific evidence that their qualities were not equal and should be concerned.

2. MATERIALS AND METHODS

2.1. Plant material

Leaves of *P. serratifolia* were collected from Mandalay Division, Myanmar, in October 2018. The taxanomic species was identified by comparing its flower and leaf characters with the references[8-10]. The voucher specimen (Ps172018) was deposited with the herbarium of the Department of Pharmacognosy, Silparkorn University, Thailand. The leaves were divided into three parts. The first part was the fresh leaves, the second part was dried in the shade, and the third part was fermented by packing in a plastic bag and place at room temperature for 10 days in the shade until the leaves turned to black color.

2.2. Preparation of essential oils

The fresh leaf sample (490 g), the dried leaf sample (244 g) and the fermented leaf sample (201 g) were separately chopped into small pieces and hydrodistillated in a Clevenger apparatus for 5 hours to obtain essential oils. The collected oils were dried over anhydrous sodium sulfate and stored at 4°C in air-tight glass containers. The yields of the essential oils of the fresh, dried and fermented leaves were 0.008, 0.139 and 0.139%, respectively.

2.3. GC-MS analysis

The essential oils were analyzed by Agilent 6890 gas chromatography equipped with Agilent technology, 5973N mass selective spectrometric detector (EIMS, electron energy, 70 eV, scanning from 40 to 500 m/z) with a quadrupole analyzer and an Agilent Chem Station data system (Agilent Technologies, U.S.A.). Two columns, fused silica capillary column (5%-phenyl)-methylpolysiloxane DB-5 (30 m x 0.32 mm ID x 0.25 µm film thickness) and Carbowax 20M polyethylene glycol (PEG) (60 m x 0.25 mm ID x 0.25 µm film thickness) were used. Ultra-high purity helium gas (99.999%) was used as a carrier gas at a flow rate of 1mL/min. The sample (1.0 µL) was injected with a splitless mode. The solvent delay for the detector was 3 minutes. The ion source temperature was 230°C and quadrupole temperature was programmed at 150ºC. For the DB-5 column, the initial oven temperature was 80°C and increased to 130°C at the 5°C/min, then increased to 280ºC at the 10ºC/min and hold for 5 min. For Carbowax 20M column, the initial oven temperature was 60ºC and increased to 220ºC at the 2ºC/min. Identification of compounds was performed by comparison of their RI relative to *n*-alkanes (C8-C26) with Adams[11], NIST Chemistry WebBook, SRD 69[12], Babushok et al[13] and Leffingwell et al[14]. Their mass spectra were also compared with libraries databases of Wiley7n.l and NIST 05.

3. RESULTS AND DISCUSSION

Essential oils of the fresh leaves of *P. serratifolia* were prepared by hydrodistillation and studied by GC-MS. Two GC columns, the non-polar DB-5, and the polar Carbowax 20M columns were used to ensure the most complete investigation of the constituents. The identification of each compound was based on its mass fragmentation pattern and RI (Retention Index) calculation comparing with data in the references. The results are shown in Table 1 and Table 2. Seventy-seven compounds were detected. Most of the identified compounds were classified into hydrocarbons (26.1% in DB-5 and 57.1% in Carbowax 20M), terpenoids (42.8% in DB-5 and 26.0% in Carbowax 20M) and phenolics (15.0% in DB-5 and 10.9% in Carbowax 20M) compound categories. Some fatty acids, apocarotenoids, and miscellaneous compounds were also detected (Table 3). The most abundant compounds were amyl vinyl carbinol (15.8% in DB-5 and 32.6% in Carbowax 20M), linalool (15.1% in DB-5 and 11.1% in Carbowax 20M), phytol (12.5% in DB-5, and 7.7% in Carbowax 20M), salicylic acid methyl ester (7.2% in DB-5 and 3.9% in Carbowax 20M) and (*E*)-caryophyllene (6.6% in DB-5 and 3.1% in Carbowax 20M). The previous study identified only 4 compounds of eugenol, eugenyl acetate, massoil and *cis*-2-oxabicyclo, 4.4.0-decane in the fresh leaf oil[1]. In this study, a very less amount of eugenol was detected (1.2% in DB5). This might be due to the different location and climate of plant origin. This study was the first report of the constituent of essential oil obtained from the leaves of this plant growing in Myanmar.

After the leaves of *P. serratifolia* were dried under the shade, increasing in the number of compounds in essential oils was observed, resulting in 82 total compounds; whereas when the leaves were allowed to ferment and turn to dark color, 90 compounds were detected (Tables 1 and Table 2). Some compounds were disappeared and some new compounds occurred as concluded in Figure 1. The compounds those lose after drying and fermentation methods were not much different. The significantly lost compounds were alpha-humulene, amyl carbinol, (*Z*)-3-hexen-1-ol and acetophenone. The new occurring compounds were mostly the small molecular weight terpenoids and aldehyde hydrocarbons compound categories, but they were detected in only trace amounts.

Table 1. Chemical constituents of the essential oils of the fresh, dried and fermented *P. serratifolia* leaves analysed by GC-MS using DB-5 column.

Compound	Fresh leaves		Dried leaves		Fermented leaves		Literature
	RI	%Relative amount	RI	%Relative amount	RI	%Relative amount	RI
alpha-Pinene	-	-	1015	0.8	-	-	939[11]
Amyl vinyl carbinol	1031	15.8	1030	6.3	1031	6.9	979[11]
Amyl ethyl ketone	-	-	-	-	1034	1.2	984[11]
beta-Myrcene	-	-	1037	8.7	1037	6.7	991[11]
Amyl ethyl carbinol	1049	6.4	-	-	1040	5.7	1002[12]
Unknown	-	-	-	-	1048	0.7	-
m-cymene	1059	0.2	-	-	-	-	1026[12]
Limonene	1069	0.3	1065	0.3	1066	0.4	1029[11]
Benzeneacetaldehyde	1079	1.5	-	-	1076	1.0	1042[11]
Unknown	-	-	-	-	1115	1.2	-
Linalool	1126	15.1	1122	6.3	1123	7.9	1097[11]
(*Z*)-beta-Terpineol	-	-	-	-	1125	0.6	1144[11]
Unknown	-	-	-	-	1144	0.4	-
p-Vinyl Anisole	1172	4.5	1172	8.9	1184	1.4	1160[12]
neo-Menthol	-	-	1190	0.6	-	-	1166[11]
Unknown	-	-	-	-	1190	1.1	-
Naphthalene	1204	0.6	-	-	-	-	1181[11]
Unknown	-	-	-	-	1205	0.5	-
alpha-Terpineol	-	-	-	-	1208	0.7	1189[11]
Unknown	-	-	1208	5.9	-	-	-
Salicylic acid methyl ester	1216	7.2	-	-	-	-	1193[13]
beta-Cyclocitral	1236	0.7	-	-	-	-	1218[13]
Nerol	1239	0.5	1237	0.3	-	-	1230[11]
5-(1'-1'-Dimethylethyl)bicycle[3,10] hexan-2-one	-	-	-	-	1256	1.7	-
p-Anisaldehyde	-	-	-	-	1260	1.9	1270[12]
Unknown	-	-	1270	1.0	-	-	-
alpha-Benzeneacetaldehyde ethylidene-	-	-	1282	0.2	1279	0.8	1279[12]
Salicylic acid ethyl ester	1282	1.1	-	-	-	-	1311[12]
Carbamic acid	-	-	1307	0.7	-	-	-
3,4,4a,5,6,8a-Hexahydro-2,5,5,8a-tetramethyl- (2.alpha.,4a.alpha.,8a.alpha)-2H-1-benzopyran	1307	0.5	-	-	-	-	-
p-Vinylgyaiacol	1323	1.1	1322	0.1	1322	0.6	1324[12]
Benzene,4-ethyl-1,2-dimethoxy-	-	-	-	-	1329	0.6	-
Unknown	-	-	-	-	1332	0.7	-
Acetanisole	-	-	1361	0.1	1362	0.8	1352[11]
Eugenol	1366	1.2	1365	0.7	1366	1.7	1359[11]
Unknown	1369	1.3	1368	0.6	-	-	-
3,4-Dimethoxystyrene	-	-	-	-	1371	0.6	1368[12]
Unknown	-	-	-	-	1384	1.4	-
(*E*)-beta-Damascenone	1392	1.4	1392	0.7	-	-	1385[11]
beta-Elemene	1400	0.3	1400	0.4	1400	0.6	1391[11]
Methyleugenol	-	-	1407	0.4	1407	0.9	1404[11]
Unknown	-	-	1413	0.4	-	-	-
Isocaryophyllene	-	-	-	-	1420	1.1	1423[12]
Unknown	-	-	1421	0.7	-	-	-
(*E*)-Caryophyllene	1436	6.6	1438	12.2	1437	12.7	1433[12]
Dihydro-beta-ionone	-	-	1447	0.4	-	-	1444[12]
Neryl acetone	-	-	1456	1.0	1456	0.6	1456[12]
(*E*)-beta-Farnesene	1459	0.4	1460	0.5	1460	0.6	1457[11]
alpha-Humulene	1467	1.6	-	-	-	-	1463[12]
1,1,4,8-Tetramethyl-(*Z,Z,Z*)-4,7,10-cycloundecatriene-	-	-	1468	2.4	1468	3.0	-
(*E*)-beta-Ionone	1491	0.9	1493	2.9	1493	1.7	1489[11]
Unknown	-	-	-	-	1499	1.1	-
alpha-Farnesene	1509	0.2	-	-	-	-	1506[12]
beta-Bisabolene	1514	3.0	1515	3.8	1515	4.8	1512[12]
Unknown	-	-	1529	0.8	1526	1.4	-
beta-Cadinene	-	-	-	-	1533	1.1	1539[11]
Unknown	-	-	1534	1.4	-	-	-
Nerolidol	1566	0.9	1566	1.3	1567	1.3	1563[11]

Table 1. Chemical constituents of the essential oils of the fresh, dried and fermented *P. serratifolia* leaves analysed by GC-MS using DB-5 column. (cont.)

Compound	Fresh leaves		Dried leaves		Fermented leaves		Literature
	RI	%Relative amount	RI	%Relative amount	RI	%Relative amount	RI
Caryophyllene oxide	1597	1.8	1598	2.6	1598	2.9	1583[11]
Humulene oxide	-	-	-	-	1628	0.4	1602[13]
3,4-Dimethyl-3-cyclohexen-1-carboxaldehyde	1629	0.3	-	-	-	-	-
beta-Tumerone	-	-	1674	1.0	-	-	1669[11]
Unknown	-	-	-	-	1678	2.4	-
Unknown	-	-	-	-	1681	2.7	-
Acorenone B	-	-	-	-	1708	4.4	1698[11]
Myristatic acid ethyl ester	1794	0.3	-	-	-	-	1794[12]
Isopropyl myristate	1826	0.3	-	-	-	-	1824[12]
Hexahydrofarnesyl acetone	1848	0.3	1844	3.6	1849	1.5	1844[12]
Isobutyl phthalate	1875	0.2	-	-	1875	0.3	1874[12]
Palmitoleic acid methyl ester	-	-	1912	0.9	1911	0.3	1912[12]
Farnesyl acetone C	1925	0.4	1926	2.0	1925	0.7	1920[13]
Isophytol	-	-	-	-	1951	0.1	1948[11]
(*Z*)-11-Hexadecenoic acid	-	-	1958	1.8	-	-	1953[12]
Unknown	1964	1.3	1963	1.0	1963	0.8	-
Palmitic acid	-	-	1978	5.0	-	-	1968[13]
(*E*)-11-Hexadecenoic acid ethyl ester	1980	0.8	-	-	1979	0.4	1974[12]
Palmitic acid ethyl ester	1990	1.2	-	-	-	-	1990[12]
Geranyl linalool isomer1	-	-	2038	0.2	-	-	-
Linoleic acid methyl ester	-	-	2098	0.4	-	-	2097[12]
Linolenic acid methyl ester	-	-	2106	1.2	2105	0.3	2108[12]
Phytol	2128	12.5	2124	7.3	2120	3.4	2122[12]
(*E*)-9-Octadecenoic acid	-	-	2146	0.4	-	-	2133[13]
Linoleic acid ethyl ester	2167	0.6	-	-	2165	0.1	2164[12]
Linolenic acid ethyl ester	2175	2.3	2174	0.4	2173	0.6	2170[12]
Geranyl linalool isomer2	-	-	2180	0.4	-	-	-
15-Methyl-heptadecanoic acid ethyl ester	2194	0.4	-	-	-	-	-
n-Docosane	-	-	-	-	2199	0.1	2200[14]
n-Tricosane	2299	0.2	2299	0.1	2299	0.1	2300[14]
n-Tetracosane	2399	0.1	2399	0.1	2399	0.1	2400[14]
n-Pentacosane	2499	0.2	2499	0.1	2499	0.2	2500[14]
Bis-(2-ethylhexyl) phthalate	2554	3.0	2552	0.1	-	-	2556[14]
n-Hexacosane	2599	0.1	2598	0.1	2598	0.2	2600[14]
n-Heptacosane	2700	0.4	2699	0.4	2698	0.3	2700[14]
Squalene	2826	0.2	-	-	2825	0.1	2847[12]
n-Nonacosane	2897	3.0	2898	0.4	2898	0.4	2900[14]

Table 2. Chemical constituents of the essential oils of the fresh, dried and fermented *P. serratifolia* leaves analysed by GC-MS using Carbowax 20M column.

Compound	Fresh leaves		Dried leaves		Fermented leaves		Literature
	RI	%Relative amount	RI	%Relative amount	RI	%Relative amount	RI
(*E*)-2-Hexenal	1271	0.6	1278	0.7	1279	0.3	1216[12]
(*E*)-beta-Ocimene	-	-	1282	0.2	1283	0.2	1250[12]
Amyl ethyl ketone	1299	7.8	1297	0.3	1298	0.7	1264[12]
p-Cymene	-	-	1307	0.2	1300	0.2	1270[13]
alpha-Terpinolen	-	-	1308	0.1	-	-	1282[13]
Amyl vinyl ketone	1325	0.3	1326	1.4	1326	0.2	1301[13]
6-Methyl-5-hepten-2-one	-	-	1355	0.1	1355	0.1	1345[12]
Amyl carbinol	1379	5.4	-	-	-	-	1371[12]
(*Z*)-3-Hexen-1-ol	1401	2.2	-	-	-	-	1373[13]
Amyl ethyl carbinol	1423	8.1	1414	2.1	1416	6.2	1392[13]
3,5,5-Trimethyl-3-cyclohexen-1-one	-	-	1425	0.1	-	-	1420[12]
Amyl vinyl carbinol	1474	32.6	1460	13.8	1461	11.5	1444[13]
(*E*)-Linalool oxide	1476	0.2	-	-	-	-	1454[13]
(*Z*)-Linalool oxide	1491	0.2	1486	0.2	1486	0.3	1474[13]
(*E*,*E*)-2,4-Heptadienal	1502	0.1	1498	0.2	1499	0.2	1491[13]
3,4,4a,5,6,8a-Hexahydro-2,5,5,8a-tetramethy-(2.alpha.,4a.alpha.,8a.alpha) 2H-1-benzopyran	1527	0.2	-	-	-	-	-

Table 2. Chemical constituents of the essential oils of the fresh, dried and fermented *P. serratifolia* leaves analysed by GC-MS using Carbowax 20M column. (cont.)

Compound	Fresh leaves		Dried leaves		Fermented leaves		Literature RI
	RI	%Relative amount	RI	%Relative amount	RI	%Relative amount	
Camphor	-	-	1531	0.1	-	-	1515[13]
(*E*)-2-Nonenal	-	-	1538	0.2	1540	0.2	1536[13]
Linalool	1554	11.1	1549	7.5	1549	5.3	1543[13]
1-Methyl-4-(1-methylethyl)-*trans*-2-cyclohexen-1-ol	-	-	-	-	1577	0.3	1571[12]
beta-Elemene	-	-	1578	0.2	-	-	1574[12]
(*E*,*Z*)-2,6-Nonadienal	-	-	1583	0.4	1585	0.6	1582[13]
(*E*)-Caryophyllene	1594	3.1	1596	14.6	1597	8.4	1599[13]
Unknown	-	-	-	-	1603	0.6	-
2-Acetylthiazole	1632	0.1	-	-	-	-	1634[12]
Acetophenone	1645	2.0	-	-	-	-	1648[12]
(*E*)-beta-Farenesene	-	-	-	-	1651	0.3	1664[13]
p-Vinyl anisole	1659	2.4	1660	13.1	1667	41.1	1670[12]
Unknown	1667	0.2	-	-	1671	0.6	-
4-Oxoisophorone	-	-	1694	0.2	1696	0.2	1690[12]
(*E*,*E*)-2,4 Nonadienal	-	-	-	-	1698	0.1	1696[13]
beta-Bisabolene	1706	1.5	1707	6.0	1706	2.7	1699[12]
Naphthalene	1717	0.2	1717	0.2	-	-	1709[12]
alpha-Farnesene	-	-	1722	0.4	1722	0.2	1744[13]
Unknown	1722	0.2	-	-	-	-	-
delta-Cadinene	-	-	1737	0.2	-	-	1756[13]
Salicylic acid methyl ester	1755	3.9	1751	0.5	1752	0.3	1768[13]
Salicylic acid ethyl ester	1788	0.4	-	-	-	-	1798[12]
Nerol	-	-	1802	0.3	1801	0.1	1795[13]
Unknown	1802	0.4	-	-	-	-	-
(*E*)-beta-Damascenone	1814	0.6	1813	0.7	1813	0.2	1821[13]
Dihydro-beta-Ionone	-	-	1827	0.3	-	-	1825[12]
Unknown	1830	0.2	-	-	-	-	-
Neryl acetone	1843	0.1	1844	1.2	1844	0.4	1835[12]
Geraniol	1852	0.7	1851	0.7	1851	0.4	1851[12]
alpha-Ionone	-	-	1853	0.6	-	-	1857[12]
4-Ethyl-1,2-dimethoxy-benzene	-	-	-	-	1870	0.4	1875[12]
Unknown	1877	0.6	1876	0.7	1877	1.1	-
(*E*,*E*,*E*)-2,4,6-Nona-trienal	-	-	1889	0.2	1889	0.4	-
Unknown	1913	0.2	-	-	-	-	-
Unknown	1923	0.2	-	-	1923	0.5	-
(*E*)-beta-Ionone	1943	0.2	1945	2.2	1944	0.4	1936[13]
Unknown	1978	0.2	-	-	-	-	-
Caryophyllene oxide	1991	0.7	1992	2.5	1991	1.0	1986[13]
Methyleugenol	-	-	2002	0.7	2002	0.5	2006[13]
4-(2,2,6-Trimethyl-7-oxabicyclo[4.1.0]hept-1-yl)-3-buten-2-one	2002	0.1	-	-	-	-	2002[12]
p-Anisaldehyde	2020	0.5	2020	1.7	2021	1.5	2011[13]
Nerolidol	2029	0.4	2029	1.4	2029	0.4	2036[13]
Myristatic acid ethyl ester	2037	0.1	-	-	-	-	2045[12]
Zingiberenol	-	-	-	-	2114	0.1	2109[12]
Hexahydro farnesyl acetone	2121	0.1	2124	4.1	2122	0.8	2129[12]
Unknown	-	-	-	-	2100	0.2	-
Acetanisole	2144	0.1	2144	0.4	2145	0.7	2148[12]
Eugenol	2151	0.6	2151	1.5	2151	1.1	2150[12]
Longiborneol	-	-	2160	0.4	2159	0.2	2157[13]
2,6,11,15-Tetramethyl-hexadeca-2,6,8,10,14-pentaene	2175	0.6	2174	1.1	2174	0.4	-
p-Vinylguaiacol	2184	0.6	-	-	2184	0.4	2180[12]
alpha-Cadinol	2191	0.1	2191	0.3	-	-	2191[12]
Unknown	-	-	2202	1.7	2202	0.5	-
Acorenone B	-	-	-	-	2217	2.4	-
Palmitoleic acid methyl ester	2226	0.1	2227	0.9	-	-	2225[12]
Palmitic acid ethyl ester	2245	0.4	-	-	2243	0.2	2235[12]
beta-Tumerone	-	-	2246	0.5	-	-	-
9-hexadecenoic ethyl ester	2268	0.3	-	-	2266	0.2	2269[12]
Unknown	-	-	-	-	2281	1.7	-

Table 2. Chemical constituents of the essential oils of the fresh, dried and fermented *P. serratifolia* leaves analysed by GC-MS using Carbowax 20M column. (cont.)

Compound	Fresh leaves		Dried leaves		Fermented leaves		Literature
	RI	%Relative amount	RI	%Relative amount	RI	%Relative amount	RI
Isophytol	-	-	2284	0.3	-	-	2293[13]
2,4-Bis(1,1-dimethylethyl)-phenol	2291	0.1	2291	0.6	2291	0.2	2316[12]
3,7,11-Trimethyl-(*E,E*)-2,6,10-dodecatrien-1-ol	-	-	-	-	2357	0.1	2366[13]
Farnesyl acetone C	2362	0.1	2363	1.2	2362	0.4	2377[13]
Ketole (1H-Indole)	-	-	2425	0.2	2425	0.2	2420[12]
Octadecanoic acid ethyl ester	2453	0.1	-	-	-	-	2450[12]
Olealic acid ethyl ester	2468	0.2	-	-	-	-	2461[12]
Linoleic acid methyl ester	-	-	2478	0.3	-	-	2480[12]
Linoleic acid ethyl ester	2515	0.2	-	-	-	-	2519[12]
Linolenic acid methyl ester	-	-	2547	1.1	2546	0.2	2550[12]
Linolenic acid ethyl ester	2584	0.7	-	-	2582	0.4	2594[12]
Phytol	2625	7.7	2620	9.0	2617	2.0	2613[13]
Dibutyl phthalate	-	-	2704	0.5	-	-	2705[12]
3-(4-Methoxyphenyl),2-propenoic acid ethyl ester	2641	0.5	-	-	-	-	-

Table 3. Category of chemical constituents of the essential oils of the fresh, dried and fermented *P. serratifolia* leaves.

Compound category	Compound sub-category	% Relative amount					
		Fresh leaves		Dried leaves		Fermented leaves	
		DB-5	Carbowax 20M	DB-5	Carbowax 20M	DB-5	Carbowax 20M
Terpenoids	Monoterpene hydrocarbons	0.5	-	9.7	0.4	7.1	0.4
	Monoterpene alcohols	15.6	11.8	7.1	8.5	9.2	6.1
	Monoterpene ketones	-	-	-	0.1	-	-
	Miscellaneous oxygenated monoterpenes	-	0.4	-	0.2	-	0.3
	Sesquiterpene hydrocarbons	12.1	4.7	19.3	21.4	23.8	11.5
	Sesquiterpenes alcohols	-	0.1	-	0.7	-	0.3
	Sesqutiterpene ketones	-	-	1.0	0.5	4.4	2.4
	Miscellaneous oxygenated sesquiterpenes	1.8	0.7	2.6	2.5	3.2	1.0
	Diterpenoid hydrocarbons	-	0.6	-	1.1	-	0.4
	Diterpenoid alcohols	12.5	7.7	7.9	9.3	3.5	2.0
	Triterpenoids	0.2	-	-	-	0.1	-
Phenolics		15.0	10.9	10.2	18.4	7.9	6.0
Fatty acids	Fatty acids	-	-	7.2	-	-	-
	Fatty acids esters	5.7	2.1	2.9	2.2	1.7	1.0
Hydrocarbons	Hydrocarbon alcohols	22.2	48.2	6.3	15.9	12.5	17.8
	Hydrocarbon aldehydes	-	0.7	-	1.7	-	1.7
	Hydrocarbon ketones	-	8.1	-	1.6	2.9	0.9
	Long chain hydrocarbons	3.9	-	1.2	-	1.2	-
Apocarotenoids	Apocarotenoids	4.9	1.7	11.9	12.1	5.7	2.8
Miscellaneous		2.8	0.2	0.9	0.9	2.7	0.2
Unknowns		2.6	2.1	11.8	2.3	13.9	5.2

The significant new abundant compounds of both the dried and the fermented leaf oils were beta-myrcene and (*Z,Z,Z*)-4,7,10-cycloundecatriene,1,1,4,8-tetramethyl. The major difference between the dried and the fermented leaf oils was the detection of fatty acids (especially palmitic acid) only in the dried leaf oil, whereas acorenone B was found only in the fermented leaf oil.

Changing in a relative amount of each constituent was the major observation after drying or fermentation (Figure 2). Comparing with essential oil of the fresh leaves, essential oils of the dried leaves and the fermented leaves possessed less amounts of monoterpenes (especially linalool), *vice versa*, a higher proportion of sesquiterpenes (e.g. (*E*)-caryophyllene, beta-bisabolene, caryophyllene oxide) were observed, especially (*E*)-caryophyllene that became the most abundant compound of the dried leaf oil (6.6-12.2% in DB-5 and 3.1-14.6% in Carbowax 20M). The amount of phytol, the only major diterpenoid compound of the fresh leaf oil, was dramatically decreased after fermentation, but changing of this compound after drying was not much significant. In overview, the amount of phenolic compounds did not change much after drying while there was a marked increase after fermentation. In detail, the amount of salicylic acid methyl ester decreased in both the dried and the fermented leaf oils, but the amount of *p*-vinyl anisole was dramatically increased after fermentation more than the drying method and became the most abundant compound of the fermented leaf oil (2.4-41.1% Carbowax 20M). On the other hand, free fatty acids were found only in the dried leaf oil. In overview, the amounts of fatty acid esters (e.g. palmitic acid ethyl ester, linolenic acid ethyl ester) and hydrocarbons (e.g. amyl ethyl carbinol, amyl vinyl carbinol, amyl ethyl ketone) tended to decrease in both the dried and the fermented leaf oils.

The relation between chemical structures of some constituents of the essential oils of the leaves of *P. serratifolia* after drying or fermentation could be explained via different reactions. beta-Myrcene which was the new detected compound of both the dried and fermented leaf oils, possesses the same chemical skeleton of acyclic monoterpenoids as linalool that concomitant dramatically decreasing in relative amount. Dehydration of linalool to form beta-myrcene might be the mechanism of this reaction but it has never been reported in the plant. There was only a report found that linalool could be conversed to beta-myrcene by microbial biotransformation using linalool dehydratase isomerase[15]. After drying and fermentation, alpha-humulene was disappeared, whereas relative amounts of (*E*)-caryophyllene and caryophyllene oxide increased. alpha-Humulene and (*E*)-caryophyllene possess a similar sesquiterpenoid skeleton and have humulyl cation as the same biosynthetic precursor[16]. Some unproven factors might affect their biosynthesis expression, and (*E*)-caryophyllene could be further oxidized to caryophyllene oxide[17]. Most of the fatty acid esters were found mainly in the fresh leaf oil (e.g. palmitic acid ethyl ester), whereas fatty acids were detected only in dried leaf oil (e.g. palmitic acid). In general, fatty acid esters have low boiling points and easily volatilized, then they might be lost during the long period of air dry and fermentation. However, at the same time, some fatty acid esters might be hydrolyzed and caused the formation of fatty acids due to endogenous or microbial enzymes[18]. Hydrolysis of palmitic acid ethyl ester to palmitic acid was the example. As same as fatty acid ester, most of hydrocarbon compounds can easily volatile, then decreasing in the amounts of amyl vinyl carbinol, amyl ethyl carbinol, amyl ethyl ketone was observed. However, some increasing in the amount of amyl vinyl ketone was detected. Functional groups were the only difference among these compounds, and the highest oxidative degree was the functional group of the amyl vinyl ketone. Therefore, the increasing of this compound was possibly due to the oxidation of its derivatives in the fresh leaves.

Apocarotenoids were the known degradative products derived from carotenoids by enzymatic and non-enzymatic oxidation. Increasing in their amounts after drying or fermentation was normally observed in many plant materials such as *Morus alba*, *M. nigra*[19], black tea and Oolong tea[20]. The result of this study was also in the same manner. After drying and fermentation, increasing in their proportion was observed. The relative amounts of apocarotenoids in the fresh leaf oil (4.9% in DB-5 and 1.7% in Carbowax 20M) were much increased in the dried leaf oil (11.9% in DB-5 and 12.1% in Carbowax 20M) than fermented leaf oil (5.7% in DB-5 and 2.8% in Carbowax 20M). Three significantly increasing compounds were beta-ionone, hexahydrofarnesyl acetone and farnesyl acetone C. Degradation mechanisms of carotenoids to form these three compounds have already been reported[19,21].

Biological activities of some major compounds of essential oils of *P. serratifolia* have been reported and supported the traditional usage of anticancer and the treatment of liver diseases. Linalool have been reported for antimicrobial, anti-inflammatory, anticancer, and antioxidant properties[22]. (*E*)-caryophyllene, palmitic acid, phytol, apocarotenoids and amyl vinyl carbinol possessed anticancer, and antioxidant activities[23-27]. beta-Myrcene was antiproliferative compound[28], whereas *p*-vinylanisole and methyl salicylate were shown to have antioxidant activity[29-30]. However, after drying or fermentation, amounts of some compounds increased and some compounds decreased. Therefore, biological activities of *P. serratifolia* leaves after drying or fermentation should be varied in potency from the fresh leaves and should be studied in more detail.

4. CONCLUSIONS

After drying and fermentation, a significant change in the chemical composition of essential oil of the leaves of *P. serratifolia* was detected. Some compounds disappeared, some new compounds occurred, and most compounds changed in their relative amounts. Dehydration, hydrolysis, and oxidation were suggested as the transformation reactions of some compounds. This result indicated the importance of the post-harvesting process on the quality of this herb. Bioactivity of its fresh, dried and fermented leaves would be further studied.

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Conflict of interest
None to declare.

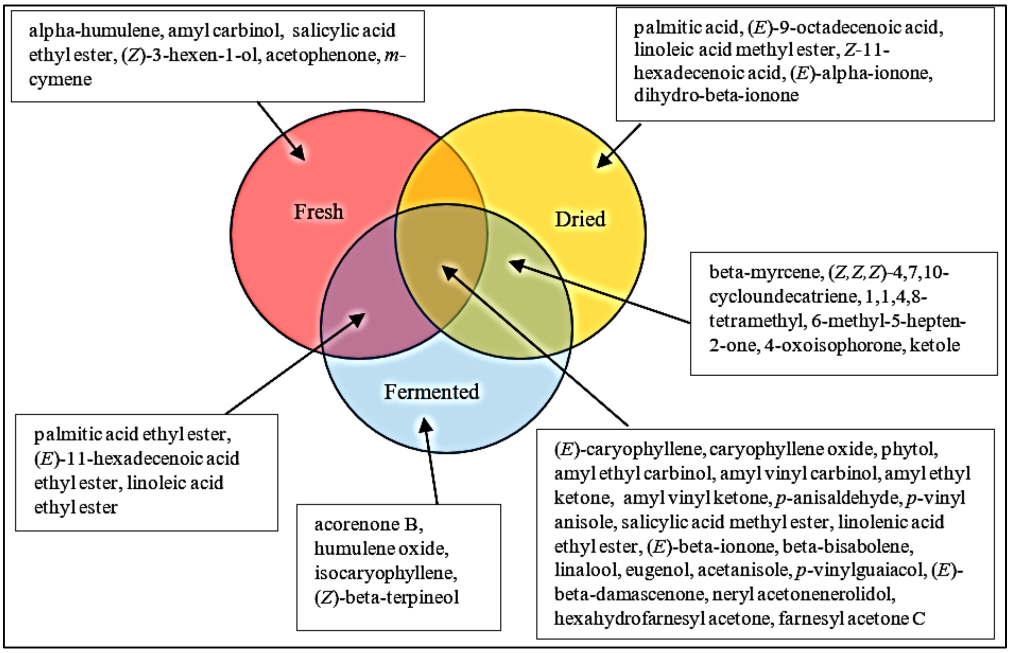

Figure 1. Comparison of some constituents of the essential oils of the fresh, dried and fermented *P. serratifolia* leaves.

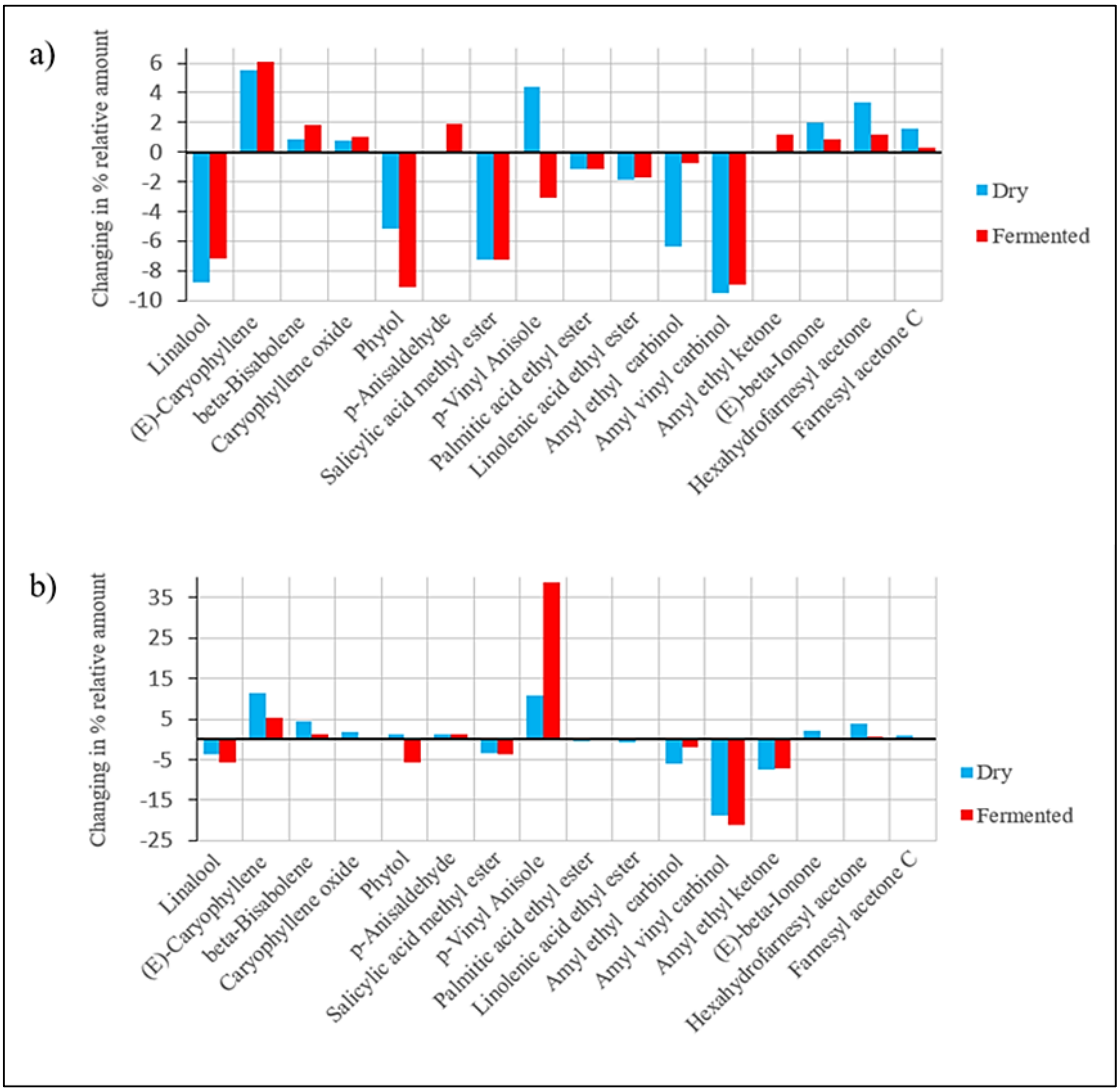

Figure 2. Changing in relative amounts of some constituents of the essential oils of the leaves of *P. serratifolia* after drying and fermentation, analysed by DB-5 (a) and Carbowax 20M (b) columns.

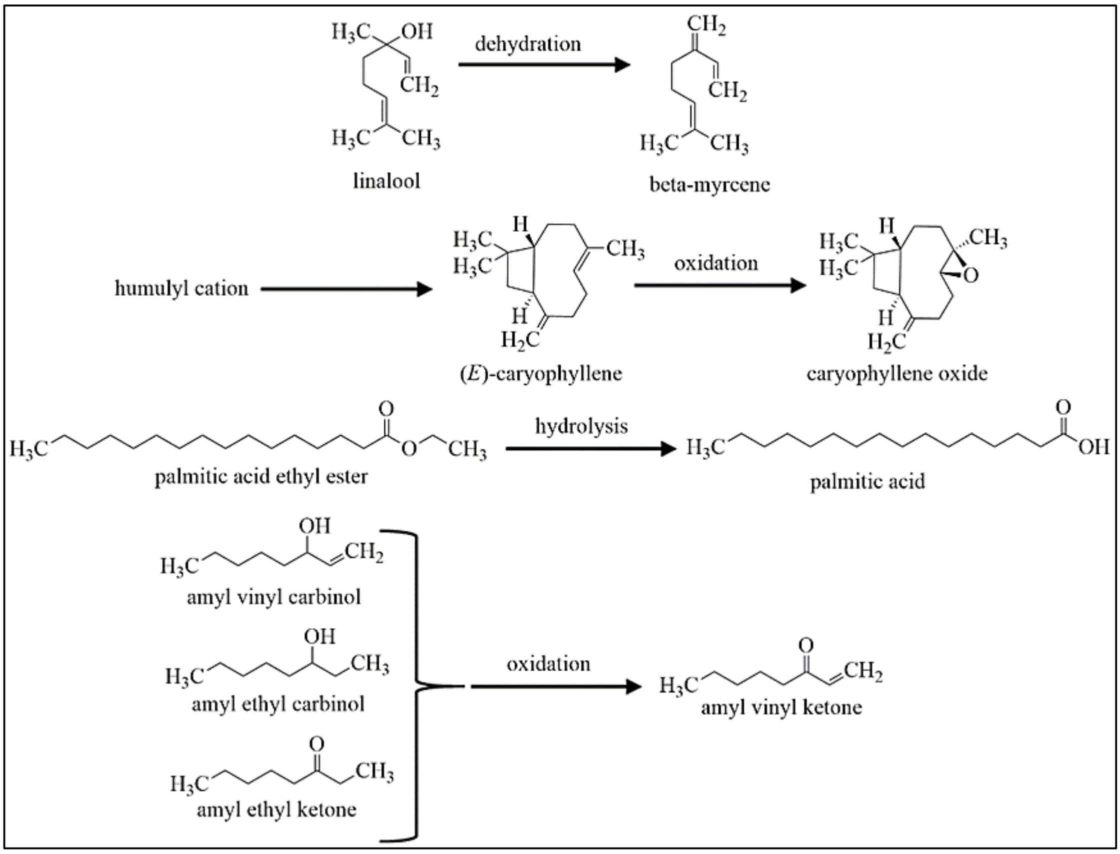

Figure 3. Proposed transformation mechanisms of some volatile compounds of the leaves of *P. serratifolia* after drying and fermentation.

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