Isolation and Identification of Some Chemical Constituents and Antioxidant Activity of *Hesperethusa crenulata R.* (Thanakha)

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Abstract

The present research deals with the investigation of chemical constituents from the stem barks of *Hesperethusa crenulata* R. (Thanakha) which have been used for remedy as well as cosmetic purpose in Myanmar. It has several scientific synonym names such as *Narigi crenulata* R. or *Hesperethusa crenulata* R. or *limonia crenulata* L. The stem barks of *Hesperethusa crenulata* R. (Thanakha) were collected from Monywa, Sagaing R egion during September to November in 2017. In order to isolate active constituents of this plant, the powdered stem barks were extracted with ethyl acetate and then, isolation of the active compounds from the defatted ethyl acetate extract was carried out by chromatographic technique at the Department of Chemistry, Kyaukse University. The isolated compounds were characterized and identified by chemical methods and modern spectroscopic methods using UV-visible, FT-IR spectrophotometer and TLC profiles. The two compounds: coumarin (compound A, 3.15 %) and flavone (compound B, 2.34 %) were isolated in this research. The antioxidant activities of both the water extract and the ethanol extract from the stem barks of Thanakha were determined by DPPH free radical scavenging assay at Department of Chemistry, University of Yangon. It was found that the ethanol extract (IC_{50} = 60.14 μg/mL) showed the higher activity than the watery extract (IC_{50} = 140.0 μg/mL) of Thanakha.

Keywords: *Hesperethusa crenulata* R., Coumarin, Flavone, Antioxidant activity

Introduction

Hesperethusa crenulata R. (Thanakha) is a tree that grown in Sri lanka, the southern and western parts of India, the north-western part of the Hymalayas and in Myanmar (CSIR, 1959). In Myanmar a fragrant durable liquid or paste is extracted from the barks that are also called Thanakha. It has several scientific synonym names such as *Narigi crenulata* R. or *Hesperethusa crenulata* R. or *limonia crenulata* L. The barks obtained from these trees are so fragrant that the ladies of Myanmar like them very much. Many local Myanmar and Thai cosmetic companies have now incorporated the stem bark powder of thanakha as a ingredient in many of their cosmetic products (Wangthong *et al*., 2010).

In Myanmar, it can be found in ShweBo, Monywa, Shinmataung, Pakokku and Taung Twin Gyi Townships. The barks are aromatic and cooling and are useful in vitiated conditions of pittas. Coumarin has been known to elicit many biological activities such as anticoagulant, dermal photosensitizing, antibacterial analgesic and hypothermal effect. Its powdered stem wood is used traditionally as a natural skin conditioner especially as facial cosmetics in Myanmar and also spread to neighbouring countries including Northern Thailand. Yellowish and sweet-smelling Thanakha wood is hard and used to make handicrafts such as trinkets, combs, boxes and many others. The root possesses much medicinal value and is used in Myanmar indigenous laxatives (Mabberley, 1997).

The objectives of this research work are to isolate and purify the isolated compounds from ethyl acetate extract of *Hesperethusa crenulata* R., to identify the classification of the isolated compounds and to evaluate antioxidant activity of this selected sample.

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Materials and Methods

Sampling of Plant Materials

The stem barks of *Hesperethusa crenulata* R. (Thanakha) were collected from Monywa City, Sagaing Region during the months of September to November in 2017. The barks were washed with water and air dried at room temperature. The dried samples were made powder by grinding mill. These samples were stored in the air-tight containers to prevent moisture changes and contamination.

Figure 1. Plant of *Hesperethusa crenulata* R.

Isolation of Phytochemical Constituents from Ethyl Acetate Extract of *Hesperethusa crenulata* R. (Thanakha)

The powder sample (20 g) was extracted with EtOAC, allowing to macerate for 1 week. Then, EtOAc extract was obtained. Ethyl acetate crude extract (1.5 g) was weighed, made a slurry with (2.5 $cm^{3)}$ of petroleum ether mixed with about twice the weight of extract with silica gel and carefully evaporated by heating with hair dryer to obtain a free following silica gel absorbed with the extract. This gel was carefully poured into the prepared silica gel and column through the long funnel and allowed to settle in the solvent lift about the silica gel column. A total of 11 fractions (F_1 ~ F_{11}) were collected from different eluents of increasing polarity: PE: EtOAc (99:1), (95:5), (7:3), (13:7), (3:2), (2:1) and (1:1) v/v. Successive fractions obtained were combined on the basis of their behavior on TLC and eleven fractions were obtained.

After removal of the solvents from each fraction, materials were obtained. Each fraction was checked on TLC with appropriate solvent systems. The solid materials provided from fractions F_1 and F_4 were purified by washing with PE, followed by crystallization.

Fraction F_1 was assigned the compound A. The compound A was provided in (3.15 % yield) (0.047 g) as deep yellow crystals. Fraction F_4 was defined as the compound B. The compound B was provided in (2.34 % yield) (0.035 g) as pale yellow color crystals.

Characterization and Identification of Isolated Compounds

The compounds isolated from *Hesperethusa crenulata* R. (Thanakha) were checked by TLC analyses. The compounds were loaded on percolated TLC silica gel plate. Then chromatography was performed in suitable solvent system. The developed chromatograms were first inspected under UV 254 nm light and sprayed with detecting agents to characterize the isolate compounds.

Some detecting reagents used for color reaction tests were 10 % $FeCl_3$ solution, I_2 vapour reagents, 10 % KOH solution and 1 % $AlCl_3$ solution. UV and FT-IR spectroscopy were recorded at Universities of Research Center, University of Yangon.

Screening of Antioxidant Activity of Watery and Ethanol Extracts from *Hesperethusa crenulata* R. (Thanakha)

The free radical scavenging activity of the stem barks of *Hesperethusa crenulata* R. (Thanakha) was measured by using DPPH free radical scavenging assay (Marinova and Batchvarov, 2011).

Procedure

DPPH radical scavenging activity was determined by UV-visible spectrophotometer (Marinova and Batchvarov, 2011). The control solution was prepared by mixing (1.5 mL) of 0.002% DPPH solution and (1.5 mL) of EtOH in the brown bottle. The sample solution was also prepared by mixing thoroughly (1.5 mL) of 0.002% DPPH solution and (1.5 mL) of test sample solution. These brown bottles were allowed to stand at room temperature and were shaken on shaker for 30 minutes. After 30 minutes, the absorbances of these solutions were measured at 517 nm using UV-vis spectrophotometer.

The antioxidant power (IC_{50}) is expressed as the test substances concentration (μg/mL) that result in a 50% reduction of initial absorbance of DPPH solution and that allows to determine the concentration. IC_{50} (50% inhibitory concentration) values were calculated by linear regressive excel program. The standard deviation was also calculated by the following equation.

Results and Discussion

Identification of Isolated Compounds from *Hesperethusa crenulata* R.

The isolated compounds were identified by chemical methods such as behavior on TLC using appropriate solvent systems and spraying reagents. In addition, spectroscopic methods such as ultraviolet (UV) and FT-IR were also employed to identify the isolated compounds.

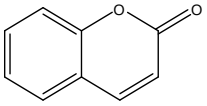
Compound A

The compound A (3.15 % yield) was isolated as a deep yellow amorphous solid from the ethyl acetate extract of *Hesperethusa crenulata* R. (Thanakha). It was found that with a silica-gel layer using solvent system of PE:EtOAc (99:1 v/v), the R_f value of the compound A was (0.50). This compound gave a black spot on TLC plate under 254 nm an UV light. It was also observed as black spot on TLC when sprayed with 10 % $FeCl_3$ solution. After TLC was dipped in 10% KOH solution, it gave brighter fluorescent color under UV 365 nm.

The UV-Vis spectrum of the compound A was taken in MeOH and wavelengths of maximum absorption were found to be 218 and 345 nm in Figure 2. The presence of several characteristic high intensity bands (220-350 nm) helps for the qualitative determination of coumarin.

In the FT-IR spectrum of the compound A, the band at 3446 cm^{-1} indicated O-H in phenolic group. The strong bands at 2956 cm^{-1}, 2924cm^{-1} and 2852 cm^{-1} were due to C-H stretching of $-CH_3$ and $-CH_2-$ groups. >C=0 stretching band was found at 1735 cm^{-1}, which is characteristic of carbonyl group in this compound. Absorption bands at 1640-1462 cm^{-1} were observed for C=C stretching

vibration of aromatic ring. 1379 cm^{-1} was due to C-H bending of -CH_3 and -CH_2- groups. In addition, C-O-C stretching of functional group was observed at 1097 and 1022 cm^{-1}. Absorption band at 802 cm^{-1} indicated C-H out-of-plane bending of cis- or trans- alkenic group in Figure 3. Due to the results of chemical tests, UV-Vis and FT-IR spectral data, the isolated compound A may be concluded as coumarin compound.



Structure of compound A (Coumarin, $C_9H_6O_2$)

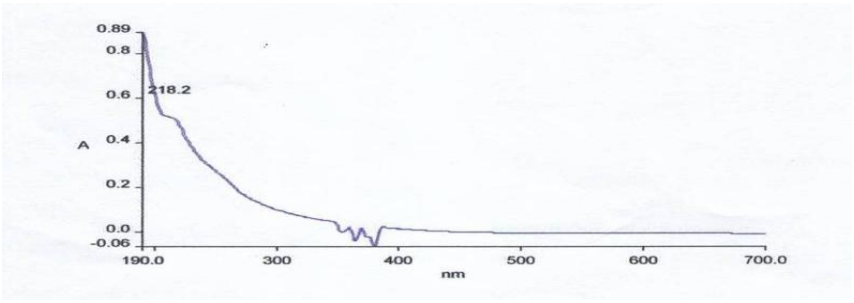


Figure 2. UV-Vis spectrum of the compound A

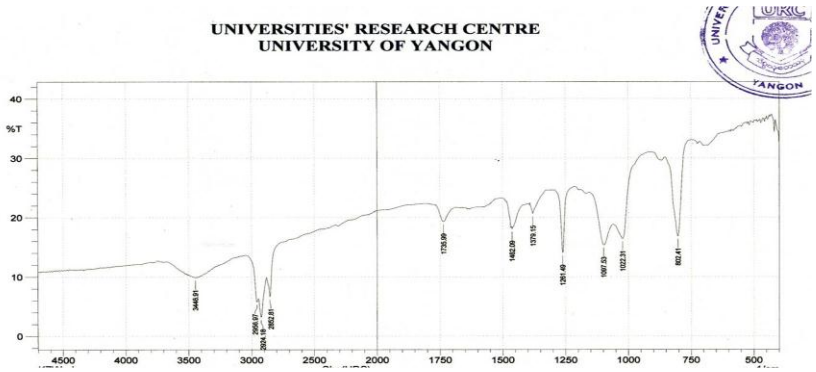


Figure 3. FT IR spectrum of the compound A

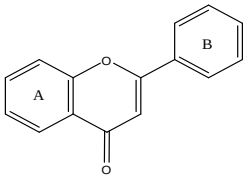
Compound B

The compound B (2.34 % yield) was isolated from the ethyl acetate extract of the bark of Thanakha after successively removing the petroleum ether. On silica gel layer using solvent system PE:EtOAc (4:1 v/v) as developing system, the compound B migrated (R_f = 0.57); this suggests a less or middle polar nature of the compound B. It gave a black spot and blue spot on TLC plate under UV 254 nm and UV 365 nm light respectively. It also showed up as a black spot by heating the TLC plate after dipping in 10 % $FeCl_3$ reagent. It was detected as pick colour appeared when mixing HCl acid and Mg turning into the compound B in the test tube. It could be revealed with 1 % $AlCl_3$ and under UV 365 nm light also as brighter fluorescent blue spot. This characteristic also suggests a flavonoid compound.

The UV-Vis spectrum of the compound B was taken in MeOH and wavelengths of maximum absorption were found to be 240, 295 and 360 nm which indicated the presence of ethylenic bond and double bond conjugated system in this compound in Figure 4.

The strong IR bounds at 3443 cm^{-1} indicated -OH in phenolic group. The bands at 2955, 2924 and 2852 cm^{-1} were due to C-H stretching of -CH_3 and -CH_2- groups. A carbonyl group is also suggested by the C=O stretching band flavone 1737

and 1641cm^{-1}. The absorption bands at 1564 and 1413 cm^{-1} were attributed to C=C stretching vibration of aromatic ring. Absorption band at 653 cm^{-1} indicated the out-of-plane C-H bending of cis- or trans- alkenic group in Figure 5. According to the chemical test, UV-Vis and FT IR spectral data, the isolated compound B may be flavone compound.



Structure of compound B (Flavone)

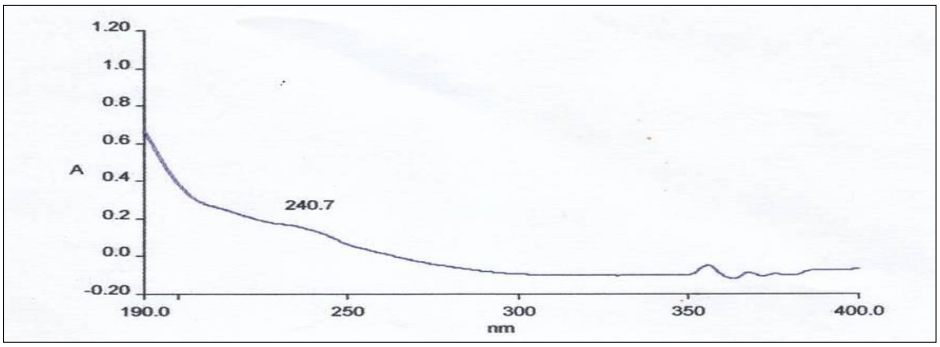


Figure 4. UV-Vis spectrum of the compound B

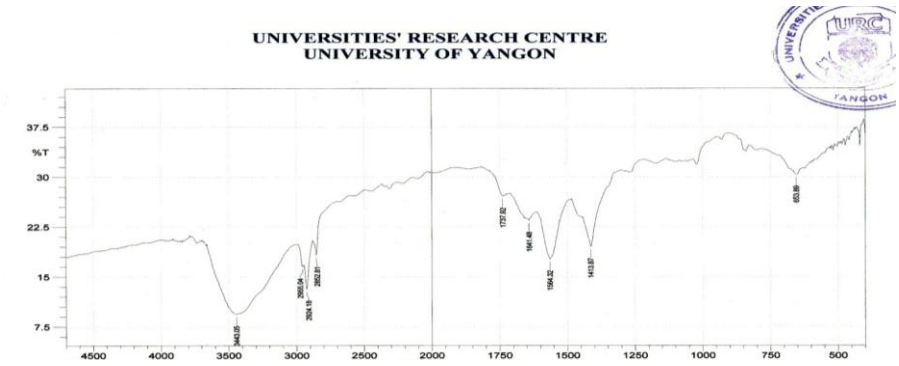


Figure 5. FT-IR spectrum of the compound B

Antioxidant Activity of Watery and Ethanol Extracts from Thanakha

The antioxidant activity of the watery and ethanol extracts of *Hesperethusa crenulata* R. (Thanakha) was studied by DPPH free radical scavenging assay (Marinova and Batchvarov, 2011). The procedure for the determination of the antioxidant activity was mentioned. The DPPH (2,2-diphenyl-1-picrylhydrazyl) assay is widely used to investigate the scavenging activities of several natural compounds such as the crude extracts of plants. DPPH radical is scavenged by antioxidant through the donation of electron forming the reduced DPPH. Sample's color changes from purple to pale yellow and which can be quantified by its decrease of absorbance at wavelength 517nm (Maw *et al*., 2011). The radical scavenging activity of juices and the crude extracts was expressed in term of % RSA and IC_{50} (50% inhibitory concentration).

From these observations, the largest radical scavenging activity to scanvage DPPH radical was observed in the ethanol extract, which inhibited 50% of free

radicals at the concentration (IC_{50}) of 60.14 µg/mL. The lowest activity was found in the watery extract, which inhibited 50% of free radical at the concentration of140 µg/mL. It can be concluded that the antioxidant potency of the ethanol extract were found to be stronger than that of the watery extract. The results are shown in Table 1 and Figures 6 and 7.

Table 1. Inhibition and IC_{50} Values of Different Extracts of Thanakha

IC_{50} = 50 % Inhibitory Concentration

Extract	Mean % Inhibition in Different Concentrations						IC_{50}
	12.5 (µg/mL)	25 (µg/mL)	50 (µg/mL)	100 (µg/mL)	200 (µg/mL)	400 (µg/mL)	
EtOH	33.09	37.75	43.24	50.30	55.45	57.52	60.14
H_2O	31.12	37.56	44.32	49.22	56.72	58.36	140

Figure 6. IC_{50} values of *H. crenulata* R.

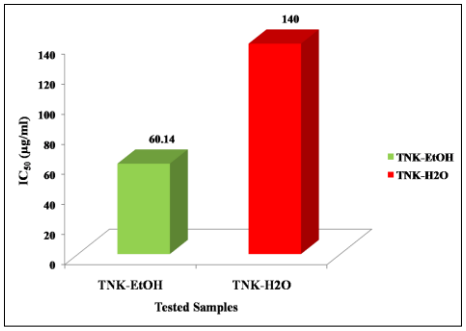


Figure 7. Mean % inhibition

Conclusion

The present investigation is an initiative for Thanakha barks in the field of natural product organic chemistry. The two compounds A (coumarin, 3.15%) and B (flavone, 2.34%) were successfully isolated by solved extraction, column and TLC methods. The structures of isolated compounds were identified by modem spectroscopic techniques. Investigation of free radical scavenging activity of different extracts was performed by 2,2-diphenyl-1-picrylhydrazyl (DPPH) Assay. It was found that the ethanol extract (IC_{50} = 60.14 µg/mL) showed the higher activity than the watery extract (IC_{50} = 140.0 µg/mL) of Thanakha.

In brief, according to above experimental data, *Hesperothusa crenulata* R. (Thanakha) barks may be used as a remedy for the antioxidant activity in Myanmar traditional medicine.

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