Isolation and Structural Identification of Quercetin from the Seed of *Aesculus assamica* Griff. (Ye-myaw)

Moe Tin Khaing[1], Khin Mar Thein[2]

Abstract

Among effective Myanmar traditional medicine, the seeds of *Aesculus assamica* Griff. (Ye-myaw) were chosen for this research work and its seeds were collected to investigate the chemical constituents. Phytochemical screening test on seeds of *Aesculus punduana* Wall ex Hiern was carried out firstly and it showed the presence of glycoside, phenolic compound, polyphenol, flavonoid, saponin, tannin, steroid and terpene. Then the antibacterial activities of crude extracts of seeds which were obtained by percolating with five different solvents such as normal hexane, chloroform, acetone, ethyl acetate and ethanol were examined by well known Agar well diffusion method. In the experiment, two prominent compounds were isolated from the ethanol extract by thin layer and column chromatography. By the color test, the resulting compound (I) indicated as a flavonoid derivative. Among six tested organisms, compound (I) showed medium activities on three organisms, *Bacillus pumalis*, *Candida albican* and *Mycobacerium* species. Then the structural identification of isolated compound was done by advanced spectroscopic methods such as ultraviolet and infrared spectroscopy and compound (I) was assigned as one of the effective flavonoid compounds, quercetin. Determination of antioxidant and other related activities of this seed should be performed in future.

Keywords: antibacterial activities, thin layer and column chromatography, organisms, ultraviolet and infrared spectroscopy

Introduction

In most countries in the world, many parts of the plants are used in traditional medicine. The plant may save many lives if they are used correctly. Plant materials are applied through developed and developing countries as home remedies. Most of Myanmar people widely used plant extract as the folk medicaments and they depend for their health on traditional medicine. In this work, one of Myanmar traditional medicinal plants, *Aesculus assamica* Griff. (Ye-myaw) was selected for chemical analysis. It belongs to the family Sapindaceae and it is native to tropical and subtropical forests. It is a genus, including about 25 species of trees and shrubs are widely distributed in Europe, America and Asia, especially Sikkin, Guangxi, Zizang, Yunnan, Bhutan, NE India, Bangladesh, Laos, Myanmar, Thailand and Vietnam. In the literature survey, the seed of *Aesculus assamica* Griff. (Ye-myaw) contain saponin with oleanolic acid, barringtogenol C and protoescigenin-21- tiglate as genins, together with glycoside of β-sitosterol, kaempferol and quercetin. Isolation of a flavonoid compound from the seeds of *Aesculus assamica* Griff. (Ye-myaw) and identification of the resulting compounds were worked out.

[1] Associate Professor, Dr, Department of Chemistry, Myitkyina University
[2] Assistant Lecturer, Department of Chemistry, Kyaukse University

Botanical Description

Figure 1. Habit of *Aesculus assamica* Griff.

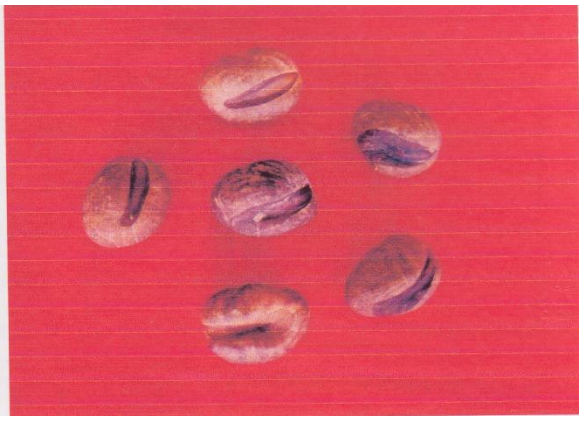


Figure 2. Seeds of *Aesculus assamica* Griff.

Family	-	Sapindaceae
Genus	-	*Aesculus*
Botanical name	-	***Aesculus assamica* Griff.**
Shan name	-	Pun Pan
Kachin name	-	We Sinni
Myanmar name	-	Ye-myaw

Perennial, tree, deciduous, stems and branches terete, brown, glabrous, when young puberulent to densely villous. Leaves palmately compounds, opposite, petiolate, petiolulate, exstipulate; petioles long, terete, brown, about 8.0 - 30.0 cm long, glabrous; leaflets 5-9, oblong -lanceolagte, dark glossy green, about 12-25 cm by 5-18 cm; glabrous above, finely pillose on veins below, cuneate to caudate at the base, crenulate to serrulate along the margin, acuminate at the apex.

Inflorescence terminal, thyrse, erect; flowers white to light yellow, showy, bisexual, zygomorphic, hypogynous, ebracteolate, bracteolate, pedicellate; bracts subulate, small. Sepals 4 or 5 *are* free or tubular. Petals 4 or *5*, white to light yellow with spotted purple near the base, when mature turned yellowish-brown, 13-22 mm by 3-7 mm. Stamens 10 (numerous), inserted on inner side of disk, free, unequal, exceeding, inner whorl of 5 complete (fertile), outerwhorl incomplete (Sterile). Ovary superior, ovoid to obovoid, 2 ovules in each locule, axile placentation; style one, terminal slender; stigma 3-lobed.

Fruits loculicidal capsule, oblongord, 7.0 cm by 4.0 cm, glabrous; pericarp leathery. Seeds depressed globose to pyriform, testa brown or glossy black, hard, leathery, hilum large.

Local Medicinal Uses of *Aesculus assamica* Griff.

The paste prepared from the dried seeds is traditionally used for the treatment of orchites, furuncles, goitre and hernia. Suspension of the seed paste in raw rice-water is used to treat inflammatory disease. Drugs prepared by baking the outer coat of the seeds with common salt are used for asthma.

Experimental

Sample Collection and Preparation

Seeds of *Aesculus assamica* Griff. were collected from Pyin Oo Lwin Township, Mandalay region, Myanmar. Then the seeds were cut into small pieces and dried in air for about one month and collected in a well-stopped bottle and used throughout the experiment.

Preliminary Phytochemical Screening

Glycoside, phenolic, polyphenol, flavonoid, saponin, steroid, terpene, alkaloid and tannin screening tests were carried out on the seeds of *Aesculus assamica* Griff. and the result obtained were shown in result and discussion.

Examination of Antibacterial Activities of Crude Extracts

For the examination of antibacterial activity, crude extracts of Yemyaw seeds were sent to DCPT (Development Centre for Pharmaceutical Technology), Insein, Yangon. The antibacterial activity of crude extract was tested by Agar well diffusion method on three selected organisms, *Bacillus subtilis*, *Staphylococcus aureus* and *Pseudomonous aeruginosa* and their activities are described in table (2). The results are shown in result and discussion.

Extraction from the Seeds of *Aesculus assamica* Griff.

300 g of the sample was percolated with 1500 ml of 95% ethanol for two months. The percolated solution was then filtered and the resulting filtrate was evaporated to crude extract. Crude extract obtained was weighed and only 2g of crude sample was used for the column separation. TLC check was applied on this crude sample and UV detector and Iodine vapour were utilized for visualization.

Separation of Crude Extract by Column Chromatography

Column chromatographic separation was performed on the ethanol extract of Ye-myaw seeds according to the following solvent systems.

1.	n-hexane:	EtOAc	(19:1 v/v)	50 ml
2.	n-hexane:	EtOAc	(9:1 v/v)	145 ml
3.	n-hexane:	EtOAc	(4:1 v/v)	50 ml
4.	n-hexane:	EtOAc	(3:2 v/v)	185 ml
5.	n-hexane:	EtOAc	(1:1 vlv)	60 ml
6.	n-hexane:	EtOAc	(2:1 v/v)	75 ml
7.	EtOAc only			20 ml

From this column separation, 133 fractions were collected and followed by TLC check. In this technique, visualizing agents were iodine vapour and UV-lamp. The fractions with the same R_f values were combined and eight combined fractions were obtained. Combined fraction (III) and (V) gave yellow crystals on evaporation. Among them, higher purity and yield correspond to combined fraction (V) (Solvent ratio = 3:2). Combined fraction (III) (Solvent ratio 4:1) also gave another isolated compound as yellow crystals.

Combined fraction (V) was then subjected to micro column separation for further purification. From this column separation, 94 fractions were collected and four combined

fractions (A, B, C, D) were obtained. Fraction (B) gave a single spot on TLC plate under UV detector. It means that this compound is in a pure state. Then this fraction was observed as pale yellow colored crystals on evaporation to dryness. These isolated compounds were applied for FT-IR spectrometric determination at Department of Chemistry, University of Mandalay.

Confirmation by Color Test

A small amount of pure crystal (MTK-1) was placed into a test tube and heated with ethanol in a water bath for about 10 minutes. Then it was tested with a few pieces of magnesium turning and a few drops of concentrated hydrochloric acid. Red color was observed and it indicated the presence of flavonoid.

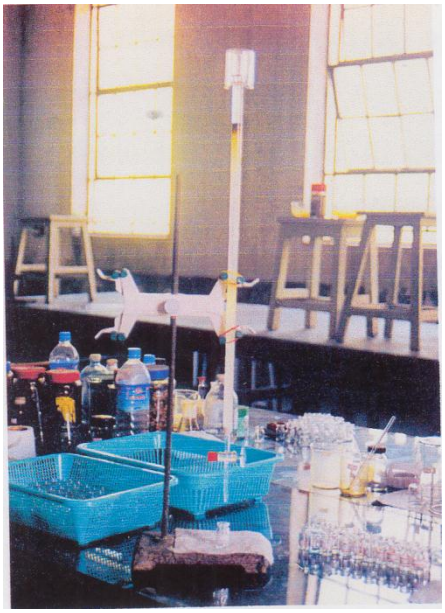


Figure 3. Column Chromatographic Separation of Seeds **of *Aesculus assamica* Griff.**

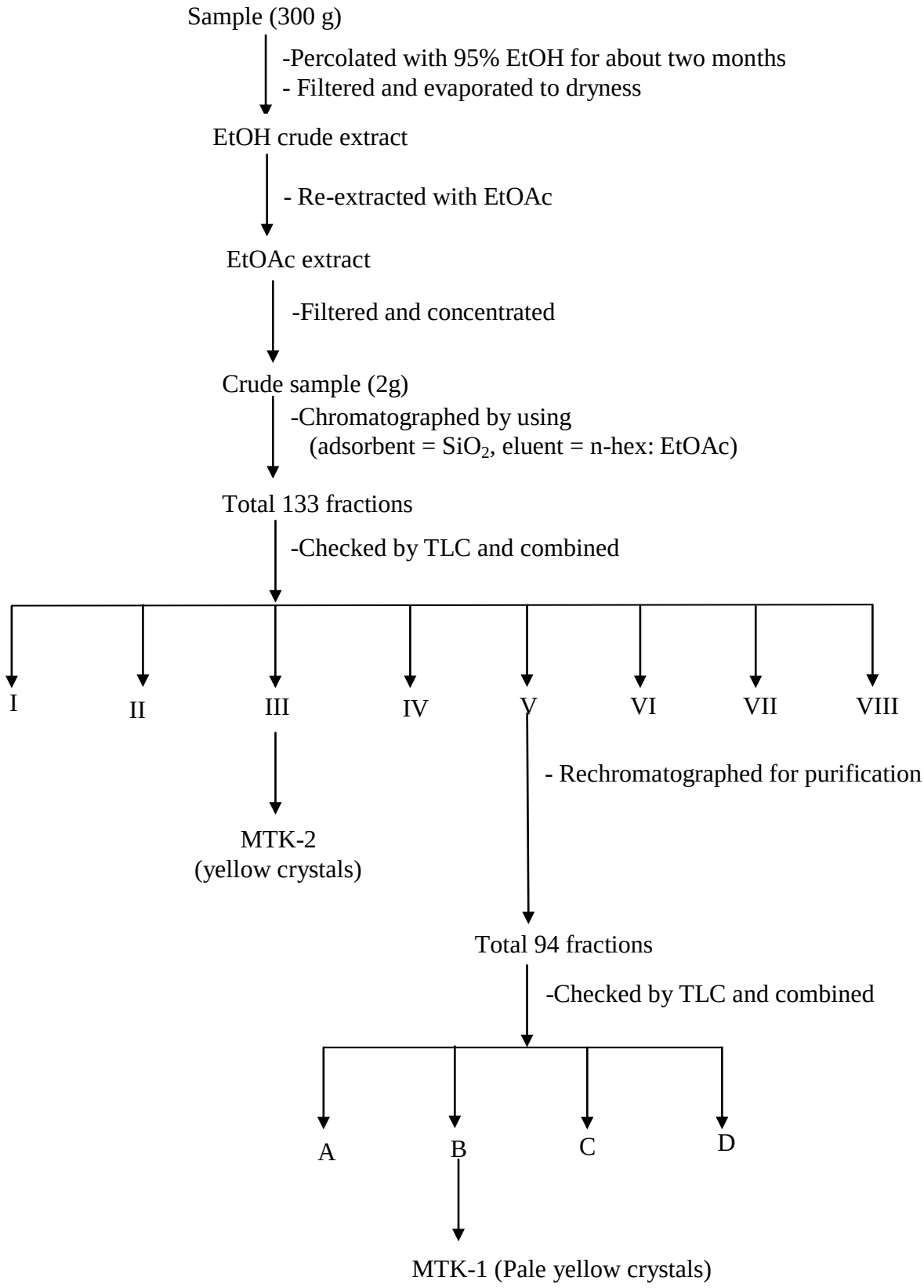
Determination of Antimicrobial Activity of Pure Isolated Compound (I)

Pure isolated compound (I) was sent to DCPT (Development centre for Pharmaceutical TEchnology), Insein, Yangon in order to examine antimicrobial activities by Agar well diffusion method. The activities given by compound (I) was shown in result and discussion section, Table (3).

Determination of FT-IR Spectra of Isolated Compounds

FT-IR spectral data of compound (I) and (II) could be determined at the Department of Chemistry, University of Mandalay. The FT-IR spectrum of compound I was compared with authentic spectrum in Fig (4).

Flow-sheet for Isolation Procedure



Results and Discussion

Table (1) Phytochemical Constituents Present in the Seeds of *Aesculus assamica* Griff. (Ye-myaw)

No.	Constituents	Reagent used	Observation	Result
1	Glycoside	10% led acetate	white ppt	+
2	Phenolic	10% $FeCl_3$	brown yellow	+
3	Polyphenol	1% $FeCl_3$ and 1% $K_3Fe(CN)_6$	greenish blue	+
4	Flavonoid	Mg tunning, HCl	red color	+
5	Saponin	Distilled water	Forth like comb	+
6	Steroid	Acetic anhydride, Conc:H_2SO_4 $CHCl_3$	green color	+
7	Terpene	Acetic anhydride, H_2SO_4, $CHCl_3$	pink color	+
8	Alkaloid	1% HCl, Mayer's reagent	No ppt	-
9	Tannin	1% $FeCl_3$	green color	+

(+) = presence, (–) = absence

Table (2) Antibacterial Activities given by Seeds of *Aesculus assamica* Griff.

Organisms	Solvents				
	n-hexane	$CHCl_3$	Acetone	EtOAc	EtOH
B. subtilis	-	13 mm (+)	15 mm (++)	20 mm (+++)	14 mm (+)
S. aureus	-	14 mm (+)	15 mm (++)	25 mm (+++)	16 mm (++)
P. aeruginosa	-	13 mm (+)	13 mm (+)	25 mm (+++)	18 mm (++)

Organisms	**Agar Well - 10 mm**
1. *Bacillus subtilis*	10 mm ~ 14 mm (+)
2. *Staphylococcus aureus*	15 mm ~ 19 mm (++)
3. *Pseudomonous aeruginosa*	20 mm above (+++)

The anti-microbial activities of this compound revealed the polar solvent (EtOAc) extract showed medium activity on three organisms, *Bacillius pumalis, Candida albi can* and *Mycobacterium* species.

Table (3) Antimicrobial Activities given by the Pure Isolated Compound (I)

	Organisms					
Solvent	*B-Sub*	S- aureus	Pseudomonas	B-pumalis	Candida	Myco-
EtOAc	(–)	14 mm (+)	(–)	16 mm (++)	16 mm (++)	15 mm (++)

"Organisms"	**Agar well -10 mm**
1. *Bacillus subtilis*	10mm ~ 14 mm (+)
2. *Staphylococcus aureus*	15mm~19 mm (++)
3. *Pseudomonas aeruginosa*	20 mm above (+++)
4. *Bacillus pumalis*	
5. *Candida albicans*	
6. *Mycobacterium* species	

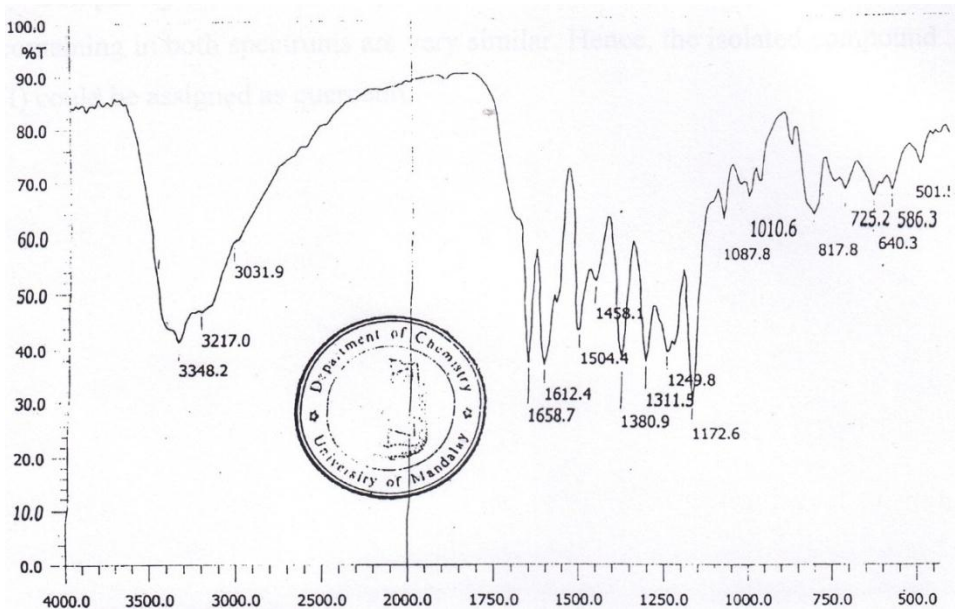
The pure form of quercetin compound (I) was isolated by thin layer and colunm chromatographic methods and by using UV detector. Compound (I) gave R_f value (0.5) with solvent system n-hexane: EtOAc (1:1).

Structural Identification of Isolated Compound (I) (MTK-1)

The FT-IR spectrum of (MTK-1) shows O-H stretching vibration band at 3348 cm^{-1}. Alkenic C-H stretching vibration band is observed at 3217 cm^{-1}. The band at 1658 cm^{-1} should be C=O stretching vibration band. One band which appears at 1612 cm^{-1} represents C = C stretching vibration band of aromatic benzene ring. Furthermore, allylic C-H in plane bending vibration can be observed at 1458 cm^{-1}. The bands at 1249 cm^{-1} and 1172 cm^{-1} imply to the C-C-O stretching bands of alcohol groups. The band which appears at 1087 cm^{-1} and 1010 cm^{-1} are due to C–O–C stretching vibrations of ether group. C–H out of plane bending vibrations of trans (or) E and Cis or Z alkene are observed at 817 cm^{-1} and 725 cm^{-1}. The functional groups observed in FT-IR spectrum are tabulated in Table (4). Then, the structural identification was done by FT-IR spectroscopic method.

Table (4) Characteristic Functional Groups Present in IR spectrum of Compound (I) (MTK-1)

No.	ν_{max} (cm^{-1})	Assignments (Functional groups)
1	3348	O–H stretching vibration of polyhydroxyl groups
2	3217	Alkenic C–H stretching vibration
3	1658	C=O stretching vibration of carbonyl group
4	1612	C=C stretching vibration of aromatic benzene ring
5	1458	C–H in plane bending vibration of allylic hydrocarbon
6	1249 and 1172	C–C–O stretching bands of alcohol groups
7	1087 and 1010	C–O–C stretching vibration of ether group
8	817 and 725	C–H out of plane bending vibration of trans or E and cis or Z alkene



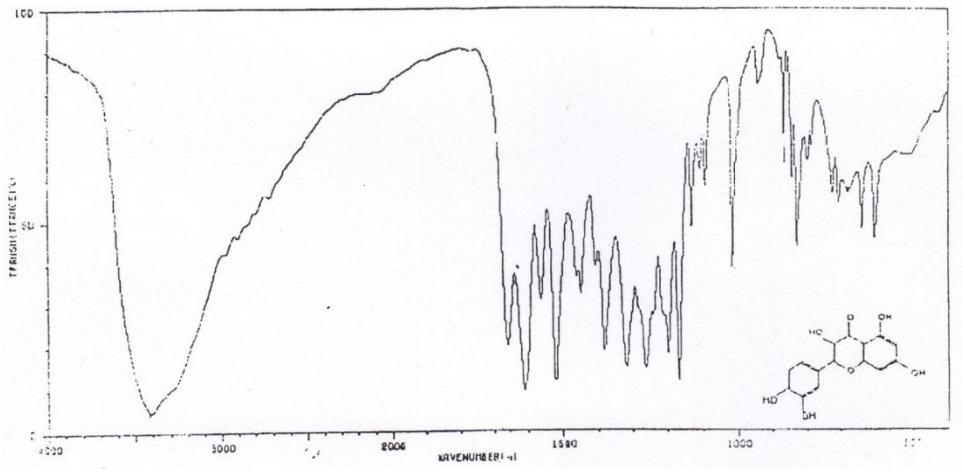


Figure 4. Comparison of FT-IR Spectrum of Compound (I) and FT-IR Spectrum of Authentic Quercetin

Comparing the two FT-IR spectrums, it is clear that the functional groups containing in both spectrums are very similar. Hence, the isolated compound (I) could be assigned as quercetin.

Table (5) Characteristic Functional Groups Present in IR spectrum of Compound (II) (MTK-2)

No.	ν_{max} (cm^{-1})	Assignments (Functional groups)
1	3409	O–H stretching vibration
2	2923 and 2854	Asymmetrical and symmetrical stretching vibration of saturated hydrocarbon
3	1720	C=O stretching vibration
4	1650 and 1604	C=C stretching vibration
5	1458	C–H in plane bending vibration of allylic hydrocarbon
6	1249 and 1188	C–C–O stretching vibration
7	972 and 690	C–H out of plane bending vibration of trans (or) E and cis (or) Z alkene

Conclusion

Ye-myaw seeds possess herbal medicinal values and therefore they were selected intentionally for the investigation of their chemical constituents, antibacterial activities, separation to individual components and their spectroscopic structural identification. Phytochemical screening of the seeds of *Aesculus assamica* Griff. was done by normal phytochemical methods which gave rise to glycoside, phenolic compound, polyphenol, flavonoid, saponin, steroid, terpene and tannin. Furthermore, the antibacterial activities of various solvent extracts such as n-hexane, chloroform, acetone, ethyl acetate and ethanol were detected. Among them, ethyl acetate extract responded the highest activity on three organisms, *Bacillus subtilis, Staphylococcus aureus* and *Pseudomonous aeruginosa.* Then ethyl acetate extract of the seeds of *Aesculus punduana* Wall was separated by advanced chromatographic technique such as thin layer and column chromatography and two isolated compounds were resulted. Among the isolated compounds, compound (I) gave medium responses towards on three organisms, *Bacillus pumalis, Candida albicans* and *Mycobacterium* species with EtOAc extract. And then the structural determination of isolated compound (I) was done by

spectroscopic methods such as ultraviolet and infrared spectroscopy. In comparison between FT-IR spectra of authentic quercetin and compound (I), the functional groups given by both spectra were very similar. Quercetin belongs to the flavonoids family and flavonoids are a group of naturally occuring compounds which are widely distributed in nature and are ubiquitous in vegetables. Therefore compound (I) could be assigned as quercetin after doing the confirmation by flavonoid color test.

The functional groups of another isolated unknown compound (compound -II) represents –OH, sp^3 hydrocarbon, alkenic group, trans (or) E and cis (or) Z alkene, allylic hydrocarbon, gem dimethyl group respectively.

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