Isolation and Identification of Elliptinol from the Roots of *Derris elliptica* Benth. (Say-Min)

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Abstract

The aim of this research is to study the chemical constituents present in the roots of Derris elliptica Benth. *called Say-min in Myanmar, which is known as natural insecticidal plant. To investigate the chemical constituents of the roots, phytochemical screening tests were performed and it showed that the presence of alkaloids, free sugar, saponins, glycoside, terpenoid, phenolic groups and flavonoid compounds. Then antibacterial activities of Say-min roots were examined with the extracts of five solvent systems on three tested microorganisms, Bacillus subtilis, Staphylococcus aureus and Pseudomonas aeruginosa by Agar Well Diffusion methods. Column chromatographic separation was performed to isolate the chemical constituents by Thin Layer Chromatographic (TLC) and Fourier Transform Infrared (FT IR) spectroscopic techniques and a pure bioactive isolated compound may be identified as elliptinol. This research work can determine the phytochemical constituents of the roots of Derris elliptica Benth. and isolate elliptinol, a derivative compound of rotenoids group which is effective as insecticidal properties.*

Keywords: Fourier Transform Infrared, Thin Layer Chromatographic, Phytochemical, Column Chromatographic Separation

1. Introduction

Pest management is becoming a major concern in almost all agricultural countries. Nowadays many types of insecticides have been used to control insect pests. However, with the development of resistant activities in insects, the threat of pesticide and bacterial contamination on food, high production and purchase costs and environmental pollution problems, plant extracts are inceasingly of interest to use alternative pest control [1].

Derris elliptica (Wall.) Benth or tuba is one of the wild plants found in hilly region of South and Southeast Asia. The root of the tuba plant was traditionally used as the fish poison in the fishing and crop pest insecticide in horticulture and agriculture [2]. This plant shows the presence of many natural toxic chemical compounds, mainly flavonoid groups including rotenone, toxicarol, elliptone, sumatrol, tephrosin, and degueline [3].

Say-min, in English name, Hone, in Botanical name, *Derris elliptica* Benth belong to the Papilinaceae Wallich family. It is a large, handsome climber, distributed in the tropics chiefly in Southeast Asia. In Myanmar it grows on the hilly regions like Chin and Kachin States and Magway Region.

As derris owes its insecticidal properties, and since Myanmar is an agricultural country, the aim of this research work is to isolate active principle from this plant and prepare plant-based insecticide for the vegetable plantations to kill agricultural pests as extension work, so that gardeners and plantation owners will have a good harvest.

2. Materials and Methods

2.1. Materials

Commercial grade ethanol, n-hexane, benzene, acetone and ethyl acetate were used. They were distilled before they were subjected to experimental applications.

2.2. Collection of Sample

In the present work, chemical investigations were carried out on the roots of *Derris elliptica* Benth. (Say-Min) shown in figures (1) (2) and (3) obtained from Saw Township, Magway Region, Myanmar. They were washed with water, cut into small pieces and then dried at room temperature. It was stored in a well stoppered-bottles and used throughout this experiment.

Figure 1. Habit of *Derris elliptica* Benth.

Figure 2. Roots of *Derris elliptica* Benth.

Figure 3. Leaves of *Derris elliptica* Benth.

Family - Fabaceae
Botanical name - *Derris elliptica* Benth.
Myanmar name - Say-Min

2.3. Extraction and Isolation on Roots of *Derris elliptica* Benth. (Say-Min)

Extraction

Roots sample 236.5 g was percolated with 1200 mL of ethanol for two months. During percolating period, the mixture was shaken frequently to achieve maximum extraction. The extracted solution was then filtered through filter paper and washed the residue with ethanol. The filtrate was evaporated with a rotatory evaporator. This crude extract thus obtained yield about 30 g. Extract sample 1.8 g was applied in the column chromatographic separation.

2.4. Phytochemical Test on Say-Min Roots Sample

Phytochemical procedures were carried out on the dried roots sample of *Derris elliptica* Benth. according to the reported method [4].

2.5. Column Chromatographic Separation

The neutral ethyl acetate extract was fractioned by column chromatography over silica gel (SiO_2, 18g). In this research work, crude extract of roots of *Derris elliptica* Benth.(Say-min) was isolated by column chromatographic method. The separated portions were checked by thin layer chromatography (TLC) and fractions with the same R_f values were combined. Visualization was carried out by UV detector to examine the conjugation system and iodine vapor for the color development. After separation for 58 h, there was a collection of (233) fractions and eight combined fractions were obtained. Only fraction II give a single spot and this isolated compound was identified by thin layer chromatography (TLC) using various solvent systems and appropriate spray reagent. From the TLC plate, the R_f value of compound was measured. Then this compound was concentrated and recrystallized in n-hexane, ethyl acetate (1:1) for two times. Pure crystal was obtained as a light yellow color and it was subjected to FT IR spectrometric determination.

3. Results and Discussion

Table 1. Results of preliminary phytochemical test on *Derris elliptica* (Say-Min) roots

No.	Test	Test Reagent	Observation	Result
1	Alkaloids	Dragendroff's	orange	+
2	Flavonoids	Conc: HCl+Mg	reddish-brown colour	+
3	Glycoside	10 % lead acetate	white ppt	+
4	Phenolic group	$FeCl_3$ + $K_3[Fe(CN)_6]$	deep blue	+
5	Polyphenol	$FeCl_3$ + $K_3[Fe(CN)_6]$	No greenish blue ppt	-
6	Saponins	Vigorously shaken	formation of frothing	+
7	Steroids	Acetic anhydrous and Conc: H_2SO_4	No green colour	-
8	Terpenoids	Acetic anhydrous and Conc: H_2SO_4	No reddish-brown colour	-

(+) = presence , (-) = absence

Roots of *Derris elliptica* Benth. were investigated for phytochemical constituents and this screening indicated the presence of alkaloids, glycoside, terpenoid, free sugar, saponins, phenolic groups and flavonoid compounds respectively.

Table 2. Antibacterial activities of roots *Derris elliptica* (Say-Min)

Sample	Solvent extracts	Zone Diameter (mm)		
		Bacillus subtilis	*Staphylococcus aureus*	*Pseudomonas aeruginosa*
Say-Min (root)	EtOAc	22 (+++)	22 (+++)	26 (+++)
	n-hexane	-	-	-
	Benzene	14 (+)	14 (+)	14 (+)
	Acetone	13 (+)	13 (+)	13 (+)
	Ethanol	15 (++)	15 (++)	17 (++)

Agar well ~ 10 mm, 10mm-14mm (low), 15mm-19mm (medium), 20 mm above (high)

In the antibacterial activities, ethyl acetate extract showed the highest responses, ethanol extract gave medium and acetone extract informed the lowest activities with three tested organisms. It was found that n-hexane extract was inactive for all tested organisms.

3.1. Identification of Isolated Compounds

The infrared spectrum of isolated compound was recorded and it showed the functional groups present in this compound.

3.2. Infrared Spectrum

The FT IR spectrum of the isolated compound from combined fraction II was described in Figure 1 and the assignments of its functional groups were tabulated in Table 3.

Table 3. Structural band assignments of isolated compound from the roots of *Derris elliptica* Benth. (Say-Min)

Observed Peaks (cm^{-1})	Band Assignments
3448 (sharp peak)	–OH stretching vibration band of alcoholic group
3085 (sharp peak)	=CH stretching vibration band of cis (or) Z alkene
2931, 2862	C–H symmetrical and unsymmetrical stretching vibration bands of sp^3 hydrocarbons
1674, 1604	C ⎓ C ring skeletal stretching vibration bands of aromatic ring
1512, 1458	=C–H in plane bending vibration band of allylic hydrocarbons
1087, 1026	C–O–C stretching vibration band of ether group
948	=C–H out of plane bending vibration band of trans (or) E alkene
817	=C–H out of plane bending vibration band of cis (or) Z alkene

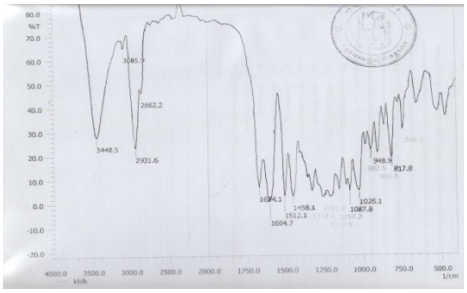
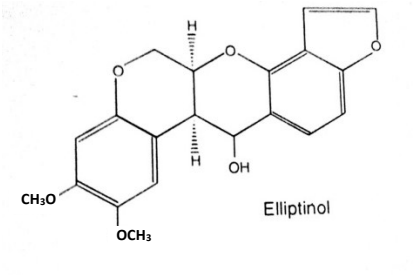


Figure 4. FT IR spectrum of isolated compound from the roots *Derris elliptica* Benth. (Say-Min)

From this spectral data, it informs the presence of the following groups

1. –OH (alcoholic) group
2. cis or Z alkene
3. sp^3 hydrocarbons
4. aromatic ring
5. allylic hydrocarbons
6. ether group
7. trans or E alkene

According to the presence of these groups and literature [5], the resulting isolated compound may be identified as elliptinol and its chemical structure was as shown below.



4. Conclusion

Derris elliptica Benth. (Say-min) is a plant which has received much attention of scientists from chemical and biological fields all over the world and this is because *Derris* root and bark extracts can be used extensively as a powerful insecticides. In this research, roots of *Derris elliptica* were investigated for phytochemical constituents and this screening indicated the presence of many important phytochemical compounds. Biological activities of roots of *Derris elliptica* were examined with ethyl acetate, n-hexane, benzene, acetone and ethanol extract on three organisms such as *Bacillus subtilis, Staphylococcus aureus* and *Pseudomonas aeruginosa.* Among these five solvent extracts, ethyl acetate showed the highest activities with all tested organisms. The crude extract was separated by column chromatographic method and it was followed by visualization of the fractions with the aid of UV detector and iodine developer. The isolated compound was purified and subjected to the FT IR measurement. According to the spectroscopic data, the roots of *Derris elliptica* contain elliptinol. At present, there are many diseases based on the residue of commercial insecticides and these problems were solved by applying the plant-based extract insecticides systematically.

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