Chemical composition of *Sarcotheca laxa* (Ridl.) Knuth essential oil and their bioactivities

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The essential oil of the leaves of *Sarcotheca laxa* (Ridl.) Knuth was analysed by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS). Thirty-two chemical components, comprising 97.4% of the total oil composition were identified. β-Caryophyllene (24.5%), δ-cadinene (13.8%), germacrene D (8.6%), bicyclogermacrene (6.5%), and aromadendrene (5.2%) were the most abundant components of the essential oils of *S. laxa*. The antioxidant and anti-inflammatory activities were determined by 2,2-diphenyl-1-picrylhydrazyl (DPPH) and lipoxygenase inhibition, respectively. The essential oil showed significant radical scavenging activity with 50% inhibition (IC_{50}) values of 75.2 mg/mL and moderate anti-inflammatory activity with an IC_{50} value of 55.7 mg/mL. To the best of our knowledge, this is the first study on the composition and bioactivities of the essential oil report concerning the genus *Sarcotheca*.

Keywords: Essential oil. *Sarcotheca laxa*. Oxolidaceae. β-caryophyllene. DPPH. Lipoxygenase

1. INTRODUCTION

The Oxalidaceae family are herbs, shrubs, or rarely trees, comprising of seven genera and about 800 species. *Sarcotheca* is one of the genus in the Oxolidaceae family. It is widespread in Peninsular Malaysia, Borneo, Sumatera, and Sulawesi [1]. In Malaysia, eleven species of *Sarcotheca* have been documented and are *S. celebica*, *S. diversifotia*, *S. ferrugenea*, S. *griffithii*, *S. glomerula*, *S. taxa*, *S. monophylla*, *S. macrophylla*, *S. ochracea*, and *S. rubrinervis* [2]. *Sarcotheca laxa* (Ridl.) Knuth is locally known as *belimbing hutan* or *pupoi* in Malaysia. This species comprised of three varieties that are S. laxa var. laxa, S. laxa var. sericea, and S. laxa var. hirsuta [3]. The tree grows in primary and secondary forests in the lowland and can reach up to 23 m of height. It can be distinguished by its oblong to elliptic or lanceolate leaves. Besides, the persistent part of the stalk is often thick and rarely longer than 2 cm long. The inflorescences are axillary and always produce 1-3 flowers on each panicle branch. The bisexual flowers are dark red and have clawed petals. The stamens are arranged in two whorls. The fruits are berries with 5 ridges, about 1.5 cm long, and become bright red or black when ripe [3]. In Malaysia, the fruits of this species are edible and have a sour taste, and are used as ingredients for curries or sweets [4]. In addition, the fruits are also used to treat cough, while the roots are traditionally used to poultice wounds [2].
Most of the genus *Sarcotheca* species are unexplored, both pharmacologically and phytochemically. Previous phytochemical investigation of *Sarcotheca* has

resulted in the isolation of flavone glycosides [5]. To the best of our knowledge, the genus *Sarcotheca* is still poorly explored as far as its essential oil composition is concerned. Essential oils from aromatic and medicinal plants have been known since antiquity to possess biological activity, most notably antibacterial, antifungal, antioxidant, anticholinesterase, anti-inflammatory properties [6].
Therefore, in a continuation of our systematic studies on pharmacologically active volatiles from Malaysian plants [7-10], it is interesting to report the essential oil composition, antioxidant, and anti-inflammatory activities of *S. laxa* for the first time from Malaysia.

2. MATERIALS AND METHODS

2.1.PLANT MATERIAL

The S. *laxa* leaves were collected from Gambang, Pahang in September 2019, and identified by Dr. Shamsul Khamis from Universiti Kebangsaan Malaysia (UKM). The voucher specimen (SK04/19) was deposited at UKMB Herbarium, Faculty of Science and Technology UKM.

2.2. ISOLATION OF ESSENTIAL OIL

The fresh leaves (500 g) were subjected to hydrodistillation in Clevenger-type apparatus for 6 hours. The essential oil obtained was dried over anhydrous magnesium sulphate and stored at 4-6°C.

2.3. ANALYSIS OF ESSENTIAL OIL

Gas chromatography (GC) analysis was performed on a Hewlett Packard 6890 series II. A gas chromatograph equipped with HP-5MS capillary column (30 m × 0.25 mm × 0.25 µm film thickness). Helium was used as a carrier gas at a flow rate of 0.7 mL/min. Injector and detector temperatures were set at 250 and 280°C, respectively. The oven temperature was kept at 50°C, then gradually raised to 280°C at 5°C/min and finally held isothermally for 15 min. Diluted samples (1/100 in diethyl ether, v/v) of 1.0 µL were injected manually (split ratio 50:1). The injection was repeated three times and the peak area percentages were reported as means ± SD of triplicates. The calculation of the peak area percentage was carried out by using the GC HP Chemstation software (Agilent Technologies).
Gas chromatography-mass spectrometry (GC-MS) chromatograms were recorded using a Hewlett Packard Model 5890A gas chromatography and a Hewlett Packard Model 5989A mass spectrometer, equipped with HP-5MS capillary column (30 m × 0.25 mm × 0.25 µm film thickness). Helium was used as carrier gas at a flow rate of 1 mL/min. The injector temperature was 250°C. The oven temperature was programmed from 50°C (5 min hold) to 280°C at 10 °C/min and finally held isothermally for 15 min. For GC-MS detection, an electron ionisation system, with ionisation energy of 70 eV was used. A scan rate of 0.5 s (cycle time: 0.2 s) was applied, covering a mass range from 50-400 amu.

2.4. IDENTIFICATION OF COMPONENTS

For the identification of essential oil components, co-injection with the standards (major components) were used, together with correspondence of retention indices and mass spectra with respect to those occurring in Adams [11], NIST 08 [12] and FFNSC2 [13] libraries. Semi-quantification of essential oil components was made by peak area normalisation considering the same response factor for all volatile components. Percentage values were the mean of three chromatographic analyses.

2.5. ANTIOXIDANT ACTIVITY - DPPH RADICAL SCAVENGING ASSAY

The free radical scavenging capacity of essential oil was evaluated according to the previously reported procedure using the stable DPPH [10]. Briefly, 10 µL of essential oil in dimethyl sulfoxide (DMSO) at different concentrations (125-7.8 µg/mL) was added to 190 µL methanol solution of DPPH (0.1 mM) in a well of 96-well plate. The mixture was shaken vigorously and allowed to stand in the dark at room temperature for 30 min. Absorbance readings were taken at 517 nm. The percentage radical scavenging activity of essential oil and standard against DPPH were calculated according to the following:

$$I\% = [(A_0 - A_1) / A_0] \times 100$$

where A_0 is the absorbance of the control (containing all reagents except the oil), and A_1 is the absorbance of the standard. Sample concentration providing 50% inhibition (IC_{50}) was calculated from the graph plotting inhibition percentage against extracts concentration. Tests were carried out in triplicate. Butylated hydroxyanisole (BHA) was used as a positive control.

2.6. ANTI-INFLAMMATORY ACTIVITY - LIPOXYGENASE ASSAY

The reagents were prepared according to the standard protocol using lipoxygenase inhibitor screening assay kit (Item No. 760700, Cayman Chemical, USA) [10]. Stock solutions of essential oil were prepared to obtain concentrations of 125-7.8 µg/mL in the respective wells. The prepared solutions were then introduced onto 96 well plates where the cells were distributed as blanks 1A-2A-1D (triplicate), positive control 1B-2B (duplicate), and 100% initial activity wells 1C-2C-2D (triplicate). The remaining wells were designated for inhibitor (essential oil) solutions in duplicate. The addition of the reagents was done according to the stan-

dard protocol, according to which, 100 µL of assay buffer was added to the blank wells and 90 µL of lipoxygenase (5-LOX) enzyme and 10 µL of assay buffer were added to positive control wells. For the 100% initial activity wells, 90 µL of lipoxygenase enzyme and 10 µL of solvent (DMSO) were added. The inhibitor (essential oil) wells were charged with 90 µL of lipoxygenase enzyme and 10 µL of respective stock (essential oil) solution. The reaction was initiated by adding 10 µL of the substrate (AA) to all wells. The plate was then shaken for 5 min on an orbital shaker. Ultimately, 100 µL of chromogen solution (prepared according to standard protocol) was added to each well to stop the enzyme catalysis. The plate was incubated for half an hour and was read at 500 nm. The percentage inhibitions (I%) of the essential oil was calculated using the following equation:

$$I\ \% = [(A_{initial\ activity} - A_{inhibitor}) / A_{initial\ activity}] \times 100$$

where $A_{initial\ activity}$ is the absorbance of 100% initial activity wells without sample and $A_{inhibitor}$ is the

Table I - Chemical composition of *Sarcotheca laxa* essential oil

No.	Components	KI[a]	KI[b]	Percentage (%)[c]	Identifications[d]
1	Limonene	1033	1034	3.5 ± 0.05	RI, MS
2	(*Z*)-β-Ocimene	1044	1042	0.2 ± 0.16	RI, MS
3	(*E*)-β-Ocimene	1055	1051	0.2 ± 0.18	RI, MS
4	Terpinolene	1080	1075	1.6 ± 0.19	RI, MS
5	Linalool	1106	1105	3.2 ± 0.22	RI, MS
6	Terpinen-4-ol	1149	1150	1.3 ± 0.17	RI, MS
7	α-Terpineol	1171	1170	1.5 ± 0.18	RI, MS
8	δ-Elemene	1336	1335	2.2 ± 0.05	RI, MS
9	α-Cubebene	1355	1351	0.5 ± 0.23	RI, MS
10	α-Ylangene	1375	1373	0.8 ± 0.25	RI, MS
11	α-Copaene	1376	1374	1.6 ± 0.25	RI, MS
12	β-Elemene	1394	1390	2.5 ± 0.12	RI, MS
13	α-Gurjunene	1402	1409	2.7 ± 0.05	RI, MS
14	β-Caryophyllene	1425	1420	24.5 ± 0.21	RI, MS, Std
15	Aromadendrene	1442	1440	5.2 ± 0.18	RI, MS, Std
16	α-Humulene	1450	1452	0.5 ± 0.02	RI, MS
17	Germacrene D	1482	1480	8.6 ± 0.14	RI, MS, Std
18	δ-Amorphene	1482	1483	2.2 ± 0.23	RI, MS
19	β-Guaiene	1490	1492	2.5 ± 0.22	RI, MS
20	β-Selinene	1490	1489	0.2 ± 0.12	RI, MS
21	Bicyclogermacrene	1500	1500	6.5 ± 0.08	RI, MS, Std
22	γ-Patchoulene	1505	1502	0.5 ± 0.02	RI, MS
23	δ-Cadinene	1520	1522	13.8 ± 0.25	RI, MS, Std
24	Germacrene B	1550	1550	0.7 ± 0.11	RI, MS
25	Spathulenol	1576	1576	0.9 ± 0.12	RI, MS
26	Globulol	1590	1590	3.1 ± 0.02	RI, MS
27	Viridiflorol	1596	1595	2.0 ± 0.15	RI, MS
28	Guaiol	1600	1600	0.2 ± 0.12	RI, MS
29	t-Muurolol	1645	1644	1.1 ± 0.03	RI, MS
30	α-Cadinol	1650	1652	1.3 ± 0.18	RI, MS
31	Bulnesol	1672	1670	1.2 ± 0.15	RI, MS
32	α-Bisabolol	1688	1685	0.6 ± 0.22	RI, MS
Group components					
Monoterpene hydrocarbons				5.5 ± 0.02	
Oxygenated monoterpenes				6.0 ± 0.05	
Sesquiterpene hydrocarbons				75.5 ± 0.18	
Oxygenated sesquiterpenes				10.4 ± 0.12	
Total identified (%)				97.4 ± 0.15	

[a] Linear retention index, experimentally determined using homologous series of C_6-C_{30} alkanes
[b] Linear retention index taken from Adams (2007) or NIST 08 (2008) and literature
[c] Relative percentage values are means of three determinations ±SD
[d] Identification methods: Std, based on comparison with authentic compounds; MS, based on comparison with Adams, FFNSC2 and NIST 08 MS databases

absorbance of essential oil/reference. Analyses were expressed as means ±SD of triplicates. A dose-response curve was plotted to determine the IC_{50} values. IC_{50} is defined as the concentration sufficient to obtain 50% of maximum anti-inflammatory activity.

2.7. STATISTICAL ANALYSIS

Data obtained from essential oil analysis and bioactivity were expressed as mean values. The statistical analyses were carried out by employing one-way ANOVA ($p<0.05$). A statistical package (SPSS version 11.0) was used for the data analysis.

3. RESULTS AND DISCUSSION

The essential oil had a spicy odour and yielded 0.25% calculated from the fresh weight of the leaves. The list of chemical components identified in the essential oil is shown in Table I. The GC and GC-MS analysis of the essential oil revealed the presence of thirty-two chemical components with the constitution of 97.4%. Sesquiterpene hydrocarbons were the most dominant components in the essential oil which constituted seventeen components, accounting for 75.5% of the total composition. They were characterised by its richness in β-caryophyllene (24.5%), δ-cadinene (13.8%), germacrene D (8.6%), bicyclogermacrene (6.5%), and aromadendrene (5.2%). Meanwhile, oxygenated sesquiterpenes were present in remarkable amounts which comprised of eight components, accounting for 10.4% of the total composition. The other minor group components detected in the essential oil were monoterpene hydrocarbons and oxygenated monoterpenes, which constituted 5.5% and 6.0%, respectively. They were dominated by limonene (3.5%) and linalool (3.2%). Meanwhile, the other components present in appreciable amounts (>2%) were globulol (3.1%), α-gurjunene (2.7%), β-elemene (2.5%), β-guaiene (2.5%), δ-elemene (2.2%), δ-amorphene (2.2%), and viridiflorol (2.0%).

β-Caryophyllene is a natural bicyclic sesquiterpene widely distributed in essential oils of various species of Myristicaceae [14], Piperaceae [15], and Lauraceae [9] families. It is found in numerous edible plants that are ingested daily, and it is approved as a food additive by the Food and Drug Administration. This compound can change the inflammatory processes in humans through the endocannabinoid system [16]. Furthermore, this compound could increase the intracellular accumulation of anticancer agents, thereby potentiating their cytotoxicity due to the absorption of 5-fluorouracil across human skin. β-Caryophyllene facilitates the passage of paclitaxel through membranes and thus potentiates its anticancer activity [17].

Following a similar line of thought, the essential oil was subjected to a preliminary test to verify the biological activity including DPPH radical scavenging assay and lipoxygenase inhibitory activity (Tab. II). Data obtained represented mean values of at least three different experiments and are expressed as mean ±SD. Statistical analysis was determined by t-test and $p<0.05$ was considered statistically significant. In this study, the ability of essential oil to scavenge DPPH radical was determined on the bases of their concentrations providing 50% inhibition (IC_{50}). The essential oil demonstrated moderate activity (IC_{50} value 75.2 µg/mL), compared to BHT (IC_{50} value 45.2 µg/mL). This could be due to the low abundance of oxygenated sesquiterpenes in the essential oil. The effect of antioxidants on DPPH radical scavenging was thought to be due to their hydrogen donating ability. It has been reported that free radical scavenging activity is greatly influenced by the phenolic components of samples [18]. Previously β-caryophyllene displayed strong antioxidant activity against DPPH radicals [19] and highly effective chain-breaking antioxidant *in vitro* lipid peroxidation and possesses significant scavenging activities against reactive oxygen species [20].

Anti-inflammatory activity was evaluated using the lipoxygenase assay. The essential oil showed moderate activity with IC_{50} value of 55.7 µg/mL, compared to quercetin (IC_{50} value 10.5 µg/mL). The high amounts of β-caryophyllene obtained in the essential oil may contribute, at least in part, to the anti-inflammatory activity ascribed to the plant. It has been reported that β-caryophyllene inhibited lipoxygenase activity [21]. Furthermore, several studies have indicated that

Table II – Biological activities of *Sarcotheca laxa* essential oil

DPPH radical scavenging assay	**Percentage inhibition (%)[a]**					**IC_{50} (µg/mL)[b]**
	7.8 µg/mL	**15.6 µg/mL**	**31.3 µg/mL**	**62.5 µg/mL**	**125 µg/mL**	
Essential oil	44.2±1.2	54.5±1.4	68.2±1.2	70.5±0.4	72.2±0.5	75.2
Butylatedhydroxytoluene	50.2±1.4	55.5±0.6	74.5±0.2	92.6±0.8	98.5±0.5	45.2
Lipogygenase assay	**Percentage inhibition (%)[a]**					**IC_{50} (µg/mL)[b]**
	7.8 µg/mL	15.6 µg/mL	31.3 µg/mL	62.5 µg/mL	125 µg/mL	
Essential oil	24.2±1.2	31.8±1.6	41.6±1.0	50.5±1.2	60.2±0.5	55.7
Quercetin	65.2±0.5	75.2±1.2	80.1±0.8	84.7±0.5	96.5±0.5	10.5

[a] Percentage inhibition (%) at concentration 7.8-125 µg/mL

[b] IC_{50} is defined as the concentration sufficient to obtain 50% of maximum activity

sesquiterpene compounds have anti-inflammatory effects on inhibiting cyclooxygenase and lipoxygenase [22].

4. CONCLUSIONS

The genus *Sarcotheca* is still poorly explored and unknown as far as its essential oil composition is concerned. This research is the first report on the essential oil composition of *S. laxa* growing in Malaysia. The GC and GC-MS analysis of the essential oil allowed us to identify β-caryophyllene as the major component. The high concentration of this component makes it a good candidate for a chemical marker for *Sarcotheca* species. In addition, the preliminary evaluation of antioxidant and lipoxygenase inhibitory activity is the first step described in the literature for this species and taken together, the data obtained here inspire more studies supporting the possibility of linking chemical contents with particular biological properties.

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BIBLIOGRAPHY

[1] R.C.K. Chung, Tree Flora of Sabah and Sarawak. Vol. 1, Sabah Forestry Department, Malaysia; Forest Research Institute Malaysia, Sarawak (1995)
[2] O.J. Mabberley, The Plant-Book. A Portable Dictionary of the Vascular Plants. 2nd Ed. Cambridge University Press (1997)
[3] I.H. Burkill, A dictionary of the economic product of Malay Peninsula. Kuala Lumpur:Ministry of Agriculture and Co-operatives (1966)
[4] J.F. Veldkamp, A revision of *Sarcotheca* Bl and *Dapania* Korth (Oxalidaceae). Blumea *15*, 519-543 (1967)
[5] R. Muharini, V. Wray, D. Lai, P. Proksch, New flavone C-glycosides from leaves of *Sarcotheca griffithii* (Hook F) Hallier F. Phytochem. Lett. *9*, 26-32 (2014)
[6] W.M.N.H.W. Salleh, F. Ahmad, H.Y. Khong, R.M. Zulkifli, Essential oil composition of Malaysian Lauraceae: A mini review. Pharm. Sci. *22*(1), 60-67 (2016)
[7] W.M.N.H.W. Salleh, M.F. Kammil, F. Ahmad, H.M. Sirat, Antioxidant and anti-inflammatory activities of essential oil and extracts of *Piper miniatum*. Nat. Prod. Commun. *10*, 2005-2008 (2015)
[8] W.M.N.H.W. Salleh, F. Ahmad, H.Y. Khong, R.M. Zulkifli, Chemical compositions and biological activities of essential oils of *Beilschmiedia glabra*. Nat. Prod. Commun. *10*, 1297-1300 (2015)
[9] W.M.N.H.W. Salleh, F. Ahmad, H.Y. Khong, R.M. Zulkifli, Comparative study of the essential oils of three *Beilschmiedia* species and their biological activities. Int. J. Food Sci. Tech. *51*, 240-249 (2016)
[10] W.M.N.H.W. Salleh, F. Ahmad, H.Y. Khong, R.M. Zulkifli, Chemical composition and biological activities of essential oil of *Beilschmiedia pulverulenta*. Pharm. Biol. *54*, 322-330 (2016)
[11] R.P. Adams, Identification of essential oil components by gas chromatography-mass spectrometry. 4th ed. Carol Stream (IL): Allured Publishing Corporation (2007)
[12] NIST 08, Mass spectral library (NIST/EPA/NIH). Gaithersburg, USA: National Institute of Standards and Technology (2008)
[13] FFNSC 2, Flavors and fragrances of natural and synthetic compounds. Mass spectral database. Japan: Shimadzu Corps (2012)
[14] W.M.N.H.W. Salleh, M.Z.A. Anuar, S. Khamis, M.A., M.D. Sul'ain, Chemical investigation and biological activities of the essential oil of *Knema kunstleri* Warb. from Malaysia. Nat. Prod. Res. in press (2019)
[15] W.M.N.H.W. Salleh, F. Ahmad, H.Y. Khong, H.M. Sirat, Chemical compositions, antioxidant and antimicrobial activities of the essential oils of *Piper officinarum* C.DC (Piperaceae). Nat. Prod. Commun. *7*(12), 1659-1662 (2012)
[16] E.J. Corey, R.B. Mitra, H. Uda, Total synthesis of d,l-caryophyllene and d,l-isocaryophyllene. J. Am. Chem. Soc. *86*(3), 485-492 (1964)
[17] J. Legault, A. Pichette, Potentiating effect of beta-caryophyllene on anticancer activity of alpha-humulene, isocaryophyllene and paclitaxel. J. Pharm. Pharmacol. *59*(12), 1643-1647 (2007)
[18] W.M.N.H.W. Salleh, F. Ahmad, H.Y. Khong, Antioxidant and anticholinesterase activities of essential oils of *Cinnamomum griffithii* and *C. macrocarpum*. Nat. Prod. Commun. *10*(8), 1465-1468 (2015)
[19] S.S. Dahham, Y.M. Tabana, M.A. Iqbal, M.B. Ahamed, M.O. Ezzat, A.S. Majid, A.M. Majid, The anticancer, antioxidant and antimicrobial properties of the sesquiterpene β-caryophyllene from the essential oil of *Aquilaria crassna*. Molecules *20*, 11808-11829 (2015)
[20] M.A. Calleja, J.M. Vieites, T.M. Meterdez, M.I. Torres, The antioxidant effect of β-caryophyllene protects rat liver from carbon tetrachloride-induced fibrosis by inhibiting hepatic stellate cell activation. Brit. J. Nutr. *109*, 394-401 (2013)
[21] G.P.P. Kamatou, R.L. van Zyl, S.F. van Vuuren,

A.M. Viljoen, A.C. Figueiredo, J.G. Barroso, L.G. Pedro, P.M. Tilney, Chemical composition, leaf trichome types and biological activities of the essential oils of four related *Salvia* species indigenous to Southern Africa. J. Essent. Oil Res. *18*, 72-79 (2006)

[22] S. Cengiz, L. Cavas, K. Yurdakoc, G. Pohnert, The sesquiterpene Caulerpenyne from Caulerpa spp. is a lipoxygenase inhibitor. Mar. Biotechnol. *13*(2), 321-326 (2011)