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Phytochemical screening and antimicrobial activity of leaf, seed and central-fruit-axis crude extract of *Swietenia macrophylla* King

MV Durai, G Balamuniappan and S Geetha

Abstract
The present study aimed to evaluate the antimicrobial activities of *Swietenia macrophylla* crude methanolic extract of leaf, seed and central-fruit-axis. The antimicrobial activity of the leaf, seed and central fruit-axis extract was evaluated against gram-positive, gram-negative bacteria and fungi based on the inhibition zone using well diffusion assay. The crude extracts of *S. macrophylla* were subjected to various phytochemical screening tests. The phytochemical tests exhibited the presences of common phytocompounds such as alkaloids, flavonoids, tannins, terpenoids, glycosides, saponins, volatile oils, amino acids and proteins as major active constituents. The SMCM seed extract had significant level of inhibitory effects on the growth of bacteria viz., *Staphylococcus aureus, E. coli* and fungi *viz*., *Fusarium sp, Helminthosporium sp* and *Alternaria sp*. The antimicrobial activity exhibited a linear relationship with extract concentrations. The seed extracts proved potential extract against fungal growth.

Keywords: Mahogany, Swietenia, Phytochemical, Crude extract, Antimicrobial

1. Introduction
Ethno medicine is the oldest method of curing diseases and infections. Various plants have been used in different parts of the world to treat human diseases and infections [1-3]. Plants are used medicinally in different countries and are a source of many potential and powerful drugs [4]. Traditional medicine using plant extracts continues to provide health coverage for over 80% of the world's population, especially in the developing world [5]. India is the largest producer of medicinal herbs [6]. Natural products of higher plants may possess a new source of antimicrobial agents with possibly novel mechanisms of action [7].
The combinations of secondary metabolites present in the plant are responsible for beneficial medicinal effects. The therapeutic actions of plants are unique to particular plant species or groups are consistent with this concept as the combinations of secondary products in a particular plant are often taxonomically distinct [8].
These bioactive compounds used as prime molecules for the development of many medicinal practices and synthetic potential antibiotics. As synthetic antibiotics have sometimes shown antagonistic effects on the host including immune-suppression, hypersensitivity and allergic reactions [9], there is need to develop antimicrobial drugs from natural origin that are much safer, less expensive and reliable. Plant based antimicrobials represent a vast untapped source for medicines and they provide enormous healing potential. They are effective in the treatment of infectious diseases with fewer side effects that are often associated with synthetic antimicrobials [10]. The systematic screening of medicinal plants with the purpose of discovering new bioactive compounds is a routine activity in many laboratories devoted to biomedical research.
The plant *Swietenia macrophylla* (Family: Meliaceae) or commonly known as big leaf mahogany, is a tropical timber tree native to Central America. There are numerous reports on the use of *Swietenia macrophylla* as antibacterial, antifungal and antiplasmodial agent [11, 12]. *Swietenia macrophylla* is also used for treating wound infection and skin condition [13]. Several studies have also been reported on the antifungal and antimalarial properties of limonoids, a major constituent of *Swietenia macrophylla* [14, 15]. While these ethnopharmacological profiles of *Swietenia macrophylla*, the present study designed to investigate the anti-infective properties of leaf, seed and central-fruit-axis extract of *Swietenia macrophylla* against selected bacteria and fungi *in vitro.*

2. Materials and Methods

2.1 Plant Material

Disease free fresh leaf, seed and central-fruit-axis samples of *Swietenia macrophylla* were collected from Mullur ($11^0$22'54.46" N; $76^0$53'38.39"E), Kothagiri, Tamil Nadu, India during the month of November, 2015. The samples were washed with tap water to remove dust and other inert materials. The cleaned samples were shade dried on the clean floor for 2days. These shade dried samples were powdered using electrical blender.

2.2 Crude Extract preparation

Thirty gram of dry powdered leaf, seed and central-fruit-axis materials were subjected to successive organic solvent extraction by refluxing in the Soxhlet apparatus each for 10 hours. In this study, the aqueous methanol (80 %) was used as solvent [16]. All the extracts were concentrated by oven-drying [17]. Each fraction was collected when no further elution of compounds was observed. The collected extracts were subject to distillation and drying in incubator. The dried extracts were stored in sterile containers in the refrigerator till further analysis.

2.3 Phyto-chemical Screening

The condensed *S. macrophylla* crude methanolic extract (SMCM) of leaf, seed and central- fruit–axis were used for preliminary qualitative screening of phytochemicals such as alkaloids (Dragendorff test & Mayer's test), tannins (Ferric chloride test), flavonoids (HCL test and Lead acetate test[18], glycosides (Fehling's test and Glacial acetic acid test), terpenoids and steroids (H_2SO_4 test), saponins (Foam test), fixed oil (Spot test), amino acids and proteins (Ninhydrin test and copper sulphate test) and terpenes (Liberman-Burchard) and tannins [19].

2.4 Organisms

In the present study, two bacteria viz., *Staphylococcus aureus (gram$^+$) and E. coli (gram$^-$)* and three fungi viz., *Fusarium sp, Helminthosporium sp and Alternaria sp* were used. The test microbes were collected from Microbiology Lab, Plant Protection Division, Institute of Forest Genetics and Tree Breeding, Coimbatore. The *S. aureus* and *E. coli* were cultured overnight at 37 °C on nutrient agar medium. The bacterial isolate from the agar plate was cultured on nutrient broth. In the same way, fungi were cultured 4 to 7 days at 37 °C on PDA broth. The test strains were maintained on separate agar and subcultured regularly (every 30 days) and stored at refrigerator following standard method [20].

2.5 Preparation of concentrations of crude extract

The crude extracts of each part of *Swietenia macrophylla* King. were dissolved in water using dimethyl sulfoxide (DMSO) in order to prepare the dilute solutions [19]. Stock solutions of 20mL of 1% were prepared from the 200 mg of extracts in 20mL of water solvent (19ml H_2O + 1 ml DMSO). The stock solution then serially diluted with water to prepare further concentrations viz., 20, 30, and 50 µg mL^{-1}. Finally, these test concentrations were stored in labelled specimen bottles for further antimicrobial activities bioassay.

2.6 Evaluation of antimicrobial activity

The antibacterial efficacy of *S. macrophylla* extracts was tested against *S. aureus* and *E. coli* with agar well diffusion method [21]. Briefly, 24-hours old broth cultures of test bacteria were swabbed on sterile nutrient agar plates using sterile cotton swab. Then, 6mm wells were punched on the agar using sterilised well dispenser. Different extracts with different concentrations were tested against these bacteria by carefully added the respectively labelled wells at fixed volume of 30µl per well. The plates were incubated at 37 °C for 24 hours in upright position, and the diameter of zone of inhibition was measured and recorded in millimetres. A control (without plant extract) was also maintained along test samples.

Potato Dextrose Agar medium was prepared and poured onto petriplates. A fungal plug was placed in the middle of plate. The crude extracts of leaf, seed and central-fruit-axis were poured into the wells (6mm) @ 30µl. The DMSO was used as negative control. Also a control was maintained without any extract. The plates were incubated at 37 °C for 3 to 4 days. The development of crescent shaped inhibition zone has shown antifungal activity of the extract. The diameter of inhibition zone was measured and recorded. Triplicates were maintained in each test. The per cent of inhibition was calculated with following method [22]:

$$\text{Inhibition (\%)} = \frac{(C-S)}{C} \times 100$$

Where, S – Absorbance of sample; C – Absorbance of control

2.7 Statistical analysis

Three replicates of each sample were used for statistical analysis and the values are reported as mean ± SD (n=3).

3. Results and Discussion

3.1 Phyto-chemicals screening test

The qualitative analyses of bioactive compounds for the three crude extracts of *S. macrophylla* have been analyzed in this study and there is wide range of phytochemical compounds present in these extracts given in the Table 1. The data reveals that significantly positive results were found for alkaloids, terpenoids and carbohydrate in both methanolic seed and leaf extracts. The significantly high tannin content was found in leaf and central-fruit axis extract. It is found that flavonoid was present in seed and central-fruit-axis while the saponin was found in seed and leaf extract. The positive result for glycosides and fixed oil was found in leaf and seed, respectively. Steroids and Amino acids have shown $^+$ve results in all extracts. Thus, the results of present study are comparable with that of others.

Table 1: Phyto-chemicals in the crude extracts of *S. macrophylla*

Sl No	Phyto-constituent	Leaf	Seed	Central-fruit-axis
1	Alkaloids	++	++	+
2	Tannins	++	+	++
3	Steroids	+	+	+
4	Terpenoids	++	++	-
5	Flavonoids	_	+	+
6	Saponins	+	+	-
7	Carbohydrate	++	++	-
8	Glycosides	+	-	-
9	Amino acid and Proteins	+	+	+
10	Oil	-	+	-

(- negative; + positive; ++ significantly positive)

3.2 Studies on anti-bacterial activity

The antimicrobial activities of the crude extracts of *S. macrophylla* were evaluated *in-vitro* using well diffusion method. The results revealed that the aqueous extracts of *S. macrophylla* had shown strong antibacterial activity on *S. aureus*, and *E. coli*. The zone of inhibition increases with increasing concentrations of the crude extract. The highest antibacterial activity was found at 50µg mL^{-1} concentration of the extracts against target organisms (Fig. 1- 6). Among crude extracts, the methanolic seed extract has shown highest inhibition zone with mean diameter of 27mm and 40.6mm on *S. aureus* and *E.coli*, respectively followed by leaf extract and central-fruit-axis (Table 2). The results of this study have a general agreement with that of earlier investigations [20, 23, 24].

Table 2: Anti-bacterial activity of *S. macrophylla* extracts

Plant parts	Inhibition zone (mean ± SE)		
	Concentration (µg/ml)	***Staphylococcus aureus*** (mm)	***Escherichia coli*** (mm)
Leaf	50	14.8±1.0	34.6±1.0
	30	10.5±0.5	20.3±1.5
	20	7.8±0.2	11.6±0.2
Seed	50	27.0±1.0	40.6±1.5
	30	19.3±0.5	31.0±1.0
	20	14.1±1.0	21.8±1.6
Central –fruit-axis	50	14.5±0.5	29.2 ±1.0
	30	11.2±0.8	11.5±0.5
	20	0.0±0.0	6.8±1.0
Control (DMSO)		-	-

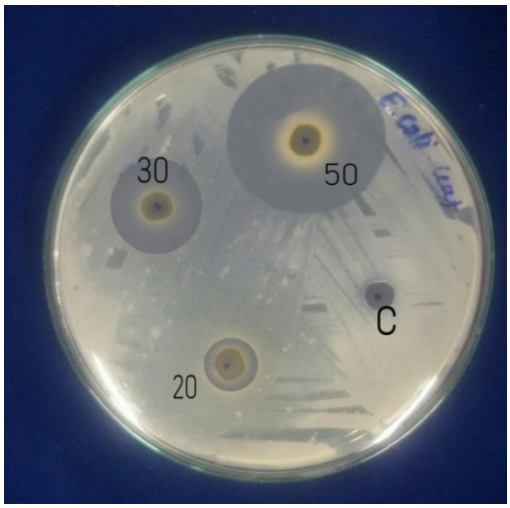

Fig 1: *E.coli* growth in leaf-extract

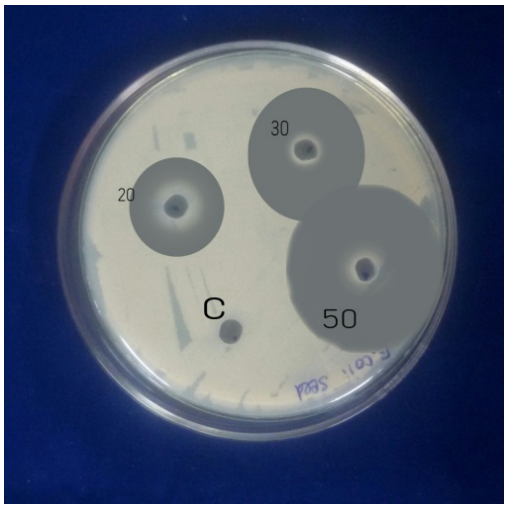

Fig 2: *E.coli* growth in seed-extract

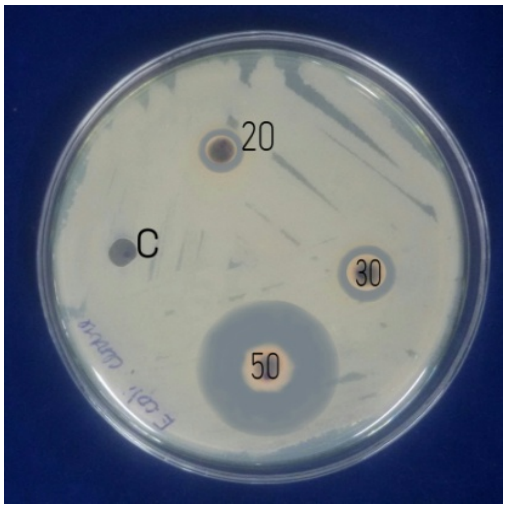

Fig 3: *E. coli* growth in central-fruit-axis

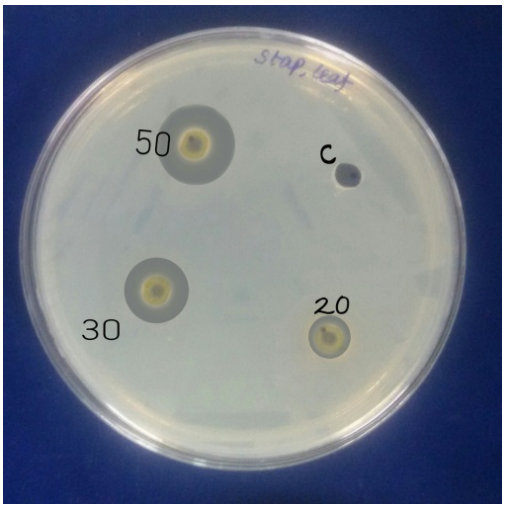

Fig 4: *S. aures* growth in leaf-extract

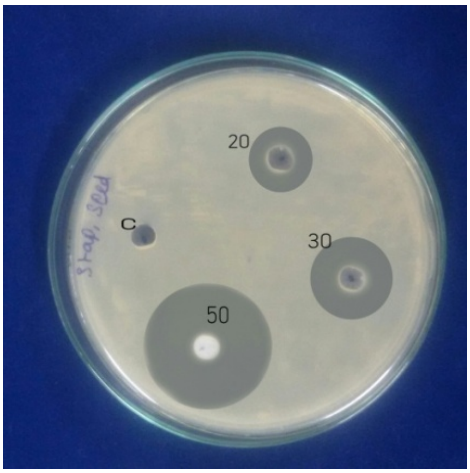

Fig 5: *S. aures* growth in seed-extract

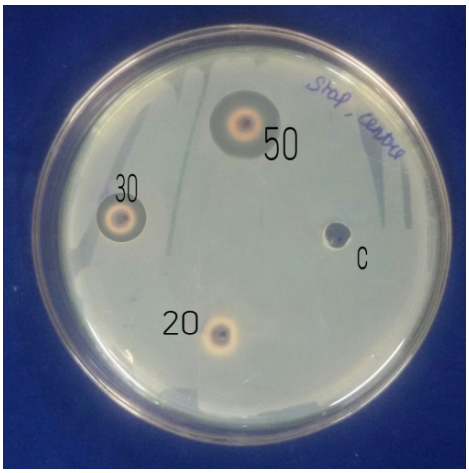

Fig 6: *S. aures* growth in central-fruit-axis

3.3 Evaluation of anti-fungal activities of crude extracts of *S. macrophylla*

The Table 3 on antifungal activity revealed that the methanolic mahogany extracts had shown positive effect against growth of fungi viz., *Fusarium sp*, *Alternaria sp* and *Helminthosporium sp* with significant inhibition zone at concentration starting from 20µl/mg to 50µl/mg. Among mahogany extracts, the seed extract was strongly inhibit growth of *Fusarium sp, Alternaria sp* and *Helminthosporium sp*, followed by leaf and central – fruit-axis extract. The antifungal activity of mahogany extract increases with concentration increase against all tested fungi (Fig 7 - 16). Thus, the seeds of *S. macrophylla* possessed marked antifungal activity. The results obtained from these studies exhibited that various parts of mahogany plant contained bioactive compounds which possess antimicrobial properties against external bacterial and fungal strains [14, 20, 23].

Table 3: Anti-fungi activity of *S. macrophylla* extracts

Type of plant extract	Inhibition zone (mean ± SE)			
	Concentration (mg/ml)	*Fusarium sp* (%)	*Alternaria sp* (%)	*Helminthosporium sp* (%)
Leaf	50	73.3 ± 20.5	72.0 ± 2.0	57.6 ± 2.0
	30	60.3 ± 3.5	60.0 ± 3.0	46.0 ± 2.0
	20	53.6 ± 1.5	51.0 ± 2.0	35.3 ± 0.5
Seed	50	75.3 ± 2.0	76.0 ± 2.0	63.3 ± 2.8
	30	54.0 ± 4.0	52.6 ± 1.5	37.0 ± 2.0
	20	43.3 ± 2.0	42.6 ± 2.5	29.3 ± 2.5
Central - fruit-axis	50	64.3 ± 0.5	59.3 ± 5.6	68.3 ± 0.5
	30	48.6 ± 1.5	51.3 ± 2.0	42.6 ± 3.0
	20	42.6 ± 2.5	43.3 ± 1.5	30.0 ± 1.0
Control (DMSO)		-	-	-

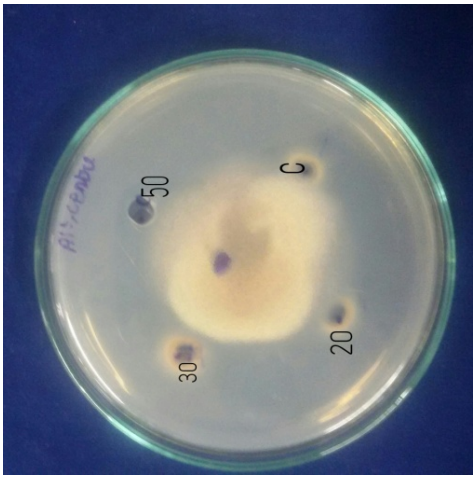

Fig 7: *Alternaria* growth in DMSO (control)

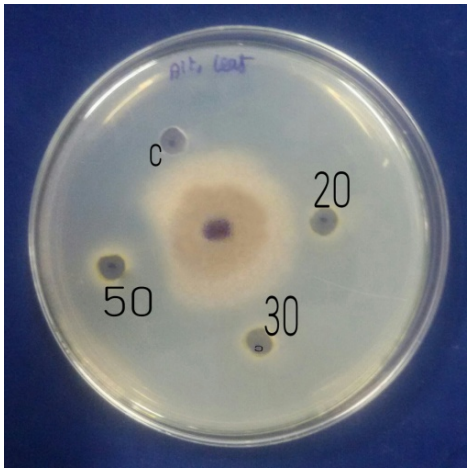

Fig 8: *Alternaria* growth in leaf-extract

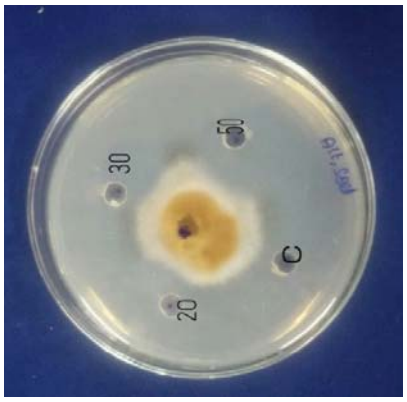

Fig 9: Alternaria growth in seed-extract

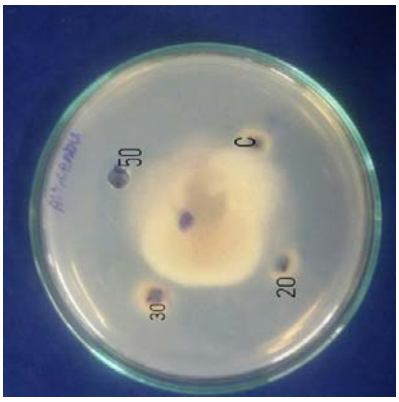

Fig 10: Alternaria growth in Central-fruit-axis

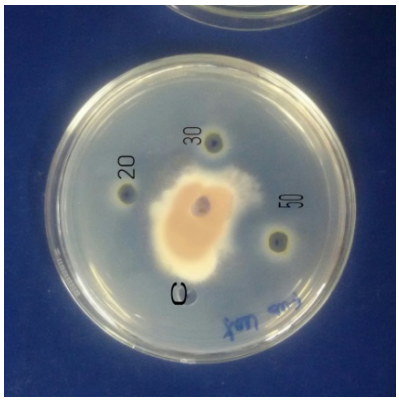

Fig 11: Fusarium growth in leaf -extract

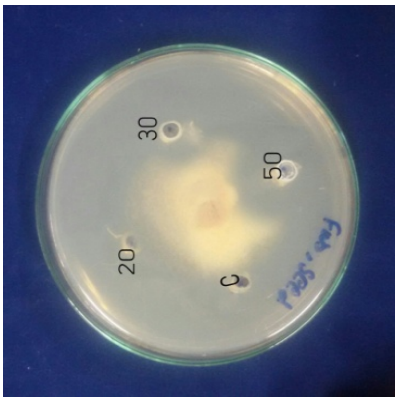

Fig 12: Fusarium growth in seed-extract

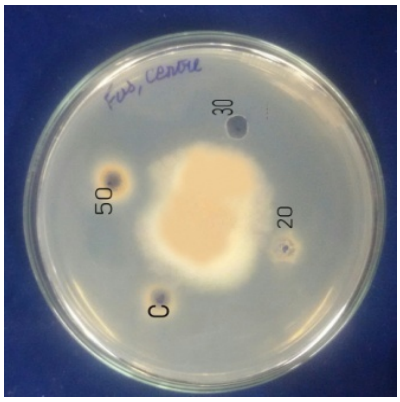

Fig 13: *Fusarium* growth in central- axis-extract

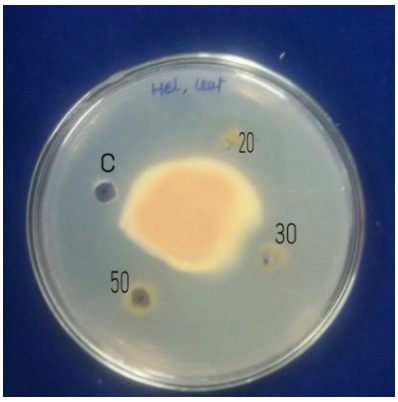

Fig 14: Helminthosporium growth in leaf- extract

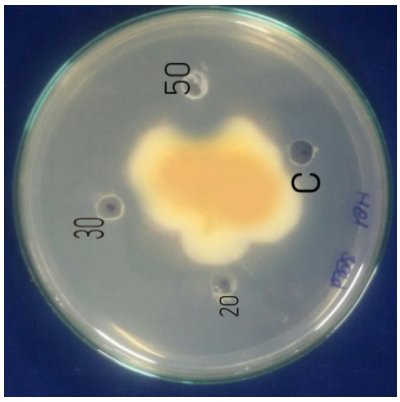

Fig 15: *Helminthosporium* sp in seed extract

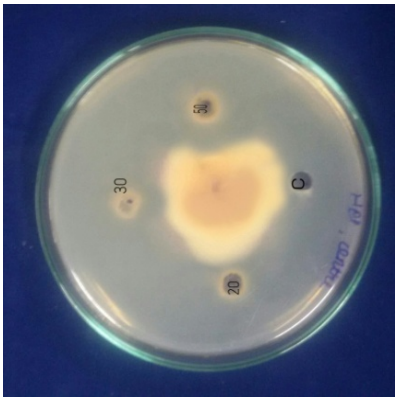

Fig 16: *Helminthosporium* sp in central – axis-extract

In the present study, a linear relationship was found between antimicrobial activities and extracts concentrations, indicating that phytochemical compounds especially limonoids and tetranortriterpenoids could be major contributors to antimicrobial activities. The antimicrobial activity of these extracts had shown moderate against bacteria and strong against fungi. Therefore, it can be concluded that SMCM leaf, central-fruit-axis and seed extracts possess a broad spectrum of activity against bacteria and fungi. These promising extracts open the possibility of finding new clinically effective antimicrobial compounds. Further purification of the active compounds and *in vivo* evaluation of antimicrobial activity along with toxicity studies of these extracts of *S. macrophylla* are therefore suggested for further studies.

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