

# INVESTIGATION ON SOME PHYTOCONSTITUENTS AND ANTIMICROBIAL ACTIVITY OF FLOWERS OF

*Markhamia Stipulata* (Wall.) Seem. (Mahlwa)

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## Abstract

The aim of the present study was to investigate the presence of phytochemical constituents and antimicrobial activity of flowers of *Markhamia stipulata* (Wall.) Seem. (Mahlwa). The preliminary phytochemical investigation indicated that alkaloids,  $\alpha$ -amino acid, carbohydrates, glycosides, organic acids, phenolic compounds, reducing sugars, saponins, steroids, tannins and terpenoids were present while cyanogenic glycosides, flavonoids and starch were absent by using the standard methods. The various crude extracts such as PE (0.66%), EtOAc (1.81%), 95% EtOH (9.19%) and H<sub>2</sub>O (2.83%) were prepared by successive solvent extraction method. By column chromatographic separation technique, one steroid compound (I, 0.0034% yield, pale green powder form, M.pt=245 °C) was isolated from EtOAc crude extract of flowers of Mahlwa. Based on FT IR spectral data, the functional groups of compound I contained hydroxyl, carbonyl and olefinic groups due to the presence of the absorption bands appeared at 3442 cm<sup>-1</sup>, 3060 cm<sup>-1</sup>, 2850 cm<sup>-1</sup>, 1691 cm<sup>-1</sup>, 1458cm<sup>-1</sup>, 1386 cm<sup>-1</sup>, 1267 cm<sup>-1</sup>, 1157 cm<sup>-1</sup>, 1095 cm<sup>-1</sup>, 1031 cm<sup>-1</sup>, 997 cm<sup>-1</sup> and 655 cm<sup>-1</sup> respectively. In the screening of antimicrobial activity of crude extracts determined by agar well diffusion method, 95% EtOH extract (inhibition zone diameter range= 14~15 mm), PE extract (inhibition zone diameter range= 11~13 mm), MeOH extract (inhibition zone diameter range= 15~17 mm), and EtOAc extract (inhibition zone diameter range= 15~20 mm) showed inhibitory effect on tested microorganisms. However, the watery extract did not exhibit antimicrobial activity.

**Keywords** : Mahlwa, *Markhamia stipulata* (Wall.) Seem.,  
phytochemical constituents, antimicrobial activity

## Introduction

Plants plays an important role in the health services as the source of medicine around the globe. Higher plants have a rich source of secondary metabolites with interesting biological activities (Kunwaret *et al.*, 2008). Drugs from the plants are easily available, less expensive, safe and efficient and rarely have side effects. Medicinal plants contain some organic compounds which provide definite physiological action on the human body and these bioactive substances include tannins, alkaloids, carbohydrates, terpenoids, steroids and flavonoids (Edoga *et al.*, 2005). *Markhamia stipulata* is a species of plant in the Bignoniaceae family. This species can be found in sparse forests and humid places at elevations from 300 m to 1700 m, growing in evergreen forest on limestone mountains in southern China. *M.stipulata* species has many potentials. Traditionally, a lot of genus have used *Markhamia* as medicines (Ali, 2011). Medicinal plants are at great interest to the researcher in the field of life science especially biotechnology where most of the pharmaceutical industries depend on the plant parts for the production of pharmaceutical drugs. Hence, the present study focused on phytochemical constituents and antimicrobial activity of the flowers of Mahlwa has been evaluated. The inhibitory effect was assessed by agar well diffusion method.

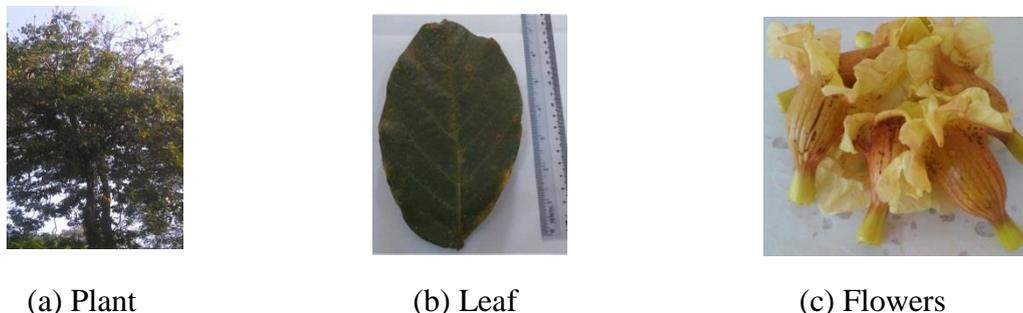
## Taxonomical Classification

Kingdom	: Plantae	Order	: Lamiales
Family	: Bignoniaceae	Genus	: <i>Markhamia</i>
Species	: <i>M.stipulata</i>	Botanical name	: <i>Markhamia stipulata</i> (Wall.) Seem.
Myanmar name	: Mahlwa	Part used	: Flowers

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## Description and Distribution

*Markhamia stipulata* (Wall.) Seem. (Mahlwa) is an evergreen tree, growing up to 25 meters tall. The bark is creamy brown. The leaves are dark green, 14-20 cm long. The flowers are dirty yellow, arranged in a many flowered inflorescence, terminal, bisexual, and pedicels are longer than 0.5 cm corolla with many large (2-3 mm) wart-like glands outside. The fruit has a long pod-like capsule, 45 to 70 cm long, splitting lengthwise into 2 sections. The seed has a long elliptic, with 2 wings. It is distributed in Vietnam, Myanmar, India, Thailand, Laos and China.



(a) Plant

(b) Leaf

(c) Flowers

Figure 1 Photographs of (a) plant (b) leaf and (c) flowers of *Markhamia stipulata* (Wall.) Seem. (Mahlwa)

## Medicinal Uses

The roots, barks, stems and leaves of *Markhamia* species have been used by traditional healers for the treatment of miscellaneous disease conditions such as microbial and parasitic diseases, anemia, diarrhea, backache, sore eyes, intercostal pain, pulmonary troubles, gout, scrotal elephantiasis, rheumatoid arthritis and external skin diseases (Adjanohoun *et al.*, 1996).

## Materials and Methods

### Collection of Plant Material

The flowers of *Markhamia stipulata* (Wall.) Seem. (Mahlwa) were collected from Mawlamyine University Campus, Mawlamyine Township, Mon State. It was taxonomically identified at the Botany Department, Mawlamyine University.

### Preparation of Plant Material

The Mahlwa flowers were cleaned and shade dried. The dried Mahlwa flowers were reduced to fine particle using a grinding machine. Then the dried powdered flowers are separately stored in air-tight containers to prevent moisture and other containments and used for further investigation.

### Phytochemical Analysis Tests

Phytochemical analysis of the isolated fractions from 95% EtOH, water, CHCl<sub>3</sub>, PE and 1% HCl extract of Mahlwa flowers for secondary metabolites such as flavonoids, phenolic compounds,  $\alpha$ - amino acids, carbohydrates, cyanogenic glycosides, glycosides, organic acids, saponins, starch, tannins, reducing sugars, terpanoids, steroids and alkaloids were done using standard methods (Harbone, 1984) (Trease and Evans, 1980) (Marini – Bettolo *et al.*, 1981) (Tin Wa, 1972).

### Preparation of Various Crude Extracts

Some crude extracts such as PE, 95% EtOH, EtOAc and H<sub>2</sub>O extracts from dried powdered of flowers of *Mahlwa* were prepared by successive solvent extraction method.

### Separation of EtOAc Crude Extracts

To isolate some organic constituents by using column chromatography, the various solvent systems were firstly determined by thin layer chromatography.

### Identification of Isolated Compounds

The isolated compound was structurally identified by UV and FT IR spectroscopic techniques.

### Screening of Antimicrobial Activity (Agar Well Diffusion Method)

In this method, ethanol, petroleum-ether, methanol, ethyl acetate and water extracts of *Mahlwa* flowers were used as the sample. Tested microorganisms were *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Candida albicans*, *Bacillus pumilus* and *E-coli*.

## Results and Discussion

### Preliminary Phytochemical Investigation

Phytochemicals are very important in medicine and constitute most of the valuable drugs. The present study reveals that *M.stipulata* flowers exhibited the presence of alkaloids,  $\alpha$ -amino acids, carbohydrates, glycosides, organic acids, phenolic compounds, reducing sugars, saponins, steroids, tannins and terpenoids. However, cyanogenic glycoside, flavonoids and starch were not observed in it. The results were presented in Table 1.

Table1 Results of Phytochemical Investigation of Flowers of *Markhamia stipulate* (Wall.) Seem. (*Mahlwa*)

No.	Test	Extract	Test Reagent	Observation	Presence	Absence
1	Alkaloids	1% HCl	(i) Dragendorff's reagent (ii) Sodium picrate (iii) Wagner's reagent (iv) Mayer's reagent	Orange ppt Yellow ppt Brown ppt White ppt	+	
2	$\alpha$ -amino acids	H <sub>2</sub> O	Ninhydrin reagent	Purple spot	+	
3	Carbohydrates	H <sub>2</sub> O	10% $\alpha$ -naphthol and H <sub>2</sub> SO <sub>4</sub>	Red ring	+	
4	Cyanogenic glycosides	H <sub>2</sub> O	Sodium picrate solution	No brick red colour		-
5	Flavonoids	EtOH	Mg tanning and conc: HCl	No pink colour		-
6	Glycosides	H <sub>2</sub> O	10% lead acetate	White ppt	+	
7	Organic acids	H <sub>2</sub> O	Bromocresol green	Colour change	+	
8	Phenolic compounds	EtOH	1% FeCl <sub>3</sub>	Colour change	+	
9	Reducing sugars	H <sub>2</sub> O	Benedict's solution	Brick-red ppt	+	
10	Saponins	H <sub>2</sub> O	Distilled water	Frothing	+	
11	Starch	H <sub>2</sub> O	1% Iodine solution	No deep blue colour		-
12	Steroids	PE	Acetic anhydride & conc: H <sub>2</sub> SO <sub>4</sub>	Green colour	+	
13	Tannins	H <sub>2</sub> O	1 % Gelatin	Colour change	+	
14	Terpenoids	CHCl <sub>3</sub>	Acetic anhydride & conc: H <sub>2</sub> SO <sub>4</sub>	Colour change	+	

### Various Crude Extracts from Flowers of *Markhamia stipulata* (Wall.) Seem. (Mahlwa)

The percent crude extracts (w/w) prepared from this selected medicinal plant were described in Table 2 and Figure 2. From these results, it was found that the non-polar constituents (PE extracts, 0.66%) of flowers of Mahlwa was obtained in lower amount than the polar components (95% EtOH extract, 9.19%). In all of these crude extracts, moderately polar components (EtOAc extract, 1.81%) was found to be the lowest: EtOAc extract (1.81%), H<sub>2</sub>O extract (2.83%), PE extract (0.66%), EtOH extract (9.19%). All of the above extracts were kept for separation.

Table 2 Yield Percent of Crude Extracts from Flowers of *Markhamia stipulata* (Wall.) Seem. (Mahlwa)

Plant Sample	Extract	Weight (g)	Yield (%)
Mahlwa flower	PE	0.46	0.66
	EtOAc	1.27	1.81
	95 % EtOH	6.43	9.19
	H <sub>2</sub> O	1.98	2.83

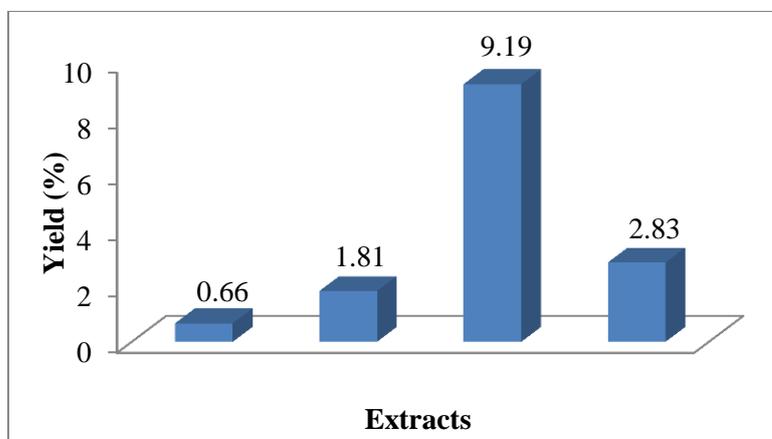


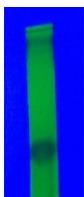
Figure 2 A bar graph showing yield percent of crude extracts from flowers of *Markhamia stipulata* (Wall.) Seem. (Mahlwa)

### Isolation of some organic compounds from EtOAc Crude Extract of Flowers of Mahlwa

From the EtOAc extract of flowers of Mahlwa, one compound (I) was isolated. Compound (I, 2.7 mg, 0.0034% yield) are pale green powder forms. The isolated compound and its TLC chromatogram were shown in Figure 3.



Compound I



under UV 254 nm

$R_f = 0.43$

Solvent system – PE :EtOAc (2:1, v/v)

Figure 3 Photographs of the isolated compound and its TLC chromatogram

### Some Physicochemical Properties of Isolated Compound I

Table 3 Some Physicochemical Properties of Isolated Compound I

Physicochemical properties	Isolated compound I	Remark
	Observation	
Solubility in	PE	-
	EtOAc	+
	EtOH	+
	MeOH	+
	CHCl <sub>3</sub>	+
	H <sub>2</sub> O	-
R <sub>f</sub> value	0.43 (PE:EtOAc = 2:1, v/v)	
Physical state	Pale green powder form	
Yield (%)	0.0034	
M.pt (°C)	245	
Liebermann-Burchard reagent	green colour	∴ It is a steroid

Some physical properties of the isolated compound I was pale green powder forms and it was soluble in ethylacetate, ethanol, methanol and chloroform but insoluble in petroleum ether and water. It also have a deep green color spot ( $R_f = 0.43$ ) on precoated silica gel plate when employed solvent system, PE : EtOAc ( 2:1, v / v). Some chemical properties of the isolated compound I was determined by treating them with Liebermann-Burchard reagent followed by heating. From the results, it was investigated that the isolated compound I may be steroid since it gave green color with Liebermann-Burchard reagent. The observations are summarized in Table 3.

### Study on UV Spectrum of Isolated Compound I

The ultraviolet-visible spectrum of the isolated compound I was measured at the Department of Chemistry, WYU (West Yangon University). The resultant spectrum and the spectral data are shown in Figure 4 and Table 4.

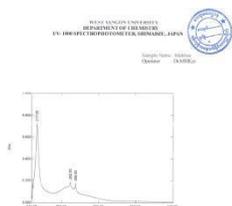


Figure 4 UV spectrum of isolated compound I from EtOAc extract of *Markhamia stipulata* (Wall.) Seem. (Mahlwa)

Table 4 UV-visible Spectral Data of Isolated Compound I

Solvent	$\lambda_{max}$ (nm)	Remark
MeOH	217, 262, 269	$\pi$ - $\pi^*$ transition ∴ C=C group is present

### Study on FTIR Spectrum of Isolated Compound I

The FTIR spectrum of the isolated compound I is shown in Figure 5 and the corresponding spectral data are depicted in Table 5. By applying FT IR spectrophotometer ( IR-Tracer 100 Shimadzu, Japan), at the Department of Chemistry, WYU. From the results, it can be deduced that the functional group of the isolated compound I contained hydroxyl, carbonyl and olefinic groups.

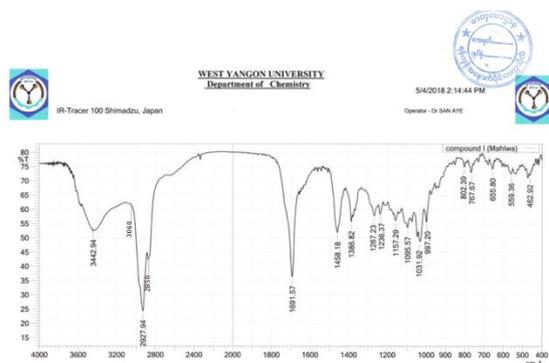


Figure 5 FT IR spectrum of isolated compound I

Table 5 Assignment for FT IR Spectral Data of Isolated Compound I

Significant frequencies (cm <sup>-1</sup> )	Assignment*
3442 (broad)	$\nu_{OH}$ (alcohol)
3060	$\nu_{-CH}$ (olefin)
2927	$\nu_{CH}$ (asymmetric) of CH <sub>3</sub> group
2850	$\nu_{CH}$ (symmetric) of CH <sub>3</sub> group
1691	$\nu_{C=O}$
1458	$\delta_{CH}$ (asymmetric) of CH <sub>3</sub> group
1386	$\delta_{CH}$ (symmetric) of CH <sub>3</sub> group
1267	$\delta_{C-OH}$ (in-plane) (alcohol)
1157, 1095, 1031	$\nu_{C-O}$ (alcohol) (or) (cyclic alcohol)
997	$\delta_{-CH}$ (out-of-plane) (olefin)
655	$\delta_{C-OH}$ (out-of-plane) (alcohol)

### Antimicrobial Activity

In this investigation, the various crude extracts were determined against six species of microorganisms such as *B.subtilis*, *S.aureus*, *P.aeruginosa*, *B.pumilus*, *C.albicans* and *E.coliby* using agar well diffusion method. The larger the inhibition zone diameters, the higher the antimicrobial activity. The inhibition zones of crude extract against six microorganisms tests are shown in Figures 6,7 and 8. The observed data are summarized in Table 6. The Mahlwa flowers of 95% EtOH extract (inhibition zone diameter range = 14 ~ 15 mm) exhibited the highest antimicrobial activity than PE extract (inhibition zone diameter range = 11 ~ 13 mm), MeOH extract (inhibition zone diameter range = 15 ~ 17 mm) and EtOAc extract (inhibition zone diameter range = 15 ~ 20 mm) showed inhibitory effect on tested microorganisms. However, H<sub>2</sub>O extract of Mahlwa flowers did not exhibit antimicrobial activity.

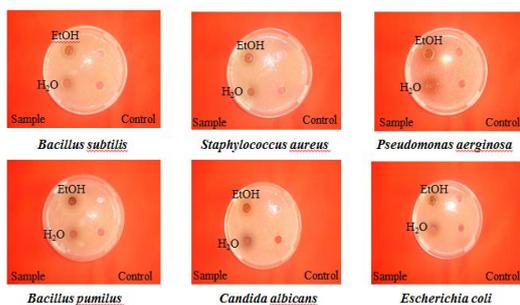
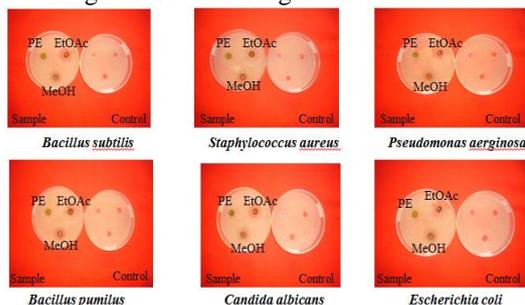
Figure 6 Inhibition zones of EtOH and H<sub>2</sub>O extracts from flowers of Mahlwa against six microorganisms

Figure 7 Inhibition zones of PE, MeOH and EtOAc extracts from flowers of Mahlwa against six microorganisms

Table 6 Inhibition Zone Diameters of Various Crude Extracts of Flowers of Mahlwa against Six Microorganisms

Organism	Inhibition Zone Diameter of Extracts (mm)				
	95 % EtOH	PE	MeOH	EtOAc	H <sub>2</sub> O
<i>B. subtilis</i>	14 (+)	12 (+)	15 (++)	20 (+++)	(-)
<i>S. aureus</i>	15 (+)	13 (+)	17 (++)	-	(-)
<i>P. aeruginosa</i>	15 (+)	11 (+)	-	20 (+++)	(-)
<i>B. pumilus</i>	15 (+)	13 (+)	-	15 (++)	(-)
<i>C. albicans</i>	15 (+)	-	-	20 (+++)	(-)
<i>E. coli</i>	14 (+)	12 (+)	-	20 (+++)	(-)

Diameter of agar well = 10 mm

10mm ~ 14 mm = (+) 20 mm above = (+++)

15mm ~ 19 mm = (++) No activity = (-)

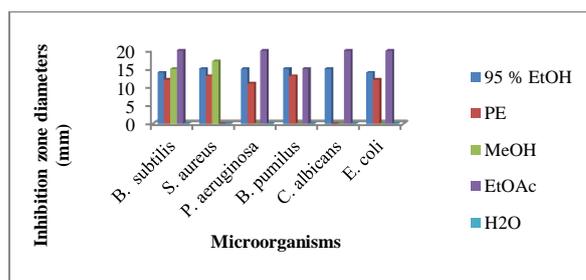


Figure 8 A bar graph of inhibition zone diameters(mm) of various crude extracts from flowers of Mahlwa against six microorganisms

## Conclusion

From the overall assessment of the present research work, the following inferences could be drawn. From the preliminary phytochemical investigate indicated that alkaloids,  $\alpha$ -amino acids, carbohydrates, glycosides, organic acids, phenolic compounds, reducing sugars, saponins, steroids, tannins and terpenoids were present while cyanogenic glycosides, flavonoids and starch were absent by using standard methods. The various crude extracts such as PE (0.66%), EtOAc (1.81%), 95% EtOH (9.19%) and H<sub>2</sub>O (2.83%) were collected from the flowers of *Mahlwa* by soxhlet extract method. From the column chromatographic separation, one steroid compound (I, 0.0034% yield, pale green powder form, M.pt = 245 °C) from EtOAc crude extract of flowers of *Mahlwa* were isolated. Based on FT IR spectral data, the functional groups of compound I contained hydroxyl, carbonyl and olefinic groups due to presence of the absorption bands appeared at 3442 cm<sup>-1</sup>, 3060 cm<sup>-1</sup>, 2850 cm<sup>-1</sup>, 1691 cm<sup>-1</sup>, 1458 cm<sup>-1</sup>, 1386 cm<sup>-1</sup>, 1267 cm<sup>-1</sup>, 1157 cm<sup>-1</sup>, 1095 cm<sup>-1</sup>, 1031 cm<sup>-1</sup>, 997 cm<sup>-1</sup> and 655 cm<sup>-1</sup> respectively. Based on the antimicrobial activity results, it was investigated that 95% EtOH extract (inhibition zone diameter range= 14~15 mm), PE extract (inhibition zone diameter range= 11~13 mm), MeOH extract (inhibition zone diameter range= 15~17 mm), and EtOAc extract (inhibition zone diameter range= 15~20 mm) showed inhibitory effect on tested microorganisms. However, the watery extract did not exhibit antimicrobial activity.

## Acknowledgements

I would like to express my sincere gratitude to Dr Aung Myat Kyaw Sein (Rector), Dr Mie Mie Sein and Dr San San Aye (Pro-Rectors), Mawlamyine University, for their permission on my research paper. I wish to express my sincere gratitude to Dr Daw Mya Thaug and Dr San San Khin (Professors, Department of Chemistry, Mawlamyine University), for their kind encouragement extended to me.

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