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The Phytochemistry Profile of Raru (*Vatica pauciflora* and *Cotylelobium melanoxylon*) and Its Potential as Diabetes Mellitus Drug

Marina Silalahi[1]

[1]Departement of Biology Education, Faculty of Education and Teacher Training, Universitas Kristen Indonesia, Cawang, 13510, Indonesia
Corresponding author: marina_biouki[at]yahoo.com; marina.silalahi[at]uki.ac.id

Abstract: ***Vatica pauciflora Blume and Cotylelobium melanoxylon Pierre have been used by Batak ethnic to cure of diabetes mellitus. Research was conducted to know the phytochemistry of Vatica pauciflora and Cotylelobium melanoxylon as initial step to bioprospection of diabetes mellitus drugs. The bark of Vatica pauciflora and obtained from Central Tapanuli (North Sumatra) and Cotylelobium melanoxylon obtained from Riau. The extraction done through maseration with ratio 1:10 w/v (raru : methanol). The phytochemistry of Vatica pauciflora and Cotylelobium melanoxylon are analysed by Thin Liquid Chromatography (TLC). The bark of the Cotylelobium melanoxylon contains triterpenoids, flavonoids, tannins, and β- sitosterol, while the bark of Vatica pauciflora have triterpenoids, flavonoids, and β-sitosterol but do not tannin.***

Keywords: *Raru, Vatica pauciflora, Cotylelobium melanoxylon, diabetes mellitus*

1. Introduction

Raru are the bark which use by the Batak ethnic to fermentation of the *Arenga pinnata* sap (*tuak*). The addition of the *raru* bark to *tuak* will be change of flavour and its colour. Based on a review of the literatures, the *raru* consist of some of species such as: *Shorea maxwelliana*, *Vatica songa*, *Garcinia* sp. (Hildebrand 1954), *Shorea faguetiana* (Erika 2005), *Cotylelobium melanoxylon* (Pasaribu et al. 2007), *Cotylelobium lanceolatum*, *Vatica perakensis* (Pasaribu 2011), *Shorea balanocarpoides* (Pasaribu et al. 2011, Pasaribu 2011), *Eurya* sp. (Anggraeni 2013; Silalahi et al. 2015).

Vatica pauciflora and *Cotylelobium melanoxylon* are the *raru* bark widely used for diabetes mellitus drugs (Silalahi 2014; Silalahi et al. 2015) and fermentation of *tuak*, so that its easy found and traded in the traditional markets in North Sumatra (Silalahi 2016). Utilization of the *raru* bark as diabetes mellitus drugs adopted from the tradition of the Batak ethnic (which is not prohibited by religion) in North Sumatra drunk of *tuak* at the night (Silalahi et al. 2015b). The people which regulary drunk of *tuak* didn,t suspect of diabetes mellitus.

Diabetes mellitus, one of the metabolic disorders characterized by chronic hyperglycemia, is a serious global health concern rapidly reaching epidemic levels (Mosa et al. 2015). The treatment of diabetes with synthetic drugs is costly and chances of side effects are high. Over the time, diabetes can damage the heart, blood vessels, eyes, kidneys, and nerves (Gaikwad et al. 2014). To curing of diabetes mellitus, the Batak ethnic have been used the medicinal plants that have bitter taste such as: *Andrographis paniculata*, *Eurycoma longifolia*, dan *Lindernia viscose* (Silalahi 2014; Silalahi et al. 2015). Wiryodidagdo et al. (2000) stated that the plants as medicine of diabetes mellitus are plant that produces compounds which can be suppress or stimulate the endocrine glands. The current diabetic therapies include use of carbohydrate digestive enzyme inhibitors (Hung et al. 2012).

Some secondary metabolites produced by medicinal plants such as terpenoids, alkaloids, flavonoids, tannins, and saponins have an activity antidiabetic. Phenols and flavonoids are phenolic compounds biosynthesized by some plants (Taiz & Zeiger 2006). Flavonoids have activity as an antidiabetic (Brahmachari 2011). Alkaloid is a class of compounds that is alkaline, containing one or more nitrogen atom (Zieggler & Facchini, 2009). Various multiple biological activities of plant-derived triterpenoids with apparent effects to diabetic complications such as: *Lagerstroemia speciosa* (Hou et al. 2009) and *Weigela subsessilis*; (Lee et al. 2010), *Protorhus longifolia* (Mosa et al. 2015). To knowing of the potential of *raru* bark as diabetes mellitus drugs, this research was conducted to analysis phytochemical bark of *raru* (*Vatica pauciflo*ra and *Cotylelobium melanoxylon*).

2. Methods

The materials used in this study are bark of *raru* (*Vatica pauciflora* obtained from Central Tapanuli and *Cotylelobium melanoxylon* obtained from Riau). The *raru* bark cut to be small pieces (1x 2 cm) and then oven-dried at 50° C until its weight is constant. The raru bark is dried pulverized to be powder.

Extraction done through the maceration methods. The powder of the *raru* barks are soaked with methanol for 3 x 24 hours with the ratio 1: 10. The raru barks solution are filtered with Whatman number 1, and then concentrated using a rotary vacuum evaporator. The extraction of raru barks of *Cotylelobium melanoxylon* (R) and *Vatica pauciflora* (S) are 6 times to each species.

Profile of phytochemical of raru barks extract screening through Thin Layer Chromatography. The eluent are used for each compounds are different for diffrent compounds (Table 1). The compounds of extract compared to Table 1. When color of extract changes after added reagens are positive (Juarna 2016 modification). To determine the type of compounds contained in the extract compared to the retention time some standard compounds. If retention time of the standart compouds similar with extract, so the extract have compounds similar to standart.

Table 1: The eluent and reagent to qualitative test of raru barks (*Cotylelobium melanoxylon* and *Vatica pauciflora*) compounds.

Compounds	Eluent	Reagen	Positive
Terpenoids	n- vanilin : etil acetate (8:2)	1% of vanillin and 10% of H2SO4.	Purple
Alkaloids	Toluene: etil acetate (7:3)	Dragendorff	Orange
Flavonoids	CHCl3: Acetone: vanillin (10:2:1)	AlCl3 10%	Yellow
Tannins	Toluene : Acetone: Formic acid (5:4:1)	FeCl3 10%	Blackish Green

3. Results

The methanol extract of raru barks contain triterpenoids, β-sitosterol, flavonoids, saponins, and tannins (Table 1) but don,t alkaloids. The bark of *Cotylelobium melanoxylon* contains triterpenoids, flavonoids, tannins and β-sitosterol, while barks of *Vatica pauciflora* don't contain tannin.

Table 2: The phytochemical profile of raru barks extract (*Cotylelobium melanoxylon* and *Vatica pauciflora*)

No samples	Samples#	Terpenoids	β-sitosterol	Alkaloids	Saponin	Tannins	Quercetin
1	RI	+	+	--	+	+	--
2	RII	+	+	--	+	+	--
3	RIII	+	+	--	+	+	--
4	SII	+	+	--	+	-	--
5	RIV	+	+	--	+	+	--
6	RV	+	+	--	+	+	--
7	RVI	+	+	--	+	+	--
8	SI	+	+	--	+	-	--
9	SIII	+	+	--	+	-	--
10	SIV	+	+	--	+	+	--
11	SV	+	+	--	+	-	--
12	SVI	+	+	--	+	-	--

R (*Cotylelobium melanoxylon*); S (*Vatica pauciflora*)

3.1. Terpenoids

The all of *raru* barks extracts (*Cotylelobium melanoxylon* and *Vatica pauciflora*) have terpenoids. The color of those extract changes to be purple when sprayed with the reagent. The samples to be purple after the vanillin are reacted with sulfuric acid caused formation trietilmethan by anisaldehid. To found the compounds terpenoids contained in the bark raru compared with standard β-sitosterol. Selection of β-sitosterol is based on the assumption that these compounds are terpenoids are most commonly found in plants. The retention time of all samples was detected by comparing the retention time with the standard β-sitosterol. The extract has the same retention time (Rf) with the standard of the compound is β-sitosterol. Based on densitometry test, those are known that β-sitosterol (Rf=0.6), which are the similar the Rf with compounds at the samples. The all samples *Cotylelobium melanoxylon* and *Vatica pauciflora* contain β-sitosterol but the its consentration are small.

3.2. Flavonoids

Phenol is one of the groups has been in the flavonoid compound. The all of samples are positive contains of flavonoids. Those compounds allegedly which resulted those extract to be reddish-brown (Figure 2). Quercetin is one of flavonoid that commonly found in plant. Those resulted the quercetin used as standard to determine of flavonoid at the samples. The test showed that the samples haven't of the similary of Rf with quercetin. Quercetins have 0.65 Rf, while the samples have 0.55 Rf. It showed that the flavonoids found in the *raru* barks didn't quercetin. To determine the types of terpenoids contained in the raru barks expected further research on flavonoids at raru barks through Gas Chromatography Mass Spectrometry (GCMS).

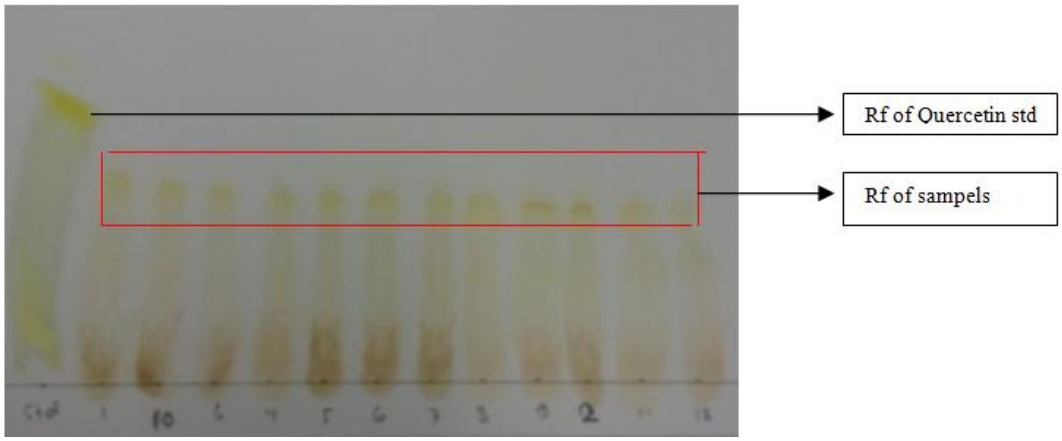

Figure 2: Retention time (Rf) of quercetin standart and samples (extract of *raru* barks)

3.3. Tannin (Phenolic)

One of the compounds belonging to the phenolic is tannin. The 7 samples of raru barks extract have tannin (Figure 3), while 5 samples haven,t contain of tannin. The all of samples haven't of tannin are *Vatica pauciflora*. Tannin allegedly implicated to the colour of *Cotylelobium melanoxylon* extract different with *Vatica pauciflora* extract. The extract of Cotylelobium melanoxylon has darker than the Vatica pauciflora extract (Figure 4).

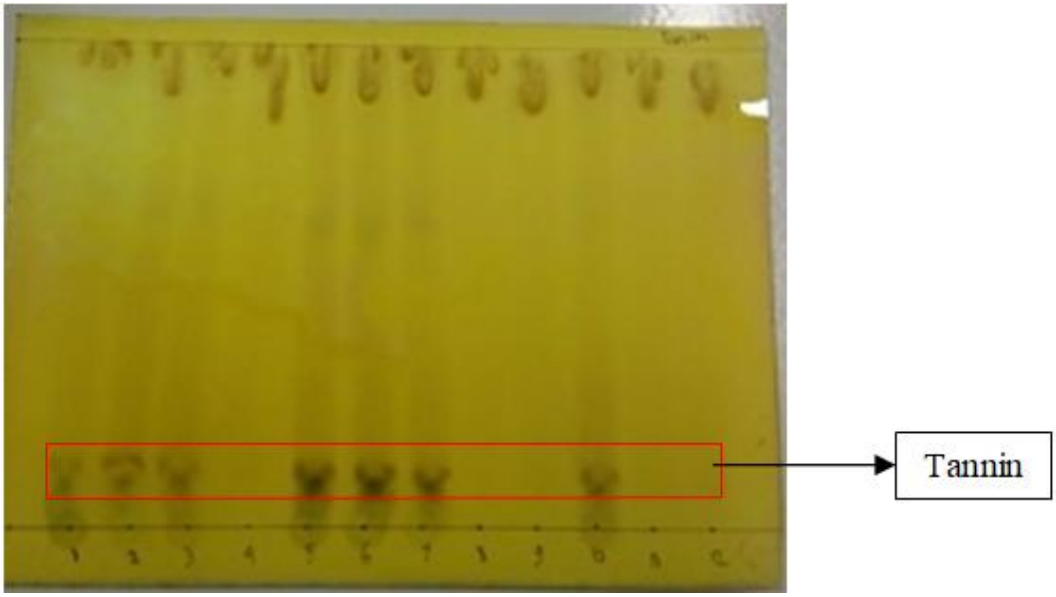

Figure 3: Profile of tannin at the raru barks extract (*Cotylelobium melanoxylon* and *Vatica pauciflora*)

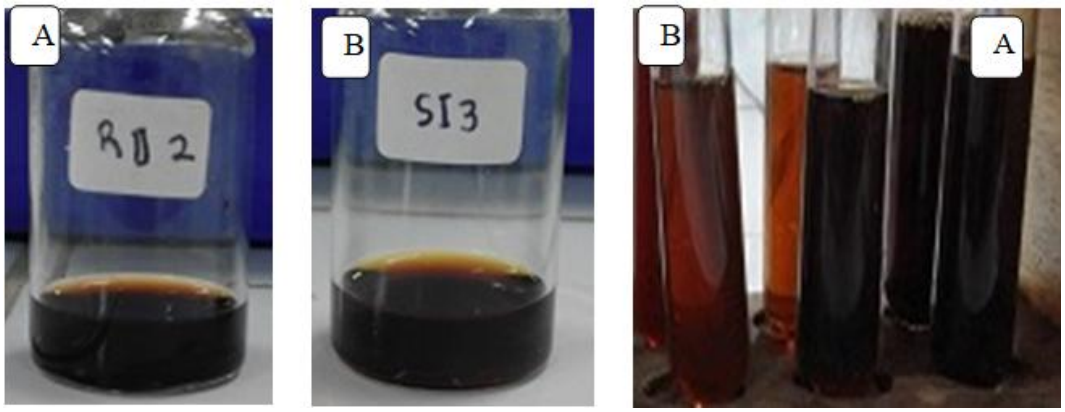

Figure 4: The colour of raru barks extract (A) *Cotylelobium melanoxylon* (B) *Vatica pauciflora*.

4. Discussion

The use of plants as traditional or modern drugs related to the content of secondary metabolites. The *raru* bark by local communities in Indonesia used as medicine of diarrhea, diabetes mellitus (Soerianegara and Lemmens, 1994; Hasanah et al., 2014; Idramsa et al. 2015; Silalahi 2015a; 2015b) and malaria (Idramsa et al. 2015).

Utilization of *raru* barks as diabetes mellitus drugs currently more prevalent than diarrhea and malaria. The empirical data shows that diabetes mellitus requiring treatment in the long term. The human believe that uses medicine from natural products (plants extract) safer than medicine sintetic for a long time. This resulted in local people are always looking for alternative treatments and herbs that are considered safer or less side effects.

Efficacy raru bark as diabetes mellitus drugs associated with the content of secondary metabolites. In this study it was found that the bark raru tested in this study include terpenoids, alkaloids, tannins, saponins, β-sitosterol, and phenol. The compounds are believed to be associated directly or indirectly with setting levels of sugar in the blood plasma. The all bark raru of Cotylelobium melanoxylon and Vatica pauciflora contains β-sitosterol but the levels are small. The β-sitosterol in the bark raru relates to the fact that the compound is one of the major components in cells (Taiz and Zeiger 2006). β-sitosterol set most phytosterol contained in plant height and spread in various organs (Gahlaut et al. 2015). Pasaribu et al. (2011) states that terpenoids are secondary metabolites found in both of the genus Vatica raru and Cotylobium. Juarna (2016) reported the same thing that all organs of plants Bischofia javanica containing β-sitosterol, but the levels are different in each organ. Further expresses the levels of β-sitosterol in plants organ allegedly associated with the function of each organ.

Alkaloid group generally has the main characteristics such as taste bitter. Alkaloids are compounds that are often stored in the leaves (Juarna 2016), which resulted in these compounds were not detected in the bark raru in this study. The abundance of alkaloids in leaf organs may be linked to the role of these compounds in plants. Alkaloids are known to have a role as a protective agent in the interaction of plants with other organisms (Aniszewski 2007).

Classified as a flavonol quercetin compound, one of six subclasses of a group of flavonoids, which are found mainly in the higher plants, but in this study was not found. Commonly found in the form of quercetin aglycone flavonoid, or compounds that do not bind to sugar. However, in nature can also be found that the compound quercetin glycosides of quercetin is one of the hydroxyl groups replaced with sugar group (Alrawaiq & Abdullah 2014). In this study, quercetin glycosides are suspected in the sample because it has a larger molecule so that its retention time is smaller dibandigkan with quercetin. In plant compounds quercetin acts as a defense against pathogenic fungi (Andersen & Markham 2006) antimicrobial (Sanzani et al. 2014). These compounds are also believed to play a role indirectly in work settings glucosidase enzyme that controls blood sugar (Riris 2013).

The number of compounds in the group of tannins found in the bark of raru related to the fact that plants produce secondary metabolites which contain a phenol group or tannin in large quantities (Taiz & Zeiger 2006). About 10,000 single compound is expected to list in phenolic compounds / tannin. Pasaribu et al. (2011) which states that the tannin is the main compound found in the bark raru. The content of tannin in the bark raru alleged medicinal properties associated with diarrhea (Soerianegara and Lemmens 1994) as well as the diabetes drug. In plants, flavonoids including tannins act as agents' alelokimia (Mierziak et al. 2014) and the color of the flower giver (Koes & Quattrocchio 1994). Meanwhile, in animals flavonoids have antioxidant properties (Chae et al., 2013; Selawa et al. 2013), and diabetes medications (Brahmachari 2011).

The *Cotylelobium melanoxylon* and *Vatica pauciflora* have terpenoids, flavonoids, β- sitosterol, so its have potential as diabetes mellitus drug. The *Cotylelobium melanoxylon* has tannin, but no in the *Vatica pauciflora*.

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