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Antimicrobial and Free Radical Scavenging Activities of the Dichloromethane Extract of *Goniothalamus umbrosus*

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Abstract: The aim of the present study, is to evaluate the antimicrobial, phenolic content and free radical scavenging properties of the dichloromethane extract of *Goniothalamus umbrosus* leaves. The antimicrobial activities were evaluated using 2 Gram-positive bacteria, Methicillin Resistant *Staphylococcus aureus* (MRSA) and *Bacillus subtilis* B 29 and 2 Gram-negative bacteria, *Pseudomonas aeruginosa* 60690 and *Salmonella choleraesuis* using disc diffusion method and compared alongside to streptomycin. Antioxidant effect and total phenolic content of the extract were also measured by DPPH assay and Folin-Ciocalteu reagent, respectively. The results have concluded that the extract explicit a broad spectrum antimicrobial activities against all tested bacteria. However, antioxidant activity is significantly different from the activity of the positive control, BHT. Accordingly, the total phenolic compounds were also, observed to be correlated positively with the low antioxidant activity revealed by the extract. As a conclusion, the promising broad spectrum antimicrobial activities of the dichloromethane extract of the leaves of *G. umbrosus* might be due a different chemical constituent (s) since, the phenolic compounds were not found richly in the extract. Further, phytochemical investigations are currently conducted to explore the active ingredients as new substance (s) in the pharmaceutical industry.

Key words: *Goniothalamus umbrosus*, antimicrobial, free radical scavenging, total phenolic contents, pharmaceutical industry

INTRODUCTION

Plants have long used by traditional healers to prevent or cure infectious conditions; western medicine is trying to duplicate their successful achievements. Studies on the antimicrobial activities of medicinal plants have clearly become a progressive trend (Mulholland, 2005). With advances in laboratory techniques, renewed interest in the field and the scientific validation of the traditional use, the possibility now exists to bring traditional medicine to such a level of recognition that it becomes an accepted alternate regimen to western healthcare systems (Vuuren, 2008). Plants are rich in a wide variety of secondary metabolites, such as tannins, terpenoids, alkaloids and flavonoids, which have demonstrated *in vitro* antimicrobial properties (Cowan, 1999).

Regardless of the presence of phyto-chemicals acetogenins and styryl-lactones in the genus Goniothalamus, only 22 species in the genus Goniothalamus (Family: Annonaceae), 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 (Tanaka *et al*., 2001), anti-malarial (Siti Najila *et al*., 2002; Ichino *et al*., 2006; Noor-Rain *et al*., 2007), anti-cancer (Hawariah and Stanslas, 1998; Inayat *et al*., 2003; Lee *et al*., 2003; Umar-Tsafe *et al*., 2004; De-Fátima *et al*., 2005; Tian *et al*., 2006), antioxidant (Likhitwitayawuid *et al*., 2006), larvicidal activity (Kabir *et al*., 2003) and inhibitory effects on platelet-activating factor properties (Jantan *et al*., 2005).

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There is an inadequate literature regarding the pharmacological properties of *G. umbrosus* by Umar-Tsafe *et al.* (2004), which investigated the *in vitro* genotoxicity of goniothalamin in Chinese hamster cell line. This present study aims to investigate the antimicrobial and antioxidant properties of *Goniothalamus umbrosus* DCM extract based on reliable *in vitro* scientific methods.

MATERIALS AND METHODS

Plant collection and extraction procedure: Leaves of *G. umbrosus* were collected freshly from Selangor State, Malaysia in 2007. The plant was identified at the Unit of Biodiversity, Institute of Bioscience, University Putra Malaysia, Malaysia. The leaves were dried and grinded into powder before cold maceration as an extraction method. Before extraction with DCM, the powdered leaves (300 g) were extracted using hexane; the remaining powdered leaves were extracted with DCM. The extraction done for 7 days with occasional shaking and the process repeated for three times. The combined DCM extracts were filtered through 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 extracts were stored in the refrigerator.

Antimicrobial activity of *G. umbrosus* DCM extract
Microbial strains: The antimicrobial activity of plant extract was evaluated using 2 g-positive bacteria, Methicillin Resistant *Staphylococcus aureus* (MRSA) and *Bacillus subtilis* B29 and 2 g-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.

Disc diffusion method: The screening of the extract antimicrobial effect was carried out by determining the zone of inhibition using paper disc (6 mm in diameter, Whatman No. 1) diffusion method (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^{-1}). The extract was dissolved in dimethyl sulphoxide which was previously tested for antimicrobial activity against all test bacteria and found to have no antimicrobial activity. The extract were diluted to concentration of 100 mg mL^{-1} 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^{-1} extract) to achieve desired concentration and placed in inoculated agar. Streptomycin (10 μg $disc^{-1}$) was used as standards. The controls were prepared using the same solvents without extract. The inoculated plates contain the test and standard discs were incubated at 37°C for 24 h.

DPPH radical scavenging antioxidant assay: Radical scavenging activity of plant extracts against stable DPPH (2, 2-diphenyl-2-picrylhydrazyl hydrate, Sigma-Aldrich Chemie, Steinheim, Germany) was determined spectrophotometrically. When DPPH reacts with an antioxidant compound, which can donate hydrogen, it is reduced. The changes in color (from deep-violet to light-yellow) were measured at 517 nm wavelength. Radical scavenging activity of extract was measured by slightly modified method of Changwei *et al.* (2008), as described below. Extract stock solutions were prepared in 100 mg mL^{-1} in ethanol. The working solution was prepared using methanol in a concentration of 500 μg mL^{-1} (Labsystems iEMS Reader MF). The solution of DPPH in methanol (2.5 mg mL^{-1}) was prepared daily, before UV measurements. Five micro liter of this solution were mixed with 100 μL extract solution 96 well plate. The samples were kept in the dark for 30 min at ambient temperature and then the decrease in absorption was measured. Absorption of blank sample containing the same amount of methanol and DPPH solution was prepared and measured daily. The experiment was carried out, in triplicate. Radical scavenging activity was calculated by the following formula:

$$\text{Inhibition (\%)} = [(A_B - A_A)/A_B] \times 100$$

Where,
A_B : Absorption of blank sample (t = 0 min)
A_A : Absorption of tested extract solution (t = 30 min)

Commercial standard antioxidant Butylated Hydroxytoluene (BHT) was also, tested against DPPH and used as a reference.

Measurement of total phenolic content: Stock solution of extract were prepared in a concentration of 20 mg mL^{-1}, a 50 μL from this solution was transferred to a test tube (n = 3). To this tube, 0.4 mL of Folin-Ciocalteu reagent (1:10) was added and the tube was shaken thoroughly (Djeridane *et al.*, 2006). After 1 min, 0.8 mL of sodium bicarbonate solution (7.5%) was added and the mixture allowed to stand for 30 min with intermittent shaking.

Absorbance was measured at 765 nm using a Shimadzu UV-Vis spectrophotometer (Mini 1240). The total phenolic content was expressed as Gallic Acid Equivalents (GAE) in mg/g extract from the calibration curve of gallic acid standard solution. For the gallic acid, the curve was established by plotting concentration (mg mL^{-1}) versus absorbance (nm) ($y = 5.145x + 0.014$; $R^2 = 0.9975$). Here y = Absorbance and x = concentration.

Statistical analysis: All data were expressed as mean ± SEM. SPSS statistical software was sued to the data with application of student t-test. Alpha probability was set as 0.05.

RESULTS AND DISCUSSION

In this study, antimicrobial and antioxidant activities of DCM extract of *G. umbrosus* were investigated, using disc diffusion method and DPPH assay, respectively. Total phenolic content of the extract was also measured,

Studies on the antimicrobial activities of medicinal plants have clearly become a progressive trend using different screening methods. 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 earlier publications used this method as a means of determining antimicrobial activity (Vuuren, 2008). The antimicrobial activities of DCM extract of *G. umbrosus* were evaluated using Gram-positive and Gram-negative bacteria and compared concurrently to streptomycin as standard antimicrobial drug. Solvents used for control and extract did not show any antimicrobial activity (the results not shown). In Table 1, the screening of the extract antimicrobial properties is presented. From these results, it was concluded that the extract explicit a broad spectrum antimicrobial activities against all tested bacteria in this study; 2 g-positive bacteria, Methicillin Resistant *S. aureus* (MRSA) and *B. subtilis* B29 and other 2 g-negative bacteria, *P. aeruginosa* 6 0690 and *S. choleraesuis*. The highest antimicrobial activity observed in this investigation is obtained towards *B. subtilis* (≈70% of streptomycin activity). The second highest activity of the extract was observed equally to be on *P. aeruginosa* and MRSA (10 mm; zone inhibition). Both MRSA and *P. aeruginosa*, which are well noted for their insusceptibility to most antibiotics (Russell, 2002), were inhibited by extract 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). The extract also showed an antimicrobial property against *Salmonella choleraesuis*. *S. choleraesuis* has an increasing impact of *S. Choleraesuis* on humans (Jean *et al*., 2006).

Table 1: Antimicrobial Activity of the DCM extract of *G. umbrosus*

	Bacterial strains			
	Diameter of inhibition (mm)			
	MRSA	PA	SC	BS
Ethyl A. extract of *G. umbrosus*	10	10	9	15
Control (Streptomycin)	20	20	23	23

[a]The screening of the extract antimicrobial 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*

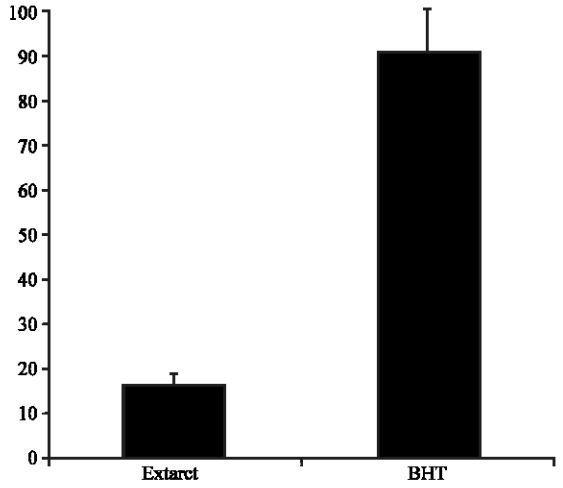

Fig. 1: DPPH absorption inhibition (%) of *G. umbrosus* DCM extract. BHT was used as a positive control

Free radical scavenging property of this extract was examined using standard assay. DPPH radical-scavenging assay was selected due to its straightforwardness, quickness, sensitivity and reproducibility (Sanja *et al*., 2008). Free radical scavenging activity associated with DCM is observed to be weak compared to the standard synthetic antioxidant used as a positive control, BHT. Whereby, the percentage inhibition of DCM extract is only observed to be 17±2.1%, which differs significantly ($p>0.05$) from BHT (Fig. 1). Similarly, phenolic content of this extract was found to be very low (1.3±0.4 mg g^{-1} extract; GAE). Polyphenols are the major plant compounds with antioxidant activity, although, they are not the only ones. The antioxidant activity of phenolic compounds is reported to be mainly due to their redox properties (Galato *et al*., 2001; Zheng and Wang, 2001), which can play an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides. Previous studies reported that antioxidant phenolic compounds have demonstrated antimicrobial activities (Gardjeva *et al*., 2007; Salomão *et al*., 2008). With regards to the current

findings, it could be understood that, the promising antimicrobial property of *G. umbrosus* might be to different type of phytochemical since the phenolic content of this extract is observed to be low.

CONCLUSION

The experiments described above demonstrated that *G. umbrosus* leaves DCM extract possesses compounds with significant broad spectrum antimicrobial effect. In addition to being a good antimicrobial, further studies are needed to be done before reaching to any solid conclusion. Efforts are being in progress to evaluate this extracts in number of other assays and to identify the active principles, responsible for their bioactivity by different spectroscopic method.

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