

# Acute Toxicity, Antimicrobial Activity, Fatty Acid Analysis and Isolation of Swietenolide from *Swietenia macrophylla* King. Seeds

Hnin Thanda Aung  
University of Mandalay  
hningthandaung07@gmail.com

Thet Nu Nge  
University of Mandalay  
thetnunge.mdy@gmail.com

## Abstract

The present study aims to evaluate phytochemical, antimicrobial and acute toxicity study, mineral contents, fatty acids components and isolation of compound of *Swietenia macrophylla* King. The phytochemical analysis by standard methods revealed that all tested phytochemical constituents except tannins were present in *Swietenia macrophylla* seeds. The seeds contain potassium with the highest amount of 0.8049% analyzed by Energy Dispersive X-ray Fluorescence Spectrometry. The ethyl acetate and ethanolic extracts showed low activity against tested microorganisms except *Pseudomonas aeruginosa* determined by agar well diffusion method. The acute toxicity test by OECD guide line showed that extract had non-toxic effect on doses of 2500 mg/kg. The fatty acids analysis by GC-MS exhibited the presence of high level of oleic acid in seeds. Furthermore, swietenolide and fatty acid fraction were isolated from *Swietenia macrophylla* seeds by silica gel column chromatography. The structure of swietenolide was confirmed by Fourier-Transform Infrared assignments and its melting point.

**Keywords** - preliminary, phytochemical, antimicrobial, pharmacological, mineral

## 1. Introduction

Plants are one of important sources of medicine and play a main role in development of new drugs. Many countries especially developing countries rely on herbal medicine for their primary health care. The therapeutic property of medicinal plant is due to the presence of bioactive compounds such as flavonoids, alkaloids, tannins, terpenoids and steroids. Medicinal plants having antimicrobial activity are essential for the development of new antimicrobial drugs. Therefore, it is important to discover safe and inexpensive antimicrobial agents from natural sources especially plants [6]. Minerals in plants are essential for good health. They help our bodies develop and function. Unsaturated fatty acids in plants are main component of a healthy diet. They reduce the risk of heart disease and lower cholesterol levels. The medicines of plant sources are expected to have very less toxicity [1]. However, medicinal plants utilized in folk medicines have been reported to show some extent of toxic effects [7]. Therefore, it is important to have proper chemical, toxicological, and safety data for the usage of plants

with traditional claims on health benefits. *Swietenia macrophylla* is widely distributed in 40 countries including in Asia and other Central American countries. Mahogany seeds increase blood circulation and lower blood pressure and have anti-inflammatory effects. The present study is aimed at evaluating the preliminary phytochemical constituents, mineral contents and fatty acid composition, isolation of pure compound, antimicrobial activity and acute toxicity study of *Swietenia macrophylla* seeds.

## 2. Materials and Methods

### 2.1. Plant Materials and Chemicals

*Swietenia macrophylla* King. seeds (Mahogany) were collected from Theingyi Market, Lanmataw Township, Yangon. The dried materials were ground in a mortar and stored in bottles until analysis. Chemicals and solvents were bought from Academy chemical shop, Yangon. Column chromatography was performed on silica gel (BW-820 MH, Fuji Silysia, Aichi, Japan).

### 2.2. Phytochemical Screening

Phytochemical screening was performed by standard methods [5] and each of the tests was quantitatively expressed as negative (-) or positive (+).

### 2.3. Analysis of Mineral Elements

The mineral compositions of the seed powder of *Swietenia macrophylla* were recorded on spectro XEPOS Energy Dispersive X-ray Fluorescence (EDXRF) Spectrometa, Germany.A.

### 2.4. Antimicrobial Activities of *Swietenia macrophylla* Seeds

Antimicrobial activities of *Swietenia macrophylla* seeds were tested in three different extracts (n-hexane, ethyl acetate and ethanol) by using agar well diffusion method against six selected organisms such as *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus pumilus*, *Candida albicans* and *Escherichia coli* at Development Center of Pharmaceutical Technology (DCPT), Insein, Yangon.

## 2.5. Acute Toxicity Study

The air dried samples (500 g) of *Swietenia macrophylla* seeds were extracted with ethanol for one month. After evaporation in vacuo, dried extract was obtained. The acute toxicity of the extract of *Swietenia macrophylla* was performed according to OECD guide line 425 [4]. This experiment involves the use of six groups of mice, which has five animals each. Both sexes of healthy Albino ICR strain mice (22 g) were selected and kept in their cages for at least five days prior to experiment to allow the acclimatization for laboratory conditions. The animals were fasted for 18 hours but were allowed with free access to water. Following period of fasting, mice were weighed and test extract was administered orally at a dose of 500 mg/kg, 1000 mg/kg, 1500 mg/kg, 2000 mg/kg and 2500 mg/kg. Distilled water was orally given to the control group. Mice were observed after dosing at least once during the first 30 minutes, periodically during the first 24 hours with special attention given during the first 4 hours and daily up to 10 days. Signs of toxicity and mortality of the mice were recorded. At the end of the test, the mice were weighed.

## 2.6. Isolation of Swietenolide from *Swietenia macrophylla* seeds

The seeds of *Swietenia macrophylla* (800 g) were percolated with ethanol (1L) for one month. The whole mixture was then filtered through filter paper and the filtrate was evaporated in vacuo at room temperature to provide (26.7 g) of dried extract. The ethanolic extract was extracted with ethyl acetate to give ethyl acetate extract (20.5 g). 1.8 g of The ethyl acetate extract was fractionated over a silica gel column, using the eluents, n-hexane : ethyl acetate (n-hexane only, 19:1 - 1:4, then ethyl acetate only) to afford 274 fractions. The fractions were combined on the basis of their TLC profiles to give 18 combined fractions (Fra-1 to Fra-18). The combined fractions (Fra-12 and Fra-18) showed one spot on TLC under UV lamp with iodine vapour. The combined fractions (Fra-12 and Fra-18) afforded white crystals of pure compound (17.3 mg). Combined fraction (Fra-1) (367.8 mg) was used for analysis of fatty acid components.

## 2.7. Analysis of Fatty Acid Components in *Swietenia macrophylla* Seeds by GC-MS

Combined fraction (Fra-1) containing fatty acids was esterified by methylation with diazomethane. The GC-MS-QP-2010 Plus equipped with Rth-5MS capillary column 100°C to 260°C at 10°C/min. The carrier gas was helium at a pressure of 92.3 Kpa. Injection volume was 0.1 µl. The MS ion source was 70 eV.

## 2.8. FT IR Assignments and Melting Point Determination

FT IR spectrum of pure compound was recorded on FT IR-410 spectrophotometer at the Department of Chemistry, University of Mandalay. The melting point of the pure compound in crystals form was determined by Stuart SMP 30 melting point apparatus.

## 3. Results and Discussion

### 3.1. Botany Description of *Swietenia macrophylla* King.

Family	Melicaceae
Botanical name	<i>Swietenia macrophylla</i> King.
English name	Mahogany
Burmese name	Mahogany
Part used	Seeds



Figure 1. The plants and seeds of *Swietenia macrophylla* King.

### 3.2. Phytochemical Screening

The phytochemical investigation revealed the presence of alkaloids, flavonoids, terpenes, sterols, glycosides, reducing sugars, lipophilics, polyphenols, saponins and phenolic compounds in the seeds extract of *Swietenia macrophylla* (Table 1).

Table 1. Phytochemical constituents of *Swietenia macrophylla*

No	Constituents	Observation	Result
1	Alkaloids	Orange ppt	+
2	Flavonoids	Reddish pink color solution	+
3	Terpenes	Pink color solution	+
4	Sterols	Blue color solution	+
5	Glycosides	White ppt	+
6	Reducing sugars	Bricked red color ppt	+
7	Lipophilics	Deep color solution	+
8	Polyphenols	Greenish blue color solution	+
9	Saponins	Formation of froth	+
10	Phenolic compounds	Purplish color solution	+

### 3.3. Analysis of Mineral Elements

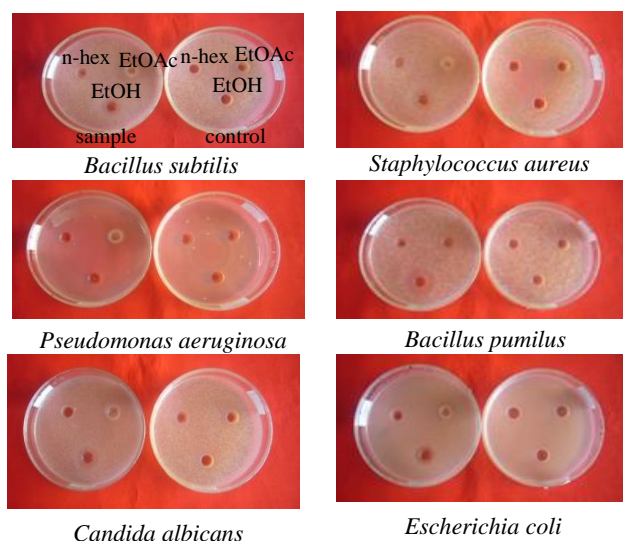
The mineral contents in the seeds powder of *Swietenia macrophlla* were potassium, phosphorus, chlorine, calcium, aluminium, sulfur, iron, titanium, rubidium, manganese, zinc, vanadium, copper and strontium (Table 2). The highest amount of potassium (0.8049%) was found in seeds powder of *Swietenia macrophlla*.

**Table 2. Minerals in *Swietenia macrophlla* seeds**

No	Elements	Results (mass %)
1	Potassium	0.8049
2	Phosphorus	0.3689
3	Chlorine	0.3578
	Calcium	0.3295
4	Aluminium	0.0606
5	Sulfur	0.05797
6	Iron	0.00809
7	Titanium	0.00624
8	Rubidium	0.00546
9	Manganese	0.00362
10	Zinc	0.00286
11	Vanadium	0.00276
12	Copper	0.00183
13	Strontium	0.00121

### 3.4. Antimicrobial Activities of *Swietenia macrophlla* Seeds

According to the antimicrobial experiment (Fig. 2), the ethyl acetate extract showed low activity against *Bacillus subtilis*, *Staphylococcus aureus*, *Bacillus pumilus*, *Candida albicans* and *Escherichia coli*. The ethanolic extract exhibited low sensitivity against all tested organisms except *Pseudomonas aeruginosa*. The n-hexane extract did not inhibit the growth of all tested organisms (Table 3).



**Figure 2. Antimicrobial activities of *Swietenia macrophlla* seeds**

**Table 3. Antimicrobial activities of three different extracts of *Swietenia macrophlla* seeds**

Test Organisms	Inhibition zone (mm)		
	n-hexane	EtOAc	MeOH
<i>Bacillus subtilis</i>	-	14 (+)	12 (+)
<i>Staphylococcus aureus</i>	-	14 (+)	11 (+)
<i>Pseudomonas aeruginosa</i>	-	-	-
<i>Bacillus pumilus</i>	-	13 (+)	11 (+)
<i>Candida albicans</i>	-	14 (+)	11 (+)
<i>Escherichia coli</i>	-	14 (+)	12 (+)

### 3.5. Acute Toxicity Study

The acute toxicity test was carried out by giving different doses of the ethanolic extract of *Swietenia macrophlla* (500 mg/kg, 1000 mg/kg, 1500 mg/kg, 2000 mg/kg and 2500 mg/kg) to the group of mice. Mice were kept under observation for 10 days. For the doses of 500 mg/kg, 1000 mg/kg, 1500 mg/kg, 2000 mg/kg and 2500 mg/kg on group of mice, the symptoms such as diarrhoea, inactivity, restlessness, aggressiveness, eye-dullness, breathing abnormalities, loss and obvious changes of body weight etc., were not observed. All groups of mice showed neither any toxic effect nor any lethal effect. Therefore, the test sample showed free from acute toxicity effect up to the dose of 2500 mg/kg and can be considered relatively safe.

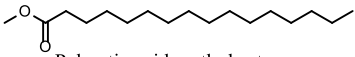
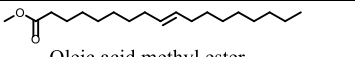
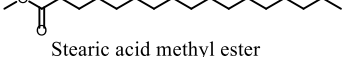
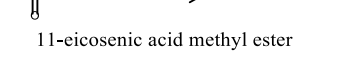
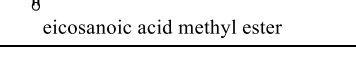
### 3.6. Isolation of Swietenolide and fatty acid fraction from *Swietenia macrophlla* Seeds

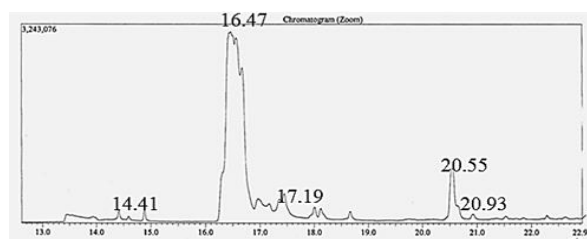
17.3 mg (white crystals) of pure compound was isolated from the seeds of *Swietenia macrophlla* by silica gel column chromatography. The yield percentage of pure compound was 0.2439% based upon ethyl acetate extract (1.8 g). 367.8 mg of Fra-1 containing fatty acids was fractionated by *Swietenia macrophlla* seeds.

### 3.7. Fatty Acid Analysis in *Swietenia macrophlla* Seeds by GC-MS

The fatty acids such as palmitic acid, oleic acid, stearic acid, 9,12-octadecadienoic acid, 11-eicosanoic acid and eicosanoic acid (Table 4) were detected in the seeds of *Swietenia macrophlla*. The *Swietenia macrophlla* seeds comprised high level of oleic acid in comparison with other fatty acids. Oleic acid is a monounsaturated fat in human diet. Monounsaturated consumption has been associated with low-density lipoprotein (LDL) cholesterol and also reduces inflammation.

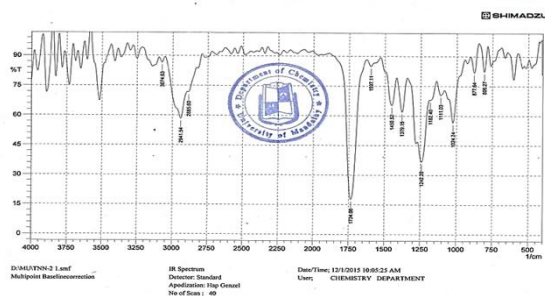
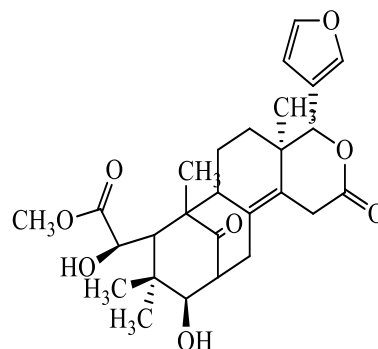
**Table 4. Methyl esters of fatty acids components in *Swietenia macrophlla* seeds**

Structure	Retention time (min)
 Palmitic acid methyl ester	14.41
 Oleic acid methyl ester	16.47
 Stearic acid methyl ester	17.19
 11-eicosenic acid methyl ester	20.55
 eicosanoic acid methyl ester	20.93

**Figure 2. Chromatogram of fatty acid analysis in *Swietenia macrophlla* seeds**

### 3.8. FT IR Assignments and Melting Point Determination

According to FT IR spectrum (Fig. 4), pure compound contains  $\text{-OH}$  group ( $\nu_{\text{OH}}$  3464.27  $\text{cm}^{-1}$ ),  $\text{sp}^2$  hydrocarbon ( $\nu$  3070.78  $\text{cm}^{-1}$ ),  $\text{sp}^3$  hydrocarbon ( $\nu_{\text{as}}$  2949.26,  $\nu_{\text{s}}$  2858.60  $\text{cm}^{-1}$ ), carbonyl groups of ester, ketone and lactone (overlapped) ( $\nu_{\text{C=O}}$  1760 -1700  $\text{cm}^{-1}$ ),  $\text{C}=\text{C}$  alkenic group ( $\nu_{\text{C=C}}$  1502.60  $\text{cm}^{-1}$ ), ether group ( $\delta$  1026.16  $\text{cm}^{-1}$ ) and trans or *E* alkenic group ( $\delta$  910.43 and 879.57  $\text{cm}^{-1}$ ) respectively. The melting point of pure compound was 174-178°C and well-matched with the literature [2]. According to FT IR assignments and melting point determination, pure compound was identified as *swietenolide* (Fig. 5).

**Figure 4. FT IR spectrum of *swietenolide* compound****Figure 5. Structure of *swietenolide* compound**

The Mahogany seeds powder reduces cholesterol, regulates blood sugar and helps to increase the immune system [3]. The seeds have been reported to have anti-inflammatory, anti-mutagenic anti-diabetics and anti-tumor activities [3]. The several health benefits of *Swietenia macrophlla* seeds are due to the presence of various phytochemical constituents in it. The high content of potassium in seeds powder of *Swietenia macrophlla* helps to reduce blood pressure. Fatty acids analysis by GC-MS showed that Mahogany seeds contain monounsaturated acids such as oleic acid, stearic acid, 11-eicosenic acid. Monounsaturated fats have a number of health benefits. They can help to lose weight, reduce the blood cholesterol level and risk of the heart disease and decrease the inflammation. The antimicrobial experiment of the extract of *Swietenia macrophlla* did not show any significant effect against six selected microorganisms. Several studies were done on toxicity analysis in order to investigate the safeness of the traditional use of medicinal plants. The present study shows administration of doses up to 2500 mg/kg did not exhibit any signs of toxicity or mortality on mice during the observation period. Therefore, lethal dose,  $\text{LD}_{50}$  of seed extract of *Swietenia macrophlla* may be considered to be greater than 2500 mg/kg. Thus, plant extract even at 2500 mg/kg may be considered as safe. However, the Mahogany seeds must be further studied for better understanding of their medicinal properties, safety and effectiveness.

### 4. Conclusion

The phytochemical analysis indicates that the seeds of this plant contain all tested phytochemicals except tannins. The three different extracts of *Swietenia macrophlla* seeds were found to have low or no antimicrobial activity against six microorganisms. The pure compound, *swietenolide* was isolated from *Swietenia macrophlla* seeds and it possesses limonin type of terpenoid compound. In the case of acute toxicity test,  $\text{LD}_{50}$  of seed extract of *Swietenia macrophlla* may be considered to be greater than 2500 mg/kg. However, further toxicity studies are required to confirm the long term effect and safe use of the seed extract. The results from this study suggest that *Swietenia macrophlla* seeds contain high content of

potassium and mono-unsaturated fatty acids and had non-toxic effect. Thus, it could be applicable for pharmacological preparations. The results of present study support the scientific evidence to the use of *Swietenia macrophylla* seeds.

## Acknowledgement

The authors are grateful to Dr Yi Yi Myint, Head of Department of Chemistry, University of Mandalay for her valuable suggestions.

## References

- [1] A. Ramzi, A. Mothana, M. Renate, R. Christiane, and L. Ulrike, "Phytochemical screening and antiviral activity of some medicinal plants from the Island Soqatra," *Phytother. Res.*, 2006, vol. 20, pp. 298–302.
- [2] H. Azhari, H. N. Mohammed, B. S. Mashitah, M. Y. Abdurahman, H. and N. M. Adam, "Bioassay-guided Isolation and Identification of Antifungal Compounds from Seeds of *Swietenia Macrophylla* King," 2016, vol. 10 (17), pp.55-62.
- [3] K. C. Chan, T. S. Tang, and H. T. Toh, "Isolation of swietenolide diacetate from *Swietenia macrophylla*," *Phytochemistry*, 1976, vol. 15, pp. 429–430.
- [4] OECD" OECD Guide line for testing of chemicals. Acute oral toxic class method, guide line no. 423. Adopted 2001 Organization for Economic Cooperation and Development, Rome 2001.
- [5] P. Tiwari, B. Kumar, M. Kaur, G. Kaur, and H. Kaur, "Phytochemical screening and extraction: a review," *International Pharmaceutica Scientia.*, 2011, vol. 1(1), pp. 98-10.
- [6] S. Koduru, D. S. Grierson, and A. J. Afolayan, "Antimicrobial activity of *Solanum aculeastrum*," *Pharm. Biol.*, 2006, vol. 44, pp. 283–286.
- [7] V. Ertekin, M. A. Selimoğlu, and S. Altinkaynak, "A combination of unusual presentations of *Datura stramonium* intoxication in a child: rhabdomyolysis and fulminant hepatitis," *J. Emerg. Med.*, 2005, vol. 28, pp. 227–228.