

Research Article

Antimicrobial and antioxidant activities of *Swietenia macrophylla* leaf extracts

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Abstract

The antimicrobial and antioxidant activities of methanol, dichloromethane and *n*-hexane extracts of *S. macrophylla* leaves were evaluated. The antimicrobial activity of the extracts was tested against four species of bacteria, *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Pseudomonas aeruginosa*, and a fungus, *Candida albicans*. The methanol and the dichloromethane extracts were found to be active against the Gram-positive bacteria tested. The methanol extract also showed antifungal properties. The antioxidant activity of *S. macrophylla* leaf extracts was evaluated using 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay in which all of the leaf extracts showed remarkable activities. At the concentration of 320 µg/mL, the methanol extract, the dichloromethane extract and the *n*-hexane extract showed 90.61%, 84.17% and 89.46% scavenging activities respectively. The extracts were found to contain a sizable amount of total phenolic, total tannin and total flavonoid contents.

Keywords: *Swietenia macrophylla*; Antimicrobial; Antioxidant; Minimum inhibitory concentration; 1,1-diphenyl-2-picrylhydrazyl.

Introduction

Swietenia macrophylla, or big-leaf mahogany, is a tree from the family Meliaceae. Its fruit, known as “sky fruit”, is used as a folk medicine in Malaysia for treating diabetes and high-blood pressure [1]. A decoction of the crushed seeds of this tree is also used to treat skin ailments and wounds [2]. The seeds are also used for diarrhea [3a]. A survey of literature showed that the organic and aqueous extracts of *S. macrophylla* seeds possess a wide array of biological properties such as anti-diabetic [3a], anti-diarrhoeal [3b], anti-inflammatory,

anti-mutagenic, anti-tumor-promoting [4], antimicrobial, antimalaria [5] and antibabesial [3c, 6].

The objective of the current study is to screen the leaf extracts of this plant for their antimicrobial and antioxidant properties. The antimicrobial activity of the leaf extracts was evaluated by agar diffusion method [7], while the antioxidant activity was determined using DPPH assay with catechin and butylated hydroxytoluene (BHT) as the positive controls.

Materials and Methods

Chemicals

Nutrient agar, HCl and Na₂CO₃ were purchased from Merck (Darmstadt), Folin-Ciocalteu's reagent, BHT, catechin, quercetin, DPPH and vanillin were purchased from Sigma-Aldrich (USA), AlCl₃ were purchased from R & M Chemicals (UK).

Materials

Leaves of *S. macrophylla* were collected on the campus of USM, Penang, Malaysia. The leaves (5 kg) were cleaned and dried in open-air at room temperature for 30 days, to approximately 20% of the initial weight. The air-dried leaves were then powdered.

Extraction procedures

The air-dried powdered leaves (1 kg) were extracted sequentially at room temperature with *n*-hexane, dichloromethane and methanol (5 L × 5 days each). The extracts were filtered with a Buchner funnel and the organic solvents were removed under reduced pressure at a temperature < 40°C, using a rotatory evaporator to give *n*-hexane extract (12.8 g), dichloromethane extract (18.2 g) and methanol extract (107.8 g).

Antimicrobial activity evaluation

Microbial culture

Bacillus subtilis, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Candida albicans* were provided by the School of Pharmaceutical Sciences, USM. Stock cultures of all microorganisms were grown in nutrient agar broth at 37°C for 24 hours. A few colonies of single bacteria were transferred into a test tube containing sterilized distilled water. The mixture was homogenized using a centrifuge. The turbidity of the solution was compared to 0.5 M McFarland standard and adjusted until the turbidity of both solutions were the same, that is, corresponding to the concentration of 1×10^8 CFU/mL [7]. All microorganisms tested were lab strains.

Preparation of extracts at different concentrations

The extracts were dissolved in DMSO (Merck) and prepared at the concentrations of 5, 10 and 20 mg/mL each.

Agar diffusion method

Nutrient agar solution was prepared by dissolving 28 g of agar powder in 1 L of distilled water and autoclaved at 121°C for 15 minutes. The agar nutrient was then cooled to a temperature between 45 to 50°C in a steam bath. Bacterial suspension (2 mL) was added to 200 mL of the nutrient agar and centrifuged. About 25 mL of the nutrient agar inoculated with bacteria was then poured onto a sterilized petri dish (90 x 15 mm) and left to solidify for 10 minutes, after which four wells were made on the agar using a 8 mm cork borer. The

wells were then filled with 60 μL of test material, at different concentrations, together with DMSO as the control. The plates were left at room temperature for one hour before incubating in an incubator at 37°C for 24 hours. After the incubation period, the plates were inspected and zones of inhibition were measured in four directions using a vernier caliper. All experiments were carried out in duplicate. Gentamicin (100 $\mu\text{g}/\text{mL}$) and amphotericin B (500 $\mu\text{g}/\text{mL}$) were used as positive controls.

Antioxidant activity evaluation

DPPH radical scavenging assay

DPPH radical scavenging activity of the leaf extracts were determined according to the method described by Kukic *et al.* [8]. First, 4.0 mL of test material at different concentrations were reacted with 0.50 mL of 1.0 mM DPPH solution and kept in the dark for 30 minutes, following which the absorbance was measured at 517 nm against a blank sample consisting of 4.0 mL of MeOH and 0.50 mL DPPH solution. DPPH scavenging activity was calculated using the equation:

$$\% \text{ DPPH radical scavenging activity} = (A_0 - A_s / A_0) \times 100 \quad (1)$$

where A_0 is the absorbance of the blank sample, and A_s is the absorbance of the test material. The IC_{50} value represented the concentration of test material that caused 50% scavenging activity [9]. Catechin and BHT were used as positive controls. The mean values were obtained from triplicate experiments. All of the test materials and reference drugs were dissolved in MeOH, and measurements taken at the concentrations of 5, 10, 20, 40, 50, 100, 150, 200 and 320 $\mu\text{g}/\text{mL}$, respectively.

Total phenolic, total tannin and total flavonoid contents

The total phenolic content was determined by using Folin-Ciocalteu method as described by Neergheen, Soobrattee, Bahorun and Aruoma [10]. First, 0.25 mL of test material was mixed with 3.75 mL of distilled water and 0.25 mL of Folin-Ciocalteu reagent. The mixture was left for 3 minutes, after which 1.25 mL of 20% Na_2CO_3 was added and the resulting mixture was shaken vigorously and heated at 40°C for 40 minutes. A blank solution was prepared by the same procedures except that the test material was replaced by 0.25 mL of MeOH. The absorbance of the mixture was measured at 685 nm against a blank [10]. Using the same procedures above, a catechin standard curve was obtained from which the total phenolic content was determined and expressed as catechin equivalents.

The total tannin content of *S. macrophylla* leaf was measured according to the procedure described by Cheng, Ye, Chen, Liu and Zhou [11]. Briefly, 1.0 mL of test material was mixed with 3.0 mL of 4% vanillin and 1.50 mL of HCl in the dark for 5 minutes. The absorbance was then measured at 500 nm against a blank sample prepared by the same procedures except that the test material was replaced by 1.0 mL of MeOH [11]. The same procedures were repeated using quercetin at different concentrations. The total tannin content was expressed as quercetin equivalents.

The total flavonoid contents of the extracts were determined by Dowd method [12], in which 5.0 mL of 2% AlCl_3 was added to 5.00 mL of test material. After 10 minutes, the absorbance was measured at 415 nm against a blank sample consisting of 5.0 mL of MeOH and 5.0 mL of test material without AlCl_3 [12]. A quercetin standard curve was obtained

simultaneously from which the total flavonoid content was determined and expressed as quercetin equivalents.

Results and Discussion

Antimicrobial activity

The antimicrobial activities of the organic extracts of *S. macrophylla* leaves were evaluated *in vitro* using agar diffusion method and expressed in terms of zone of inhibition (mm). The results shown in Table 1 indicated that the MeOH and the dichloromethane extracts were active against the Gram-positive bacteria tested, with inhibition zones ranging from 11.55 ± 0.15 mm to 21.45 ± 0.97 mm. The MeOH extract showed an impressive activity with inhibition zones of 21.45 ± 0.97 mm and 16.10 ± 0.13 mm respectively towards *Staphylococcus aureus* and *Bacillus subtilis*, which were higher than that of 100 µg/mL Gentamicin. However, the former two extracts showed a limited activity against *Escherichia coli* and *Pseudomonas aeruginosa*. The MeOH extract showed inhibition zone of 14.25 ± 0.35 mm against *Pseudomonas aeruginosa* at the highest concentration tested (20 mg/mL), while the dichloromethane extract showed partial inhibition zones ranging from 11.60 ± 0.39 mm to 12.80 ± 0.74 mm towards *Escherichia coli*. The MeOH extract also showed inhibitory effect against *Candida albicans*, with inhibition zones of 12.05 ± 0.10 mm and 11.50 ± 0.13 mm respectively at the concentrations of 20 mg/mL and 10 mg/mL. On the other hand, the *n*-hexane extract was found to be inactive as an antimicrobial agent towards all of the microorganisms tested.

The antimicrobial activity displayed by organic extracts of *S. macrophylla* leaves may thus justify some of the traditional claims of the medicinal properties of this tree in providing cure for health problems such as skin ailments, wounds and diarrhea [2, 3a, 13].

Table 1. Antimicrobial activity of *Swietenia macrophylla* leaf extracts.

Values are expressed as means \pm standard deviation ($n = 2$)

Sample	Concentration (mg/mL)	Inhibition zone in diameter (mm)				
		<i>Staphylococcus aureus</i>	<i>Bacillus subtilis</i>	<i>Escherichia coli</i>	<i>Pseudomonas aeruginosa</i>	<i>Candida albicans</i>
MeOH extract	20	21.45 ± 0.97	16.10 ± 0.13	-	14.25 ± 0.35	12.05 ± 0.10
	10	16.50 ± 0.65	12.50 ± 0.13	-	-	11.50 ± 0.13
	5	13.65 ± 0.51	10.45 ± 0.22	-	-	-
Dichloromethane extract	20	14.45 ± 0.23	15.50 ± 0.15	(12.80 ± 0.74)	-	-
	10	13.35 ± 0.25	13.30 ± 0.18	(11.60 ± 0.39)	-	-
	5	12.60 ± 0.28	11.55 ± 0.15	-	-	-
<i>n</i> -Hexane extract	20	-	-	-	-	-
	10	-	-	-	-	-
	5	-	-	-	-	-
Reference Control		18.10 ± 0.18	15.90 ± 0.91	17.50 ± 0.28	10.20 ± 0.41	12.30 ± 0.13
		-	-	-	-	-

Control: DMSO; References: Gentamicin (100 µg/mL) for *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Pseudomonas aeruginosa*; Amphotericin B (500 µg/mL) for *Candida albicans*. (): partial inhibition. -: no inhibition.

DPPH[•] radical scavenging activity

The antioxidant activities of the extracts were evaluated using DPPH[•] assay. The results (Fig. 1.) showed that all of the leaf extracts exhibited good scavenging activity ranging from $27.60 \pm 2.04\%$ to $90.61 \pm 0.09\%$ when the concentrations of the extracts were increased

gradually from 10 $\mu\text{g/mL}$ to 320 $\mu\text{g/mL}$. The methanol extract was the strongest radical scavenger ($\sim 91\%$ scavenging) followed by *n*-hexane extract ($\sim 89\%$ scavenging) and dichloromethane extract ($\sim 84\%$ scavenging) at the concentration of 320 $\mu\text{g/mL}$. At the highest concentration tested (320 $\mu\text{g/mL}$), the scavenging activity of methanol extract ($90.61 \pm 0.09\%$ scavenging) was higher than that of catechin ($86.50 \pm 1.37\%$ scavenging) and was comparable to BHT ($90.04 \pm 0.39\%$ scavenging). Based on the plotted graph of concentration versus corresponding scavenging activity, the IC_{50} values of methanol extract, dichloromethane extract and *n*-hexane extract were determined as $7.67 \pm 0.29 \mu\text{g/mL}$, $15.00 \pm 0.50 \mu\text{g/mL}$ and $26.00 \pm 1.73 \mu\text{g/mL}$ respectively (Fig. 2.). It was observed that the IC_{50} value of MeOH extract was lower than that of catechin ($\text{IC}_{50} = 7.83 \pm 0.29 \mu\text{g/mL}$), implying that it is a stronger radical scavenger than catechin.

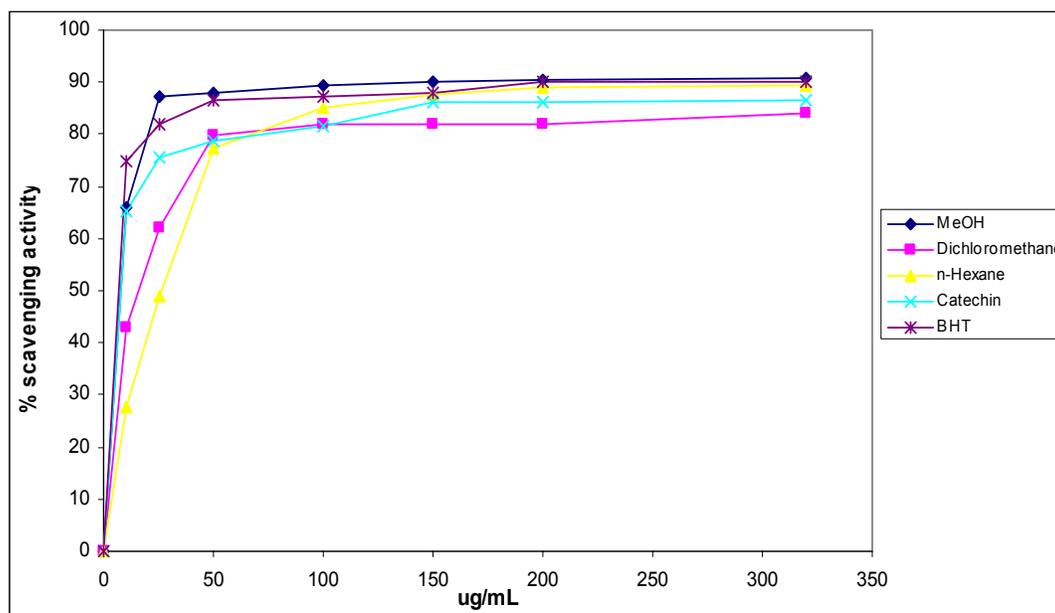


Figure 1. DPPH scavenging activity of *S. macrophylla* leaf extracts.

Values are expressed as means \pm standard deviation ($n = 3$)

Total phenolic, total tannin and total flavonoid contents

The total phenolic content of the *S. macrophylla* leaf extracts was measured spectrophotometrically using Folin-Ciocalteu reagent at 685 nm, and the results were expressed as catechin equivalents (CAE) using the standard curve of catechin ($R^2 = 0.9955$). The total phenolic content of the leaf extracts (Table 2) were found to range from 63.37 to 259.34 mg CAE/g dry weight, with the MeOH extract showing the highest phenolic content (259.34 ± 1.50 mg CAE/g dry weight), followed by the dichloromethane extract (111.92 ± 1.32 mg CAE/g dry weight) and the *n*-hexane extract (63.37 ± 0.58 mg CAE/g dry weight), at the concentration of 1000 $\mu\text{g/mL}$.

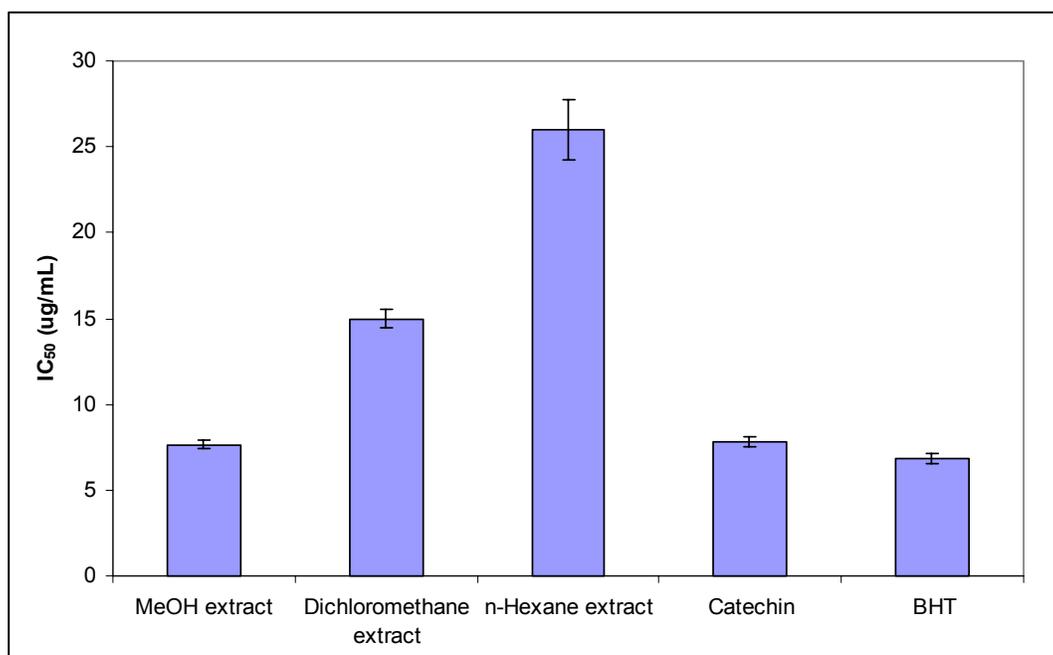


Figure 2. IC₅₀ value of extracts of *S. macrophylla* leaves.
Values are expressed as means \pm standard deviation ($n = 3$)

The total tannin content of *S. macrophylla* leaf extracts (Table 2), measured using a standard curve generated by quercetin ($R^2 = 0.9836$) were observed to range from 18.67 to 90.33 in terms of quercetin equivalents (QE)/g dry weight. The MeOH extract showed the highest content (90.33 ± 3.33 mg QE/g dry weight of extract) followed by the dichloromethane extract (33.67 ± 6.01 mg QE/g dry weight of extract) and the *n*-hexane extract (18.67 ± 5.00 mg QE/g dry weight of extract).

A similar order was observed for the total flavonoid content of the extracts (Table 2), with the MeOH extract showing the highest total flavonoid content (26.64 ± 0.21 mg QE/g dry weight), followed by the dichloromethane extract (15.20 ± 0.18 mg QE/g dry weight) and the *n*-hexane extract (6.50 ± 0.19 mg QE/g dry weight).

Table 2. Total phenolic, tannin and flavonoid contents of *S. macrophylla* leaf extracts.
Values are expressed as means \pm standard deviation ($n = 3$)

	MeOH extract	Dichloromethane extract	<i>n</i> -Hexane extract
Total phenolic content ^a	259.34 ± 1.50	111.92 ± 1.32	63.37 ± 0.58
Total tannin content ^b	90.33 ± 3.33	33.67 ± 6.01	18.67 ± 5.00
Total flavonoid content ^b	26.64 ± 0.21	15.20 ± 0.18	6.50 ± 0.19

^a mg CAE/g dry weight of extract.

^b mg QE/g dry weight of extract.

Overall, the total contents of phenolics, tannins and flavonoids of the extracts were found to decrease in the order MeOH extract > dichloromethane extract > *n*-hexane extract.

Conclusions

The present study has evaluated the antimicrobial and antioxidant activities of different organic extracts of *S. macrophylla* leaves and found the methanol extract to be the most active extract. The higher activity displayed by this extract could be attributed to its higher content of bioactive constituents or to the presence of certain polar bioactive components that were absent in the other less polar extracts. The remarkable antioxidant activities exhibited by all the extracts tested could be associated with the presence of phenolics such as tannins and flavonoids within them.

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