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Publication Type	Local publication
Publisher (Journal name, issue no., page no etc.)	Universities Research Journal, Vol.9, No.2
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Extraction and Isolation of Flavonoid from *Persea americana* Miller

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Abstract

The fruits of *Persea americana* Miller (avocado) were collected from Pyin Oo Lwin Township during the year 2012-2015. Preliminary phytochemical examination for flavonoid was studied on the fruit pulp. The cultivar of marketable *Persea americana* Miller was selected and extraction and isolation of the compound flavonoid were done by thin layer chromatography using suitable solvent ratios, ultraviolet and infrared spectroscopic techniques. According to spectroscopic data, the compound glycoflavonoid (0.045g, 0.0074%) was obtained.

Key words: *Persea americana* Miller, Glycoflavonoid

Introduction

The avocado fruit is an important food in South America and is nutritious with high levels of mainly unsaturated oils, minerals, vitamins and reasonable levels of protein. The oil is evidently similar in composition to olive oil (Anonymous 2012).

Avocado or alligator pear is one of the finest salad fruits, although some people regard it as an acquired taste. The fresh, smooth, buttery pulp is eaten and it is the most nutritious of all fruits. Its consumption is increasing in the United States and the United Kingdom. The pulp, which may be preserved by freezing, is used as a sandwich filling or spread, and in ice creams and milk shakes (Purseglove 1968).

The avocado has been used not only for food but also for medicinal purposes. In *in vitro* studies, the vegetable totum of the avocado (all of the constituent phytochemicals) inhibits growth and stimulates apoptosis of tumor cells, the Seminar in Cancer Biology concluded in 2007 (Beles 2011). Clinical feeding studies in humans have shown that avocado oil can reduce blood cholesterol (Anonymous 2012).

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However, the goodness of avocado does not stop with the health benefits. It is widely used in various beauty treatments and for maintaining beautiful skin and hair. The reason of avocados' such widespread popularity is because they contain for more vitamins, minerals and nourishing oils than any other fruit.

Avocados can nourish dry hair through a lack of vitamins A and E. The avocado rich in oil is a great treatment for dry, frizzy hair and it can penetrate deep into each strand to strengthen and moisturize brittle hair.

Flavonoids are widely distributed in plants. They are known to be responsible for the yellow or red / blue pigmentations in flowers and also provide protection from attack by microorganisms and insects. The widespread distribution of flavonoids, their variety, and their relatively low toxicity compared to other active plant metabolites has led to many animals, including humans, ingesting significant quantities in their diet without problems (Bello *et al*. 2011).

Many of these flavonoids are biologically active compounds that many have disease preventing properties. In addition to their potent antioxidant properties, their mechanisms of action may include specific inhibition of enzymes or binding to receptors. Most of the flavonoids in plants are found in the glycoside form, which means they are attached to a sugar residue (Schmid *et al.* 2003).

Several flavonoids are currently used in pharmaceutical, cosmetic and food preparations (Ardhaoui *et al.* 2004; Vorsa *et al.* 2007; Birbara 2011 as cited in Danihelova *et al.* 2012). Flavonoids have protective and cleaning effect to skin, antiaging properties, pigment-saving activity and supportive mechanical strength to hair (Ghoul *et al.* 2006 as cited in Danihelova *et al.* 2012).

Flavonoids form major constituents of the human diet as they contribute to the flavour and colour of many fruits and vegetables. They have beneficial antioxidant (Kiefer *et al.* 2010).

Flavonoids have a wide range of biological effects *in vitro*, including antioxidant, anti-inflammatory, antiallergic, antiulcer, antibiotic and anticarcinogenic properties. Epidemiological evidence suggests that high consumption of plant-derived flavonoids may provide protection against coronary heart disease, stroke and lung cancer. A major role of flavonoids in both plants and humans is protection against oxidative stress promoted by free radical species. Since oxidative stress plays a major role in many chronic diseases, it is thought that increased consumption of flavonoid rich food may reduce the incidence and mortality rates of chronic diseases.

Flavonoids are polyphenolic compounds that constitute a large group of secondary plant metabolites (Howard *et al.* 2004).

Flavonoids with glucose side chains are called glucoflavonoids or glucosides (glycoside if the sugar is not specified), while the flavonoid component without sugar is called an aglycone. Flavonoid are mainly water-soluble compounds (Raaman 2006). Flavonoid compounds and the related coumarins usually occur in plants as glycosides in which one or more of the phenolic hydroxyl groups are combined with sugar residues (Ikan 1991).

Flavonoids are antioxidants present in plant foods. They occur mainly as glycosides, i.e. linked with various sugars (Peter *et al.* 1999). There is a basic structure which is common to all flavonoids; there is a great diversity of flavonoids due to different hydroxylation and glycosylation positions. Most flavonoids exist as glycosides in plant sources (Pikulski & Brodbelt 2003).

The basic structure of flavonoids is derived from the C_{15} body of flavone. They differ from other phenolic substances in the degree of oxidation of their central pyran ring. And, very fundamentally, also in their biological properties. While some classes (the flavonones, for example) are colourless, the members of other classes (the anthocyanes, for example) are always coloured and known as pigments of flowers or other plant parts (Raaman 2006).

The flavonoid compounds can be regarded as $C_6 - C_3 - C_6$ compounds, in which each C_6 moiety is a benzene ring, the variation in the state of oxidation of the connecting C_3 moiety determining the properties and class of each such compound (Ikan 1991).

Flavonoids are mainly water soluble compounds. Flavonoids contain conjugated aromatic systems and thus show intense absorption bands in the UV and visible region of the spectrum. The flavonoids are most commonly known for their antioxidant activity (Nyein Mon Wai 2011).

The flavonoids also have antioxidant, antiallergenic, antiinflammatory and neuropharmacological activities, thus contributing to human health (Lee *et al.* 2007, Bischoff 2008 as cited in Masada *et al.* 2009).

In *vivo* studies showed that the flavonoid was very effective in brightening the skin tone and had an even stronger effect on the fading of age-spots, resulting in a more even skin tone (Kiefer *et al.* 2010).

The present study is concerned with the investigation of phytochemical constituents. The main aims are to investigate the presence of phytochemical, to extract and isolate the bioactive compound flavonoid

from the fruit pulp of *Persea americana* Miller, and to identify it by chromatographic and spectroscopic analysis. The objective of the present research work is to accomplish the information on phytochemical and bioactive compound of edible fruit *Persea americana* Miller growing in Myanmar.

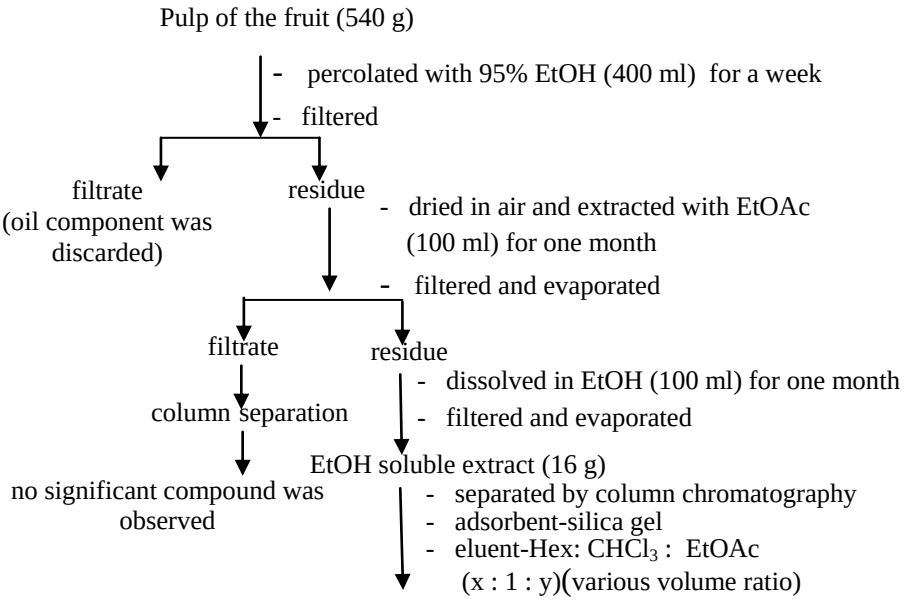
Materials and Methods

Test for Flavonoid

The two gram sample was extracted with ethanol 10 ml for ten minutes and filtered. The filtrate was added with 10 drops of concentrated hydrochloric acid and then three pieces of magnesium were added. The solution was boiled for a few minutes. The changes of colour to pink or red showed the presence of flavonoid.

Extraction and Isolation

The process for extraction and isolation was shown in Figure 1.



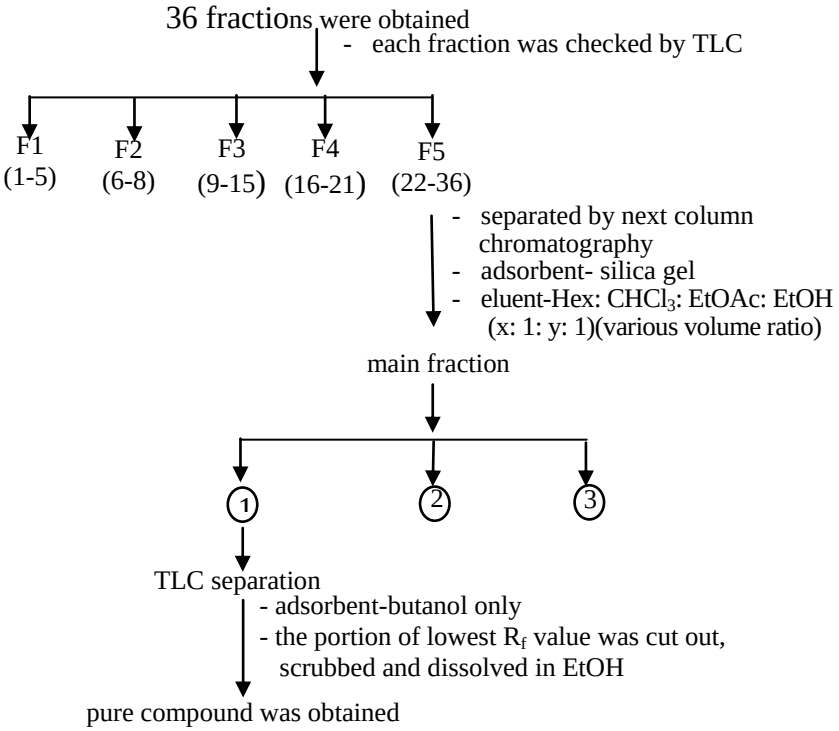


Figure 1. Extraction and Isolation of Glycoflavonoid from Fruit Pulp of *Persea americana* Miller

Identification of the Combined Fractions by Thin Layer Chromatography

The eluent collecting in each bottle was concentrated by evaporation. Fractions were checked by thin layer chromatograms using n-hexane: ethyl acetate with the suitable solvent ratios. Fractions with the same R_f value were combined. The R_f value was obtained using the following formula.

$$\text{Relative flow } (R_f) = \frac{\text{distance migrated by an analyte (DA)}}{\text{distance migrated by the solvent (DS)}}$$

Identification by Ultraviolet Spectroscopy

The isolated compound was dissolved in ethanol. The wavelength of maximum absorption for the sample compound was measured by using

Shimadzu UV-1800 series spectrophotometer at the Department of Chemistry, Universities' Research Center, Mandalay.

Identification by Fourier Transform Infrared Spectroscopy

The infrared spectrum of the isolated compound was examined by using Shimadzu FTIR-8400 spectrophotometer at the Department of Chemistry, Mandalay University.

Results

Preliminary Phytochemical Investigation of *Persea americana* Miller

The preliminary phytochemical investigation for presence of flavonoid in the pulp of fruits was carried out. The test for flavonoid indicated pink colour. Therefore flavonoid was present.

Identification by Thin Layer Chromatography

The isolated pure compound was checked by TLC as shown in Figure (3). The R_f value was 0.5 (EtOAc : n-hexane= 1:2). The physical state of the compound was found to be yellow amorphous and weighed 0.045 g. The yield percent accounted for 0.0074%.

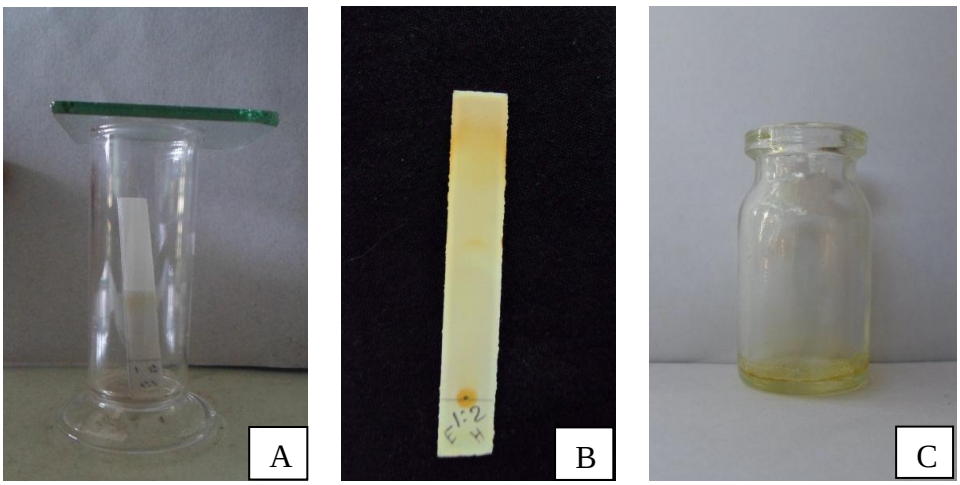


Figure 2. Identification of pure compound by using Thin Layer Chromatography

A. TLC Chamber B. TLC Plate

C. Yellow Amorphous Pure Compound

Identification by Ultraviolet Spectroscopy

The isolated compound was dissolved in ethanol. The wavelength of maximum absorption for the sample compound was measured by using Shimadzu UV-1800 series spectrophotometer at the Department of Chemistry, Universities' Research Center, Mandalay. According to the UV spectral data, the λ max of isolated compound showed at 205,260,409, 664 and they were shown in Figure (3) and Table (1).

Identification by Fourier Transform Infrared Spectroscopy

The infrared spectrum of the isolated compound was identified by using Shimadzu FTIR-8400 spectrophotometer at the Department of Chemistry, Mandalay University.

Identification of the Functional Group of Isolated Compound

The peak at 3400 cm^{-1} indicates O-H stretching frequency of alcohol and phenol group. The C-H stretching frequency of C=C-H group appears at 3037 cm^{-1}. Asymmetrial C-C-H stretching frequency was observed at 2935 cm^{-1} and symmetrical C-C-H stretching frequency was observed at 2891 cm^{-1}. The band which appears at 1714 cm^{-1} shows the stretching frequency of C=O carbonyl group in the compound. The C=O stretching mixed with hemiacetal of the aldosugar appears at 1645 cm^{-1}. The peak at 1570 cm^{-1} represent, the C=C alkenic and aromatic group. The peak found at 1406 cm^{-1} exhibits in plane bending vibration frequency of C-H group. The C-O-C and C-C-O asymmetric stretching vibration appear at 1249 cm^{-1}. The peak at 1058 cm^{-1} is due to the C-O-C and C-C-O symmetric stretching vibration. The C=C-H out of plane bending vibration of Z (C=C-H alkenic group) appears at 898 cm^{-1}. The peak at 821 cm^{-1} shows the C=C-H out of plane bending vibration of C=C-H alkene. The O-H out of plane bending vibration appears at 705 cm^{-1}. The FTIR spectrum of isolated compound was shown in Figure (4) and Table (2).

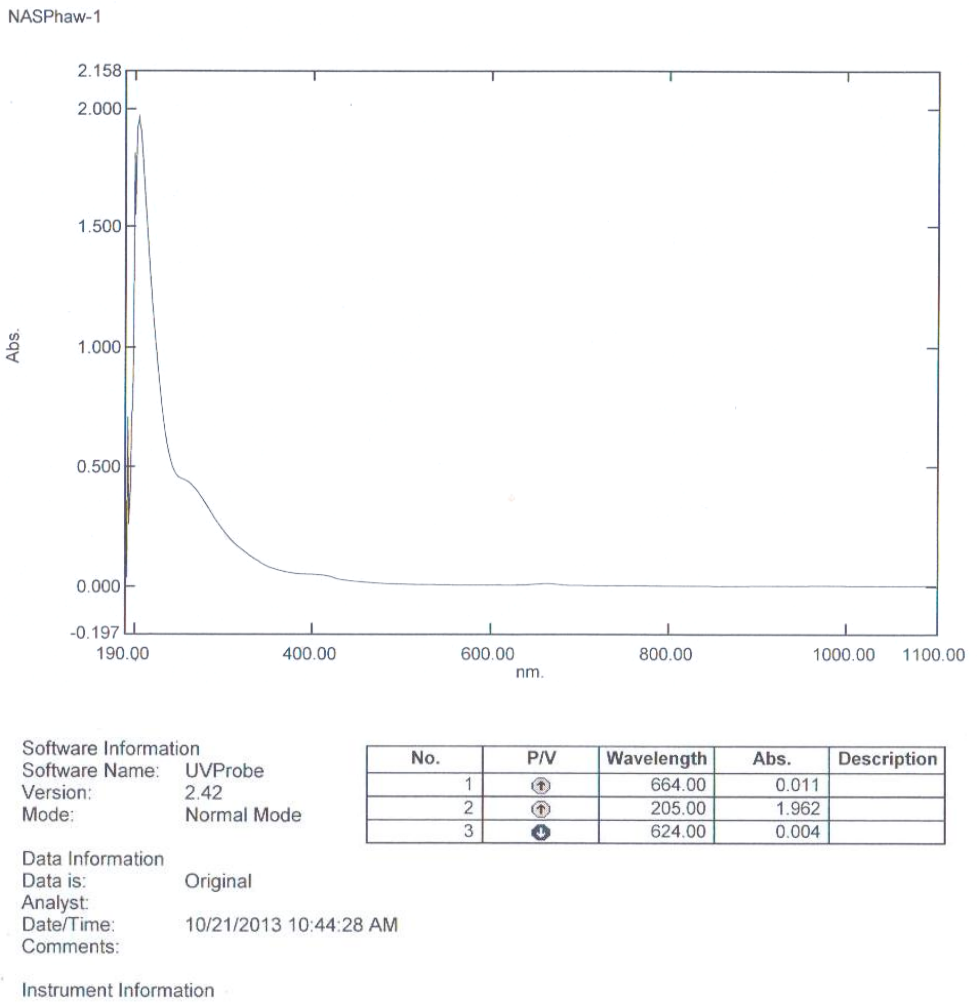


Figure 3. Ultraviolet and visible spectrum of isolated compound

Table 1. UV-Vis spectral data of isolated compound

Compound	λ max nm (nanometer)		Reference
	Observed	Literature	
Isolated Compound	205, 260, 409, 664	255, 268, 409	Masterova *et al*. 1989

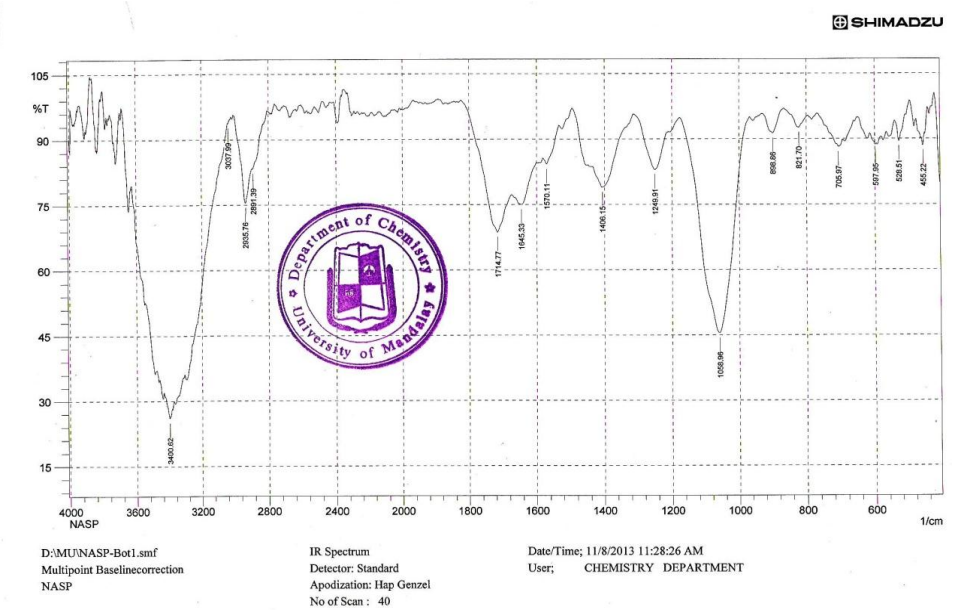


Figure 4. FTIR spectrum of isolated compound

Table 2. FTIR Spectrum data of isolated compound

No.	Wave number cm^{-1}		Functional group
	Observed Wavelength number	Literature Masterova *et al.* (1989)	
1	3400	3400	O–H stretching frequency alcohol and phenol group
2	3037	–	C–H stretching frequency of C = C–H group
3	2935	2900	C–C–H stretching frequency of asymmetrical
4	2891	–	C–C–H stretching frequency of symmetrical
5	1714	–	C=O stretching frequency of carbonyl group
6	1645	1660, 1600	C=O stretching mixed with hemiacetal of the aldosugar
7	1570	1500	C = C alkenic and aromatic group
8	1406	1445, 1430	C–H in plane bending vibration frequency
9	1249	1360, 1210	C–O–C and C–C–O asymmetric stretching vibration
10	1058	1130	C–O–C and C–C–O symmetric stretching vibration
11	898	925	C = C–H out of plane bending vibration of Z (C = C–H alkene)
12	821	810	C = C–H out of plane bending vibration (C = C–H alkene)
13	705	–	O–H out of plane bending vibration

Discussion and Conclusion

In the present study, the phytochemical investigation was carried out on the pulp of *Persea americana* Miller. Extraction, isolation and identification of glycoflavonoid compound were performed on the fruit of the marketable *Persea americana* Miller. According to phytochemical investigation, *Persea americana* Miller (Avocado) contains flavonoid compound. Flavonoids represent large group of plant pigments. These polyphenolic compounds may be found in the nature as active components of fruits, vegetables and other plants and derived products. Due to established biological effects they are attractive substances for many areas of human life. Many flavonoids are nowadays used in pharmaceutical, cosmetic and food preparations (Danihelova *et al.* 2012).

Flavonoids are polyphenolic phytochemicals which occur in edible fruits and vegetables. Flavonoids are potent water-soluble super antioxidants and free radical scavengers. They prevent oxidative cell damage, have strong anticancer activity and protect against all stages of carcinogenesis. Flavonoids in intestinal tract lower the risk of heart disease, inflammation and represent the most common and widely distributed groups of plant phenolic compounds. Flavonoids in leaves and fruits of *Persea americana* Miller could be behind anti-inflammatory, anti-cancer and anti-hypertensive property of the plant (Arukwe *et al*. 2012).

According to phytochemical investigation, the fruit pulp of avocado contains flavonoid. Therefore, avocado has the antioxidant properties and it is also the fruit which is useful in pharmaceutical, cosmetic and food preparations.

Isolation of flavonoid compounds from the fruit pulp of *Persea americana* Miller was carried out by using column chromatography. The isolated compound was identified by TLC behavior, UV and IR spectroscopic methods.

According to thin layer chromatogram, R_f value showed 0.61 (Ice *et al.* 1950) and 0.45 (Masterova *et al*. 1989). The isolated compound gave only one spot with R_f value 0.5 by using EtOAc: n-hexane (1:2) solvent system. It was also found that the isolated compound was the UV active compound. Raaman (2006) stated that the majority of flavonoids only appear during examination in UV light. UV spectrum of isolated compound showed λmax (wavelength of maximum absorption) values 205, 260, 409 and 664 nm, respectively. The UV spectrum described by Masterova *et al.* (1989) showed at 255,268,409 nm.

Masterova *et al*. (1989) described that FTIR spectrum appeared at 3400, 2900, 1660, 1600, 1500, 1445, 1430, 1360, 1210, 1130, 925 and 810 cm^{-1}. Gupta & Singh (1991) stated that IR spectrum showed hydroxyl absorption at 3700-3300 cm^{-1}, bands at 1600 and 1660 cm^{-1} due to the presence of an α, β-unsaturated function (C-4 of flavonoid), other peaks at 2930 cm^{-1} due to methylene groups and peaks at 1500 –1600 cm^{-1} due to an aromatic ring. A broad carbonyl stretching band in the region 1100 – 1600 cm^{-1}, suggested a glycosidic nature. In the present study, due to the presence of functional groups which appear at 3400, 3037, 2935, 1714, 1645 and 1249 cm^{-1}, the isolated compound can be assumed to be glycoflavonoid.

The flavonoid glycosides are a group of chemical compounds which are occurring in small but significant amounts in fruits and vegetables. Since they are rich in antioxidants, flavonoid glycosides are believed to help boost a person's immune system. With such bolstered immune function, it is thought people can protect themselves from serious illnesses. For this reason, a diet rich in foods that contain flavonoid glycosides may benefit many people (Atta *et al.* 2013). According to Yeol Lee *et al.* (2009), glycoflavonoids are well known for their biological activities that can be used to protect against cardiovascular disease and cancer. Some flavonoid glycosides are prepared synthetically or by biotransformations, usually for pharmaceutical purposes (Kren & Martinkova 2001).

Therefore, *Persea americana* Miller (avocado fruit) is a very effective fruit in all sectors such as nutrition, cosmetics and health of mankind due to the presence of phytocosmetic compound, glycoflavonoid.

Acknowledgements

We would like to express my deep gratitude to Dr Nu Nu Yee, Professor and Head, Department of Botany, University of Mandalay, for her permission to carry out this research work.

Our special thanks were due to Dr Hla Myo Min, Lecturer, Department of Chemistry, Kalay University for his kind help in extraction, isolation and identification of chemical studies.

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