Pharmacogn J. 2018; 10(1):60-63
A Multifaceted Journal in the field of Natural Products and Pharmacognosy
www.phcogj.com | www.journalonweb.com/pj | www.phcog.net

Original Article

Qualitative and Quantitative Test of Total Flavonoid Buni Fruit (*Antidesma bunius* (L.) Spreng) with UV-Vis Spectrophotometry Method

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ABSTRACT
The aim of this research is to determine of total flavonoid content in the Buni fruit (*Antidesma bunius* L. Spreng) extract. The extract was produced by stratified maceration method with the different solvent, i.e *n*-Hexane, Ethyl acetate and ethanol. The analysis of chemical compound using chemical reagent and Thin Layer Chromatography (TLC) method. The method is used to determines total flavonoid contains Buni fruit (*Antidesma bunius* L.) extract was based on the amount of Rutin Equivalent (RE) were used. The result shows that the flavonoid content higher in the *n*-Hexane extract is 10.72 %, then ethyl acetate extract is 7.9 % and 3.56 % ethanol extract was counted to or as a Rutin.
Key words: *Antidesma bunius* L. Spreng, Flavonoid content, Spectrophotometry UV-VIS.

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History
- Submission Date: 01-06-2017;
- Review completed: 11-09-2017;
- Accepted Date: 20-11-2017

DOI : 10.5530/pj.2018.1.12

Article Available online
http://www.phcogj.com/v10/i1

INTRODUCTION

Oxidative stress has been associated with the neuro-degenerative disorder, Alzheimer's disease, type 1 and type 2 diabetes,[1] cancer, and cardiovascular disease. Epidemiology study showed that many constituents on fruits can protect human body against oxidative stress. Consume the natural antioxidant can keep our body health.[2]

Flavonoids are class of low molecular weight compounds that assembled by a polyphenols skeleton.[3] Flavonoids can prevent injury caused by free radicals in various ways. One way is the direct scavenging of free radicals. Flavonoids are oxidized by radicals, resulting in a more stable, less reactive radical. In other words, flavonoids stabilize the reactive oxygen species by reacting with the reactive compound of the radical.[4]

Antidesma bunius (L.) Spreng (buni fruits) may be shrubby, 3-8 m high, or may reach up to 15-30 m. Buni fruits contain anthocyanin because is his red to purple (violet). According to research Samappito and Butkhup revealed that the fruit of Mao Luang or buni fruits (*A. bunius* L.) is a kind of medicinal plants where many villagers in Northeast Thailand use juices of ripe fruits to heal their health problems on diabetes, dysentery, indigestion and constipation and contained some enormous amount of flavonoids chemical compounds, ie, catechin, procyanidin B1 and procyanidin B2[5] and also two groups of organic acids, i.e major and minor group where these chemical compounds possess its important role as protective agents against fungus and uv irradiation.[6] Buni fruits are rich in nutritional components such as carbohydrates, sugars, organic acids, proteins, minerals, vitamins. In previous research has shown that methanolic extract buni fruit (*A. bunius* L.) that grows in India show high anti-oxidant activity, with an average IC_{50} value of 100.08 mg/mL, when compared with the other fruits.[7]

Based on these descriptions, needs further research on content flavonoids in buni fruits to add scientific data so it can be justified

EXPERIMENTAL METHOD

Sample Preparation

Buni fruit (*A. bunius* L.) sample obtained in Enrekang district. The sample is then cleaned of dirt by using running water and then dried with aerated. After dried, the powered samples, then it was extracted by maceration gradual method.

Buni fruit powder (*A. bunius* L.) weighed 200 grams and then put in a maceration container. *n*-hexane solvent as much as 500 mL, was added to the maceration container until all the powder samples submerged, and then cover tightly. Maceration container stored in a protected place out of direct sunlight for 3 × 24 hours while stirring periodically. The mixture was filtered and maceration again with *n*-hexane as much as 1 L for 3 × 24 hours. The mixture is then filtered and immersed with 700 mL of ethyl acetate for 3 × 24 hours, then the mixture was filtered and the residue maceration again with 900 mL of ethyl acetate for 3 × 24 hours. Last, immersed in ethanol as much as 1 L for 3 × 24 hours. The mixture was filtered and maceration again with ethanol as much as 1 L for 3 × 24 hours. Collected liquid extract then

Cite this article: Hamidu L, Ahmad AR, Najib A. Qualitative and Quantitative Test of Total Flavonoid Buni Fruit (*Antidesma bunius* (L.) Spreng) with UV-Vis Spectrophotometry Method. Pharmacog J. 2018;10(1):60-3.

concentrated by evaporator simple method to obtain the *n*-hexane, ethyl acetate and ethanol viscous extract.

Qualitative Flavonoids Test

The Thin Layer Chromatography (TLC) method chosen because it has several excesses such as speed and sensitivity.[8] Spotting obtained detected under UV_{366} nm light and using spray reagent. Reagents used for flavonoids is $AlCl_3$ and citroborat. Then observed again under UV_{366} nm light. $AlCl_3$ gives yellow color[8] whereas citroborat reagent gives something positive will fluoresce yellow-green. Each *n*-hexane, ethyl acetate and ethanol extract of buni fruit diluted with methanol and then spotted on the TLC plate. The plate take in the chamber that contains the eluent chloroform: acetone (1 : 1). Spotting observed under UV_{366} nm. Then sprayed with a specific reagent. Reagent commonly used for identification of flavonoids as reagents spray in the TLC is $AlCl_3$ and citroborat which will give a yellow color.

Determination of Total Flavonoids
Preparation of Rutin Solution

In the study, total flavonoid content was determined using a modified method based on the procedure of Chang *et al.*[9] Weighed 10 mg of rutin and dissolved in 10 mL of methanol p.a (1000 ppm). Taken 5 mL stock solution, then added volume to 50 mL with methanol PA (100 ppm). To the stock solution of 100 ppm rutin standard then created a series of concentration is 20 ppm, 30 ppm, 40 ppm, 50 ppm and 60 ppm. The each standard solution (1 mL) was added with 0.2 mL $AlCl_3$ 10%, 0.2 mL potassium acetate 1M, 3.0 mL methanol p.a and 5.6 mL aqua bidestillata, and then incubated for 30 minutes. Furthermore, absorbance was measured at maximum wavelength 415 nm.[10]

Preparation of Sample Solution

Weighed 10 mg of rutin and dissolved in 10 mL of methanol p.a (1000 ppm). The each rutin solution (1 mL) was added with 0.2 mL $AlCl_3$ 10%, 0.2 mL potassium acetate 1M, 3.0 mL methanol p.a and 5.6 mL aqua-bidestilata, and then incubated for 30 minutes. Furthermore, absorbance was measured in 415 nm wavelength. The sample solution made in three replications.

Table 1: The results of calculations percent yield of *n*-hexane, ethyl acetate and ethanol extract of buni fruit (*A. bunius* L.)

Solvent	Weight of sample (g)	Weight of extract (g)	Rendamen (%)
n-Hexane	200	7.6094	3.8047
Ethyl acetate	200	2.0017	1.0009
Ethanol	200	3.5718	1.7859

RESULT AND DISCUSSION

The sample used in this study is the buni fruit (*A. bunius* L.). Where the berry fruits containing anthocyanin because of his red to purple (violet) colour. According to research Samappito and Butkhup revealed that the fruit of Mao Luang or buni fruits (*A. bunius* L.) is a kind of medicinal plants where many villagers in Northeast Thailand use juices of ripe fruits to heal their health problems on diabetes, dysentery, indigestion and constipation and contained some enormous amount of flavonoids chemical compounds, ie, catechin, procyanidin B1 and procyanidin B2[5] and also two groups of organic acids, i.e major and minor group where these chemical compounds possess its important role as protective agents against fungus and uv irradiation.[6] Buni fruits are rich in nutritional components such as carbohydrates, sugars, organic acids, proteins, minerals, vitamins. In previous research has shown that methanolic extract buni fruit (*A. bunius* L.) that grows in India show high antioxidant activity, with an average IC_{50} value of 100.08 mg/mL, when compared with the other fruits.[7]

The extraction method used in this study is maceration gradual method. Maceration is the process of extracting using solvent with several mixing at room temperatures.

The solvent used is *n*-hexane, ethyl acetate and ethanol. These solvents in accordance with the level of polarity, started in nonpolar, semi polar and polar. This is intent on to maximize extraction of flavonoid compounds extract of buni fruits (*A. bunius* L.).

After obtained the yield results percentation, furthermore to identify the class of chemical compounds. The purpose is to identify groups of chemical compounds to determine the flavonoid compounds contained in *n*-hexane, ethyl acetate and ethanol extracts of buni fruits. Identification of chemical compounds using the TLC method with eluent Chloroform: Acetone (1: 1). Then sprayed with reagent citroborat and $AlCl_3$.

Description: Stationary phase = Silica gel F_{254} (7 × 1 cm)

Mobile phase = Chloroform: Aceton (1 : 1)

(a) UV_{366} detection and spray reagent $AlCl_3$

(b) UV_{366} detection and spray reagent citroborac

1. *n*-Hexane Extract
2. Ethyl Acetate Extract
3. Ethanol Extract

From the results of phytochemical screening on extract of buni fruit showed that each extract of *n*-hexane, ethyl acetate and ethanol contain secondary metabolites such as flavonoids.

Flavonoids can form a bond in the position of the other with a mixture of boric acid and citric acid on heating, and is known by citroborat reagent. Color/fluorescence that is formed is yellow-green yellow fluorescence under light UV_{366} nm. This is a result of the reaction between citroborat with flavonoid group to form a complex between the hydroxyl

Table 2: Total flavonoid content measurement buni fruit extract (*A. bunius* L. Spreng)

Sample	Replication	Absorbance (y)	First Flavonoid Contents (mg/mL)	Total Flavonoid (g. RE/g. extract)	% Flavonoid Content
n-Heksan	1	0.313	0.1033	0.1072	10.72%
	2	0.335	0.1111		
Ethyl Acetate Extract	1	0.232	0.0744	0.079	7.9%
	2	0.258	0.0836		
Ethanol Extract	1	0.124	0.0358	0.0356	3.56 %
	2	0.123	0.0354		

Based on the results of the calculation of total flavonoid content of each buni fruit extract that has been carried out, showing that the total flavonoid content of *n*-hexane extracts by 10.72%, the ethyl acetate extract of 7.9% and the ethanol extract of 3.56%.

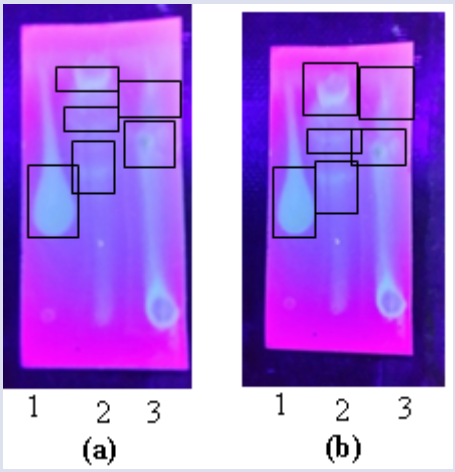

Figure 1: Qualitative test flavonoid *n*-hexane, ethyl acetate and ethanolextract of buni fruit (*A. bunius* L.)

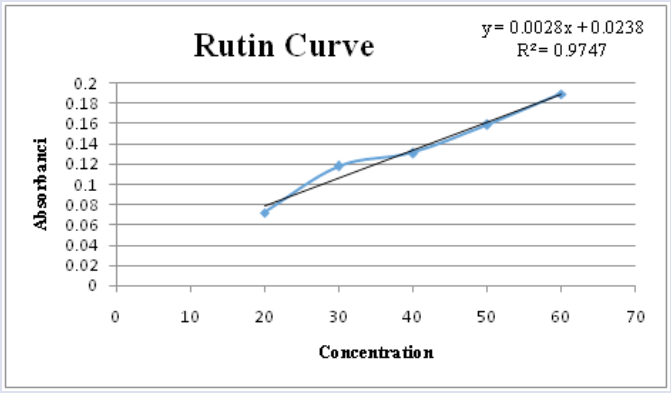

Figure 1: Qualitative test flavonoid *n*-hexane, ethyl acetate and ethanolextract of buni fruit (*A. bunius* L.)

and ketone neighboring acid resistant or with groups that are not acid resistant ortohydroxyl.[11,12] Flavonoids will indicate extinction spots on UV_{254} nm, whereas at UV_{366} nm spots will fluoresce dark yellow, green or blue.[13]a portion of phenylalanine ammonia lyase, chalcone synthase, glucosyl transferase, and all of the trans-cinnamate 4-monooxygenase and NADH Cytochrome c reductase (the last an endoplasmic reticulum marker After being sprayed with $AlCl_3$, flavonoid compounds will give yellow.[8]

The quantitative analysis of flavonoids can use UV-Vis spectrophotometer. Ultraviolet absorption spectra and absorption appears to be a single most useful way to identify the structure of flavonoids.[12] Flavonoids contain aromatic conjugated system that can show strong absorption band in the UV-Vis.[8]

Analysis of total flavonoid content using the colorimetric method of Chang *et al.*[9] measured the UV-Vis spectrophotometry. Stage of making a standard solution, namely by using a standard solution rutin. Standard solution rutin used because the flavonoids found in plants, most commonly in the form of quercetin glycosides such as 3-rutinosida or rutin compound.[10]

Quantitative analysis begins with created the series of concentration rutin solutions with modified method based on the procedure of Chang *et al.*[9] i.e 20 ppm, 30 ppm, 40 ppm, 50 ppm and 60 ppm. Each standard solution (1 mL) was added with 0.2 mL $AlCl_3$ 10%, which serves to give effect bathochromic by a shift towards a wavelength longer, thus altering the wavelength of a rutin standard to get into range λ ultraviolet and λ visible, and there is also the effect of hyperchromic or increase the intensity of a standard solution regularly produce color thick yellow, so the color reaction which formed can be observed and measured on a UV-Visible spectrophotometer. Then added 0.2 mL potassium acetate 1M, which serves as a stabilizer, so that bathochromic effects that occur can be maintained, added 3.0 mL methanol p.a which serves as a solvent and 5.6 mL aquabidestilata, and then incubated for 30 minutes. It is intended that the reaction between the rutin standard with reagents used can take place perfectly.

In the sample absorbance measurements, weighed 10 mg of rutin standard and dissolved in 10 mL of methanol p.a (1000 ppm). Each standard solution (1 mL) was added with 0.2 mL $AlCl_3$ 10%, 0.2 mL potassium acetate 1M, 3.0 mL methanol p.a and 5.6 mL aqua bidestilata, and then incubated for 30 minutes. Absorbance measurement rutin solution running begins with a wavelength suitable for rutin solution. The running showed that the maximum wave running rutin standards turned away at λ 418 nm.

The results of the rutin solution absorbance measurement with several concentrations showed that a linear relationship between the absorbance at a concentration that is equal to 0.9747. The magnitude of this linearity approaching a value of one, so that it can be said that the absorbance is directly proportional to the concentration and follow the linear regression equation is as follows: Y = bx + a. From the calculations, the value of the intercept of 0.0028 and a slope value of 0.0238, so the equation of the standard curve is y = 0.0028xn + 0.0238. This equation is used as a comparison in the quantitative analysis on the measurement of the content of rutin flavonoid compounds to *n*-hexane, ethyl acetate and ethanol extract of buni fruit.

CONCLUSION

Based on research that has been conducted, buni fruit (*A. bunius* (L.) Spreng) contains flavonoids with the percentage of *n*-hexane extract concentration by 10.72%, the ethyl acetate extract of 7.9% and the ethanol extract of 3.56% counted towards or as rutin.

CONFLICT OF INTEREST

There is no conflict of interest.

ABBREVIATION USED

TLC: Thin Layer Chromatography; **UV**: Ultraviolet; **Vis:** Visible; **$AlCl_3$**: Aluminium Chloride.

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GRAPHICAL ABSTRACT

SUMMARY

- This paper reported content of flavonoid buni fruit (*Antidesma bunius* L. Spreng)
- Total flavonoid content were determined by reference to the chang method using a UV-VIS spectrophotometer

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Cite this article: Hamidu L, Ahmad AR, Najib A. Qualitative and Quantitative Test of Total Flavonoid Buni Fruit (*Antidesma bunius* (L.) Spreng) with UV-Vis Spectrophotometry Method. Pharmacog J. 2018;10(1):60-3.