

# Isolation and Structural Identification of Echitamine Compound from the Stem Bark of *Alstonia scholaris* R.Br (Taung-ma-yo)

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## Abstract

*Alstonia scholaris* R.Br, commonly known as devil's tree, is an important medicinal plant. In indigenous medicine, the drug of *Alstonia scholaris* R.Br would fully replace quinine in malignant tertian fever. This research work focused on the isolation and structural identification of echitamine compound from the stem bark of Taung-ma-yo. The stem bark of *Alstonia scholaris* R.Br was collected from Pyin-Oo-Lwin Township, Mandalay Region. The preliminary detection of phytochemical compounds present in the sample was carried out by phytochemical tests. The antimicrobial activities of five solvent extracts were investigated by applying Agar-well diffusion method on six organisms. Isolation of echitamine compound may be performed by using column chromatography and checked by TLC. The isolated pure compound, echitamine was confirmed by melting point determination and phytochemical test. The structure of pure compound was also identified by using Fourier Transform Infrared (FT-IR) spectroscopy.

**Keywords:** *Alstonia scholaris* R.Br, Agar-well diffusion, column chromatography, TLC, FT-IR

## Introduction

*Alstonia scholaris* R.Br belongs to "Apocynaceae" family. It is a large evergreen tree with a straight trunk. It is a well-known medicinal plant in Myanmar. This tree is found in Indo-Malaysian region, in sub-Himalayan tract-from Jumna eastward, Ceylon, Malay, Central America, tropical Africa, Australia, Pacific region, Philippines, Southeast Asia, Southern China and ascending to 3000 feet. It is widely cultivated throughout India and abundantly found in Bengal. (Wealth of India, 1948)

It also naturally grows in all parts of Myanmar, especially in the west coast forests and lower Myanmar. Although it grows in abundance in cold regions, it is a warm tree and is cultivated as a shade tree.

The stem bark of Taung-ma-yo is dark-grey in outer and yellow inner, rather rough, thick and exudes a bitter milky sap. When dried, this milky sap becomes dark. The outer bark is always more bitter than the inner bark. The genus contained in the stem bark is a source of a remedy against malaria. The tastes of this tree are bitter, hot and astringent. All parts of the tree contain a milky sap, rich in poisonous alkaloids and possesses medicinal value. (Nagathein Ashin, 1972) (Dr. KM Nadkarni, 1954)

According to literature, chemical constituents of the stem bark of Taung-ma-yo are several alkaloids such as Echitamine or Ditaine, Ditamine, Echitenine, Echitamidine, Alstonine, Porphyrin, Porphyrine, Alsonine and Alstonidine together with Triterpene,  $\alpha$ -Amyrin, Lupeol, Stigma Sterol, Several Fatty Acids and Resinous Substances. (Merck Index, 1960)

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## Botanical Description

Family name : Apocynaceae

Botanical name : *Alstonia scholaris* R.Br

English name : Devil's Tree

Myanmar name : Taung-ma-yo, Taung-me-oke, Let-pan-kha, Seg-gyi

Part used : Stem-bark (J.L.C.H Van Valkenburg, 2002) (WHO Regional Office for the Western Pacific, 1990)



Figure 1. The tree, flower and stem bark of *Alstonia scholaris* R.Br (Taung-ma-yo)

## Aim and Objectives

The aim of this research work is to conduct the isolation and structural identification of echitamine compound from stem bark of Taung-ma-yo.

The objectives of the present research work are;

1. To collect the sample (stem bark of Taung-ma-yo)
2. To carry out phytochemical screening
3. To test the antimicrobial activity by Agar-well diffusion method
4. To percolate and to extract the sample with 95 % ethanol
5. To isolate the alkaloid compound, mainly echitamine by column chromatography and TLC

## Experimental

### Materials and Instruments

#### Materials

Commercial grade reagents and solvents were used for analytical preparative thin layer chromatography and it was performed by using precoated silica gel (Merk,Co. Inc., Kieselgel 60 F<sub>254</sub>). Silica gel (70- 230 mesh ASTM) was used for column chromatography.

#### Instruments

1. FT-IR Spectrometer (Shimadzu, Japan)
2. UV lamp (Lambda - 40, Perkin- Elmer Co., England)
3. The apparatus for extraction and chromatography were used with common laboratory tools.

#### Sample Collection

The stem bark of *Alstonia scholaris* R.Br was collected from Pyin-Oo-Lwin Township, Mandalay Region. This stem bark was cut into small pieces and air dried. The air dried crude sample was stored in air-tight glass bottles to prevent moisture changes, contamination and causing the growth of mould and used throughout the experiment.

### Preliminary Phytochemical Tests

Preliminary phytochemical tests on the stem bark of Taung-ma-yo was carried out by phytochemical methods to indicate the presence of general classes of phytoconstituents. These were identified by characteristic colour changes using standard procedures. (J.B. Harbone, 1984)

### Antimicrobial Activities of Taung-ma -yo

Antimicrobial activities of Taung-ma -yo were checked by Agar-well diffusion method in Microbiological Assay at D.C.P.T, Yangon.

### Extraction of Crude Sample

About 300g of stem bark of Taung-ma-yo sample was percolated with 1500 mL of 95 % ethanol for about two months. During the percolation, the crude mixture was frequently shaken to achieve the maximum extraction of sample. Then the extracted solution was filtered through Whatman No.1 filter paper. The obtained solution was concentrated. This ethanol extract was reextracted with ethyl acetate and checked by TLC plate with various solvent systems to choose the proper solvent system for isolation.

### Isolation of EtOAc Extract by Column Chromatography

#### Materials

Column size : (1.5 x 40) cm  
Adsorbent : Silica gel plate  
Developer : UV and Iodine  
Flow rate : 0.4 mL/min  
Eluting solvent : n-hexane : ethyl acetate

(1) n-hexane only

(2) 19 : 1

(3) 9 : 1

(4) 4 : 1

(5) 7 : 3

(6) 3 : 2

(7) 1 : 1

(8) 2 : 3

(9) 1 : 2

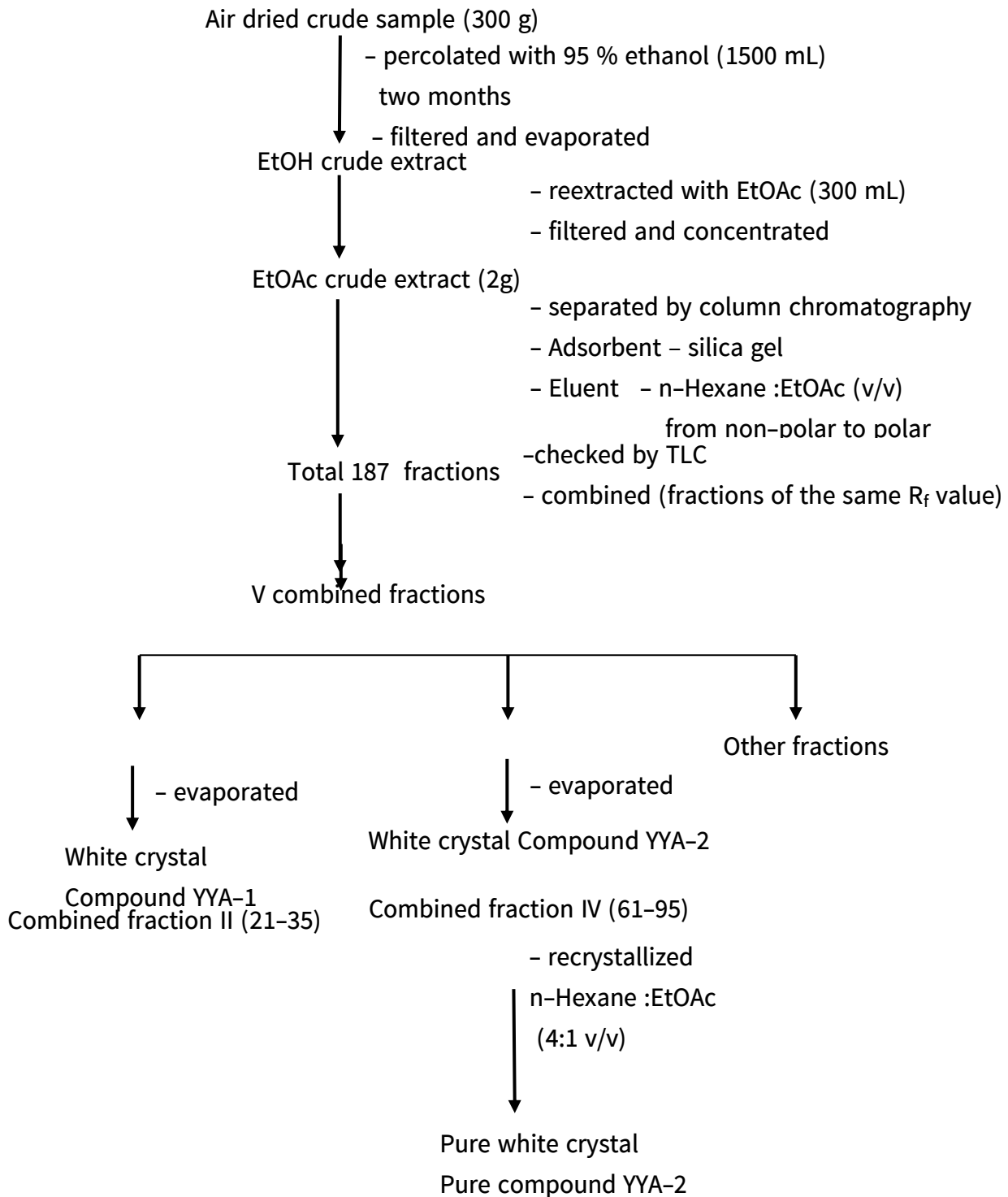
(10) 1 : 3

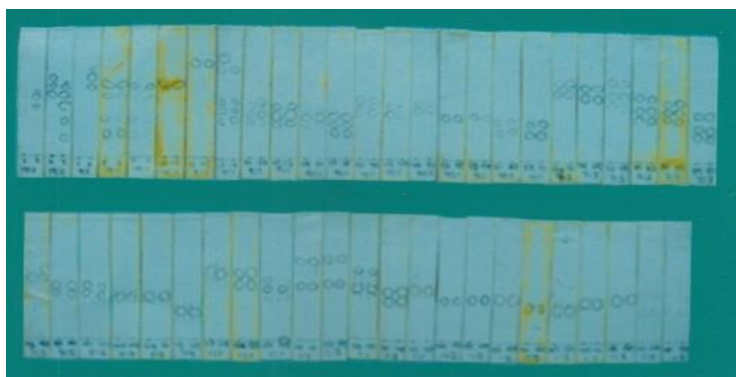
(11) ethyl acetate only



Figure 2. Column chromatographic separation of EtOAc extract

## Method





**Figure 3. Thin Layer Chromatograms of each fraction**

### **Recrystallization of Compound YYA -2**

Compound YYA-1 was not chosen for chemical analysis due to polarity of selected compound. Compound YYA -2 was chosen for identification of echitamine compound. Compound YYA-2 was dissolved in solvent mixture which consists of normal-hexane and ethyl acetate (4 : 1 v/v). Then it was recrystallized by lower step ratio stored in the refrigerator for one night.

### **Determination of Melting Point of Pure Compound YYA-2**

A small amount of crystal was inserted into a capillary tube and it was attached to the thermometer by using a small rubber ring and it was inserted into a round-bottomed flask containing concentrated sulphuric acid. The thermometer was supported by a cork. Then the flask was gently heated.

### **Alkaloid Test for Pure Compound YYA -2**

A small quantity of pure compound YYA-2 was introduced into a test tube and heated with 1% hydrochloric acid on a water-bath for about 10 minutes, and then allowed to cool and filtered. To the filtrate, a few drops of Dragendorff's reagent was added. (J.B. Harbone, 1984)

### **Structural Identification of Pure Compound YYA-2**

Fourier Transform Infrared (FT-IR) Spectrum of pure compound YYA-2 isolated from stem bark of *Alstonia scholaris* R.Br was carried out by using FT-IR spectrometry at Department of Chemistry, University of Mandalay.

## **Results and Discussion**

### **Preliminary Phytochemical Tests**

Table (1) Results of Phytochemical Test on Stem Bark of Taung-ma-yo

Test	Extract	Test Reagent	Observation	Result
Alkaloid	1 % HCl	Dragendorff's	Orange ppt	+
		Mayer's	Cream ppt	+
		Hager's	Yellow ppt	+
Glycoside	Distilled water	10 % lead acetate	Yellow ppt	+
Saponin	Distilled water	Vigorously shaken	Froth like comb	+
Reducing sugar	Distilled water	Benedict's solution	Red ppt	+
Phenolic compound	Distilled water	10 % FeCl <sub>3</sub>	Purplish colour	+
Polyphenol	EtOH	Mix: of 1 % FeCl <sub>3</sub> + 1 % K <sub>3</sub> [Fe(CN) <sub>6</sub> ]	Green blue colour	+
Terpene	EtOH	1 mL CHCl <sub>3</sub> + 1 mL acetic anhydride + 1 mL conc: H <sub>2</sub> SO <sub>4</sub>	Pink colour	+
Flavonoid	EtOH	Conc: HCl (5 dps) and 3 pieces of Mg	Pink colour	+

(+) = presence (-) = absence

According to this table, the crude extract contained alkaloid, glycoside, saponin, reducing sugar, phenolic compound, polyphenol, terpene and flavonoid, respectively.

### Antimicrobial Activities of Stem Bark of Taung-ma-yo

Table (2) Results of Antimicrobial Activities

Sample	Solvent	Organisms					
		I	II	III	IV	V	VI
Stem bark of <i>Alstonia scholaris</i> R.Br	n-hexane	-	-	-	-	-	-
	Chloroform	15mm (++)	15mm (++)	15mm (++)	12mm (+)	-	15mm (++)
	Acetone	16mm (++)	15mm (++)	12mm (+)	15mm (++)	12mm (+)	15mm (++)
	Ethyl acetate	28mm (+++)	22mm (+++)	23mm (+++)	23mm (+++)	26mm (+++)	22mm (+++)
	Ethanol	12mm (+)	14mm (+)	15mm (++)	15mm (++)	12mm (+)	14mm (+)

Organisms

Agar well - 10 mm

- I = *Bacillus subtilis* 10 mm ~ 14 mm (+) Lower activity  
 II = *Staphylococcus aureus* 15 mm ~ 19 mm (++) Moderate activity  
 III = *Pseudomonas aeruginosa* 20 mm above (+++) Highest activity  
 IV = *Bacillus pumalis*  
 V = *Candida albicans*  
 VI = *Mycobacterium* species

From the antimicrobial activities, the ethyl acetate extract of crude sample showed highest activity on all six organisms. n-Hexane extract gave no activity for all six organisms. The chloroform, acetone and ethanol extracts showed moderate and lower activities respectively.

From this investigation, it may be inferred that chloroform, acetone, ethyl acetate and ethanol extracts can be effective in the formation of medicine for the treatment of diseases namely intestinal disorders, eye-infection, skin and bone infections, meningitis, osteomyelitis, endocarditis, pneumonia, otitis media and externa, candidiasis thrush, urinary tract infection, tuberculosis and leprosy.

#### Isolation of Echitamine Compound by Column Chromatography

After isolation, the fraction IV (61-95) gave pure compound YYA-2 (120 mg) as white crystal. This pure compound YYA-2 may be alkaloid compound (echitamine).

Table (3) R<sub>f</sub> values and yield percent of pure compound YYA-2

Compound	R <sub>f</sub>	Solvent System n-hexane : EtOAc	Yield Percent
YYA-2 (61-95)	0.55	7:3 v/v	0.04 %

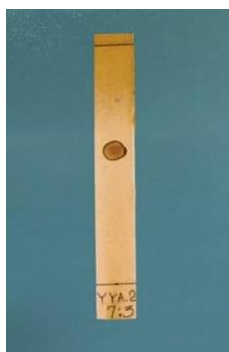


Figure 4. Thin layer chromatogram of pure compound YYA-2

#### Recrystallization of Compound YYA-2

After the compound YYA-2 was recrystallized by lower step ratio stored in the refrigerator for one night, the white crystal appeared. The obtained white crystal was filtered off to yield the pure compound YYA-2.

#### Determination of Melting Point of Pure Compound YYA-2

The pure crystal in the capillary tube was melted down at 285-287°C. It agrees with the literature value of melting point of echitamine compound.

#### Alkaloid Test for Pure Compound YYA -2

Alkaloid test for pure compound YYA -2 gave the orange precipitate by using Dragendorff's reagent. This indicates that the pure compound YYA-2 may be an alkaloid.

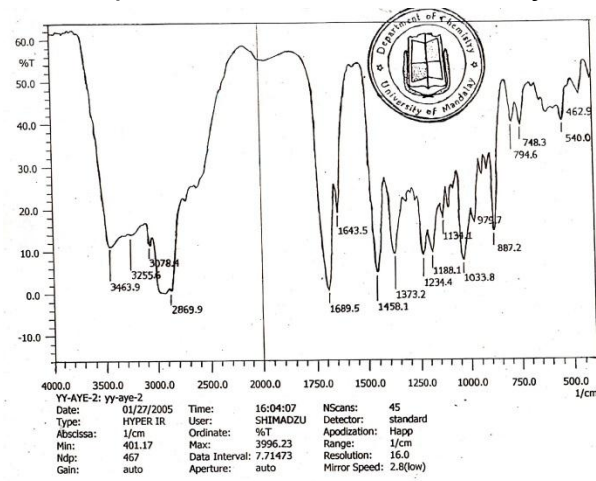


**Figure 5. Alkaloid test for pure compound YYA-2**

According to determination of melting point and alkaloid test, the pure compound YYA-2 may be an alkaloid compound (echitamine).

**Structural Identification of Isolated Pure Compound YYA-2**

The structure of pure compound YYA- 2 was confirmed by FT-IR spectrum (Figure 6).



**Figure 6. FT-IR Spectrum of YYA-2**

**Table (4) Characteristic absorption frequencies of compound YYA-2**

Observed wave number (cm <sup>-1</sup> )	Possible assigned and remark
3463.9	O-H stretching frequency of alcohol
3255.6	N-H stretching frequency of amine
3078.4	aromatic C-H stretching frequency of aromatic ring
2869.9	sp <sup>3</sup> C-H stretching frequency of saturated hydrocarbon
1689.5	$\text{>C=O}$ stretching frequency of ester
1643.5	C ----- C ring skeletal stretching vibration of benzene ring
1458.1	C-H in plane bending vibration of allylic group
1373.2	O-H in plane bending frequency of alcohol
1234.4	C-O-C stretching vibration of ester
1188.1	C-O stretching vibration of 2° alcohol
1134.1	C-N stretching vibration of amine
1033.8	C-O stretching vibration of 1° alcohol
979.7	C-H out of plane bending vibration of trans or E-alkene
887.2	C-H out of plane bending vibration of trisubstituted alkene
794.6	C-H out of plane bending vibration of cis or z-alkene



The existence of the two O-H functional groups (1° alcohol and 2° alcohol) and 2° amine could be confirmed at 1033.8 cm<sup>-1</sup>, 1188.1 cm<sup>-1</sup> and 1134.1 cm<sup>-1</sup>. It was resembled supported by the high intensity of the O-H and N-H band at 3463.9 cm<sup>-1</sup> and 3255.6 cm<sup>-1</sup>. Moreover, the occurrence of the ester C=O group was observed at 1689.5 cm<sup>-1</sup>, which results from intramolecular hydrogen bonding.

According to above evidence, the compound YYA-2 may be an alkaloid compound, echitamine.

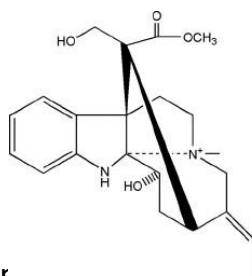
### Echitamine

Molecular

Formula = C<sub>22</sub>H<sub>29</sub>N<sub>2</sub>O<sub>4</sub><sup>+</sup>

Molecular weight = 385.48 gmol<sup>-1</sup>

Melting point = 286°C (±1)



Echitamine is a special type of iridoid alkaloid and is not a protoplasmic poison. In Ayurveda, it is used as a bitter and as an astringent herb for treating skin disorders, malarial fever, chronic dysentery, diarrhea and in snake bite. The ethanolic extract also showed cytotoxic activity. It contains echitamine and loganin as major compounds and could potentially be used as an anti-irritation agent.

### Conclusion

In this research work, the ethanol and ethyl acetate crude extracts of stem bark of Taung-ma-yo (*Alstonia scholaris* R.Br) were used for investigation.

From the preliminary phytochemical tests, the crude sample gave positive test for alkaloid, glycoside, saponin, reducing sugar, phenolic compound, polyphenol, terpene and flavonoid respectively.

According to the antimicrobial activities, the ethyl acetate extract of crude sample showed highest activity on all six organisms. n-Hexane extract gave no activity for all six organisms. The acetone and ethanol extracts showed moderate and lower activities respectively.

The pure compound YYA-2 gave positive test for alkaloid. According to the FT-IR spectral data and melting point (285-287°C) of pure compound YYA-2, this pure compound YYA-2 was found to be same as literature value of echitamine (286°C). Therefore, the pure compound YYA-2 may be an alkaloid compound, echitamine.

The stem bark of Taung-ma-yo is also used in bowel complaint; mixed with water it is applied to ulcers and as an application in rheumatic pains. It is valuable in debility and after effects of fever also in chronic diarrhoea, dysentery, catarrhal fever, skin disorders, malarial fever, and in snake bite. In the indigenous medicine, the drug of *Alstonia scholaris* R.Br would fully replace quinine in malignant tertian fever. Medicinal uses agreed with the antimicrobial activities. Therefore, the stem bark of *Alstonia scholaris* R.Br could potentially be used in medical applications.

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## Online Materials

<https://www.cec.gov>fungal,can>  
<https://en.m.wikipedia.org/wiki>  
<https://wickhamlabs.co.uk,fact-she...>

