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Full Length Research Paper

Free radical scavenging and antibacterial activities of Malaysian guttiferae plants

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Guttiferae family is well known to have wide range of biological activities. The family is distributed throughout Malaysia, especially the genus of *Calophyllum* and *Garcinia*. Some of the species shows interesting bioactivity, such as *Calophyllum lanigerum* found in Borneo is exhibited anti- human immunodeficiency virus (HIV) properties. The study was conducted to determine the antioxidant and antibacterial activities of the extracts of selected Guttiferae collected in Malaysia. About 23 extract from different part of nine plants namely *Calophyllum canum, Calophyllum depressinervosum, Calophyllum macrocarpum, Calophyllum teysmanii, Calophyllum symingtonianum, Garcinia griffithii, Garcinia prainiana, Garcinia malaccensis* and *Mesua grandis* were tested for their free radical scavenging and antibacterial activities. Free radical scavenging activity was screened using dot blots 1, 1 -diphenyl-2-picrylhydrazyl (DPPH) staining and followed by DPPH free radical reaction. The antibacterial activity of these plant extracts was tested *in vitro* by using disc diffusion method and minimum inhibitory concentration (MIC). *n*-Hexane extract of the stem bark of *G. griffithii* showed higher scavenging activity with an IC_{50} of 0.09 mg/ml followed by *n*-hexane extract of the leaves of *G. griffithii* with an IC_{50} of 0.098 mg/ml. The antibacterial activity of the extracts revealed that the *n*-hexane extract of the stem bark of *C. canum, C. teysmanii* and *M. grandis* have the highest antibacterial effect with same MIC value at 0.25 mg/ml on the *Bacillus cereus.*

Key words: Antibacterial, disc diffusion method, DPPH, free radical scavenging guttiferae.

INTRODUCTION

According to Sultanbawa (1980); Bennet and Lee (1989), the guttiferae family contains over 1000 species, mainly restricted to the tropics except for the genus *Hypericum* which occurred widely in tropical region. Majority of these plants are trees or shrubs and some of them yield useful timber. Some of these genera and species are found to be endemic to certain land masses, for example, *Kielmeyera* is confined to the South Africa continent, *Symphonia* and *Pentadesma* are confined to Africa. Whitemore (1973) divided the guttiferae family into 4 genera and the number of each genus were found in Peninsular Malaysia and they are as follow: *Garcinia* (Kandis) (49 spp), *Calophyllum* (Bintangor) (45 spp), *Mesua* (Penaga) (23 spp) and *Mammea* (4 spp). Extensive phytochemical studies have documented that guttiferae is rich in a variety of oxygenated and prenylated polyphenols. Many compounds such as xanthones, benzophenones,

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Abbreviations: HIV, Human immunodeficiency virus; **DPPH,** 1,1 -diphenyl-2-picrylhydrazyl; **MIC,** minimum inhibitory concentration; **DCM,** dichloromethane; **ATCC,** American type cell culture; **MHB,** mueller-hinton broth; **UV/VIS,** ultraviolet-visible.

biflavonoids, neoflavonoids, coumarins, triterpenoids and steroids have been isolated from various guttiferae species. The xanthones are usually found in this family and consider as a chemotaxanomic interest. In the review by Sultanbawa (1980), 95 xanthones from guttiferae have been listed. Since then there has been a steady stream of reports in which more than 80 new xanthones which have been characterized from ca. 60 species of Guttiferae (Bennet and Lee, 1989). The objective of the study was to determine the free radical scavenging and antibacterial activities of guttiferae plants collected from Malaysia. 23 extracts from different part of the nine plants namely *C. canum, C. depressinervosum, C. macrocarpum, C. teysmanii, C. symingtonianum, G. griffithii, G. prainiana, G. malaccensis* and *M. grandis* were tested for their activities.

MATERIALS AND METHODS

Plant materials

The plant materials were collected from Pahang and Malacca, Malaysia (Table 1). Voucher specimens were deposited in the herbarium of Kulliyyah Pharmacy, International Islamic University Malaysia.

Plant extraction

The fresh samples were washed, dried and ground to fine powders. All samples were extracted using soxhlet apparatus with *n*-hexane, dichloromethane (DCM) or ethyl acetate and methanol, consecutively. The extracts were concentrated *in vacuo* to obtain the crude extracts. Samples were stored at -4°C for further use.

Bacterial culture

The bacteria were obtained from American Type Cell Culture Collection (ATCC). Four bacterial strains were used in this study, Gram-negative bacteria, *Escherichia coli* ATCC35218, *Pseudomonas aeruginosa* ATCC27853, Gram-positive bacteria, *Staphylococcus aureus* ATCC25923, *B. cereus* ATCC117788.

DPPH free radical scavenging activity

The free radical scavenging effects of the extracts and the positive controls on DPPH were determined using the method describe by Blois (1958) with a slight modifications. 1.9 ml of freshly prepared DPPH solution (0.004%) in methanol was added to 100 µl of each sample in methanol at various concentrations (5, 2.5, 1.25, 0.625, 0.3125, 0.1563 and 0.0781 mg/ml) and (1, 0.5, 0.25, 0.125 and 0.0625 mg/ml) for the positive control (α-tocopherol). After 30 min reaction, the absorbance was measured at 515 nm using UV/VIS spectrophotometer. All experiments were performed in triplicates. The capability to scavenge the DPPH radical was calculated using the following equation:

$$\text{Percent inhibition} = \left(\frac{ADPPH - Asample}{ADPPH}\right) x\ 100$$

Where: A_{DPPH} = The absorbance of DPPH; A_{sample} = The absorbance of the sample.

Antibacterial activity

Disc diffusion method

The agar disc diffusion method was employed for the determination of antibacterial activities of the extracts. Briefly, inoculums' containing 10^7 CFU/ml was spread on Mueller- Hinton agar plates for bacteria. Sterile filter papers (6 mm diameter) containing the 2 mg/ml for each compound (200 µg/disc), standard antibiotics (chloramphenicol-10 µg/disc) or negative control (methanol) were laid down on the surface of inoculated agar plate by using sterile forceps. The plates were incubated at 37°C for 24 h. Each compound was tested in triplicate and the zone of inhibition was measured in millimeter diameter.

Micro dilution method

MIC was performed as previously described by Qaralleh et al. (2010), with slight modification. MIC was measured by determining the smallest amount of compound or standard antibiotic needed to inhibit the growth of tested bacteria. This was done using 96 well plates and performed on Versa Max™ Tunable micro plate reader. The assay plates were filled with Mueller-Hinton broth medium (MHB) containing different concentrations of compounds, streptomycin sulphate or methanol and the tested bacteria (10^7 CFU/mL). After 24 h incubation periods at 37°C, the turbidity in each well was measured at 600 nm.

RESULTS AND DISCUSSION

Radical scavenging activity

The free radical scavenging activity of the extracts depends on the ability of the antioxidant compounds to donor their hydrogen atoms and the structural conformation of the compound (Mammadov et al., 2009). The test is based on the colour change observed when the reaction between DPPH free radicals and antioxidant molecules takes place. The use of the DPPH· in antioxidant reaction (Figure 1) has been widely popular among researchers for the evaluation of free radical scavenging activity on extracts from plant, food material or on pure compounds (Kondo et al., 2004). The degree of discoloration indicates the scavenging potential of the extract by transferring proton to DPPH free radical. The antioxidant activity of *M. grandis, G. griffithii, G. prainiana, G. malaccensis, C. teysmanii, C. macrocarpum, C. symingtonianum, C. canum* and *C. depressinervosum* was investigated using the DPPH free radical-scavenging assay. The results of the free radical scavenging activities were calculated as percent inhibitions which in turn were used to determine the IC_{50} of all the extracts and positive controls. The low IC_{50} value indicated high antioxidant activity. All of the extracts and controls showed antioxidant activities with different IC_{50}. The IC_{50} values were directly calculated from linear

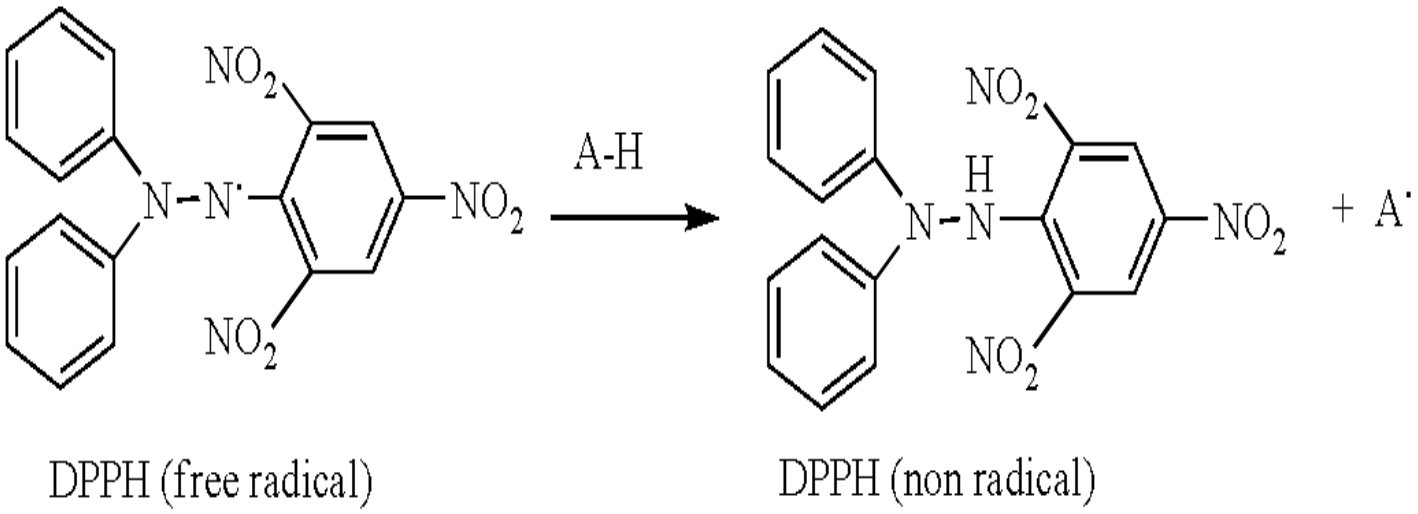

Figure 1. DPPH antioxidant reaction.

Table 1. Location of collected plants.

Plant	Location
C. canum	*Pelindung* reserved forest, Pahang
C. depressinervosum	*Pandan* river, Pahang
C. macrocarpum	Botanical Garden, Malacca
C. symingtonianum	*Pandan* river, Pahang
C. teysmanii	*Teluk Cempedak*, Pahang
G. griffithii	Botanical Garden, Pahang
G. prainiana	Botanical Garden, Pahang
G. malaccensis	Botanical Garden, Pahang
M. grandis	Botanical Garden, Pahang

regression equation of the extracts and standards. Table 2 shows all the calculated IC_{50} values of the standards and extracts. *n*-Hexane extract of the stem bark of *G. griffithii* showed higher scavenging activity with an IC_{50} of 0.09 mg/ ml followed by *n*-hexane extract of the leaves of *G. griffithii* with an IC_{50} of 0.098 mg/ml. The antioxidant activity of *G. griffithii* might be related to its chemical constituents of benzophenone and xanthones which are present in the extract (Nguyen et al., 2005).

Antibacterial activity

In this study, the results obtained from the antibacterial test indicated that the *n*-hexane extracts of the stem bark of *C. canum*, *C. depressinervosum*, *C. teysmanii, M. grandis* and the leaves of *C. canum* inhibit the growth of the gram-positive bacteria namely *S. aureus* and *B. cereus*. Further investigation of the antibacterial effects of the extracts revealed that the *n*-hexane extracts of the stem bark of *C. canum, C. teysmanii and M. grandis* have higher antibacterial effect with the same MIC value of 0.25 mg/ml on the *B. cereus*. Compared to the all extracts, the positive control has a low MIC value (0.2 mg/ml) for both gram-positive bacteria (Table 4). The antibacterial effects of the extracts and chloramphenicol (positive control) on pathogenic bacteria are presented in Table 3. The extracts at 10 mg/ml concentration were effective on some tested bacteria. *n*-Hexane extracts of the leaves and stem bark of *C. canum*, the stem bark of *C. depresinervosum*, *C. teysmanii* and *M. grandis* gave inhibition zone on *B. cereus* and *S. aureus*. The MIC was conducted for all the extracts that showed inhibition zone in the disc diffusion test. The results indicated that the gram-positive bacteria were more susceptible to the extracts compared to the gram-negative bacteria. It is possibly because of the presence of outer membrane that serves as an effective barrier in gram-negative bacteria (Nikaido, 1999). In contrast, gram-negative bacteria showed the least susceptibility to all extracts. The susceptibility of the gram-negative bacteria could be attributed to their intrinsic properties that are related to

Table 2. The IC_{50} of free radical scavenging activity of plant extracts.

Sample (part)	Extracts	IC_{50} (mg/ml)
G. griffithii (stem bark)	DCM	0.22
	n-Hexane	0.09
	Methanol	0.51
G. griffithii (leaves)	DCM	0.11
	n-Hexane	0.098
	Methanol	1.7
G. prainiana (twig)	Methanol	2.9
	Ethyl acetate	1.9
G. malaccensis (stem bark)	DCM	2.1
	Methanol	3.5
M. grandis (stem bark)	Methanol	0.25
	n-Hexane	0.47
C. teysmanii (stem bark)	Methanol	0.12
	n-Hexane	0.51
C. macrocarpum (stem bark)	Methanol	0.23
C. depressinervosum (stem bark)	Methanol	0.27
	Ethyl acetate	0.23
	n-Hexane	0.38
C. symingtonianum (stem bark)	Methanol	0.22
	n-Hexane	0.42
	Ethyl acetate	0.2
C. canum (stem bark)	*n*-Hexane	0.26
C. canum (leaves)	*n*-Hexane	0.27
Alpha-tocopherol		0.56

Table 3. Antibacterial activities of the guttiferae plant *n*-hexane extracts.

Bacteria	Zone of inhibition (mm)			*C. teysmanii*	*M. grandis*	Chloramphenicol
	C. canum (S)	*C. canum* (L)	*C. depressinervosum*			
E. coli	-	-	-	-	-	37
P. aeruginosa	-	-	-	-	-	33
B. cereus	11	-	10	13	14	26
S. aureus	8	9	6	-	12	24.5

Note: S = stem bark; L = leaves; - = no inhibition.

the permeability cell surface and extracts.

Conclusion

M. grandis, G. griffithii, G. prainiana, G. malaccensis, C. teysmanii, C. macrocarpum, C. symingtonianum, C. canum and *C. depressinervosum* exhibited different range of free radical scavenging and antibacterial activities. *G. griffithii* exhibited the highest radical scavenging activity against DPPH free radical. *B. cereus* is the most sensitive bacteria to the extracts of the stem bark of *C. canum*, *C. depressinervosum*, *C. teysmanii, M. grandis* and the leaves of *C. canum*. The obtained results may provide a support to utilize the plants in traditional medicine. Most of the tested plants are not scientifically investigated in term of their chemistry and bioactivity.

Table 4. The MIC value for the extracts.

Bacteria	MIC (mg/mL)					
	***C. canum* (S)**	***C. canum* (L)**	***C. depressinervosum* (S)**	***C. teysmanii* (S)**	***M. grandis* (S)**	**Chloramphenicol**
B. cereus	0.25	n.d	0.5	0.25	0.25	0.02
S. aureus	0.5	0.5	1	n.d	0.5	0.02

(S), stem bark; (L), leaves; n.d: not determined.

Based on this, it is recommended to carry out further study on isolation of active principles present in the plants.

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