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Chemical compositions and antimicrobial activity of twig essential oils from three *Xylopia* (Annonaceae) species

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The chemical composition of twig essential oils of *Xylopia malayana*, *Xylopia elliptica* and *Xylopia fusca* were analyzed using GC and GCMS. Both *X. malayana* (12.9%) and *X. fusca* (11.8%) twig oils contained spathulenol as the major constituent while *X. elliptica* twig oil was found to be rich in terpinen-4-ol (11.9%). The antimicrobial activities of the essential oils were evaluated against six gram positive bacteria, two gram negative bacteria and yeast by using the broth microdilution method. The twig oil of *X. elliptica* at a concentration ranging from 156 to 625 µg/ml was found to be active against all bacterial and yeast strains tested. Both *X. malayana* and *X. fusca* twig oils demonstrated weak activity toward all microorganisms tested with the MIC value of 5000 µg/ml. Gram negative bacteria's, *Pseudomonas aeruginosa* and *Escherichia coli* seemed to be resistant to the essential oils tested. *α*-Pinene showed moderate to strong inhibitory effect against all the microorganism strains tested with the MIC value ranging from 325 to 2500 µg/ml, as compared to the other standard compounds which showed weak inhibitory even at the highest concentrations used. This finding demonstrated that the twig oil of *X. elliptica* possesses antimicrobial activity which may be useful and potential ingredient in the production of health care products.

Key words: *Xylopia* twigs, essential oils, GC & GCMS analysis, antimicrobial activity.

INTRODUCTION

The family Annonaceae comprises about 120 genera and more than 2000 species (Leboeuf et al., 1982) and is distributed mainly in South America, Paleotropical Africa and Southeast Asia. The plants of the family Annonaceae can be divided into three categories which are trees, shrubs, woody climbers and commonly found in the

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lowland forest (Kochummen, 1997). The shape of the plant's leaf is simple and alternate. The bark is smooth, brown or dark green in colors and fragrant. The flowers are solitary or clusters and possessing a strong aromatic smell while the fruits are normally oblong in shape, stalked or sessile. Many members of Annonaceae family are used by local native in traditional medicine for the treatment of skin diseases, fever and stomachaches (Wiart, 2006). Phytochemical studies on the Annonaceae plants resulted in the isolation of several compounds for examples isoquinoline alkaline alkaloids (Mukhtar et al., 2000) and acetogenins (Alali et al., 1999) which have been found to possess antimicrobial, insecticidal, antimalarial and antioxidant properties (Iwu et al., 1999). The genus *Xylopia* comprises about 170 species and they are widely distributed throughout Southeast Asia, Australia and Melanesia (Hyland and Whiffin, 1993). In Malaysia, eight species namely *Xylopia malayana*, *Xylopia caudata*, *Xylopia ferrugineae*, *Xylopia magna*, *Xylopia fusca*, *Xylopia elliptica*, *Xylopia stenopetala* and *Xylopia subdehiscens* have been documented by Kochummen (1972). The plants are usually shrubs or small trees in nature and commonly found in lowland, peat swap and mountain forests forest. The secondary metabolites from *Xylopia* species have been known for their great usage in folk medicine, food flavoring and medical industries (Leboeuf et al., 1982). Besides, the essential oils of some *Xylopia* sp. are also used in traditional medicine as a remedy in the treatment of stomach-ache and intestines. *Xylopia* species are sources of volatile components, diterpenes and alkaloid, which have been reported to possess antimicrobial, anti-inflammantory and antimalarial properties. Volatile constituents and bioactivity studies are available in the literature on *Xylopia aethiopica* (Issakou et al., 2014; Sylvain et al, 2014; Vyry et al, 2014), *Xylopia longifolia* (Fourier et al, 1993), *Xylopia parviflora* (Woguem et al, 2014; Samuel et al., 2015), *Xylopia aromatica* (Lago et al, 2003) and *Xylopia langsdorfiana* (Correia et al., 2015).

X. malayana or locally known as Jangkang is a small tree up to 20 m high and occurs in the northern part of East Malaysia. The morphology of leaves are elliptic-oblong whilst flowers are normally small and aromatic. In Peninsular Malaysia, the leaves of *X. malayana* are traditionally be used for treatment after childbirth (Kamarudin, 1988). The chemical constituent and antimicrobial activity of essential oils from the leaf of *X. malayana* have been previously reported by Nor Azah et al. (1996). *X. elliptica* or lilan is a medium sized tree which could grow up to 8 to 10 m tall and is one of the endemic plants. The leaves are membranous and blade elliptic while the flowers are axillary with oblong fruits. *X. fusca* is widely distributed in Southeast Asia. It is a medium to large trees with 125 m in height and commonly found in peat swamp forest. *X. fusca* is known as jangkang paya. In traditional medicine practice, the plant used as an abortifacient and as an antidote for insect-bites. The wood of *X. fusca* is used in making a pineapple box whilst the barks of this plant are also used for walls of huts (Whitmore, 1972). The chemical composition of the leaf essential oils from *X. malayana*, *X. fusca* and *X. elliptica* have been examined by Siti Humeirah et al. (2010) but no information is available on the twig oil of these plants. Therefore, in this study, the chemical compositions of essential oils of twig part from *X. malayana*, *X. elliptica* and *X. fusca* as well as their antimicrobial properties were explored.

MATERIALS AND METHODS

Plant

The twig parts of *X. malayana* (FRI 54729), *X. elliptica* (FRI 54736) and *X. fusca* (FRI 54733) were randomly collected from Pasoh Forest Reserve, Negeri Sembilan, Malaysia on November 2007. All the plant materials were identified by Mr. Kamarudin Salleh from Forest Research Institute Malaysia (FRIM) and the voucher specimens were deposited at the FRIM herbarium

Extraction of essential oils

The plant material were cut into small pieces, weighed (200 to 400 g) and hydrodistilled separately in clavenger type apparatus for 6 h. The oily layer obtained from each plant material were separated, dried over anhydrous sodium sulfate and stored at 4°C until used. The yield (%) of essential oil was also determined by Azeotropic methods (British Pharmacopoeia, 2004).

GC and GCMS analysis

The chemical constituents of essential oils were analysed by GC and GCMS. GC analysis was carried out using Shimadzu GC2010 (FID) chromatography with a CBP-5 column (25 mm × 0.25 µm × 0.25 mm film thickness). GCMS analysis was performed using a Agilent GCMS 7890A/5975C series MS with a HP-5MS column (30 mm × 0.25 µm × 0.25 mm film thickness). The components of essential oils were identified by comparison of their retention indices with literature values (Adams, 2001) and confirmed by comparison of their mass spectral data with those from the Wiley (1990), HPCH 2205.L and NIST05a.L libraries. The Kovats indices (Kovats, 1965) of each component were determined relative to the retention time of a series of n alkanes (C_8-C_{20}). The relative proportions of the chemical compounds were expressed as percentages obtained by peak-area normalization, all relative response factors being taken as one.

Antimicrobial assay

The twig oils of *X. malayana, X. elliptica* and *X. fusca* were screened for their antimicrobial activity against nine strains of microorganisms including six gram positive bacteria *Staphylococcus aureus* ATCC 25923 (MSSA); *S. aureus* ATCC 33591 (MRSA); *S. aureus* ATCC 70069 (VISA); *Vancomycin Resistant S. aureus* (VRSA156), *Vancomycin Intermediate S. aureus* (VISA24) and *S. epidermidis* ATCC 12228. The gram negative bacteria including *Pseudomonas aeruginosa* ATCC 25668, *Escherichia coli* ATCC 10536 and yeast *Candida albicans* ATCC 10231 using the Minimum Inhibitory Concentration (MIC)

method as described previously by Saiful et al. (2008). The gram positive and negative bacteria and yeast were purchased from American Type Culture Collection (ATCC) while VRSA156 and VISA24 were lab passage derived mutants from clinical MRSA isolate.

Minimum inhibitory concentration (MIC)

The minimum inhibitory concentration (MIC) values of twig oils were determined using the double-broth microdilution method. Briefly, the well A was loaded with essential oils stock solution (100 mg/ml) and topped up with respectively broth. The mixture in well A was mixed thoroughly and transferring to well B to H which are already contained with respectively broth. The same procedure (well A) was repeated for well B to H to create a serial doubling dilution of the oils with concentration ranging from 10 to 0.078 mg/ml. Finally, a suspension containing 10^8 colony-forming units (CFU)/ml which are equivalent to McFarland standard tube no. 0.5 used (Vandepitte et al., 1991) were inoculated into the mixture to produce a final volume of 100 µl. Oxacillin and cyclohexamide were used as positive control whiles DMSO-broth mixture (5%) was used as a negative control. The experiments were performed in triplicates and repeated twice. The lowest concentration which produces no visible growth or remains clear after observed through the macroscopic evaluation was taken as the MIC. The activity was categorized as strong (MIC ≤ 1000 µg/ml), moderate (999 µg/ml < MIC < 4900 µg/ml) and weak (MIC ≥ 5000 µg/ml) (Ibrahim et al., 2009).

RESULTS AND DISCUSSION

Essential oil compositions

The twig parts of *X. malayana* (354.3 g), *X. fusca* (252.2 g) and *X. elliptica* (400.0 g) were extracted by hydrodistillation and yielded 0.08, 0.08 and 0.05% (v/w) of oils respectively. Both twig oils from *X. elliptica* and *X. malayana* were yellowish in color while the twig oil from *X. fusca* was colorless. The yield percentages of essential oils from fresh plant materials of *X. malayana* varied greatly from the previous study by Nor Azah et al. (1996). The twigs of *X. malayana* on the earlier study yielded 0.40% of essential oils. According to Yang et al. (2002), the variation factor of essential oil contents could be due to extraction time and the ratio of water and twig used in the study. The chemical composition of the twig oils are shown in Table 1. GC/MS analysis resulted in the identification of 29 (61.2% of the total oil), 17 (45.3% of the total oil) and 33 (79.7% of the total oil) components from the twig oils of *X. malayana*, *X. fusca* and *X. elliptica,* respectively. Components were mainly made up of monoterpenes hydrocarbon, oxygenated monoterpenes, sesquiterpenes hydrocarbon and oxygenated sesquiterpenes. The major constituents of the twig oil from *X. malayana* were spathulenol (12.9%), cyperotundone (4.9%), elemol (8.3%) and α-eudesmol (5.9%). Other components were γ-gurjunene (2.0%), α-terpineol (1.9%), β-pinene (1.5%) and β-phellandrene (0.9%). Spathulenol (36.0%) are identified as major component in the twig oil of *X. malayana*, previously mentioned by Nor Azah et al. (1996). However, in the present study, spathulenol was constituted only 12.9% of the total *X. malayana* twig oil. Differences in the chemical composition of *X. malayana* twig oils 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 plant studied (Cimanga et al., 2002). On the other hand, *X. elliptica* twig oil was found to be rich in terpinen-4-ol (11.9%), ρ-cymene (7.4%), spathulenol (6.3%), α-cadinol (6.8%), guaiol (4.5%) and linalool (4.2%). The twig oil was also made up of α-pinene (3.2%), 1,8-cineole (2.7%), α-terpineol (1.8%), sabinene (2.5%) and β-elemene (0.8%). The twig oil of *X. fusca* was mainly made up of spathulenol (11.8%) and α-cadinol (8.2%) as the major components. Other components present in appreciable amount were 1-epi cubenol (3.6%), vulgarone B (4.8%) and caryophyllene oxide (2.0%).

Several studies on the chemical composition of the essential oils of other *Xylopia* species have shown that monoterpenes and sesquiterpenes are the major constituents of essential oils. Spathulenol, which was detected in the twig oils of the *X. elliptica X. fusca* and *X. malayana* was identified as the major component in the *X. aromatica* (Founier et al., 1994) and *Xylopia emarginata* (Lago et al, 2003) essential oils. Both monoterpenoid, *p*-cymene and α-pinene were detected as–major constituents in the essential oil of *Xylopia sericea* (Lemos et al., 1992). Other chemical constituents for example terpinen-4-ol, β-pinene, δ-cadinene, δ-cymene and α-copaene were also found in other part of *Xylopia* species Nor Azah et al. (1996); Lemos et al. (1992). However, the essential oils obtained from *X. fusca* contained certain compounds which are not observed in the other oils namely; isopulegyl acetate, gymnomitrol, 14-hydroxy-δ-cadinene and 14-hydroxy-α-muurolene which could be considered unique to the species. The presence of some terpene type compounds in appreciable amounts supported the use of these compounds as chemical marker in the species identification purposes. Phytochemical analysis of the oils indicated that the chemical constituents represent a valuable chemotaxonomic characterization for supporting the identification of the genus *Xylopia* (Siti Humeirah et al., 2010).

Antimicrobial activity

The antimicrobial assays of the twig oils of *X. malayana, X. fusca* and *X. elliptica*, together with eight reference standards which are α-pinene, geranyl acetate, linalool, limonene, geraniol, terpinen-4-ol, 1,8-cineole and β-pinene were carried out by broth microdilution method against nine different strains of microorganisms. According to the results in Table 2, the twig oil from *X. elliptica* have been shown to possess the strongest

Table 1. Chemical compositions (%) of the twig essential oil from *X. elliptica*, *X. fusca* and *X. malayana*..

Compound	Retention index (RI)	Twig oils concentration (%)			Method identification
		X. elliptica	*X. fusca*	*X. malayana*	
α- pinene	935	3.2	-	0.4	RI,MS, CO
sabinene	977	2.5	-	-	RI,MS
β-pinene	980	3.2	-	1.5	RI,MS,CO
myrcene	989	0.5	-	-	RI,MS,CO
ρ-cymene	1023	7.4	-	0.2	RI,MS
β-phellandrene	1029	1.6	-	0.9	RI,MS
1,8-cineole	1037	2.7	-	-	RI,MS,CO
(Z)-β-ocimene	1038	0.7	-	-	RI,MS
γ-terpinene	1059	-	-	0.3	RI,MS
terpinolene	1085	-	-	0.2	RI,MS
linalool	1099	4.2	-	0.8	RI,MS,CO
cis-ρ-menth-2-en-1-ol	1121	0.4	-	-	RI,MS
trans-ρ-menth-2-en-1-ol	1141	0.6	-	-	RI,MS
trans-pinocarveol	1144	-	-	0.8	RI,MS
pinocarvone	1163	-	-	0.5	RI,MS
para-metha-1,5-diene-8-ol	1175	-	-	0.3	RI,MS
terpinen-4-ol	1177	11.9	0.1	1.4	RI,MS,CO
α- terpineol	1192	1.8	-	1.9	RI,MS
cis-piperitol	1194	0.3	-	-	RI,MS
myrtenal	1201	-	-	0.8	RI,MS
verbenone	1210	-	0.1	0.1	RI,MS
trans-carveol	1232	-	0.1	0.1	RI,MS
5-hydroxy-isobornyl isobutanoate	1252	0.3	-	-	RI,MS
bornyl acetate	1280	0.4	-	1	RI,MS,CO
δ–elemene ε	1333	0.3	-	-	RI,MS
α-terpinyl acetate	1344	0.8	-	-	RI,MS
α-copaene	1367	0.7	0.1	0.3	RI,MS
β-elemene	1386	0.8	0.1	0.5	RI,MS
cyperene	1396	1.2	-	-	RI,MS
β-caryophyllene	1415	-	0.1	0.2	RI,MS
δ–copaene	1435	0.3	-	-	RI,MS
γ-cadinene	1494	0.4	-	-	RI,MS
bicyclogermacrene	1498	-	-	1.2	RI,MS
4-epi-cis-dihydroagarofuran	1502	-	-	0.9	RI,MS
α-murolene	1512	-	2	-	RI,MS

Table 1. Contd.

δ–cadinene	1518	1.1	2.1	0.9	RI,MS
maaliol	1555	0.3	-	-	RI,MS
elemol	1561	2	-	8.3	RI,MS
spathulenol	1584	6.3	11.8	12.9	RI,MS
viridiflorol	1590	4.3	-	-	RI,MS
caryophyllene oxide	1597	-	2	-	RI,MS
guaiol	1601	4.5	-	-	RI,MS
γ-gurjunene	1607	-	-	2	RI,MS
14-hydroxy-α-muurolene	1621	-	3.1	-	RI,MS
1-epi-cubenol	1634	-	3.6	-	RI,MS
cyperotundone	1638	1	-	4.9	RI,MS
β-cedrene	1644	6.5	-	-	RI,MS
α-cadinol	1661	6.8	8.2	-	RI,MS
α-eudesmol	1663	-	-	5.9	RI,MS
bulnesol	1671	0.7	-	-	RI,MS
isopulegyl acetate	1674	-	3.1	-	RI,MS
vulgarone B	1692	-	4.8	2.6	RI,MS
gymnomitrol	1855	-	2.2	-	RI,MS
14-hydroxy-δ-cadinene	1875	-	1.8	-	RI,MS

Percentages were calculated based on results obtained from gas chromatography on CBP-5 column; RI = retention indices; MS= mass fragmentation; CO= co-chromatography with authentic sample.

antibacterial and antifungal activity against all gram positive bacteria and yeast strains tested with the MIC values ranging from 156 µg/ml to 625 µg/ml. Gram negative bacteria's, *P. aeruginosa* and *E.coli* seemed to be resistant to the essential oils tested. Both *X. fusca* and *X. malayana* twig oils however showed less inhibitory effect on the growth of bacteria and yeast strains even at the highest doses (5000 µg/ml) used. The results indicated the gram positive bacteria are more sensitive to plant oil than gram negative bacteria due to their restrictive outer membrane barrier which are generally less susceptible and destabilization. The inhibitory effects of *X. elliptica* twig oil on bacterial strains tested could be related to the present of α-pinene (Magwa et al., 2006), terpinen-4-ol (Barel et al., 1991) and *ρ*-cymene which have been reported their high level of antimicrobial activity. In addition, it is also possible that the other minor or trace compounds might be involved in some types of antimicrobial synergism with other active components of essential oil. Various studies have reported antimicrobial activities for essential oils from *Xylopia* species. Lemos et al. (1992) reported the essential oil of *X. sericea* which contains 1,8-cineole as the major constituents showed effective antifungal activity against *Candida* strains. The essential oils of *Xylopia longifolia* from France were also found to possess antibacterial and antifungal activity (Fourier et al., 1993). The antimicrobial activities of essential oil from *Xylopia parvifloral* and *Xylopia aethiopica* from Africa have been reported by Bakarnga et al. (2014) and Woguem et al. (2014).

Beside essential oil, the antimicrobial properties of selected standard compounds were also conducted in the present study. Based on the results summarized in Table 2, the antibacterial and antifungal activity of the five reference standards indicated that α-pinene had the moderate to strong inhibitory effect against all

Table 2. Minimum Inhibitory Concentration (µg/ml) of twig oils from three *Xylopia* species and standard compounds.

Species/ Standards	Parts	sa2	sa3	sa7	VISA	VRSA	Se	Pa	Ec	Ca
X. elliptica	twig	625	625	625	312	312	156	>5000	>5000	312
X. fusca	twig	>5000	>5000	>5000	>5000	>5000	>5000	>5000	>5000	>5000
X. malayana	twig	5000	5000	5000	5000	5000	>5000	>5000	>5000	>5000
Standard compounds										
α-pinene		625	2500	1250	1250	1250	625	2500	325	325
β-pinene		>5000	>5000	>5000	>5000	>5000	>5000	>5000	>5000	>5000
linalool		5000	5000	5000	5000	5000	5000	5000	2500	2500
limonene		5000	5000	5000	5000	5000	5000	5000	5000	5000
Geranyl acetate		5000	5000	5000	5000	5000	5000	5000	5000	5000
1,8-cineole		>5000	>5000	>5000	>5000	>5000	>5000	>5000	>5000	>5000
Terpinen-4-ol		5000	5000	5000	5000	5000	2500	2500	625	2500
Geraniol		5000	5000	5000	2500	5000	1250	2500	1250	2500
Positive control										
Streptomycin sulfate		NT	NT	NT	NT	NT	156	2500	2500	NT
Oxacillin		312	312	625	2500	1250	NT	NT	NT	NT
Cycloheximide		NT	NT	NT	NT	NT	NT	NT	NT	1250

Sa2, *Staphylococcus aureus* ATCC 25923 (MSSA); Sa3, *S. aureus* ATCC 33591 (MRSA/VSSA); Sa7, *S. aureus* ATCC 70069 (MRSA); VRSA156, Vancomycin Resistant *S. aureus*; VISA24, *Vancomycin Intermediate S. aureus*; Se, *S. epidermidis* ATCC 12228 ; Pse, *Pseudomonas aeruginosa* ATCC 25668; Ec, *Escherichia coli* ATCC 10536; Ca, *Candida albicans* ATCC 10231; NT, Not Test.

bacterial and yeast growth at the concentration of 325 to 2500 µg/ml. *Xylopia epidermidis*, *E. coli* and *C. albicans* were most susceptible to the compound investigated and hence support the earlier finding on α-pinene's significant antimicrobial potential (Dorman and Deans, 2000). The reference standards of β-pinene and 1,8-cineole however inhibited weak (MIC values of 5000 µg/ml) antibacterial and antifungal activity against all microorganisms tested. Terpinen-4-ol, the most abundant component in the twig oil of *X. elliptica*, has been shown to have a moderate to strong antibacterial activity against *P. aeruginosa* and *E-coli* strains with the MIC values of 2500 and 625 µg/ml. Terpinen-4-ol however showed weak inhibitory effect against MRSA strains tested. The results however was not in agreement with a previous study reported by Barel et al. (1991) which found the bacteriostatic effects of tested compound, terpinen-4-ol against several microorganisms. Linalool was found to be less effective to all microorganisms tested with the MIC values ranging from 1250 to 5000 µg/ml.

Conclusion

The characteristics of the essential oils from three *Xylopia* species are represent a valuable chemotaxonomic tool for the identification of the genus *Xylopia.* Considering the relatively high yield of monoterpenoids and bioactive component in the oils, *Xylopia* could be a potential new source of fragrance ingredient for food, pharmaceutical and cosmetic industries.

Conflict of interests

The author(s) did not declare any conflict of interest.

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