Short Note

Chemical composition and cytotoxicity of *Garcinia urophylla* Scort. ex King essential oil

Keith Katrina Adward[1]
Wan Mohd Nuzul Hakimi Wan Salleh[1,2] ✉
Abubakar Siddiq Salihu[1,3]
Nurunajah Ab Ghani[4,5]
Mohd Hafiz Arzmi[6]
Shamsul Khamis[2,7]

[1] Department of Chemistry
Faculty of Science and Mathematics
Universiti Pendidikan Sultan Idris
Tanjong Malim, Perak, Malaysia
[2] Fraser's Hill Research Center
Universiti Kebangsaan Malaysia
Bangi, Selangor, Malaysia
[3] Department of Pure and Industrial Chemistry
Faculty of Natural and Applied Sciences
Umaru Musa Yar'adua University
Katsina, Nigeria
[4] Atta-ur-Rahman Institute for Natural Product Discovery (AuRIns)
Universiti Teknologi MARA
Puncak Alam, Selangor, Malaysia
[5] Faculty of Applied Sciences
Universiti Teknologi MARA
Shah Alam, Selangor, Malaysia
[6] Department of Fundamental Dental & Medical Sciences
Kulliyyah of Dentistry, International Islamic University Malaysia
Kuantan, Pahang, Malaysia
[7] Department of Biological Sciences and Biotechnology
Faculty of Science and Technology
Universiti Kebangsaan Malaysia
Bangi, Selangor, Malaysia

✉ CORRESPONDING AUTHOR:
E-mail : wmnhakimi@fsmt.upsi.edu.my
Tel. : +605-4507123
https://orcid.org/0000-0003-1408-229X

Received: March 7, 2023
Accepted: September 8, 2023

Essential oils, a volatile mixture derived from plants, have shown a wide biological activity, and have been used as ancient remedies for the treatment of various diseases. The objective of this study was to investigate the chemical composition and cytotoxicity of the essential oil obtained from *Garcinia urophylla* leaves collected from Malaysia. Eighteen components were identified using gas chromatography-flame ionisation detection (GC-FID) and gas chromatography/mass spectrometry (GC-MS), which represent 99.9% of the essential oil. The major components were β-caryophyllene (56.2%), α-humulene (26.3%), and α-gurjunene (6.3%). The cytotoxicity of essential oil was evaluated using an MTT assay. The essential oil exhibited cytotoxicity against three cancer cell lines which are HepG2, MCF7, and A549 with the IC50 values of 71.5, 56.2, and 68.5 µg/mL, respectively. To the best of our knowledge, this is the first report on the essential oil composition obtained from *Garcinia urophylla*, which may have implications on the pharmaceutical and therapeutic applications of Garcinia genus essential oils.
Keywords: Essential oil; *Garcinia urophylla*; β-caryophyllene; cytotoxicity

1. INTRODUCTION

Essential oils have been widely studied as anticancer drugs in recent years. They are secondary metabolites with a key role in plant protection, consisting primarily of terpenes with a volatile nature and a diverse array of chemical structures. Essential oils exhibit a wide range of bioactivities, most notably antibacterial, antifungal, and antioxidant properties and have long been utilised for treating various human ailments and diseases [1-5]. In recent years, essential oils have been introduced as alternatives to the well-known side effects caused by synthetic chemotherapeutic drugs. In addition, chemical compounds from plants have been reported to prevent carcinogenic processes by cell arrest, induce both inner and outer apoptosis pathways, inhibit the mutagen in cells, and reduce oxidative stress in cells [6]. Therefore, the cytotoxicity of essential oils against different cell lines is necessary.

Clusiaceae are a tropical family mainly composed of latex with about 40 genera. In Malaysia, four genera and 121 species of the Clusiaceae are found namely, *Garcinia* (49 sp.), *Calophyllum* (45 sp.), *Mesua* (23 sp.) and *Mammea* (4 sp.) in different habitats [7]. *Garcinia* is an economically important genus of Clusiaceae consisting of about 400 species within palaeotropical regions concentrated mainly in Southeast Asia and secondarily in India and West Africa. Species of this genus are typically small to medium dioecious evergreen fruit trees, occasionally shrubs, usually with hard timber and abundant latex. The fruits, latex (gum and resin), timber, leaves, and roots of several species are of economic and medicinal value [8]. The major classes of biomolecules reported to be present in these plants include xanthones, benzophenones, flavonoids, phloroglucinols, fatty acids, and terpenoids [9]. Additionally, important pharmacological properties such as antioxidant, anticancer, anti-di-

abetic, anti-inflammatory, cardioprotective, neuroprotective, antimicrobial, and hepatoprotective activities along with the toxicological studies of the *Garcinia* have been documented [10].

G. urophylla, locally known as 'kandis hutan' in Malaysia, which are native to Peninsular Malaysia, Thailand, and India. It is a shrub and grows primarily in the wet tropical biome. The fruits are traditionally used to treat stomach-ache and the leaves are used to treat fever [11]. Previously, the stem extract of *G. urophylla* was found active against human tumour cell lines, representing tumours of the breast (MCF-7), lung (NCI-H460) and prostate (DU-145) with IC_{50} values of 8.0, 37.0, and 32.0 µg/mL, respectively [12]. Meanwhile, the leaf extract of *G. urophylla* showed a strong nitric oxide inhibitory activity in RAW 264.7 macrophage cell line with IC_{50} value 22.0 µg/mL [12]. Another study revealed the isolation of xanthones in the phytochemical investigation of *G. urophylla* leaves [13].

As part of a systematic evaluation of Malaysian *Garcinia* species [14-17], we present the chemical composition and cytotoxicity of the essential oil extracted from the leaves of *G. urophylla*. Literature searches have revealed no reports on the composition of leaf oil in this species.

2. MATERIALS AND METHODS

2.1 PLANT MATERIAL

The samples of *G. urophylla* were collected from Fraser Hill, Pahang (January 2023) and were identified by Shamsul Khamis from Universiti Kebangsaan (UKM). The voucher specimen (SA30-39) was deposited at Herbarium of UKM.

2.2 ISOLATION OF ESSENTIAL OIL

The fresh leaves of *G. urophylla* (400 g) were subjected to hydro distillation for 4 hours in a Clevenger-type apparatus. The obtained essential oil was dried over anhydrous magnesium sulphate and stored at 4-6°C.

2.3 ANALYSIS OF ESSENTIAL OIL

Gas chromatography-flame ionisation detection (GC-FID) analysis was performed on a Hewlett Packard 6890 series II A gas chromatograph 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 increased to 280°C at 5°C/min rate and finally held isothermally for 15 min. Diluted samples (1.0 µL, 1/100 v/v in diethyl ether) were injected manually (split ratio 50:1). Injection was repeated three times and peak area percentages were reported as means ±SD of triplicate. Peak area percentages were calculated from flame ionisation detection (FID) using GC HP Chemstation software (Agilent Technologies).

Gas chromatography-mass spectrometry (GC-MS) chromatograms were recorded using a gas chromatograph Hewlett Packard Model 5890A and a Hewlett Packard Model 5989A mass spectrometer. The GC was equipped with HP-5 column. Helium was used as the 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 250°C at 10°C/min and finally held isothermally for 15 min. For GC-MS detection, an electron ionization system with an 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 ESSENTIAL OIL COMPONENTS

The essential oil components were identified by co-injection with standards (major components: β-caryophyllene, α-humulene, and α-gurjunene) and their comparison with reported retention indices and mass spectra found in Adams, NIST 08 and FFNSC2 libraries [18]. Semi-quantification of essential oil components was made by peak area normalisation considering the same response factor for all volatile components.

2.5 CYTOTOXICITY

Cytotoxic examination of the essential oil was carried out using the MTT assay [19]. Briefly, the cells were diluted in a 96-well microplate (5×10^4 cells per well of 200 µL mixture). The samples (1-100 µg/mL) and the positive control, doxorubicin (0.05-1.56 µg/mL), were added to the cells and incubated at 37°C for 48 h with 5% CO_2. MTT (20 µL) was added to the wells and incubation was continued at 37°C for 4 h. Absorbance was recorded at 540/720 nm using a Spark multimode reader (Tecan). Each experiment was repeated in triplicate. The inhibitory percentage (%) = $[1 - OD_{sample}/OD_{conc}] \times 100\%$; where OD_{sample} and OD_{conc} were the optical densities of the samples and the control, respectively. Data obtained from the cytotoxicity are expressed as mean values. 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

Hydrodistillation by fresh *G. urophylla* leaf produced (w/w) 0.29% of essential oil. A summary of the chemical components identified in the essential oil is shown in Table I. GC and GC-MS analysis (Figure 1) of the essential oil successfully revealed the existence of 18 chemical components, representing 99.9% of the total essential oil composition. Sesquiterpene hydrocarbons were the most dominant group in the essential oil components, accounting for 96.1% of the total composition, respectively. The essential oil composition was demonstrated by its richness in β-caryophyl-

Table I - Chemical components identified from *Garcinia urophylla* essential oil

No	Components	KI[a]	KI[b]	Percentage (%)	Identification[c]
1	α-Pinene	934	935	0.2	RI, MS
2	*o*-Cymene	1022	1020	0.4	RI, MS
3	γ-Terpinene	1052	1055	0.2	RI, MS
4	Terpinen-4-ol	1169	1166	1.9	RI, MS
5	α-Copaene	1374	1372	0.8	RI, MS
6	β-Elemene	1389	1390	0.6	RI, MS
7	**α-Gurjunene**	1405	1405	**6.3**	RI, MS, Std
8	**β-Caryophyllene**	1420	1420	**56.2**	RI, MS, Std
9	**α-Humulene**	1436	1435	**26.3**	RI, MS, Std
10	Alloaromadendrene	1460	1462	2.2	RI, MS
11	γ-Gurjunene	1470	1470	0.7	RI, MS
12	α-Selinene	1490	1491	0.6	RI, MS
13	Viridiflorene	1505	1504	1.0	RI, MS
14	(*E*,*E*)-α-Farnesene	1505	1506	0.3	RI, MS
15	δ-Cadinene	1524	1522	1.1	RI, MS
16	Palustrol	1567	1565	0.3	RI, MS
17	Caryophyllene oxide	1582	1580	0.5	RI, MS
18	*neo*-Intermedeol	1658	1658	0.3	RI, MS
	Monoterpene hydrocarbons			**0.8**	
	Oxygenated monoterpenes			**1.9**	
	Sesquiterpene hydrocarbons			**96.1**	
	Oxygenated sesquiterpenes			**1.1**	
	Total identified			**99.9**	

RI: based on comparison of calculated RI with those reported in Adams
MS: based on comparison with Wiley, Adams, FFNSC2, and NIST08 MS databases
Std: based on comparison with standard compounds
[a] Linear retention index experimentally determined using homologous series of C6-C30 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.

lene (56.2%), α-humulene (26.3%), and α-gurjunene (6.3%).

In comparison to the previous studies, β-caryophyllene has also been reported as a major component in Malaysian *Garcinia* species such as from the leaf oils of *G. nigrolineata* (25.2%) [20], *G. gummi-gutta* (53.82%) [21], and *G. celebica* (5.85%) [22]. In addition, Indian *Garcinia* species were also reported the high content of β-caryophyllene which are from the leaf oil of *G. morella* (69.6%), *G. assamica* (31.0%), *G. lanceifolia* (15.9%), *G. xanthochymus* (15.7%), *G. pedunculata* (9.8%), *G. dulcis* (9.2%) [23], *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%) [24]. Furthermore, it was also reported from *G. quaesita* (Sri Lanka: leaf oil 6.7%), *G. zeylanica* (Sri Lanka: leaf oil 12.9%) [25], *G. huillensis* (Zimbabwe: fruit oil 12.6%) [26], and *G.*

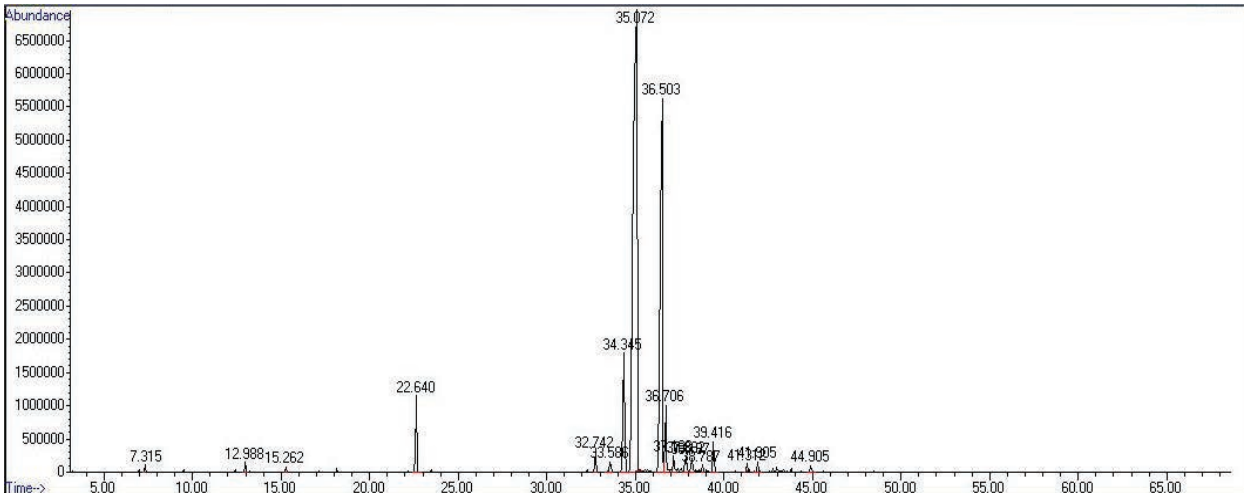

Figure - 1 Chromatogram of *Garcinia urophylla* essential oil

Table II – Cytotoxicity of *Garcinia urophylla* essential oil

Cell lines	IC_{50} value (µg/mL)	
	Essential oil	Doxorubicin
Human liver cancer HepG2	71.5	0.76
Human breast cancer MCF7	56.2	0.20
Human lung carcinoma cancer A549	68.5	0.95

mangostana (Nigeria: leaf oil 17.3%, stem-bark oil 21.1%) [27]. These subtle differences in the chemical components may be attributed to the differences in environmental and genetic factors, chemotype, and nutritional status of the plants, which may influence their oil composition [28].

β-Caryophyllene 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 [29]. 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 [30].

Following a similar line of thought, the essential oil was subjected to a preliminary test to verify the cytotoxicity effect using the MTT assay. The results are shown in Table II. Doxorubicin remarkably inhibited the growth of the studied cancer cell lines with lower IC_{50} values compared to the essential oil. The best inhibitory result of the essential oil with IC_{50} value of 56.2 µg/mL reported against the MCF7 cell line. The cytotoxicity of the essential oil may be attributed to the presence of the major component in the sample. Previous studies have revealed that sesquiterpenes exhibited cytotoxic effects in several cancer cell lines [31]. Previously, β-caryophyllene was reported to show cytotoxicity on breast carcinoma cells [32]. Besides, the treatment of β-caryophyllene alone with human tumour cell lines has stimulated apoptosis and suppressed tumour growth [30]. Furthermore, in studies on *G. atroviridis* [33] and *G. celebica* [22] essential oils, the high content of sesquiterpenes was found in the leaf oil and exhibited potent cytotoxicity on human breast cancer cells. According to the results, the potent cytotoxicity of *G. urophylla* essential oil could be due to the major occurrence of sesquiterpenes, which accounted for 96.1% of the total oil.

4. CONCLUSION

Essential oils from aromatic plants are recognised as a perfect source of food additives and pharmaceuticals. This study of *G. urophylla* essential oil has revealed the existence of sesquiterpene hydrocarbons as the main component of the group, dominated by β-caryophyllene, α-humulene, and α-gurjunene. The essential oil also revealed a significant cytotoxic effect, mainly against MCF7 MCF-7 human breast cancer cell line with IC_{50} value 56.2 µg/mL. Hence, the species could be a source of natural products for further research into the development of chemopreventive or cosmetic agents.

Acknowledgements

This research was supported by the Fundamental University Research Grant (GPUF2022) [2022-0130-102-01]. The authors also would like to thank the Department of Chemistry, Faculty of Science and Mathematics, UPSI for providing the research facilities.

References

[1] W.M.N.H.W. Salleh, N.A. Ghani, A.B.M. Abdel-Aal. Chemical composition and anticholinesterase activity of *Lepisanthes rubiginosa* (Roxb.) Leenh. essential oil. Z. Naturforsch. C J. Biosci. 77(11-12), 525-529 (2022).

[2] A.A.H. Yahaya, W.M.N.H.W. Salleh, N.A. Ghani, S. Khamis, N.S. Rezali, M.A.A. Juhari. Chemical composition and bioactivities of *Magnolia candollii* H.Keng essential oil. Z. Naturforsch. C J. Biosci. 77(11-12), 519-523 (2022).

[3] W.M.N.H.W. Salleh, S. Khamis. Chemical composition and anticholinesterase inhibitory activity of *Pavetta graciliflora* Wall. ex Ridl. essential oil. Z. Naturforsch. C J. Biosci. 75, 467-71 (2020).

[4] W.M.N.H.W. Salleh, F. Ahmad, K.H. Yen, R.M. Zulkifli. Chemical composition and biological activities of essential oil of *Beilschmiedia pulverulenta*. Pharm. Biol. 54(2), 322-330 (2016).

[5] 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(11), 2005-2008 (2015).

[6] K. Blowman, M. Magalhaes, M.F.L. Lemos, C. Cabral, I.M. Pires. Anticancer properties of essential oils and other natural products. Evid. Based Complement. Altern. Med. 3149362 (2018).

[7] E.J.H. Corner. Wayside trees of Malaya. Vol. 1. Singapore: Government Printing Office, 1952.

[8] B. Liu, X. Zhang, R.W. Bussmann, R.H. Hart, P. Li, Y. Bai, C. Long. *Garcinia* in Southern China: Ethnobotany, Management and Niche Modeling. Econ. Bot. 70(4), 416-430 (2016).

[9] O. Chantarasriwong, A. Batova, W. Chavasiri, E.A. Theodorakis. Chemistry and biology of the caged *Garcinia* xanthones. Chem. Eur. J. 16, 9944-9962 (2010).

[10] D. Obolskiy, I. Pischel, N. Siriwatanametanon, M. Heinrich. *Garcinia mangostana* L.: A phytochemical and pharmacological review. Phytother. Res. 23, 1047-1065 (2009).

[11] I.H. Burkill. A dictionary of the economic product of Malay Peninsula. Kuala Lumpur: Ministry of Agriculture and Co-operatives, 1966.
[12] M.L. Jabit, F.S. Wahyuni, R. Khalid, D.A. Israf, K. Shaari, N.H. Lajis, J. Stanslas. Cytotoxic and nitric oxide inhibitory activities of methanol extracts of *Garcinia* species. Pharm. Biol. 47(11), 1019-1026 (2009).
[13] R.M. Khalid, Md.L. Jabit, F. Abas, J. Stanslas, K. Shaari, N.H. Lajis. Cytotoxic xanthones from the leaves of *Garcinia urophylla*. Nat. Prod. Commun. 2(3), 271-276 (2007).
[14] W.M.N.H.W. Salleh, S. Khamis. Essential oil composition of *Garcinia nigrolineata* Planch. ex T.Anderson (Clusiaceae). Agric. Conspec. Sci. 86(4), 375-378 (2022).
[15] W.M.N.H.W. Salleh, S. On, F. Ahmad, H.M. Sirat, M. Taher, S.D. Sarker, L. Nahar. A new xanthone and a new benzophenone from the roots of *Garcinia hombroniana.* Phytochem. Lett. 35, 216-219 (2020).
[16] W.M.N.H.W. Salleh, N.S.A.N. Sazali, F. Ahmad. Biflavonoids from the leaves and stem bark of *Garcinia griffithii* and their biological activities. Marmara Pharm. J. 21(4), 889-897 (2017).
[17] S. On, W.M.N.H.W. Salleh, F. Ahmad, H.M. Sirat, M. Taher. Cytotoxicity of triterpenes from the leaves of *Garcinia prainiana* King (Guttiferae). Marmara Pharm. J. 21(1), 127-131 (2017).
[18] R.P. Adams. Identification of essential oil components by gas chromatography-mass spectrometry. 4th ed. Carol Stream (IL): Allured Publishing Corporation, 2007.
[19] W.M.N.H.W. Salleh, N.A. Muhammad, N.A. Ghani, M.H. Jauri. Volatile components of *Dillenia reticulata* King (Dilleniaceae) essential oil and their cytotoxicity. Bull. Chem. Soc. Ethiop. 37(1), 245-249 (2023).
[20] W.M.N.H.W. Salleh, S. Khamis. Essential oil composition of *Garcinia nigrolineata* Planch. ex T. Anderson (Clusiaceae). Agric. Conspec. Sci. 86(4), 375-378 (2021).
[21] W.N. Tan, W.Y. Tong, C.R. Leong, N.N.S. Nik Mohamed Kamal, M. Muhamad, J.W. Lim, M. Khairuddean, M.B. Her Man. Chemical composition of essential oil of *Garcinia gummi-gutta* and its antimicrobial and cytotoxic activities. J. Essent. Oil Bear. Pl. 23(4), 832-842 (2020).
[22] W.N. Tan, Z.H. Tan, N.I. Zulkifli, N.N.S. Nik Mohamed Kamal, N.A.S. Rozman, W.Y. Tong, C.R. Leong, J.W. Lim. Sesquiterpenes rich essential oil from *Garcinia celebica* L. and its cytotoxic and antimicrobial activities. Nat. Prod. Res. 34(23), 3404-3408 (2020).
[23] L.N. Menon, P.S. Shameer, J. Sarma, K.B. Rameshkumar. Profiles of volatile chemicals from the leaves of six *Garcinia* species from North East India. Nat. Prod. Res. 35(13), 2269-2273 (2019).
[24] K.B. Rameshkumar. Diversity of *Garcinia* species in the Western Ghats: Phytochemical perspective. Jawaharlal Nehru Tropical Botanic Garden and Research Institute, Kerala, India, 2016.
[25] S. Kokilananthan, V.P. Bulugahapitiya, H. Manawadu, C.S. Gangabadage. Investigations of chemical compositions and antioxidative potential of essential oils isolated from the leaves of two *Garcinia* species. J. Adv. Pharm. Tech. Res. 14(1), 12-17 (2023).
[26] L.S. Chagonda, J.C. Chalchat. The essential oil of the fruit of *Garcinia huillensis* Welw. ex. Oliv. from Zimbabwe. Flavour Fragr. J. 20(3), 313-315 (2005).
[27] S. Aboaba, A. Akande, G. Flamini. Chemical composition, toxicity and antibacterial activity of the essential oils of *Garcinia mangostana* from Nigeria. J. Essent. Oil Bear. Pl. 17(1), 78-86 (2014).
[28] K. Marjo, P. Eija, E.S. James. Variation in volatile compounds from tansy (*Tanacetum vulgare* L.) related to genetic and morphological differences of genotypes. Biochem. Syst. Ecol. 29, 267-285 (2001).
[29] 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).
[30] 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).
[31] K. Celik, B. Togar, H. Turkez, N. Taspinar. In vitro cytotoxic, genotoxic, and oxidative effects of acyclic sesquiterpene farnesene. Turk. J. Biol. 38, 253-259 (2014).
[32] Y. Asakawa, Y. Noma. Biotransformation of sesquiterpenoids in: Comprehensive Natural Products II: Chemistry and Biology. Mander L and Liu HW. (eds.) Elsevier, United Kingdom, 2010.
[33] W.N. Tan, J.Q. Lim, F. Afiqah, N.N.S.N.M. Kamal, F.A.A. Aziz, W.Y. Tong, C.R. Leong, J.W. Lim. Chemical composition and cytotoxic activity of *Garcinia atroviridis* Griff. ex T. Anders. essential oils in combination with tamoxifen. Nat. Prod. Res. 32, 854-858 (2018).