

Determination of Constituents, Antibacterial Activity and Partial Structure of a Pure Biologically Active Organic Compound Isolated from Taung let-pan (*Bombax Insigne*)

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Abstract

In this research work, a pure bioactive organic compound was isolated from the stem bark of Taung let-pan. This sample was collected from Tada U Township in Mandalay Region. Before analyzing, phytochemical screening of this plant was carried out. Antibacterial activities of this plant in various solvents were examined by means of Agar well diffusion method. In addition, the active compound (KW) could be isolated from Taung let -pan applying column chromatography. Then, thin layer chromatographic separation method was used for its purity. The isolated compound could be identified by FT-IR spectrum.

Keywords: *Bombax insigne*, phytochemical screening, antibacterial activity, column chromatography, thin layer chromatography

Introduction

The plant kingdom constitutes an invaluable source of new chemical products which may be important due to their biological properties and well-known because of their potential use in medicine.

As chemical techniques become improved, the active constituents are isolated from plants and are structurally characterized, and any organic compound is synthesized in the laboratory to support the useful drugs as a tool in medicine. Sometimes more active and better tolerated drugs were produced by chemical modification (semi synthesis) or by total synthesis of analogue of active principles.

For several years, plants remained in use as a source of conventional medicine for the cure of many diseased conditions. Majority of the plants contain a good amount of phytochemicals having strong antioxidant activities which can be used for medicinal purposes (Razail et al., 2008). Ailments due to microbial infections are the foremost cause of death throughout the globe because of the multidrug resistant bacteria.

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Pandya.DJ et al.,(2010), investigated that qualitative and quantitative microscopic evaluation of the leaf material and establishment of physicochemical and phytochemical evaluation in the research entitled “Pharmacognostic Study and Establishment of Quality Parameters of Leaves of *Bombax insigne*”. There is no research in the determination of pure compound regarded to the stem bark of Taung let-pan in Myanmar. That is why how to use the indigeneous medicine which was extracted from the stem bark of Taung let-pan is the cause of analysis on isolation of pure organic compound from it.

Bombax insigne that belongs to the family Bombacaceae, is recognized with the common names of silk cotton tree and salmalia. It is considered as a noteworthy medicinal plant in Indian tropical and subtropical regions and is also found in many countries including Laos, Myanmar, Vietnam and Pakistan. Indian traditional systems of medicine describe its use for the treatment of many diseases such as sexual incapacity, to stop bleeding from wounds and against vaginal infections. (Anandarajagopal et al., 2013)

Botanical Description

Scientific Name	:	<i>Bombax insigne</i> Wall.
Family	:	Bombacaceae
Myanmar Name	:	Taung let-pan
Flowering period	:	February to April
Parts Used	:	Resin, Leaves, Stem bark, Thorns, Seeds, Flowers
Medicinal Uses	:	Treatment of diuretic, dysenteric, emetic, leprosy, diarrheal, wounds, acne, skin blemish and pigmentation, cold and cough, hotness and inflammation asthma (powder stem).



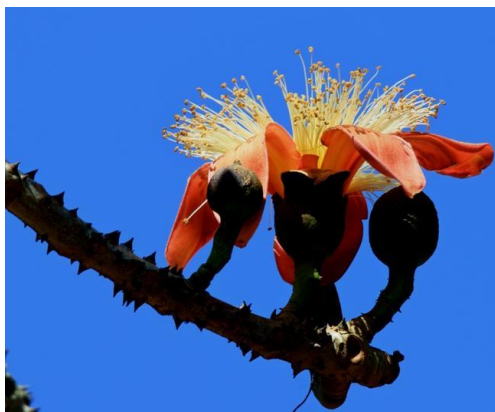


Figure 1. Stem bark and flower of habit of *Bombax insigne* (Taung let-pan)

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 F₂₅₄) silica gel (70– 230 mesh ASTM) was used for column chromatography. Iodine vapour and UV detector were used for location of the spot on the TLC plates.

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 barks of Taung let-pan were collected from Tada U Township in Mandalay Region. These stem barks are chopped into pieces and allowed to air dry for two months. The air dried sample were ground to powder, stored in well stoppered bottle and used throughout the experiments.

Preliminary Phytochemical Screening of Taung let-pan Stem Bark

The phytochemical screening of crude extract was carried out by using J.B Harbone methods (3rd Edt, (1998)).

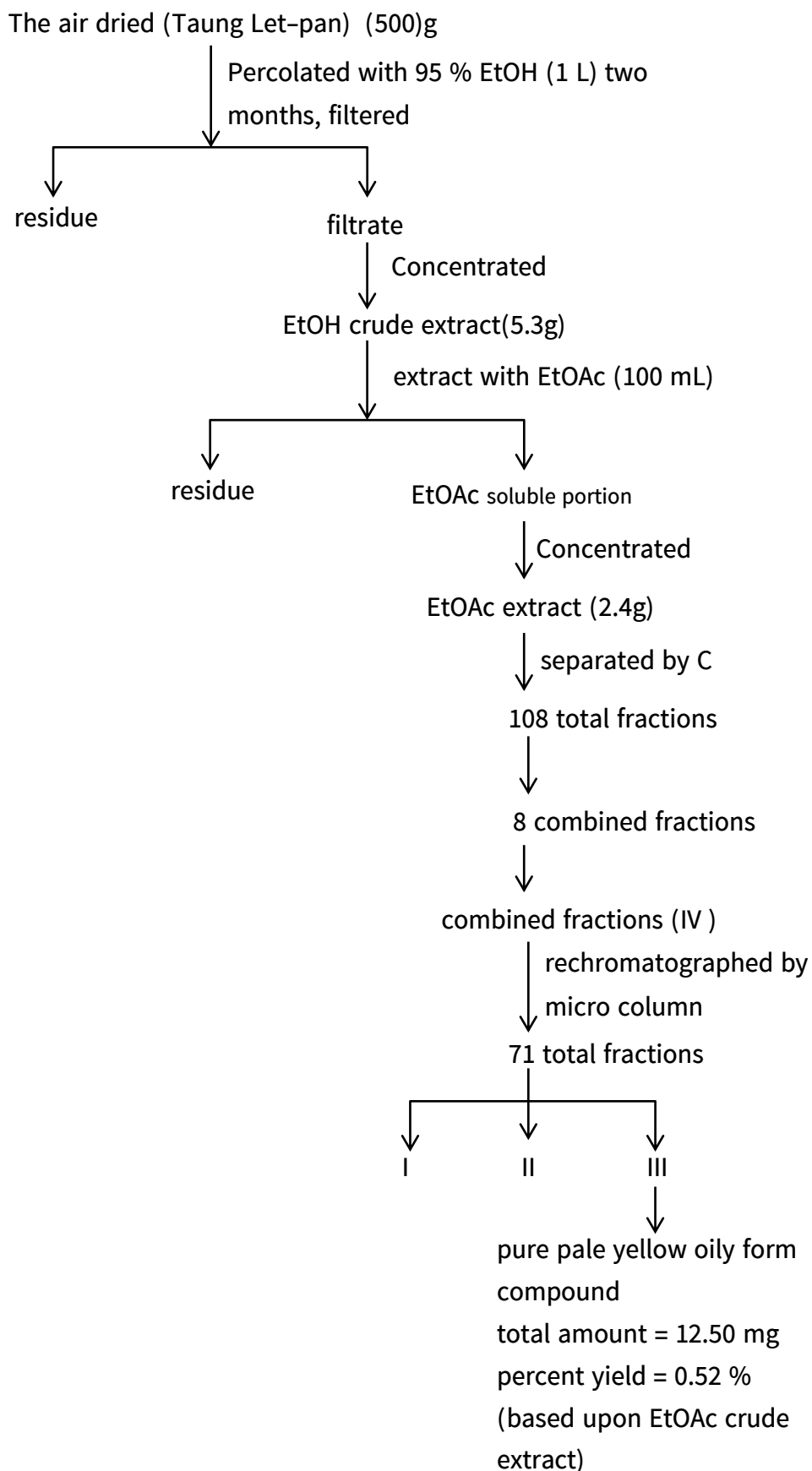
Antibacterial Activities of Taung let-pan Stem Bark

The different kinds of solvent extracts were checked for antibacterial activities by using agar well diffusion method.

Extraction and Isolation of Pure Compound from Taung let-pan Stem Bark

Ethanol(95 %) percolated solution was filtered and concentrated by using rotatory evaporator. The residue was dissolved in 100 mL of ethyl acetate solution. Ethyl acetate crude extract (2.4g) was separated by column chromatographic method by using silica gel with EtOAc and n-hexane with various ratios as eluents. Totally 108 fractions were obtained. Each fraction was checked by TLC and the same R_f value of the fractions were combined. Eight combined fractions were obtained. The combined fractions (IV) (59-71,45 mg) were further rechromatographed by micro column. Then 71 fractions were obtained. The combined fraction (III) (51-57, 12.5 mg) was obtained. The total yield of pure compound was 0.52 % based on the ethyl acetate crude extract. The functional groups of the pure compound were investigated by FT-IR spectrum.

Flow Sheet for extraction and isolation of unknown compound



Results and Discussion

Preliminary Phytochemical Screening of Taung let-pan Stem Bark

Table (I) Phytochemical Screening of Taung let-pan Stem Bark

No.	Constituents	Reagent Used	Observation	Inference
1.	Alkaloid	(I) Dragendroff's reagent (2)Mayer's reagent	Orange ppt Cream color ppt	-
2.	Flavonoid	Conc HCl + Mg + EtOH	No. pink (or) green color	+
3.	Phenolic	10% FeCl ₃	Green ppt	+
4.	Polyphenol	1%FeCl ₃ +1%K ₃ [Fe(CN) ₆]	Blue green color	+
5.	Glycoside	10% lead acetate	White ppt	+
6.	Sugar	Benedict's solution	Red ppt	+
7.	Lipophilic	0.5 N KOH	No deep color	-
8.	Saponin	NaHCO ₃	No frothing	-
9.	Tannin	Conc: H ₂ SO ₄ ,1% FeCl ₃	Yellowish-brown	+
10.	Terpene	CHCl ₃ , (CH ₃ CO) ₂ O, Conc: H ₂ SO ₄	Red color	+

Note: (+) = presence of constituent

(-) = absence of constituent

According to table (1), the stem bark of Taung let-pan was found to be flavonoid, phenolic compound, polyphenol, glycoside, sugar, tannin and terpene respectively.

Antibacterial Activities of Taung let-pan Stem Bark

The antibacterial activities of the crude extracts with various solvent systems are recorded in Table 2.

Table (2) Antibacterial Activities of Taung let-pan Stem Bark

Sample	Organisms	Solvents				
		n-hexane	pet-ether	CHCl ₃	EtOAc	EtOH
Taung let-pan	I	++	+	++	+++	+++
	II	++	+	++	+++	+++
	III	++	+	++	+++	+++

Solvents

Agar well - 10mm

10 mm ~ 14 mm (+) (+) = Low activity
 15 mm ~ 19 mm (++) (++) = Moderate activity
 20 mm above (+++)

Organism; I = *Bacillus subtilis*
 II = *Staphylococcus aureus*
 III = *Pseudomonous aeruginosa*

In this table, EtOAc and EtOH extract of Taung-let-Pan respond the highest activities on three microorganisms. According to the results of antibacterial activities, this sample can be used for the following treatment: such as diuretic, dysenteric, emetic, leprosy, diarrheal, wounds, acne, skin blemish and pigmentation, cold and cough, hotness and inflammation asthma (powder stem).

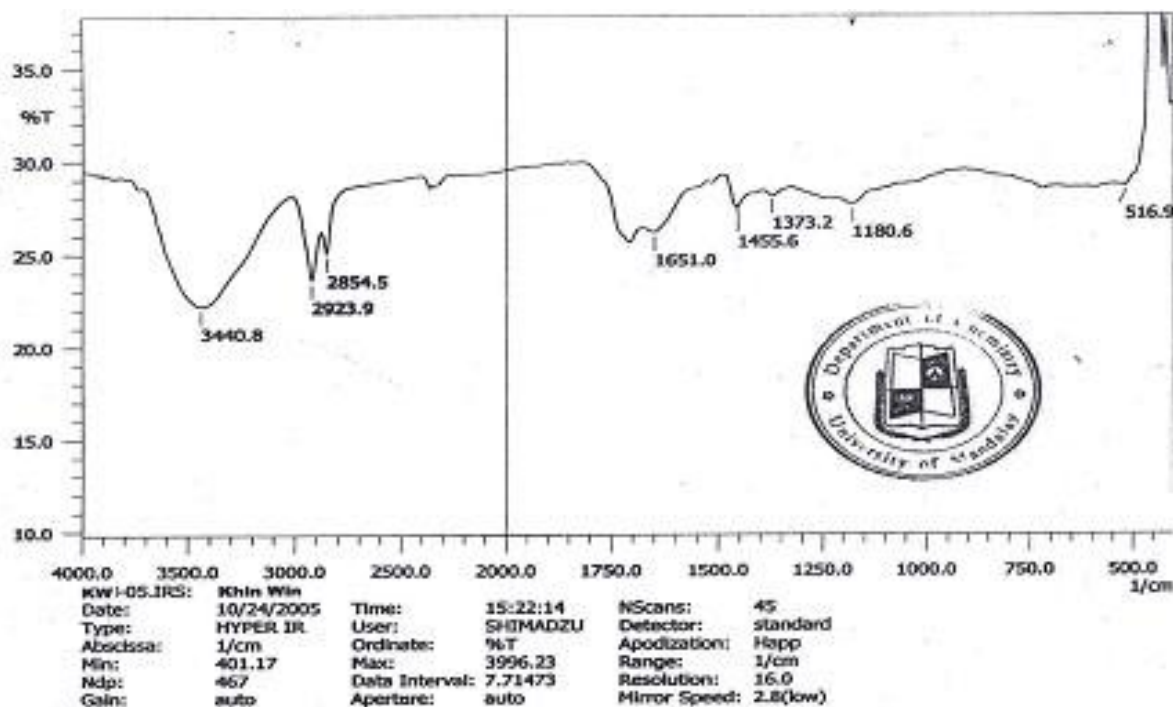


Figure 2. FT-IR Spectrum of Pure Organic Compound (Taung let-pan)

Determination of functional Groups in Isolated Pure Compound

FT-IR spectrum identified the functional groups at the Department of Chemistry, University of Mandalay and it is shown in Figure (2). This spectrum represents some prominent functional groups containing in this pure compound. In FT-IR spectrum, the broad band which appears at 3440.8 cm^{-1} should be -OH stretching vibration of alcohol group. The peak at 2923.9 cm^{-1} and 2854.5 cm^{-1} indicate the asymmetric and symmetric stretching vibration of sp^3 hydro-carbon. Moreover, C-H out of plane bending vibration of gem-dimethyl group is also observed at 1455.6 cm^{-1} . The band at 1373.2 cm^{-1} implies the C-O stretching vibration of secondary alcohol. On the other hand, C-O-C stretching vibration of ether group could be detected at 1180.6 cm^{-1} . The functional groups containing in this pure compound are tabulated in Table 3.

Table (3) Functional Groups presented in FT-IR Spectrum of pure organic compound from Taung let-pan stem bark

No	Wave Number (cm ⁻¹)	Functional Groups
1	3440.8	O-H Stretching vibration (broad band)
2	2923.9, 2854.5	Symmetric and asymmetric sp ³ C-H Stretching vibration of sp ³ hydrocarbon
3	1651	C = C Stretching vibration of alkenic group
4	1455.6	sp ³ -CH out of plane bending vibration of gem-dimethyl group
5.	1373.2	C-O stretching vibration of secondary alcohol
6.	1180.6	C-O-C ether group

Conclusion

The stem bark of Taung let-pan was collected from Tada U Township in Mandalay Region.

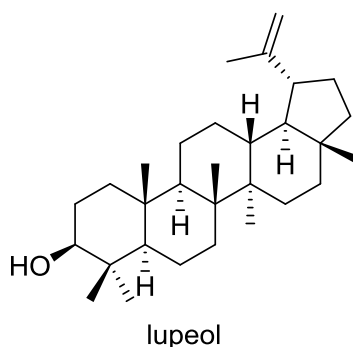
In this research work, the phytochemical tests and antibacterial activities of the stem bark of Taung let-pan were performed. The sample responded the presence of flavonoid, phenolic compound, polyphenol, glycoside, sugar, tannin and terpene. In addition, the antibacterial activities of the crude extract in various solvent system were tested by agar well diffusion method on three selected organisms. According to the results of antibacterial activities, the ethanol and ethyl acetate extract of Taung let-pan gave rise to high activities on all tested organisms.

A pure compound was isolated by using column and thin layer chromatography. The yield percent of this compound was found to be 0.52 % based upon the crude extract.

The FT-IR spectrum of this compound responded the presence of alcohol, alicyclic hydrocarbon, sp^3 hydrocarbon, allylic hydrocarbon, gem dimethyl and Trans or E alkenic functional groups, respectively.

Isolation and characterization resulted in the identification of the compound from the extract of stem bark of Taung let-pan. This compound could be lupeol which possesses potent hypotensive activity.

The molecular formula of lupeol is $C_{30}H_{50}O$. It is a triterpenoid compound which has strong antioxidant, antimutagenic, anti-inflammatory and antiangiogenic activity. In this regard, further studies need to be carried out to explore Taung let-pan for its potential in preventing and treating diseases.



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