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Full Length Research Paper

GC/MS determination of bioactive components and antibacterial properties of *Goniothalamus umbrosus* extracts

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In this study, the antibacterial activity and chemical composition of *Goniothalamus umbrosus* leaves extracts were evaluated. The antibacterial activity was investigated using two gram-positive bacteria, Methicillin resistant *Staphylococcus aureus* (MRSA) and *Bacillus subtilis* B29, and two gram-negative bacteria, *Pseudomonas aeruginosa* 60690 and *Salmonella choleraesuis*. The activity was tested using disc diffusion and minimum inhibitory concentration assays. The chemical compositions of the ethyl acetate extract of *G. umbrosus* were investigated using Shimadzu gas chromatography–mass spectrometry (GC-17A) while the mass spectra of the compounds found in the extract was matched with the library. The results showed that the extracts demonstrated broad spectrum antibacterial effects against all tested bacteria. GC/MS analysis of ethyl acetate extract of *G. umbrosus* revealed the existence of 1-butyl-2-cyclohexen-1-ol (46.84%), benzaldehyde (4.42%) and globulol (4.07%). The results of this study offer a platform of using *G. umbrosus* as herbal alternative for the current synthetic antimicrobial agents.

Key words: *Goniothalamus umbrosus*, GC/MS, antibacterial properties.

INTRODUCTION

Plants are a rich source of secondary metabolites with interesting biological activities. In general, these secondary metabolites are an important source with a variety of structural arrangements and properties (de-Fátima et al., 2006). Distinguished examples of these compounds include flavonoids, phenols and phenolic glycosides, saponins and cyanogenic glycosides (Shahidi 2000 and Shahidi, et al., 2008). Natural products from microbial sources have been the primary source of antibiotics, but with the increasing recognition of herbal medicine as an alternative form of health care, the screening of medicinal plants for active compounds has become very significant because these may serve as talented sources of book antibiotic prototypes (Meurer-Grimes et al., 1996; Koduru et al., 2006). It has been shown that *in vitro* screening methods could provide the needed preliminary observations necessary to select crude plant extracts with potentially useful properties for further chemical and pharmacological investigations (Mathekaga and Meyer, 1998).

Goniothalamus umbrosus is a plant described firstly by James Sinclair in 1955 (Mat-Salleh et al., 2000). In some parts of Southeast Asia, the water extract from this plant is a part of the diverse traditional medication that is used by indigenous folk (Ahmad et al., 1991). It has been

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used widely in post-partum decoction or in abortion (Mat-Salleh et al., 2000). Irrespective of the presence of cytotoxic acetogenins and styryl-lactones in the genus Goniothalamus (Family: Annonaceae), only 22 out of 160 species (13.7%) have so far been investigated (Wiart, 2007). This genus is known to possess versatile biological activities such as immunosuppressive and anti-inflammatory, anti-malarial (Abdel-Wahab et al., 2009), anti-cancer (Umar-Tsafe, et al., 2004), antioxidant and inhibitory effects on platelet-activating factor properties (Jantan et al., 2005).

To the best of our knowledge, there is only one scientific report published regarding only one biological property of *G. umbrosus* by Umar-Tsafe et al. (2004) which studied the genotoxicity of goniothalamin in CHO cell line.

However, this compound has been isolated from different species of *Goniothalamus* genus and scientific investigation in order to determine the therapeutic potential of *G. umbrosus* is limited except for our recent reports that specifically studied the biological activities of this certain species, *G. umbrosus* (Abdel-Wahab et al., 2009a; Abdel-Wahab et al., 2009b). Therefore, the aim of this study was to evaluate phenolic content, antioxidant and antibacterial activities of ethyl acetate and methanol extracts of *G. umbrosus.*

MATERIALS AND METHODS

Plant material and extraction procedure

Leaves of *G. umbrosus* were bought freshly from Puchong, Selangor, Malaysia in October 2007. The plant was identified at Unit of Biodiversity, Laboratory of Natural Products, Institute of Bioscience, University Putra Malaysia. The leaves were dried and ground into a powder form before cold maceration as an extraction method. Before extraction with ethyl acetate and methanol, the powdered leaves (300 g) were defatted using hexane and dichloromethane to remove non-polar chemical constituents. The extraction was done for 7 days with occasional manual shaking and the process repeated for three times for each solvent (3 X 7). The combined extracts for each solvent were filtered through a Whatman® No. 41 filter paper (pore size 20 - 25 µm) and dried under vacuum using a rotary evaporator and kept at 4°C until required. The ethyl acetate and methanol extracts were stored in the refrigerator for further assays.

Antibacterial activity of *G. umbrosus*

The antibacterial activity of ethyl acetate extract of *G. umbrosus* leaves was evaluated using two gram-positive bacteria, Methicillin resistant *Staphylococcus aureus* (MRSA) and *Bacillus subtilis* B29, and two gram-negative bacteria, *Pseudomonas aeruginosa* 60690 and *Salmonella choleraesuis*. All bacterial strains were obtained from the Laboratory of Molecular Biomedicine, Institute of Bioscience, University Putra Malaysia, Serdang, Malaysia. The screening of the extract antibacterial effect was carried out by determining the zone of inhibition using paper disc (6 mm in diameter, Whatman No. 1) diffusion method (Rath et al., 1999; Sahoo et al., 2006). The obtained microorganism strains were inoculated in a Petri dish containing nutrient broth at 37°C for 24 h and were referred as seeded broth.

The density of the bacterial suspension was standardized by standard method and the concentrations of the cultures were adjusted turbidometrically at wavelength of 600 nm to 500,000 - 1000,000 colony forming unit per ml (CFU/ml). The extract was dissolved in dimethylsulfoxide which was previously tested for antibacterial activity against all test bacteria and found to have no antibacterial activity. The extract was diluted to concentration of 100 mg/ml and finally sterilized by filtration using 0.45 µm millipore filters. The sterile discs were impregnated with extract solution (0.05 ml from 100 mg/ml extract) to achieve desired concentration and placed in inoculated agar.

Streptomycin (10 µg/disc) was used as standard and it was prepared using the same solvents used for tested extract to assure exclusion of variability due to usage of different solvents. The inoculated plates contain the test and standard discs were incubated at 37°C for 24 h.

Minimum inhibitory concentration (MIC)

The lowest inhibitory concentrations of the plant extracts against the sensitive organisms were estimated using the agar disc method (ADM). Innocula of one milliliter of plant extract was poured into each Petri-dish and the agar was later poured and permitted to set. Wells were bored using the sterile 3 mm cork borer. Serial dilutions of the extracts were added into the marked wells. The plates were incubated at 37°C for 24 h. The growth was observed to determine the sensitivity of each organism using clear zones of no microbial growth. The least concentration of the plant extract that had inhibitory effect was taken as the minimum inhibitory concentration (MIC) of that plant extract against such organisms.

Gas chromatography–mass spectrometry (GC/MS) and mass spectrometer analysis

Since the ethyl acetate extract shows the better antimicrobial activity, GC/MS analysis of this extract (EAE) was performed using a Shimadzu GC/MS (GC-17A) equipped with a ZB-1 MS fused silica capillary column (30 m × 0.25 mm i.d., film thickness 0.25 µm). For GC/MS detection, an electron ionization system with ionization energy of 70 eV was used. Helium gas was used as the carrier gas at a constant flow rate of 1 ml/min. Injector and MS transfer line temperature were set at 260 and 320°C respectively. The oven temperature was programmed from 60 - 320°C at 3°C/min, increase then held isothermal for 11 min and finally raised to 320°C at 10°C/min. Diluted samples (1/100, v/v in methanol) of 1.0 µl were injected manually in the split less mode. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. Mass spectrometer: Shimadzu GC/MS (GC-17A) system recording at 70 eV; scan time 1.5 s; mass range 40–300 amu. Software adopted to handle mass spectra and chromatograms was a ChemStation.

RESULTS AND DISCUSSION

Antibacterial activity of extracts of *G. umbrosus* leaves

The antibacterial activities of extracts of *G. umbrosus* were evaluated using gram-positive and gram-negative bacteria. The solvents used for control and all extract dissolving did not show any antibacterial activity (the results not shown). In Table 1, the screening of the ex-

Table 1. Antibacterial Activity of the ethyl acetate extract of *G. umbrosus.*

Extracts	MRSA[a]		PA[a]		SC[a]		BS[a]	
	Inhibition zone (mm)	MIC (mg/ml)	Inhibition zone (mm)	MIC (mg/ml)	Inhibition zone (mm)	MIC (mg/ml)	Inhibition zone (mm)	MIC (mg/ml)
Ethyl Acetate	10	5	10	4.5	11	4	14	3.5
Methanol	8	5	8	5	8	5	7	5
Streptomycin	20		20		23		23	

[a]The screening of the extracts antibacterial effect was carried out by determining the zone of inhibition using paper disc (6 mm in diameter, Whatman No. 1) diffusion method (n=2). MRSA: Methicillin Resistant *Staphylococcus aureus*, PA: *Pseudomonas aeruginosa*, SC: *Salmonella choleraesuis* and BS: *Bacillus subtilis*. MIC: Minimum inhibitory concentration.

Table 2. Total ionic chromatogram (GC–MS) of ethyl acetate extract of *G. umbrosus* obtained with 70 eV using a HP-5MS column (30 m×0.25 mm) with He gas as the carrier.

Compound no.	Retention time	%	Compound	Similarity (%)
1	6.78	4.42	Benzaldehyde	91
2	8.14	0.18	Methanone, dicyclopropyl-	80
3	8.167	0.49	Benzeneacetaldehyde	74
4	8.692	0.40	Benzoyformic acid	89
5	8.92	2.95	1,2,3-Propanetriol, diacetate	95
6	10.092	0.63	Benzoic acid	88
7	10.208	0.29	α-Propyl benzenemethanol	79
8	10.65	0.39	cyclohexane, 2-methyl, acetate, cis	77
9	11.239	6.86	1, 2,3-Propanetriol diacetate	97
10	11.442	0.24	Ethanone, 2-hydroxy-1-phenyl-	92
11	12.592	0.32	Triacetin	89
12	13.158	0.14	2-Propenoic acid, 3-phenyl-	73
13	13.317	0.31	2-Ethenyl-2,8,8-trimethyl-4-methylidenebicyclo[5.2.0]nonane	81
14	13.975	0.31	3-Phenyl-2-propenoic acid	81
15	14.075	0.36	2-Bromo-4-methyl-pentane	82
16	14.408	0.19	Diphenyl ether	59
17	14.508	0.48	Naphthalene, 1,2,3,5,6,7,8,8a-octahydro-1, 8a-dimethyl-7-(1-methylethenyl)-, [1R-(1.alpha., 7.beta., 8a.alpha.)]-	85
18	14.767	0.78	Caryophyllene	88
19	14.843	0.86	Naphthalene,decahydro-4a-methyl-1-methylene-7-(1-methylethenyl)-, [4aR-(4a.alpha., 7.alpha.,8a.beta.)]-	88
20	15.062	0.93	Cedrene	82
21	15.915	0.92	Spathulenol	82
22	15.992	0.68	Ledol	81
23	16.084	0.62	Phenol, 2,6-dimethoxy-4-(2-propenyl)-	78
24	16.425	0.59	Humulane-1,6-dien-3-o1	75
25	16.508	1.16	Alpha.-Bisabolol	78
26	16.733	1.27	τ-Muurolol	84
27	16.852	1.65	α-Cadinol	87
28	16.941	4.07	Globulol	86
29	17.695	1.94	Kauran-18-a1, 17(acetyloxy)-, (4.beta)-	81
30	18.244	1.28	Buten-2-one, 4-(3-hydroxy-6, 6-dimethyl-2-methyelenecyclohexyl)-	73
31	18.593	1.27	3-Eicosyne	86
32	18.709	1.02	Perhydrocyclopropa[e]azulene-4,5,6-triol, 1,1,4,6-tetramethyl	74
33	19.331	0.99	Aromadendrene oxide-(2)	81
34	19.975	1.88	n-Hexadecanoic acid	85
35	20.017	1.81	Tetradecanoic acid	85

Table 2. Contd.

36	20.067	0.75	Dibutyl phthalate	82
37	21.443	1.97	1-Pentene, 3,3,4-trimethyl-5-phenyl-	77
38	21.817	1.00	2-Hydroxy cyclopentadecanone	80
39	22.002	46.84	1-butyl-2-Cyclohexen-1-ol	79
40	29.208	0.37	γ-tocopherol	69
41	29.792	0.41	Vitamin E	78
42	29.992	5.5	Bicyclo[2.2.1]heptane, 2-cyclopropylidene-1, 7,7,-trimethyl-	78

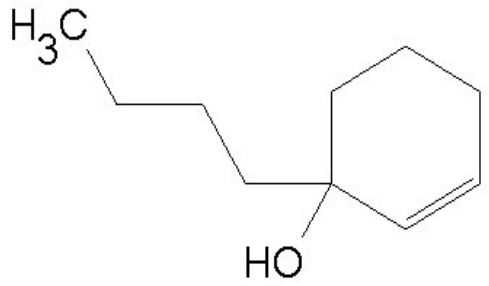

Figure 1. 1-Butyl-2-cyclohexen-1-ol.

tracts' antibacterial effects was summarized. Ethyl acetate extract of the leaves of *G. umbrosus* at concentration of 5 mg/disc showed a broad spectrum of antibacterial activity against all tested bacteria, but in variable degree and the diameter of the inhibition zone is 10 mm for Methicillin resistant *S. aureus*, MRSA, 14 mm for *B. subtilis* B29, 10 mm for *P. aeruginosa* 60690 and 11 mm for *S. choleraesuis*. On the other hand, the methanolic extract at a concentration of 5 mg/disc also demonstrated a broad spectrum of antibacterial activity against all organisms, but to different extent and the diameter of the inhibition zone is 8 mm for Methicillin resistant *S. aureus*, MRSA, *P. aeruginosa* 60690 and 11 mm for *S. choleraesuis*; and 7 mm for *B. subtilis* B29.

Studies on the antibacterial activities of medicinal plants have clearly become a progressive trend using different screening method. Disc diffusion method was the first method of choice, possibly due to its simplicity and capability to analyze a large number of test samples. Many publications have used this method as a means of determining antibacterial activity (Van-Vuuren, 2008). Both extracts of this study showed a broad spectrum of antimicrobial activity against all bacterial strains used in this research. Both MRSA and *P. aeruginosa*, which are well noted for their lack of susceptibility to most antibiotics, were effectively inhibited by the extracts with a remarkable activity. *P. aeruginosa* is known to have a high level of intrinsic resistance to virtually all known antimicrobials and antibiotics, due to a very restrictive outer membrane barrier (Mann et al., 2000). A previous study has confirmed the antibacterial activity of *G. Scortechinii* (Wiart, 2007). In addition to that, minimum antibacterial inhibitory concentrations, of the ethyl acetate and methanol extracts of *G. umbrosus* towards Methicillin resistant *S. aureus*, MRSA, *B. subtilis* B29, *P. aeruginosa* 60690 and *S. choleraesuis* were obtained using agar disc method.

Minimum inhibitory concentration of the extract was obtained using ADM. The lower concentration was revealed by ethyl acetate extract on *B. subtilis* (3.5 mg/ml).

Results of the GC/MS analysis showed that at least 42 compounds were present in ethyl acetate extract of *G. umbrosus.* These compounds were identified through mass spectrometry attached with GC. The mass spectra of these compounds were matched with those found in the NIST/NBS spectral database and the data are given in Table 2. The fragmentation pattern of the major compound (retention time: 22.002 min) was found to be rather similar to that of 1-butyl-2-Cyclohexen-1-ol with similarity index of 79%. This compound is observed to consist about 46.84% as a relative percentage amount. Benzaldehyde was also detected (4.42%), however this compound is known to possess an antimicrobial activity (Ramos-Nino et al., 1998). Sesquiterpene alcohol, globulol, (4.07%) was also detected in this ethyl acetate extract of GU. Previous study has shown that globulol having antimicrobial activity (Tan et al., 2008).

In conclusion, the ethyl acetate and methanol extracts of *G. umbrosus* leaves have demonstrated a broad spectrum antibacterial activity against all tested bacteria. Ethyl acetate showed the strongest antibacterial activity compared to the methanol extract. Gas chromatography and mass spectroscopy analysis showed the existence of various compounds with variable chemical structures.

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