Rapid communication

Natasa Mohd Shakri, Wan Mohd Nuzul Hakimi Wan Salleh*, Shamsul Khamis, Nor Azah Mohamad Ali and Muhammad Helmi Nadri

Composition of the essential oils of three Malaysian *Xylopia* species (Annonaceae)

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Abstract: The rich and diversified Malaysian flora represents an excellent resource of new chemical structures with biological activities. The genus *Xylopia* L. includes aromatic plants that have both nutritional and medicinal uses. This study aims to contribute with information about the volatile components of three *Xylopia* species essential oils: *Xylopia frutescens*, *Xylopia ferruginea*, and *Xylopia magna*. In this study, essential oils were extracted from the leaves by a hydrodistillation process. The identification of the essential oil components was performed by gas chromatography (GC-FID) and gas chromatography–coupled mass spectrometry (GC-MS). The major components of the essential oils from *X. frutescens* were bicyclogermacrene (22.8%), germacrene D (14.2%), elemol (12.8%), and guaiol (12.8%), whereas components of the essential oils from *X. magna* were germacrene D (35.9%), bicyclogermacrene (22.8%), and spathulenol (11.1%). The *X. ferruginea* oil was dominated by bicyclogermacrene (23.6%), elemol (13.7%), guaiol (13.4%), and germacrene D (12.3%).

Keywords: Annonaceae; essential oil; *X. ferruginea*; *X. magna*; *Xylopia frutescens*.

***Corresponding author: Wan Mohd Nuzul Hakimi Wan Salleh**, Department of Chemistry, Faculty of Science and Mathematics, Universiti Pendidikan Sultan Idris (UPSI), 35900 Tanjong Malim, Perak, Malaysia, E-mail: wmnhakimi@fsmt.upsi.edu.my. https://orcid.org/0000-0003-1408-229X
Natasa Mohd Shakri, Department of Chemistry, Faculty of Science and Mathematics, Universiti Pendidikan Sultan Idris (UPSI), 35900 Tanjong Malim, Perak, Malaysia
Shamsul Khamis, School of Environmental and Natural Sciences, Faculty of Science and Technology, Universiti Kebangsaan Malaysia, 43600 Bangi, Selangor, Malaysia
Nor Azah Mohamad Ali, Natural Products Division, Forest Research Institute Malaysia (FRIM), 52109 Kepong, Selangor, Malaysia
Muhammad Helmi Nadri, Innovation Centre in Agritechnology (ICA), Universiti Teknologi Malaysia, 84600 Pagoh, Johor, Malaysia

1 Introduction

Essential oils are natural, complex, multicomponent systems composed mainly of terpenes in addition to some other nonterpene components. Essential oils and their constituents possess various biological activities, including antioxidant, antimicrobial, antifungal, antiinflammatory, antityrosinase, anticholinesterase, and anticancer activities [1, 2]. Historically, essential oils have been widely used in food as condiments, cosmetic industries, as well as for human health care [3, 4]. Numerous members of the Annonaceae family are odorous: the presence of essential oils, mainly containing terpene compounds, is responsible for the fragrance. *Xylopia* is an important genus among the Asian Annonaceae, comprises approximately 14 species in Malaysia [5]. It is widely distributed in tropical and subtropical regions of the Americas, Africa, Asia, and Oceania [6]. The plants are usually shrubs or small trees in nature and commonly found in lowland, peat swamp, and mountain forest [7]. Some *Xylopia* species are used to treat fever, cough, and various skin infections [8].

Many *Xylopia* species have been investigated chemically and were shown to possess volatile components [9–16]. As a continuation of essential oil studies on *Xylopia*, we describe here the volatile components in the essential oils of three *Xylopia* species, which are *Xylopia frutescens*, *Xylopia ferruginea*, and *Xylopia magna*.

X. frutescens Aubl. is a tree commonly known in Malaysia as *jangkang betina*. In folk medicine, the seeds are used for rheumatism and inflammation treatment to improve digestion and as antidiarrheal [9]. Some essential oil studies on the essential oils from *X. frutescens* have been previously reported, mainly from Brazil [17–21].

X. ferruginea (Hook.f. & Thomson) Baill. locally known as *jangkang bukit* or *banitan merah* is an endemic plant of Malaysia. This species has a unique signature of a stilted root that is rusty in color. In folk medicine, a decoction of the stem barks of this plant was used to stop vomiting and to treat antispasmodic disease [8]. Literature reviews

indicated that only one study has been investigated for its essential oil composition [22].

X. magna Maingay ex Hook.f. & Thomson is locally known as *jangkang* in Malaysia. However, there has been no information of this plant in traditional or folk medicine practice, including its essential oil composition.

2 Material and methods

2.1 Plant materials

The fresh samples of *X. ferruginea* (SK252/19), *X. frutescens* (SK25/19), and *X. magna* (SK58/19) were collected from Gambang, Pahang, in September 2019 and identified by Dr. Shamsul Khamis, a vascular plant taxonomist from Universiti Kebangsaan Malaysia (UKM). The voucher specimens were deposited at Herbarium of UKM (UKMB).

2.2 Extraction of essential oils

The fresh leaves of each sample (300 g) were chopped into small pieces and continuously subjected to a hydrodistillation process. The process was carried out in a Clevenger-type apparatus for 5 h in order to maximize the yield of essential oils [23]. The essential oils obtained were dried over anhydrous magnesium sulfate and stored at 4–6 C. The oil yield (%) was calculated based on the fresh weight (w/w).

2.3 Analysis of essential oils

Gas chromatography (GC-FID) analysis was performed on Shimadzu GC-2010 Plus (Shimadzu) gas chromatograph. Two types ofcapillary columns with different polarities were used, HP-5MS or DB-Wax capillary column (Agilent) (30 m × 0.25 mm × 0.25 μm film thickness). Helium was used as a carrier gas at a flow rate of 1.0 mL/min. The flame ionization detector (FID) temperatures were set at 250 (injector) and 280 C (detector), 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. The diluted samples (1/100 in diethyl ether, v/v) of 1.0 μL were injected manually (split ratio 10:1). Gas chromatography–coupled mass spectrometry (GC-MS) chromatograms were recorded using Agilent GC-MS 7890A/5975C Series MSD (70 eV direct inlet) with a 30 m × 0.25 mm internal diameter × 0.25 μm film thickness HP-5MS or DB-Wax capillary column. 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 250°C at 10 C/min 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 to 400 amu.

2.4 Identification of components

For identification of the essential oil components, coinjection with the standards (major components) was used, together with correspondence of retention indices (relative to the retention times of *n*-alkanes from C_6 to C_{30}) and mass spectra with respect to those reported in the study by Adams [24], NIST08 [25], and FFNSC2 [26] libraries. Semi-quantification of the essential oil components was undertaken 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-FID analysis of representative authentic compounds. Percentages values were the mean of three chromatographic analyses.

3 Results and discussion

Hydrodistillation of the fresh leaves of *X. frutescens*, *X. ferruginea*, and *X. magna* gave pale yellow oils with a pungent smell in mean yields of 0.15, 0.18. and 0.12% (w/w), respectively. The lists of components identified in the oils are shown in Table 1. Figure 1 shows the total ion chromatograms of the essential oils.

The essential oil of *X. frutescens* revealed the presence of 28 components with a percentage of 97.7%. The essential oil showed a high concentration of sesquiterpene hydrocarbons (56.8%). The oil was characterized by its richness in bicyclogermacrene (22.8%), germacrene D (14.2%), elemol (12.8%), and guaiol (12.8%). Previous studies on the essential oil compositions of the leaf, stem bark, and fruit from *X. frutescens* essential oils have been reported [17–22]. Particularly, caryophyllene (31.48%), δ-cadinol (27.4%), germacrene D (24.2%), α-cubebene (25.2%), bicyclogermacrene (23.23%), linalool (12.1%), and β-pinene (8.0%) were the predominance components identified in the previous *X. frutescens* oils. In *X. ferruginea* oil, a total of 26 components were detected with the constitution of 97.3%. The oil was made up predominantly of sesquiterpene hydrocarbons, constituting about 53.0% of the oil. The most abundant components were bicyclogermacrene (23.6%), elemol (13.7%), guaiol (13.4%), and germacrene D (12.3%). In a previous study, Ali [22] successfully identified β-pinene (40.7%) as the major monoterpene component in the leaf oil of *X. ferruginea*. The chemical differences could be attributed to different collecting time, climate effect on the plants which are growing from the different habitat, method of extraction used, and geographic origin of the plant studied [4]. In the case of *X. magna*, the leaf oil consisted of 15 components, representing 90.2%. Germacrene D (35.9%), bicyclogermacrene (22.8%), and spathulenol (11.1%) were found to be the main components in this oil. To the best of our knowledge, this is the first report on the essential oil composition of *X. magna* oil.

Based on the studies of the essential oil composition of *Xylopia* species, most of them consisted mainly of sesquiterpenes. In addition, most of the major components of these essential oils gave similar components, which

Table 1: Chemical composition of the essential oils of three *Xylopia* species.

No.	Components	KI[a]	KI[b]	Percentage (%)[c]			Methods[d]
				XFRT	XFRG	XMGN	
1	α-pinene	930	1032	0.4 ± 0.1	0.6 ± 0.1		RI, MS, Std
2	Sabinene	970	1135		1.7 ± 0.2		RI, MS
3	β-pinene	972	1125	1.2 ± 0.1			RI, MS
4	α-terpinolene	1088	1295			0.6 ± 0.1	RI, MS
	Monoterpene hydrocarbons			**1.6**	**2.3**	**0.6**	
5	δ-elemene	1332	1570	2.6 ± 0.2	2.8 ± 0.2		RI, MS
6	Isoledene	1375	1520		0.9 ± 0.1		RI, MS
7	β-maaliene	1380	1582	2.2 ± 0.1			RI, MS
8	β-elemene	1385	1595	3.7 ± 0.2	4.2 ± 0.3		RI, MS
9	Longifolene	1405	1575		2.8 ± 0.2		RI, MS
10	α-gurjunene	1410	1530			0.9 ± 0.1	RI, MS
11	β-caryophyllene	1415	1605	0.9 ± 0.2	0.9 ± 0.1	0.7 ± 0.1	RI, MS
12	γ-elemene	1435	1635	0.2 ± 0.1			RI, MS
13	Aromadendrene	1440	1650	0.4 ± 0.2	0.8 ± 0.1	3.1 ± 0.2	RI, MS
14	α-humulene	1450	1660	0.3 ± 0.1	0.4 ± 0.1		RI, MS
15	Alloaromadendrene	1460	1662	0.9 ± .2			RI, MS
16	γ-gurjunene	1475	1668			1.2 ± 0.1	RI, MS
17	α-elemene	1478	1680	2.9 ± 0.1			RI, MS
18	γ-muurolene	1480	1685	0.9 ± 0.1	0.8 ± 0.1		RI, MS
19	α-amorphene	1485	1690			0.8 ± 0.1	RI, MS
20	Germacrene D	1486	1725	14.2 ± 0.3	12.3 ± 0.2	35.9 ± 0.2	RI, MS, Std
21	β-selinene	1490	1710	0.6 ± 0.1	0.6 ± 0.1		RI, MS
22	β-guaiene	1495	1665		0.4 ± 0.1		RI, MS
23	Valencene	1496	1734	0.7 ± 0.1			RI, MS
24	Bicyclogermacrene	1502	1735	22.8 ± 0.2	23.6 ± 0.2	22.8 ± 0.2	RI, MS, Std
25	α-muurolene	1505	1725	0.5 ± 0.1		0.9 ± 0.1	RI, MS
26	Germacrene A	1510	1750	0.1 ± 0.1	0.1 ± 0.1		RI, MS
27	γ-cadinene	1512	1752	1.3 ± 0.1	1.1 ± 0.1	0.9 ± 0.1	RI, MS
28	δ-cadinene	1520	1750	1.6 ± 0.1	1.3 ± 0.1	3.7 ± 0.3	RI, MS
	Sesquiterpene hydrocarbons			**56.8**	**53.0**	**70.9**	
29	Elemol	1550	2065	12.8 ± 0.2	13.7 ± 0.2	2.7 ± 0.1	RI, MS, Std
30	Spathulenol	1575	2125	3.8 ± 0.3	2.7 ± 0.1	11.1 ± 0.2	RI, MS, Std
31	Globulol	1590	2060	2.1 ± 0.1	1.8 ± 0.1		RI, MS
32	Viridiflorol	1592	2102	1.1 ± 0.1	1.0 ± 0.1		RI, MS
33	Guaiol	1600	2075	12.8 ± 0.2	13.4 ± 0.2	3.0 ± 0.3	RI, MS, Std
34	γ-eudesmol	1628	2180		2.2 ± 0.1		RI, MS
35	β-eudesmol	1650	2245	1.5 ± 0.2	1.5 ± 0.2		RI, MS
36	α-eudesmol	1655	2230	2.1 ± 0.2	2.3 ± 0.2		RI, MS
37	Bulnesol	1670	2200	3.1 ± 0.3	3.4 ± 0.3		RI, MS
	Oxygenated sesquiterpenes			**39.3**	**42.0**	**16.8**	
38	Kaurene	2035	2235			1.9 ± 0.1	RI, MS
	Diterpene					**1.9**	
	Total identified			**97.7**	**97.3**	**90.2**	

XFRT–*X. frutescens*; XFRG–*X. ferruginea*; XMGN–*X. magna*.

[a]Linear retention index (KI) on HP-5MS column, based on comparison with those reported in the study by Adams [24].

[b]Linear retention index (KI) on DB-Wax column, based on comparison with those reported in FFNSC2 [25] and NIST08 [26].

[c]Relative percentage values are means of three determinations ± SD.

[d]Identification methods: Std, standard, based on comparison with authentic compounds; MS, mass spectroscopy; RI, retention index, based on comparison with Wiley, Adams, FFNSC2, and NIST08 MS databases.

include bicyclogermacrene, germacrene D, elemol, and guaiol. Germacrene D which was present in large amount in *X. magna* was also found in the oils of *Xylopia laevigata* (leaf oil: 43.62%) [11], *Xylopia langsdorffiana* (leaf oil: 22.90%) [27], *Xylopia fusca* (leaf oil: 17.0%) [28], and *Xylopia aethiopica* (leaf oil: 24.50%) [29]. On the other

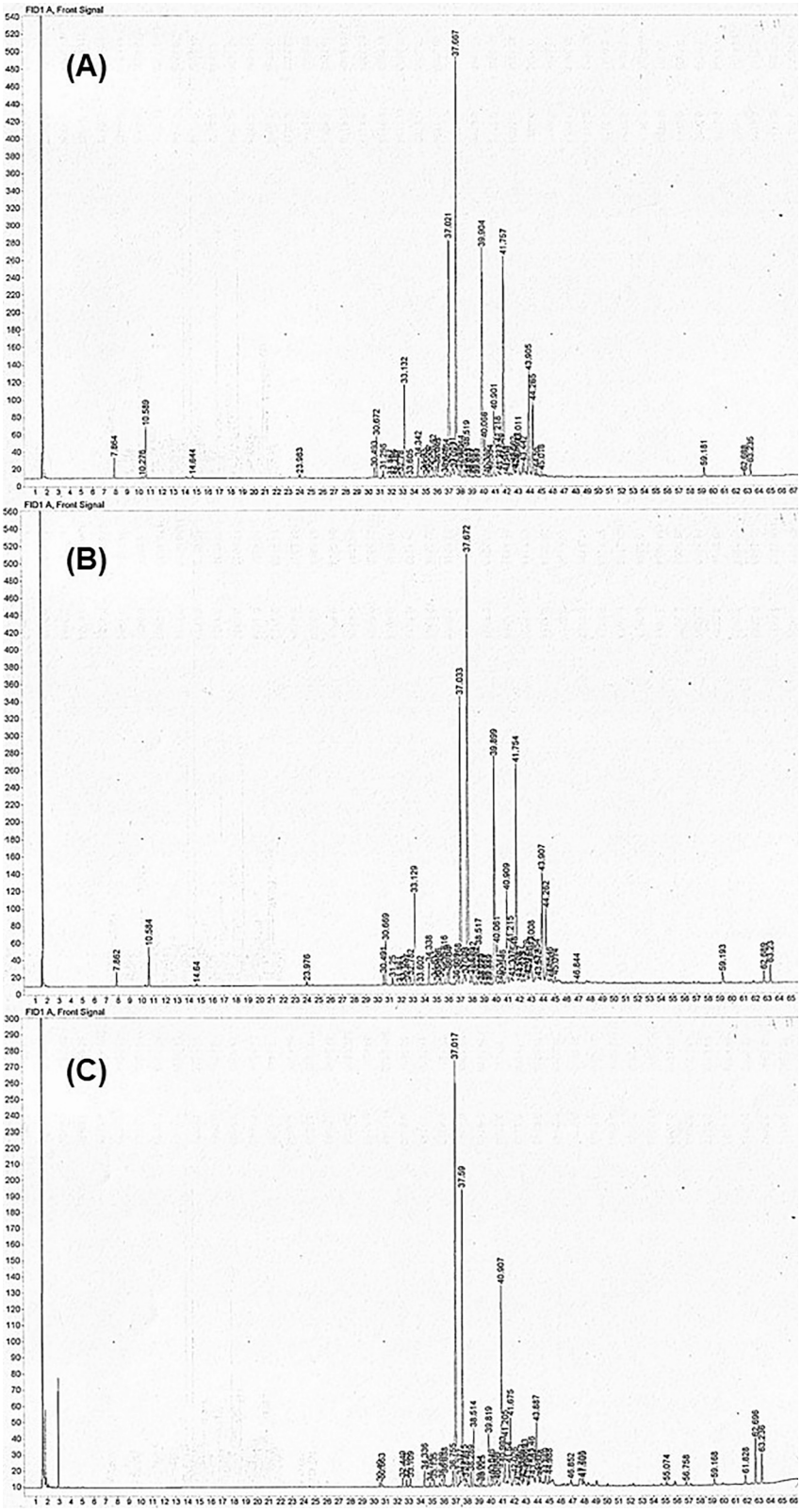

Figure 1: Total ion chromatograms (TICs) of (A) *X. frutescens*, (B) *X. ferruginea*, and (C) *X. magna* essential oils.

hand, bicyclogermacrene as the main component in *X. frutescens* and *X. ferruginea* oils had made up the bulk of the oils of *Xylopia ochrantha* (leaf oil: 25.18%) [10], *Xylopia aromatica* (leaf oil: 36.5%) [30], *Xylopia elliptica* (leaf oil: 11.50%) [28], and *Xylopia maccraea* (leaf oil: 34.0%) [31]. Spathulenol was found as a major component from the oils

of *Xylopia malayana* (twig oil: 36.0%) [32], *X. aromatica* (leaf oil: 27.11%) [12], *Xylopia sericea* (fruit oil: 16.42%) [16], and *Xylopia longifolia* (flower oil: 36.10%) [33]. Monoterpenoids were also found in other *Xylopia* oils. The identified components were limonene (*X. laevigata*) [14], 1,8-cineole (*X. sericea*) [34], α-pinene (*X. langsdorffiana*) [15], *p*-cymene (*X. longifolia*) [33], terpinen-4-ol (*X. elliptica*) [32], sabinene (*Xylopia villosa*) [35], and (*Z*)-β-ocimene (*Xylopia quintasii*) [36]. According to previous studies, analysis of the essential oil of *Xylopia* species revealed a high content of germacrene D, bicyclogermacrene, (*E*)-2caryophyllene, limonene, α-pinene, and β-pinene. It is evident that there are variations between different species with a different origin.

4 Conclusion

This study reports the chemical profiles of the essential oils from three Malaysian *Xylopia* species containing bicyclogermacrene, germacrene D, elemol, guaiol, and spathulenol as the most abundant components. 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. This study also provides valuable and useful information and indications for further exploring the potential nutraceutical and pharmaceutical applications of the genus *Xylopia*.

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