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Scopoletin from *Hymenodictyon orixense* (Roxb.) Mabb.

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Abstract

Hymenodictyon orixense (Roxb). Mabb. (Ku-than) is belonging to the family Rubiaceae. It is widely distributed in Bago, Mandalay and Yangon of Myanmar. The botanical descriptions of this plant was classified, identified to confirm by the literature references. The morphological characters and preliminary phytochemical tests of the samples are described in this study. The main constituent, scopoletin was isolated from the methanolic extract of the stem bark and identified from UV, FT-IR and ^{1}H NMR spectral data.

Keywords : Morphological characters, phytochemical tests, scopoletin

Introduction

Hymenodictyon orixense (Roxb.)Mabb, belonging to the family Rubiaceae is widely distributed throughout the greater part of India and is abundant and native to tropical Asia and Africa. In Myanmar *H. orixense* (Roxb.) Mabb. is used in indigenous medicine and grows wild from Bago Yoma to the hill regions of upper Myanmar. It produces alkaloids and scopoletin used as febrifuge, serves in native medicine as substitute for quinine, potential inhibitors of tumor promotion, good for throat and appetite. Myanmar traditional medicine system was established since several hundred years ago. Nowadays, Myanmar traditional and herbal medicines are being popular and accepted by people. It is observed that Ku-than is a less known plant ingredients that is commonly used in Myanmar by the traditional medicine practitioners. Although Ku-than was not a common ingredient like *Cinchona* which was used for the treatment of malaria, the constituents of the plants should be investigated due to it similar or alternative use in the treatment of malaria as *Cinchona*. Therefore, the present study aims to investigate and to identify the chemical constituents of barks of ku-than.

Materials and Methods

The specimens of *Hymenodictyon orixense.*(Roxb.)Mabb. used in this study were collected from South Nawin Forest Area of Pauk-Khaung region,

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Pyay District, during the flowering and fruiting seasons which is from July to November.

The plants samples were identified and classified according to the standard method. Specimens were preserved as herbarium sheets and kept in the Botany Department of Yangon University.

The preliminary phytochemical tests such as the test for Alkaloids, Carbohydrate, Saponins, Tannins, Resin, Steroids, Amino Acids, Phenolic compounds, Glycosides, Flavonoids, Quinones, Cyanogenic Glycosides were determined by the methods of Physicochemical Standards of Unani Formulations (1986); Khalid, (1989) and Marini Bettolo et al ., (1981).

Fifty grams of dried bark powder was extracted with 200ml of methanol for 30 minutes under reflux on the water bath. The filtrate was evaporated to dryness on the water bath, and residue was dissolved in an appropriate volume of methanol and used for TLC studies. (Harborne, 1989).

Thin Layer Chromatography was employed to detect the chemical constituents present in the methanol extract (Stahl and Shild, 1981).

Individual phytoconstituents from the fractions were isolated by preparative thin layer chromatography (Harborne, 1989).

The UV spectrum of isolated compound was recorded with a Shimadzu UV-240, UV-visible spectrophotometer (Japan) at the Asia Research Centre, University of Yangon. A small amount of each sample was dissolved in methanol ($2cm^3$). This solution was introduced into a cuvette with capacity of $2cm^3$ and UV spectrum was taken (The Merck Index, 1996).

The infrared spectrum of isolated compound was recorded by using Shimadzu FT-IR-8400 Fourier Transform Infrared Spectrophotometer, at the University Research Centre, University of Yangon (Stahl & Shild, 1981).

The ^1H NMR spectrum of isolated compound was recorded by means of a Bruker 300 MHz spectrometer at the Department of Chemistry, University of Peradeniya, Srilanka. The spectrum was run on the $CDCl_3$ + CD_3OD solution of sample.

Results and Discussion

Diagnostic characters of the plants

- Habits : deciduous trees, 10 to 16m in height; the barks soft, brownish-gray
- Leaves : simple, opposite and decussates, elliptic to obovate, both surface softly, pubescent, the tips acute, the margin entire, the base cuneate; stipulate margin with blackish-gray serrate; petiole length unequal
- Inflorescences : paniculate thryrses, drooping, peduncle cylindrical
- Flowers : bracteate, bracteolate., pedicellate, bisexual, complete, actinomorphic, pentamerous
- Calyx : 5 lobes, green colour
- Corolla : 5 lobes, pale green to yellowish colour, tubular - urceolate
- Androecium : 5, anther dithecous, dorsifixed, inserted, epipetalous
- Gynaecium : pistil 1, the style long, the stigma globose, ovary bicapellary - 2 loculed, axile plecentation
- Fruit : loculicidally, capsule
- Seeds : golden - brown, ellipsoidal, winged

Flowering and fruiting periods from June to November

Myanmar Name Ku - than or Ku - San

It is conservational status is common in mixed deciduous forests of Myanmar.

Table 1. Phytochemical investigations in sun-dried barks of *H. orixense* (Roxb.) Mabb.

No.	Test	Extract	Test reagent	Requirements	Result
1.	Alkaloids	1% HCL	1. Mayer's solution	White ppt	+
			2. Dragendroffs' solution	Orange ppt	+
			3. Wagner's solution	Brown ppt	+
			4. Sodium picrate	Yellow ppt	+
2.	α - amino acid	H_2O	Ninhydrin	Violet colour	-

No.	Test	Extract	Test reagent	Requirements	Result
3.	Carbohydrate	H_2O	Benedict's solution	Brick-red colour	+
4.	Cyanogenic glycoside	H_2O	Toulene	Brick-red colour	-
5.	Flavnoid	MeOH	Dilution HCL, Mg turning	Pink colour	+
6.	Glycoside	MeOH	NaOH solution	Yellow colour	+
7.	Phenolic compounds	MeOH	10% lead acetate	Yellow colour	+
8.	Resin		Acetic anhydrite, concentration H_2SO_4	Red - violet	+
9.	Saponin		Distilled water	Frothing	+
10.	Starch		KI solution	Blue colour	-
11.	Steroid	P.E	Acetic anhydrite concentration H_2SO_4	Greenish - blue colour	+
12.	Tannin	H_2O	1% $FeCl_3$ and 1% gelatin	White ppt	+
13.	Quinone	H_2O	10% HCL solution ether: chloroform, 10% NaOH	Red colour	+

P.E = Petroleum ether, MeOH = Methanol

The phytochemical screening of *H. orixense* (Roxb.) Mabb. resulted that alkaloids, carbohydrate, flavonoid, glycoside, phenolic compound, resin, saponin, steroid, tannin and quinone were present. Amino acid, cyanogenic glycoside and starch were not estimated in the bark of this plant.

Crude Scopoletin Extracts

Fifty grams of dried bark powdered of *H. orixense* (Roxb.) were refluxed for 30 minutes with 200ml of mathenol, then filtered. The filtered and filtrate were evaporated to dryness on the water-bath, and the residue dissolved in 3-5 drops of methanol and used for TLC studies.

Detection of Scopoletin by Thin Layer Chromatography

The crude extract of bark *H. orixense* (Roxb.) exhibited the presence of scopoletin by TLC test, using ethylacetate: methanol: distilled water. The compound appeared as a blue-violet fluorescent band under UV 254nm light as silica gel plate. Further the flourescence emission measured in UV-254 spectrophotometer confirmed the presence of coumarines in the bark extract of this plant.

Characterization of Isolated Scopoletin by Thin Layer Chromatography

The scopoletin isolated from the bark of *H. orixense* (Roxb.) was dissolved in MeOH and banded on precoated silica gel with referenses. Scopoletin in TLC plates were developed with the help of solvent systems such as Ethylacetate: Methanol: Distilled water (100:17:13)v/v and Ethylacetate: Formic acid: Distilled water (67:7:26)v/v. After the plate had dried, the bands were observed under UV Light at 254nm as blue-violet fluorescent bands as well as using 5 or 10% ethanolic KOH reagent as yellowish-green bands. The R_f value of the isolated scopoletin was comparable with the literature value (cf. Table. 2).

In the UV spectrum (Fig. 1) of isolated compound, showed strong absorption band at 254nm similar to a coumarin comparable with that reported in literature (The Merck Index, 1996) for scopoletin (Table. 2).

Table 2. Comparison of Thin Layer Chromatographic and Ultra-Violet Spectral Data of Reference and Isolated Scopoletin from *H. orixense.*(Roxb.)Mabb.

	TLC	**UV absorption peaks**
Reference data for scopoletin	Colour-blue-violet flourescence with UV_{254}* R_f = 0.76, Solvent-EtOAc: HCO_2H : D/W=67 : 7 : 26**, needles/ prisms from chloroform/ acetic acid,	Wavelength of maximum adsorption **230, 254, 260, 298, 346** nm

	TLC	UV absorption peaks
	moderately sol. in chloroform, insol. in benzene, yellowish green with spray reagent- 10% ethanolic	
Scopoletin isolated from *H.orixense.*(Roxb.) Mabb.	Colour-blue-violet fluorescence with UV_{254}* R_f = 0.83 , Solvent-EtoAc: HCO_2H: D/W=67 : 7 : 26** , needles/prisms from chloroform/ acetic acid, moderately sol. in chloroform, insol. in benzene, yellowish green with spray reagent- 10% ethanolic KOH***	Wavelength of maximum adsorption **214, 227, 251 (sh), 259 (sh), 284 (sh), 297, 349** nm

*The Merck Index (1996) ** Stahl & Schild (1981) ***Harborne (1989)

Characterization of Isolated Compound by FT-IR Spectroscopy

The FT-IR spectrum (FIG. 2) of isolated compound showed the characteristic absorptions of functional groups present in the structure of scopoletin (Table. 3).

FT-IR spectrum of isolated compound was taken as KBr pellet samples. Phenolic group in this compound gave bands at 3436.58 cm^{-1} (ν O - H) and 1205.69 cm^{-1} (ν C - O). The carboxyl C = O stretching at 1706.15 cm^{-1} in the FT-IR spectrum also supports the conjugated structure. The aromatic methoxyl group bands appeared at 2923.84 and 2825.18 cm^{-1} (ν C -

H). The aromatic C C ring stretching bands were observed as strong bands at 1616.93 and 1508.96 cm^{-1}.

Characterization of Isolated Compound by ^1H NMR Spectroscopy

^1H NMR spectrum (FIG. 3 and Table. 4) of isolated scopoletin has shown the 2 olefinic and 2 aromatic protons in it: the 2 olefinic protons of lactone ring as doublets at 7.68 and 6.24 with coupling constant of 9.6 Hz and 2 para protons of aromatic ring as singlets at 6.90 and 6.87. The aromatic methoxy group could not be observed since the methyl protons of CD_3OD solvent given signal in the same position. The phenolic hydroxyl group also did not show up due to deuterium exchange with OD group of CD_3OD.

Table 3. Standard Assignment of Infrared Spectral Data of Isolated Scopoletin from *Hymenodictyon orixense.*(Roxb.)Mabb.

Wavenumber(cm^{-1})	Assignment	
	Group	Vibration Mode
3436.58	ϕ - OH	ν O - H
2923.84 2852.18	- OCH_3	ν C - H
1706.15	δ - Lactone in coumarin	ν C = O
1616.93 1508.96	Aromatic	ν C --- C
1425.18	- OH	δ C - OH (associated)
1384.98	- CH_3	δ_s C - H
1262.61 1237.01 1205.69	ϕ - O - CH_3 ϕ - OH	ν_{as} ar - C - O - C - al & ν C - O
1168.82	δ Lactone in coumarin	ν C - O

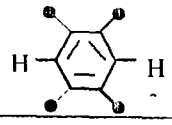
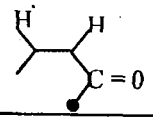
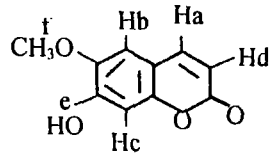
Wavenumber(cm^{-1})	Assignment	
	Group	Vibration Mode
1027.04	ϕ OCH_3	ν_s ar - C - O - C - al
848.77		δ_{oop} ar C - H
791.02		δ_{oop} ar C - H

Table 4. ^1H NMR Signal Assignment of Isolated Scopoletin from *Hymenodictyon orixense.*(Roxb.)Mabb.



(ppm)	Integration	Multiplicity	Assignment
7.68	1 H	d (J_{ad} = 9.6 Hz)	a
6.90	1 H	singlet	b
6.87	1 H	singlet	c
6.24	1 H	d (J_{ad} = 9.6 Hz)	d

The morphological characters of the plants were agreed with Hooker (1880), Hutchinson (1963) and Backer (1965).

The phytochemical tests provided the highlight on their chemical constituents of the sample.

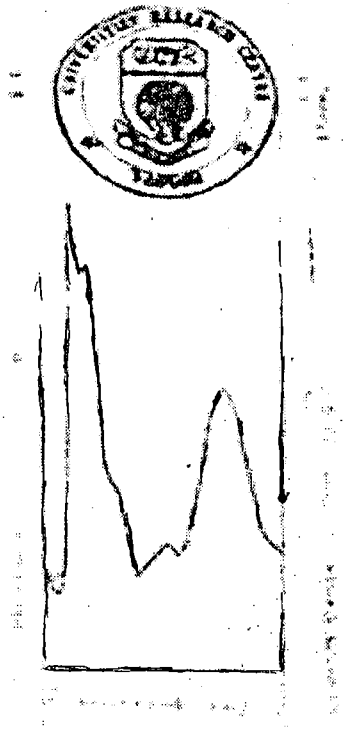
Chemical constituents of Ku-than was isolated in this study. The extraction of crude methanolic extracts are carried out by reference procedures with an expectation to isolate scopoletin constituents. Only one constituent could be isolated by these procedures. Structural elucidation by

UV, FT-IR and ^{1}HNMR, reveals that the isolated substances is scopoletin. The amount of scopoletin in bark is important to assess the medicinal property of the bark (Schedules, 2001). This is in agreement with the report by Rastogi & Mehrotra, (1991).

Chemical studies on the plant Ku-than revealed the presence of scopoletin as the major constituent of stem bark of *H. orixense* (Roxb.) The scopoletins belonging to coumarim group have been used as analgesic, reduction of blood pressure and anti - imflimatory (Noni, 2005).

Conclusion

From the overall assessments of the present work, it can be deduced that the samples were identified to confirm as family Rubiaceae by using available literature. The phytochemical tests were show the highlight on their chemical constituents and how to be prepare the methods from plants with easily. Scopletin have been isolated from active methanolic extract and identified by UV, FT-IR and ^{1}H NMR spectroscopic methods



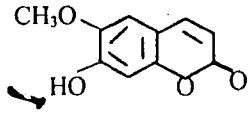


Fig (1) UV Spectrum of Isolated Scopoletin

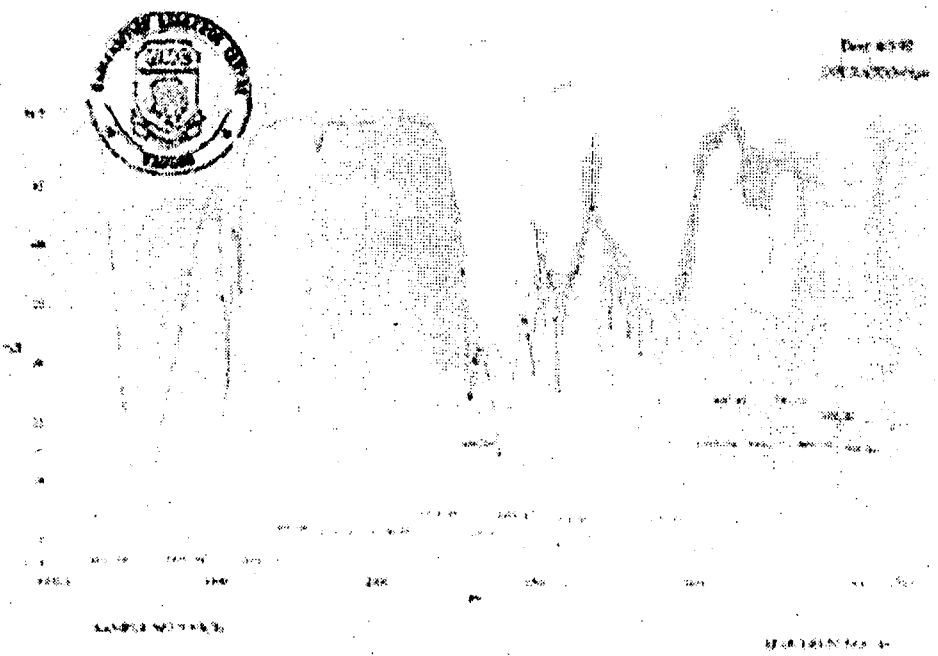


Fig (2) FT-IR Spectrum of Isolated Scopoletin

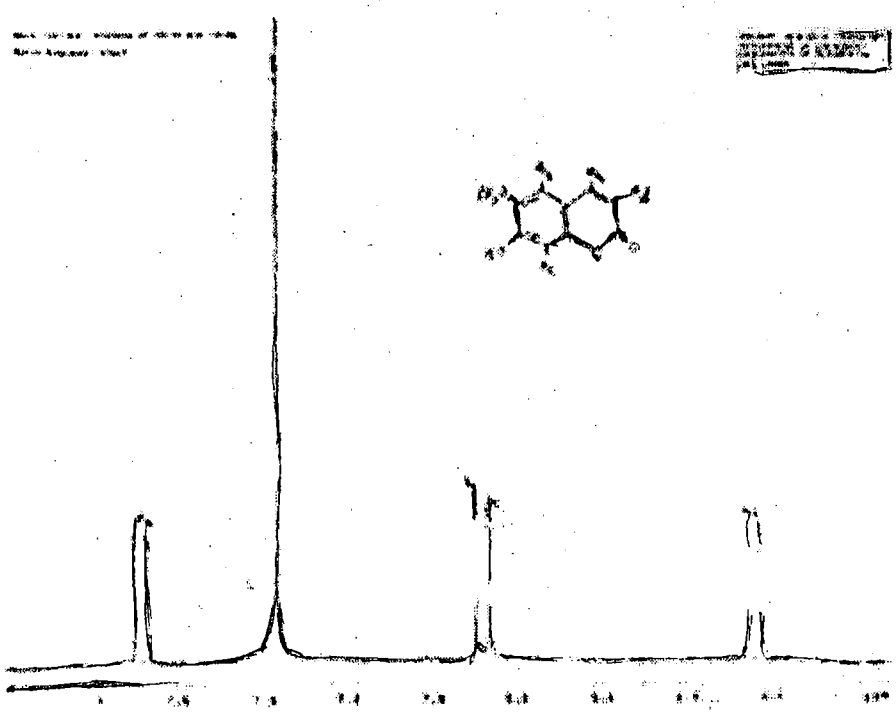


Fig (3) ^{1}H NMR Spectrum of Isolated Scopoletin

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