Essential Oil Composition of *Garcinia nigrolineata* Planch. ex T. Anderson (Clusiaceae)

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Summary

The present study aims to examine the chemical composition of the essential oils of the leaves of *Garcinia nigrolineata*. The essential oil was extracted by hydrodistillation and characterized by gas chromatography (GC-FID) and gas chromatography-mass spectrometry (GC-MS). In total, 37 chemical components were identified in the essential oil which made up 98.3 % of the total oil composition. The essential oil is composed mainly of β-caryophyllene (25.2 %), α-humulene (12.8 %), valencene (6.2 %), α-cadinol (5.8 %), and germacrene D (5.5 %). To the best of the authors' knowledge, this is the initial study that has looked into the essential oil composition of *Garcinia nigrolineata* collected from Malaysia.

Key words

Essential oil, *Garcinia nigrolineata,* Clusiaceae, hydrodistillation, β-caryophyllene

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Received: October 31, 2020 | Accepted: January 7, 2021

Introduction

The genus *Garcinia* is the biggest genus in the family of Clusiaceae. *Garcinia* species grow primarily in tropical Asia, Southern Africa, and Polynesia (Corner, 1952). There are about 450 species of *Garcinia* worldwide, with about 50 species found in the lowland and mountains of Peninsular Malaysia (Pangsuban et al., 2009). Many *Garcinia* species are valued for their edible fruits, such as *Garcinia mangostana*, which is widely cultivated and its sweet pleasant-tasting fruits are eaten fresh or in processed food products like juices (Liu et al., 2016). In addition, various plant parts of *Garcinia* species have been used to treat abdominal pain, dysentery, diarrhea, suppuration, infections, leucorrhoea, chronic ulcer, and gonorrhoea in traditional ways (Jamila et al., 2016).

Garcinia nigrolineata commonly known as *kandis hutan*, is a tropical fruit that is commonly cultivated throughout Singapore, Malaysia, Thailand, and Myanmar. The edible fruit is gathered from the wild for local use, whilst the tree is grown in Southeast Asia both for its delicious fruits and also as a rootstock for the mangosteen, *Garcinia mangostana*. Apparently, the leaves and fruits of *Garcinia* species have been used as an ingredient for weight loss products. Previous phytochemical investigations resulted in the isolation of xanthones along with minor compounds including benzopyran, biphenyl, benzoquinone, and isoflavone derivatives, and some of these compounds have shown antibacterial activity (Raksat et al., 2019; Rukachaisirikul et al., 2003a, 2003b, 2005). In continuation of our systematic studies on pharmacologically active volatiles from Malaysian plants (Salleh et al., 2012, 2014a, 2014b, 2015, 2016), we report in this study the chemical composition of the leaves of *G. nigrolineata*.

Material and Methods

Plant Material and Isolation of the Essential Oil

The leaves of *G. nigrolineata* were collected from Behrang, Perak in September 2019. The species were identified by Shamsul Khamis from Universiti Kebangsaan Malaysia (UKM). The voucher specimens (SK01/18) were deposited at UKMB Herbarium. The fresh mature leaves of *G. nigrolineata* (250 g) were weighed and then subjected to hydrodistillation using a Clevenger-type apparatus for 4 hours (Salleh et al., 2012). The obtained oil was then dried using anhydrous magnesium sulfate, weighed, and stored in dry amber vials at 4 °C until analysis. The average yield of oil was calculated as percentage weight by weight (% w/w) of the fresh plant.

Analysis of the Essential Oil

Gas chromatography (GC-FID) analysis was performed on an Agilent Technologies 7890B and an Agilent 7890B FID equipped with HP-5 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.

Gas chromatography-mass spectrometry (GC-MS) chromatograms were recorded using Agilent Technologies 7890A and Agilent 5975 GC MSD equipped with HP-5MS column (30 m long, 0.25 µm thickness, and 0.25 mm inner diameter). Helium was used as a carrier gas at a flow rate of 1 mL min^{-1}. The injector temperature was 250 °C. The oven temperature was programmed from 50 °C (5 min hold) to 250 °C at 10 °C min^{-1} and finally held isothermally for 15 min. For GC-MS detection, an electron ionization system with ionization 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.

Identification of Essential Oil Components

For identification of essential oil components, co-injections with the standards (major components: β-caryophyllene, α-humulene, valencene, α-cadinol, and germacrene D) were used, together with correspondence of retention indices and mass spectra with respect to those occurring in Adams, Wiley, NIST 08 and FFNSC2 libraries (Adams, 1995). The semi-quantification of essential oil components was made by peak area normalization considering the same response factor for all volatile components. Quantification was done by the external standard method using calibration curves generated by running GC analysis of representative authentic compounds.

Results and Discussion

Hydrodistillation from the fresh leaf of *G. nigrolineata* yielded 0.12 % (w/w). The list of chemical components identified in the essential oils is shown in Table 1. The essential oil of *G. nigrolineata* revealed the presence of 37 components with a total percentage of 98.3 %. The oil was characterized by a high concentration of sesquiterpenes hydrocarbons (60.0 %) followed by oxygenated sesquiterpenes (26.9 %). The oil showed richness in β-caryophyllene (25.2 %), α-humulene (12.8 %), valencene (6.2 %), α-cadinol (5.8 %), and germacrene D (5.5 %). Other components identified in noteworthy levels (>2.0 %) were α-muurolol (3.5 %), caryophyllene oxide (3.2 %), δ-cadinene (3.2 %), β-selinene (2.6 %), limonene (2.5 %), linalool (2.5 %), eudesma-4(15),7-dien-1-β-ol (2.4 %), selin-11-en-4-α-ol (2.2 %), terpinene-4-ol (2.0 %), and α-terpineol (2.0 %). In comparison to the previous study, β-caryophyllene has been reported from other *Garcinia* species, mainly from India. It has been identified from the leaves oil of *G. morella* (69.6 %), *G. assamica* (31.0 %), *G. lanceifolia* (15.9 %), *G. xanthochymus* (15.7 %), *G. pedunculata* (9.8 %), and *G. dulcis* (9.2 %) (Menon et al., 2019). In addition, β-caryophyllene was also found in North East *Garcinia* species such as *G. imberti* (38.1 %), *G. rubro-echinata* (37.9 %), *G. talbotii* (30.4 %), *G. wightii* (19.0 %), *G. indica* (18.6 %), and *G. pushpangadaniana* (11.4 %) leaf oils (Rameshkumar, 2016). β-Caryophyllene has been described as a defence constituent to herbivore attack (Wang et al., 2009). It possesses several biological activities including anti-inflammatory, anticarcinogenic, anti-fibrotic, anxiolytic, anesthetic, anticancer, antioxidant and antimicrobial (Klauke et al., 2014; Dahham et al., 2015).

Table 1. Chemical components identified from *G. nigrolineata* essential oil

No	Components	KI[a]	KI[b]	Percentage (%)	Identifications[c]
1	Myrcene	987	988	0.2	RI, MS
2	α-Terpinene	1015	1014	0.4	RI, MS
3	Limonene	1025	1025	2.5	RI, MS, Std
4	1,8-Cineole	1026	1025	1.2	RI, MS
5	(*Z*)-β-Ocimene	1035	1035	0.2	RI, MS
6	(*E*)-β-Ocimene	1045	1045	0.2	RI, MS
7	Linalool	1095	1092	2.5	RI, MS, Std
8	Terpinene-4-ol	1174	1172	2.0	RI, MS
9	α-Terpineol	1186	1185	2.0	RI, MS
10	Nerol	1215	1210	0.2	RI, MS
11	α-Ylangene	1372	1372	0.2	RI, MS
12	α-Copaene	1375	1374	0.4	RI, MS
13	β-Bourbonene	1382	1380	0.2	RI, MS
14	α-Cedrene	1405	1409	0.3	RI, MS
15	α-Gurjunene	1410	1410	0.5	RI, MS
16	β-Caryophyllene	1415	1417	25.2	RI, MS, Std
17	α-Humulene	1450	1452	12.8	RI, MS, Std
18	β-Selinene	1472	1470	2.6	RI, MS
19	Germacrene D	1485	1484	5.5	RI, MS, Std
20	Valencene	1492	1490	6.2	RI, MS
21	α-Selinene	1494	1495	0.4	RI, MS
22	γ-Cadinene	1511	1510	1.0	RI, MS
23	δ-Cadinene	1520	1522	3.2	RI, MS
24	α-Calacorene	1542	1548	1.0	RI, MS
25	Germacrene B	1550	1550	0.5	RI, MS
26	Elemol	1558	1556	1.2	RI, MS
27	(*E*)-Nerolidol	1565	1565	2.8	RI, MS
28	Caryophyllene oxide	1582	1580	3.2	RI, MS
29	Gleenol	1585	1585	1.2	RI, MS
30	Thujopsan-2-α-ol	1587	1589	1.2	RI, MS
31	Guaiol	1602	1600	1.4	RI, MS
32	Humulene epoxide	1605	1606	0.5	RI, MS
33	1-*epi*-Cubenol	1624	1626	1.5	RI, MS
34	α-Muurolol	1645	1645	3.5	RI, MS
35	α-Cadinol	1650	1652	5.8	RI, MS, Std
36	Selin-11-en-4-α-ol	1653	1654	2.2	RI, MS
37	Eudesma-4(15),7-dien-1-β-ol	1688	1682	2.4	RI, MS
	Monoterpene hydrocarbons			4.7	
	Oxygenated monoterpenes			6.7	
	Sesquiterpene hydrocarbons			60.0	
	Oxygenated sesquiterpenes			26.9	
	Total identified (%)			98.3	

[a] Linear retention index experimentally determined using homologous series of C_6-C_{30} alkanes
[b] Linear retention index taken from Adams, Wiley or NIST08 and literature
[c] Quantification was done by the external standard method using calibration curves generated by running GC analysis of representative authentic compounds

Conclusions

The present study is the first report of the leaf volatile components of *Garcinia nigrolineata* from Malaysia. The present report identifies the studied *Garcinia* species as valuable natural sources of essential oils having biologically active sesquiterpenes in significant quantity. The next step will be to evaluate the biological activities of the essential oil in order to valorize this species with a special ecological character.

Acknowledgements

The authors would like to thank the Department of Chemistry, Faculty of Science and Mathematics, University Pendidikan Sultan Idris (UPSI) for research facilities.

References

Adams R. P. (1995). Identification of Essential Oil Components by Gas Chromatography/Mass Spectroscopy. Allured Publishing Co. Carol Stream, Illinois

Corner E. J. H. (1952). Wayside trees of Malaya. Vol. 1. Singapore: Government Printing Office.

Dahham S., Tabana Y., Iqbal M., Ahamed M., Ezzat M., Majid A., Majid A. (2015). The ßßAnticancer, Antioxidant and Antimicrobial Properties of the Sesquiterpene ß-caryophyllene from the Essential Oil of *Aquilaria crassna*. Molecules 20 (7): 11808-11829. doi: 10.3390/molecules200711808

Jamila N., Khan N., Khan I., Khan A.A., Khan S.N. (2016). A Bioactive Cycloartane Triterpene from *Garcinia hombroniana*. Nat Prod Res 30: 1388-1397. doi: 10.1080/14786419.2015.1060594

Klauke A. L., Racz I., Pradier B., Markert A., Zimmer A.M., Gertsch J., Zimmer A. (2014). The Cannabinoid CB2 Receptor-Selective Phytocannabinoid *ß*-Caryophyllene Exerts Analgesic Effects in Mouse Models of Inflammatory and Neuropathic Pain. Eur Neuropsychopharmacol 24 (4): 608-620. doi: 10.1016/j.euroneuro.2013.10.008

Liu B., Zhang X., Bussmann R.W., Hart R.H., Li P., Bai Y., Long C. (2016). *Garcinia* in Southern China: Ethnobotany, Management and Niche Modeling. Econ Bot 70 (4): 416-430. doi: 10.1007/s12231-016-9360-0

Menon L. N., Shameer P. S., Sarma J., Rameshkumar K. B. (2019). Profiles of Volatile Chemicals from the Leaves of Six *Garcinia* Species from North East India. Nat Prod Res 35 (13): 2269-2273. doi:10.1080/14786419.2019.1667349

Pangsuban S., Bamroongrugsa N., Kanchanapoom K., Nualsri C. (2009). Facultative Apomixis in *Garcinia atroviridis* (Clusiaceae) and Effects of Different Pollination Regimes on Reproductive Success. Trop Life Sci Res 20: 89-108

Raksat A., Maneerat W., Andersen R.J., Pyne S.G., Laphookhieo S. (2019). A Tocotrienol Quinone Dimer and Xanthones from the Leaf Extract of *Garcinia nigrolineata*. Fitoterapia 136: 104175. doi: 10.1016/j.fitote.2019.104175

Rameshkumar K.B. (2016). Diversity of *Garcinia* Species in the Western Ghats: Phytochemical Perspective. Jawaharlal Nehru Tropical Botanic Garden and Research Institute, Kerala, India. Available at: https://jntbgri.res.in/downloads/jntbgri_diversity_of_garcinia.pdf [Accessed: 15 September 2020].

Rukachaisirikul V., Kamkaew M., Sukavisit D., Phongpaichit S., Sawangchote P., Taylor W.C. (2003a). Antibacterial Xanthones from the Leaves of *Garcinia nigrolineata*. J Nat Prod 66 (12): 1531-1535. doi: 10.1021/np0303254

Rukachaisirikul V., Ritthiwigrom T., Pinsa A., Sawangchote P., Taylor W. C. (2003b). Xanthones from the Stem Bark of *Garcinia nigrolineata*. Phytochemistry 64 (6): 1149-1156. doi: 10.1016/s0031-9422(03)00502-8

Rukachaisirikul V., Tadpetch K., Watthanaphanit A., Saengsanae N., Phongpaichit S. (2005). Benzopyran, Biphenyl, and Tetraoxygenated Xanthone Derivatives from the Twigs of *Garcinia nigrolineata*. J Nat Prod 68 (8): 1218-1221. doi: 10.1021/np058050a

Salleh W. M. N. H. W, Ahmad F., Khong H. Y. (2014a). Chemical Compositions and Antimicrobial Activity of the Essential Oils of *Piper abbreviatum, P. erecticaule* and *P. lanatum* (Piperaceae). Nat Prod Commun 9(12): 1795-1798. doi: 10.1177/1934578X1400901235

Salleh W. M. N. H. W, Ahmad F., Khong H.Y. (2014b). Chemical Composition of *Piper stylosum* Miq. and *Piper ribesioides* Wall. Essential Oils and Their Antioxidant, Antimicrobial and Tyrosinase Inhibition Activities. Bol Latinoam Caribe Plantas Med Aromat 13 (5): 488-497

Salleh W. M. N. H. W., Ahmad F., Khong H. Y., Sirat H. M. (2012). Chemical Compositions and Antibacterial Activity of the Leaf and Stem Oils of *Piper porphyrophyllum* (Lindl.) N.E.Br. Excli J 11: 399-406

Salleh W. M. N. H. W., Ahmad F., Khong H. Y., Zulkifli R. M. (2015). Chemical Compositions and Biological Activities of Essential Oils of *Beilschmiedia glabra*. Nat Prod Commun 10 (7): 1297-1300. doi: 10.1177/1934578X1501000740

Salleh W. M. N. H. W, Ahmad F., Khong H. Y., Zulkifli R. M. (2016). Chemical Composition and Biological Activities of Essential Oil of *Beilschmiedia pulverulenta*. Pharm Biol 54 (2): 322-330. doi: 10.3109/13880209.2015.1037003

Wang R., Peng S., Zeng R., Ding L. W., Xu Z. (2009). Cloning, Expression and Wounding Induction of β-Caryophyllene Synthase Gene from Mikania micrantha H.B.K. and Allelopathic Potential of β-Caryophyllene. Allelopath J 24: 35-44

aCS86_42