African Journal of Biotechnology Vol. 9(34), pp. 5511-5515, 23 August, 2010
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2010 Academic Journals

Full Length Research Paper

Chemical constituents and antimicrobial activity of *Goniothalamus macrophyllus* (Annonaceae) from Pasoh Forest Reserve, Malaysia

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Accepted 17 May, 2010

The essential oils from the twig and root of *Goniothalamus macrophyllus* were obtained by hydrodistillation and subjected to Gas Chromatography (GC-FID) and Gas Chromatography/Mass Spectrometry (GC-MS) using CBP-5 capillary column in order to determine their chemical composition. Both twig and root oils and four reference standard compounds (α-pinene, linalool, geraniol and geranyl acetate) were evaluated for their antimicrobial properties against gram positive and gram negative bacteria, yeast and dermatophyte fungi using broth microdilution methods. The GCMS analysis revealed twenty-one and fourteen compounds from twig and root oils which represented 90.0 and 42.5% of the total oils, respectively. The oils were found to possess the following major components: twig: geranyl acetate (45.5%), geraniol (17.0%), linalool (12.7%) and camphene (7.5%); root: cyperene (9.8%), geranyl acetate (9.4%), geraniol (3.4%) and linalool (2.6%). Other components present in appreciable amounts in both essential oils were α-pinene (0.8%) and benzaldehyde (0.5%). The root oil exhibited the most notable inhibitory activity (0.3 mg/ml) against Vancomycin intermediate-resistance *Staphylococcus aureus* (*VISA 24*), *Staphylococcus epidermidis* and *Candida albicans*. α-pinene meanwhile inhibited the bacteria and fungal growth at 0.3 and 2.5 mg/ml. With regards to antimicrobial potential, α-pinene superceeds linalool, geraniol and geranyl acetate, respectively.

Key words: *Goniothalamus macrophyllus*, Annonaceae, essential oils, geranyl acetate, cyperene, geraniol, linalool, α-pinene, antimicrobial activity.

INTRODUCTION

The genus *Goniothalamus (*Annonaceae) comprises some 115 species of aromatic trees and shrubs and mostly distributed in Asia and Australia (Leboeuf et al., 1982). According to Burkill (1966), many plants of this genus have been widely used as a source of fiber and timber on a local scale. The ethnobotanical studies of *Goniothalamus* plants have been well documented. The most common medicinal usage of *Goniothalamus* sp. relates to abortion and post-natal treatments. Perry (1980) reported that some of the species from this genus have been used by tribal people from different countries in treating fever, scabies and rheumatisms. Chemical investigations of several *Goniothalamus* species have led to the isolation of various compounds including acetogenins, aporphine

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alkaloids and notablystyryl-lactone derivatives (Blazquez et al., 1999; Zhang et al., 1999; Cao et al., 1998; Alali et al., 1999) which have been found to possess strong antimicrobial, larvicidal and antimalarial activities (Ee et al., 1998; Khan et al., 1999; Wirasathien et al., 1997).

Goniothalamus macrophyllus is a shrub or small tree which could grow up to 8 m tall. It is locally known as "gajah beranak", "penawar hitam" or "monsoi" (Wiart, 2000). In Malaysia, the decoctions of *G. macrophyllus* root are used for the treatment of colds, fever and malaria while the leaves are externally applied on swellings (Burkill and Haniff, 1930). The burnt leaves of *G. macrophyllus* have also been noted to be fragrant and are an effective mosquito repellent. Goniothalamin and goniothalamin oxide, two compounds isolated from the aerial parts of *G. macrophyllus* have been found to possess embryotoxic properties (Sam et al., 1987). This is the first systematic chemical and antimicrobial study on the twig and root oil of *G. macrophyllus*.

MATERIALS AND METHODS

Plant material and extraction

Twigs and roots of *G. macrophyllus* (FRI 54727) were collected in November 2007 from Pasoh Forest Reserve, Negeri Sembilan, Malaysia. The voucher specimens were identified by Mr. Kamarudin Salleh from the Forest Research Institute Malaysia (FRIM), Malaysia. All the plant materials were air dried for two days and cut into small pieces and subjected to water distillation using a Clevenger-type apparatus for 6 h to obtain the essential oils. The oils were separated from the water layer by drying over with anhydrous sodium sulphate. The oil yields were calculated based on the dry weight of the plant material and then stored at 4°C prior to further evaluation.

Analysis of essential oils

The chemical constituents of the twig and root oils were analyzed by GC and GCMS. The GC analysis was performed on a Shimadzu GC-2010 gas chromatograph equipped with a flame ionization detector (FID) and CBP-5 (25 m x 0.25 mm; 0.25 µm film thickness) capillary column. The oven temperature was programmed from 60°C (10 min) to 230°C at a rate of 3°C/min. Injector and detector temperature were set up at 220 and 280°C, respectively. Helium was the carrier gas and the volume of oil injected was 1.0 µl. The peak areas and retention times were measured by electronic integration. The GCMS analyses were performed on Agilent GCMS 7890A/5975C Series MSD (70 eV direct inlet) equipped with HP-5MS fused silica capillary column (30 m x 0.25 mm; 0.25 µm film thickness). The column and injector temperature were the same as those for GC. The mass range was 50 - 550 in the full scan mode with a rate of 2.91 scans/ s and the total scan time was 67.7 min.

Identification of components

The resulting essential oil components were identified by comparison of their retention indices with literature values (Adams, 2001; Jennings and Shibamoto, 1980) and also confirmed by comparison of their mass spectral data with those from the Wiley, HPCH 2205.L and NIST05a.L mass spectral database. Whenever possible, the data was confirmed by co-chromatography with authentic samples or with published data. The retention indices (RI) of the components were determined relative to the retention times of a series of *n-alkanes* (C_8-C_{30}). The relative proportions of the chemical compounds were expressed as percentages obtained by peak-area normalization, all relative response factors being taken as one.

Microbial strains used in this study

Eleven isolates were used in this study representing the Gram positive bacteria *Staphylococcus aureus* ATCC 25923 (MSSA), *S. aureus* ATCC 33591 (MRSA), *S. aureus* ATCC 70069 (VISA) and *Staphylococcus epidermidis* ATCC 12228, Vancomycin intermediate-resistant *S. aureus* (VISA24) and Vancomycin resistant *S. aureus* (VRSA15), the Gram negative bacteria (*Escherichia coli* ATCC 10536 and *Pseudomonas aeruginosa* ATCC 25668), *Candida albicans* ATCC 10231 and dermatophytes (*Tricophyton rubrum* ATCC 28188 and *Microsporum cannis* ATCC 36299). The *S. epidermidis*, Gram negative bacteria, yeast and dermatophytes strains were purchased from the American Type Culture Collection (ATCC). VISA24 and VRSA15 were lab passage derived mutants from a clinical MRSA isolate.

Inhibitory evaluation

The Minimum Inhibitory Concentration (MIC) of *G. macrophyllus* twig and root oils as well as four standard compounds were determined by double-broth microdilution methods, as described previously (Saiful et al., 2006). Both Tryptic Soy broth (TSB) and Sobouraud Dextrose broth (SDB) media were used for bacteria and fungi, respectively. In brief, test samples (essential oils and standards) at the concentration ranging from 10 to 0.078 mg/ml were prepared in a sterile 96-well microtitre–plate. Overnight broth cultures of each target microbe were adjusted accordingly to obtain a suspension containing 10^8 CFU/ml. The plates were then incubated for 24 h at 37°C for bacteria and at 26°C for yeast and fungi. The MIC value was defined as the lowest concentration producing no visible growth (absence of turbidity and or precipitation) as observed through the naked eye. The experiments were performed in triplicates and repeated twice. Streptomysin sulphate, oxacillin and cyclohexamide were used as positive controls whilst 5% DMSO-broth mixture was used as the negative control. All the results were recorded as the mean concentration of triplicate. The standards were selected based on their availability and their presence in the essential oils studied.

RESULTS AND DISCUSSION

Essential oil

The twig and root oils of *G. macrophyllus* were obtained by hydro-distillation and yielded 0.14 and 0.05% of oils, respectively. Both twig and root oils were yellowish in color with a characteristic woody odor. The chemical compositions of the essential oil are listed in Table 1. Twenty-one components representing 90.0% of the total oil compositions were identified from the twig oil. According to the gas chromatogram, among the 21 constituents, 87.5% were identified as monoterpenoids. The most abundant component of the twig oil was geranyl acetate (45.5%). Other major monoterpenoids present in the oils were geraniol (17.0%), linalool (12.7%), camphene (7.5%),

Table 1. The chemical constituents (%) of the essential oils of *G. macrophyllus*.

Number	Name of compounds	Retention indices (RI)	Concentration (%)		Methods of identification
			Twig	Root	
1.	α-pinene	935	0.8	0.8	RI,MS,CO
2	Camphene	949	7.5	7.5	RI,MS
3	Benzaldehyde	963	0.5	0.5	RI,MS
4	β-pinene	975	0.2	-	RI,MS,CO
5	Myrcene	988	0.3	-	RI,MS
6	α-phellandrene	998	0.1	-	RI,MS
7	δ-3-carene	1004	0.1	-	RI,MS
8	Limonene	1037	0.1	-	RI,MS,CO
9	(E)-β-ocimene	1048	0.1	-	RI,MS
10	γ-terpinene	1058	0.1	-	RI,MS
11	Terpinolene	1085	0.3	-	RI,MS,CO
12	Linalool	1110	12.7	2.6	RI,MS,CO
13	Camphor	1145	0.4	0.7	RI,MS,CO
14	Camphene hydrate	1152	-	1.2	RI,MS
15	α-terpineole	1194	1.2	-	RI,MS
16	Nerol	1230	0.2	-	RI,MS
17	Geraniol	1270	17.0	3.4	RI,MS,CO
18	Bornyl acetate	1284	0.3	2.5	RI,MS
19	α-copaene	1358	0.1	-	RI,MS
20	Geranyl acetate	1375	45.5	9.4	RI,MS,CO
21	β-elemene	1383	-	0.8	RI,MS
22	Cyperene	1400	2.4	9.8	RI,MS
23	β-Caryophyllene	1412	0.1	0.6	RI,MS
24	Gleenol	1582	-	0.6	RI,MS
25	1-epi-cubenol	1627	-	2.1	RI,MS

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

cyperene (2.4%), α-terpineol (1.2%), α-pinene (0.8%) and benzaldehyde (0.5%). The sesquiterpenoids identified in the twig oil were representing only 2.5% of the oil with cyperene (2.4%) detected as the major compound. The other sesquiterpenoid identified was β-caryophyllene which accounted for 0.2% of the oil.

The chemical constituents of the root oil of *G. macrophyllus* was analyzed by GCMS and resulted in the identification of fourteen compounds which represented 42.5% of the total oil compositions. The oils were made up predominantly of monoterpenoids, constituting 28.6% of the oil whereas sesquiterpenoids constituted only 13.9% of the oil. The principal components of the root oil were cyperene (9.8%), geranyl acetate (9.4%), camphene (7.5%), geraniol (3.4%), bornyl acetate (2.5%) and linalool (2.6%). Other compounds that were present in appreciable amount were camphene hydrate (1.2%), α-pinene (0.8%), camphor (0.7%), β-caryophyllene (0.6%) and benzaldehyde (0.5%).

Overall, the results obtained illustrated that the oil compositions between the twig and root are quite similiar. However, camphene hydrate (1.2%), β-elemene (0.8%), gleenol (0.6%) and 1-epi-cubenol (2.1%) identified in the root oils were not detected in the twig oils of *G. macrophyllus*. In both twig and root samples, the total amounts of the monoterpenes was higher than the sesquiterpenes with geranyl acetate (9.4 - 45.5%), geraniol (3.4 - 17.0%) and linalool (12.7 - 2.6%) being the main components. These four compounds have earlier been identified in *G. macrophyllus* and *Goniothalamus boornensis* bark oil as the main components by Fasihuddin et al. (2007) and Ibrahim et al. (2005). A recent study by Hisham et al. (2006) on the essential oil compositions of *Goniothalamus cardiopetalus* bark part showed that the oil contained both monoterpenoid and sesquiterpenoid in significant amounts with linalool (11.7%), camphor (3.9%) and bornyl acetate (3.9%) as the major constituents. Therefore, components of twig and root oils of *G. macrophyllus* found in this study are at least similar in its monoterpenoid and sesquiterpenoid content with the components

Table 2. The minimum inhibitory concentrations (MIC) of essential oils of *G. macrophhyllus* and four reference standards (mg/ml).

Samples	MIC										
	***Sa*2**	***Sa*3**	***Sa*7**	***VRSA* 15**	***VISA* 24**	***Se***	***Pse***	***Ec***	***Ca***	***Tr***	***Mc***
Twig	5	5	5	2.5	2.5	2.5	>5	2.5	2.5	2.5	2.5
Root	1.2	1.2	2.5	0.6	0.3	0.3	>5	>5	0.3	2.5	2.5
α-pinene	0.6	2.5	1.2	1.2	1.2	0.3	2.5	2.5	0.3	0.3	0.3
Geraniol	5	5	5	5	2.5	1.2	2.5	1.2	2.5	2.5	2.5
Geranyl acetate	5	>5	>5	>5	>5	5	>5	5	>5	>5	>5
Linalool	>5	>5	>5	5	>5	5	>5	2.5	2.5	2.5	2.5
Oxacillin	0.3	0.3	0.6	2.5	1.2	NT	NT	NT	NT	NT	NT
Streptomycin sulfate	NT	NT	NT	NT	NT	0.1	2.5	2.5	NT	NT	NT
Cyclohexamide	NT	NT	NT	NT	NT	NT	NT	NT	1.2	1.2	2.5

*Sa*2 = *S. aureus* ATCC 25923 (MSSA); *Sa*3 = *S. aureus* ATCC 33591 (MRSA); *Sa*7= *S. aureus* ATCC 70069 (MRSA); VRSA15 = Vancomycin-resistant *S. aureus*; VISA24 = Vancomycin intermediate-resistance *S. aureus*; *Se* = *S. epidermidis* ATCC 12228; *Pse* = *Pseudomonas aeruginosa* ATCC 25668; *Ec* = *Escherichia coli* ATCC 10536; *Ca* = *C. albicans* ATCC 10231; *Tr* =*T. rubrum* ATCC 28188; *Mc* = *M. cannis* ATCC 36299; NT = not tested.

of other *Goniothalamus* species.

Antimicrobial activity

The antimicrobial activity of root and twig oils of *G. macrophyllus* together with four of its reference standards: α-pinene, linalool, geraniol and geranyl acetate were investigated by broth microdilution method and the results are shown in Table 2. The oils were found to demonstrate notable antimicrobial activity with the MIC values below the cut off point of 1 mg/ml (Gibbon, 2004). The root oils were considerably more active than the twig oil in inhibiting all the bacteria and fungal strains except *P. aeruginosa*. The gram negative bacteria with their restrictive outer membrane are generally less susceptible to the essential oils tested (Nikaido and Vaara, 1985; Mann et al., 2000). The root oils had the strongest inhibitory effect against *VISA*24, *S. epidermidis* and *C. albicans* with the MIC values of 0.3 mg/ml. The twig oils demonstrated moderate to weak activity toward all bacterial strains tested with the MIC values ranging from 2.5 to 5 mg/ml. Both dermatophytes, *T. rubrum* and *M. canni*s however showed similar susceptibility to the twig and root oils with the MIC values of 2.5 mg/ml.

The antibacterial and antifungal activity of the four reference standards indicated α-pinene strong to moderate inhibitory effect against bacteria and fungal growth (concentration of 0.3 to 2.5 mg/ml). The *S. epidermidis*, *C. albicans*, *T. rubrum* and *M. cannis* were most susceptible to the compound investigated and hence support the earlier finding on α-pinene's significant antimicrobial potential (Dorman and Deans, 2000). Scoring MIC at 5 mg/ml, geranyl acetate's antimicrobial activity on the other hand, is negligible despite its abundance in both twig and root oils. Linalool and geraniol were found to be less effective to all microorganisms tested with the MIC values ranging from 1.2 to 5 mg/ml. The inhibitory activities as observed from the twig and root oils could be due to other compound and or group of compounds working synergistically. This would possibly explain the vast difference in their respective inhibitory potentials. Subject to further pharmacodynamic and safety evaluations findings, results of this study support the possible use of *G. macrophyllus* oils as one of the active ingredients for personal care products. In addition, chemical investigation of the *G. macrophyllus* essential oils can also be used as chemotaxonomic guide for the identification of other *Goniothalamus* species.

ACKNOWLEDGMENTS

This research was supported by Ministry of Science, Technology and Innovation (MOSTI), Malaysia fund no: 02-03-10SF0047. The authors would like to thank Kamarudin Salleh for his help in collecting and identifying plant material and also to the staff of Medicinal Plants Division of Forest Research Institutes especially Abd. Majid Jalil, Mazurah Mohd. Isa, Hannan Abdul Wahab, Mohd Faridz, Z.P., Nik Yasmin, N. Y. and Ahmad Abdul Qayyum, M. for their technical assistance.

Abbreviations

GC-FID, Gas Chromatography-Flame ionization detector; **GC-MS,** gas chromatography/mass spectrometry; **TSB,** tryptic soy broth; **SDB,** sobouraud dextrose broth; **DMSO,** dimethyl sulfoxide.

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