Research Article

PRELIMINARY STUDIES ON ANTIMICROBIAL ACTIVITY OF *SWIETENIA MACROPHYLLA* LEAF EXTRACT

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ABSTRACT

The plant *Swietenia macrophylla belonging* to the family Meliaceae is a traditionally important plant with more medicinal properties. Phytochemical investigation of crude extracts of *Swietenia macrophylla* resulted in the identification of various chemical constituents. The crude extracts were also tested against human pathogenic bacteria and fungal strains. Based on minimum inhibitory concentration (MIC) of the present study, the plant extract has antibacterial activity as well as good antifungal activity. This study supports the traditional claim and usefulness of the plant.

Keywords: *Swietenia macrophylla*, Antibacterial, Antifungal, Extract.

INTRODUCTION

Antibacterial drugs are the greatest contribution of the 20th century in developing countries, where infective diseases predominate. With advancement in medicinal chemistry, a wide range of natural, semi synthetic and synthetic antimicrobials are available for treating infectious diseases in human and animals[1]. Natural antibacterial agents are called as 'antibiotics'. These are substances produced by microorganisms, which suppress the growth or kill other microorganisms at very low concentrations. In recent years, drug resistance to human pathogenic bacteria has been commonly reported from all over the world[2]. Resistance to antimicrobial agents has resulted in morbidity and mortality from treatment failures and increased health care costs.

The plant *Swietenia macrophylla belonging* to the family Meliaceae is a lofty, evergreen large tree native to tropical America, Mexico and South America. This tree is mainly cultivated at tropical zones, such as India, Malaysia, and Southern China[3]. It contains alkaloids, terpenoids, antraquinones, cardiac glycosides, saponins, phenols, flavonoids, volatile oils, phospholipid and long chain unsaturated acid. These plant specially contains 45 limonoids like swietenine, swietenolide, swietemahonin, khayasin, andirobin, augustineolide, 7-deacetoxy-7-oxogedunin, proceranolide and 6-O-acetyl swietonolide, 2-hydroxy-3-O-tigloylswietenolide, swiemahogins A and B belonging to the structural classes andirobin, gendunin, mexicanolide and phragmalin, triterpens, tetranortriterpenes, and chlorogenic acid[4-6]. It also contains triterpenoid, tetranortriterpenoid, swietenine dimeric triterpenoid. Traditionally, leaves have been used for the treatment of diarrhoea, febrifuge, colds, and cataract. The seed have been used for the treatment of leishmaniasis, cancer, amoebiasis, coughs, chest pains, intestinal parasitism, hypertension, diabetes and malaria[3, 7]. The seeds of *S. macrophylla* have been reported to have anti-inflammatory, antimutagenecity and antitumor activity[8-10]. Paste of seeds of *Swietenia macrophylla* are traditionally used by the local healers of India for curing skin diseases and infections being caused by wounds. The bark has been used as an astringent for wound[11].

Despite the availability of a range of synthetic antibiotics, the infectious diseases continue to be a major health problem worldwide. The development of widespread antibiotic resistance among pathogens and undesirable side effects associated with the continued use of synthetic drugs has stimulated a renewed interest in the alternative therapeutics. Therefore, it is essential to search substances that can either inhibit the growth of pathogens or kill them and have no or least toxicity to host cells are considered candidates for developing new antimicrobial drugs. To overcome the problems associated with antimicrobials, medicinal plants would be the best source to obtain a variety of drugs as an alternative treatment for diseases. Therefore, the present study was undertaken to explore the antibacterial potential of various extracts of *S. macrophylla* leaf against some pathogenic bacteria like *Escherichia coli, Pseudomonas aeruginosa, Bacillus subtilis,* Methicillin-resistant *Staphylococcus aureus* (MRSA).

MATERIALS AND METHODS

Collection of plant material

The plant material, *Swietenia macrophylla* leaves was collected from Penang Botanical Garden, Malaysia in the month of January 2011. The plant material was authenticated by botanist Botanical garden Penang, Malaysia. A Voucher specimen has been deposited in AIMST University, Malaysia.

Extraction

All the leaves are initially blended into coarse then into powder form by using Waring Commercial Blender in low

power. The powder is sieved using Laboratory Test Sieve, aperture size 1.70 mm. About 250 gm of the powdered plant leaves were packed inside the Soxhlet extractor and was successively extracted with ether, chloroform, ethanol and water. The extracts so collected were distilled on a water bath at atmospheric pressure and the last traces of solvent were removed using vacuum. The extract is dried in freeze drier. A semisolid extract of various solvents were used for the phytochemical and antimicrobial activity.

Phytochemical studies

The extracts were tested by preliminary phytochemical screening for the presence of tannins, alkaloids, anthraquinones, cyanogenetic glycosides, saponin glycosides, reducing sugars and flavonoids using the conventional standard protocols for detecting the presence of different chemical constituents in the plant extract[12].

Microorganism used

Antimicrobial activity were tested using gram positive bacteria namely E. coli (NCIM 269) and *Pseudomonas aeruginosa* (NCIM 2036), MRSA, *Bacillus Subtilis* and fungi *Candida albicans* (MTCC 227), *Aspergillus flavus* (MTCC 2206), *Aspergillus niger* (MTCC 281) and *Trichophyton mentagrophytes* (CAS 66) were obtained from University Science Malaysia, Penang, Malaysia and Department of Biotechnology, AIMST University, Malaysia.

Well Diffusion Method

Well diffusion method was used for the determination of antimicrobial activity of the extract[13]. The drug Chlorampenicol 200µg/ml was used as a reference drug. Briefly, a suspension of the tested organism was swabbed on Mueller- Hinton Agar (MHA) in order to obtain a lawn culture. After 20 minutes of inoculation of test plates, plates were punched to make the well of 6mm diameter with the help of sterile cork borer. Five wells were made in each plate. Three different concentrations of each extract, 5%, 10% and 20% is poured into the well in assay plates, each for 100µl. The other two wells were filled with control and standard antibiotic. The plates were incubated overnight at 37°C, and all plates were observed for the zone of inhibition; diameter of these zones was measured in millimetres by using ruler. The zone for inhibition was measured in millimeters and compared with standard drug[14-16]. The control consists of filter paper disc covered with dimethyl formamide and evaporated to dryness.

MIC Assay

The agar dilution method recommended by National Committee for Clinical Laboratory Standards was used[13]. A final concentration ranging from 83-333µg/ml was prepared in Sabourauds dextrose agar (SDA) slant to check antifungal activity. 5ml of extract at different concentrations were taken into sterile test tube and mixed with 1 ml of each fungus to be tested. Then 0.5 ml of mixture (culture with extract) was added to 2.5 ml of SDA in the tubes. Afterwards all the tubes were incubated at 30˚C for 15 days. The tubes were observed for visible growth of fungi. The highest dilution showing no visible growth was regarded as minimal inhibitory concentration[14, 15].

RESULTS AND DISCUSSION

In this research project we prepared four different extracts of *Swietenia macrophylla.* The preliminary phytochemical analysis was carried out for petroleum ether, chloroform, ethanol and aqueous extracts. The phytochemical screening result showed in table 1 indicates the presence of alkaloids, flavonoids, terpenoids, tannins, glycosides, reducing sugars and anthraquinones. It has been reported that flavonoids[16], terpenoids[17] and tannins[18] has antibacterial activity against various pathogenic bacterial mainly against *Staphylococcus aureus*, *Pseudomonas aeruginosa, Streptococcus pyogens, Salmonella typhi and Escherichia coli etc.* Similarly, in the present study, due to the presence of flavonoids, terpenoids and tannins we decided to evaluate antibacterial activity of this plant. There has been a close relationship between flavonoids structure and antibacterial activity. In this study, the petroleum ether and chloroform extracts showed good antibacterial activity due to the presence of flavonoids and terpenoids. Tannins exhibited antibacterial activities against all the tested microorganisms. *S. aureus* was the most resistant to tannins isolated from the plant material followed by *Streptococcus pyogens, Salmonella typhi, Escherichia coli, Proteus vulgari and Pseudomonas aeruginosa*[19].

Table 1: Results of phytochemical screening

Constituents	Types of extracts			
	Petroleum Ether	Chloroform	Ethanol	Water/aqueous
Saponins	_	_	_	+
Flavonoids	+	+	_	_
Tannins	_	_	+	+
Glycosides	_	_	_	_
Reducing Sugar	_	+	_	+
Alkaloids	+	_	_	_
Anthraquinones	_	_	_	+
Terpenoids	+	_	+	_

+ : present; -: absent.

Table 2: Antimicrobial activity of *Swietenia macrophylla* leaf extracts.

Sample	Concentration (mg/mL)	Zone of inhibition in diameter (mm)			
		MRSA	*Bacillus subtilis*	*Escherichia coli*	*Pseudomonas aeruginosa*
Aqueous extract	20	-	10.50 ± 0.12	10.0 ± 0.95	-
	10	-	10.50 ± 0.13	10.20± 0.17	-
	5	-	8.0 ± 0.18	9.80 ± 0.92	-
Ethanolic extract	20	12.20 ±0.97	11.70 ± 0.24	10.0± 0.70	10.50 ± 0.42
	10	11.50 ± 0.67	10.80 ± 0.21	9.60 ± 0.90	10.0 ± 0.31
	5	11.60 ± 0.91	10.10 ± 0.42	8.40 ± 0.12	9.60 ± 0.99
Chloroform extract	20	-	7.20 ± 0.22	-	-
	10	-	10.45 ± 0.82	-	-
	5	-	8.30 ± 0.13	-	-
Petroleum ether extract	20	16.50 ± 0.25	16.65 ± 0.41	21.50 ± 0.97	20 ± 0.91
	10	15.40 ± 0.15	15.70 ± 0.56	19.80 ± 0.97	17 ± 0.20
	5	14.20 ± 0.17	15.55 ± 0.99	17.0 ± 0.11	12 ± 0.32
Reference standard		**12.30 ± 0.22**	**15.10 ± 0.20**	**35.20 ± 0.97**	**24 ± 0.10**
Control	**-**	**-**	**-**	**-**	**-**

Values are expressed as means ± standard deviation (*n* = 3); Control: DMF (dimethyl formamide); Reference standard: Chloramphenicol 1000 µg/mL; (-): no inhibition; *MRSA:* methicillin-resistant *staphylococcus aureus*

Table 3: Anti fungal activity of *Swietenia macrophylla* leaf extracts

Extract concentration		*Candida spp*	*A.flavus*	*A.niger*	*T.mentogrophytes*
Aqueous extract	83	+	+	+	+
	166	+	+	+	+
	333	-	-	+	-
Ethanolic extract	83	+	+	+	+
	166	+	+	-	-
	333	-	+	-	-
Chloroform extract	83	+	+	+	+
	166	-	-	-	-
	333	-	-	-	-
Petroleum ether extract	83	+	+	+	+
	166	+	+	+	+
	333	-	-	-	-

+ : present; -: absent.

In the present study, it was found to have antibacterial activity in both ethanolic and aqueous extract, due to the presence of tannins. The petroleum ether and ethanolic extract showed good antibacterial activity due to the presence of terpenoids. The second scope of the research was carried out to check the antimicrobial activity of the extracts. Each extracts were prepared into (5%, 10%, 20%) concentration and they were subjected to antimicrobial test using diffusion method in presence of an antibiotic (Chloramphenicol 200 µg/ml) in four bacterial strains *Bacillus subtilis, Pseudomonas aeruginosa, Escherichia coli* and *Methicillin-resistant Staphylococcus aureus*. DMF was used as negative control. The results of antimicrobial test are given in table 2.

All the extracts had shown a distinct zone of inhibition against *Bacillus subtilis.* In depth, zone of inhibition was high for petroleum ether and ethanolic extract. The antibacterial activity of petroleum ether extract is effective against most microorganisms particularly in *Bacillus subtilis* and *Methicillin-resistant Staphylococcus aureus* (MRSA). Ethanolic extract, on the other hand, has shown significant antibacterial activity against *Methicillin-resistant Staphylococcus aureus* (MRSA) when compared to its standard reference. A dose dependent antibacterial activity was observed for petroleum ether extract. Significantly higher antibacterial activity was observed for petroleum ether extract against *Bacillus subtilis and MRSA*. Extracts also has got significant antifungal activity and results are tabulated in Table 3. All the tested organisms namely *Candida species, Aspergillus flavus, Aspergillus niger* and *Trichophyton mentagrophyte* responded well to the leaf extract with MIC value of 83(µg/ml) by agar dilution method. The solvents used for solubility purpose not exploited any antimicrobial activity.

CONCLUSION

Based on the observations of the study zone of inhibition, all the extract are effective against *Bacilli subtilis.* It also has been found that ethanolic and petroleum ether extract have shown higher antibacterial activity against the chosen microorganism. The ethanolic and petroleum ether extract shown good antibacterial activity at high concentration (20mg/ml) stating that it showed a dose

dependent antibacterial effect. It is concluded that *Swietenia macrophylla* leaves are proven to be a very useful medicinal plant as an antibacterial agent. Other pharmacological properties and its mechanisms of action of *Swietenia macrophylla* leaves are yet to be explored.

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