THE PHYTOCHEMISTRY PROFILE OF *RARU* (*Vatica pauciflora* AND *Cotylelobium melanoxylon*) AND ITS POTENTIAL AS DIABETES MELLITUS DRUGS*

Marina Silalahi[1]

[1]Departement of Biology Education, Faculty of Education and Teacher Training, Universitas Kristen Indonesia, Cawang, 13510, Indonesia
Corresponding author: marina_biouki@yahoo.com

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 barks of *Vatica pauciflora* is obtained from Central Tapanuli (North Sumatra) and *Cotylelobium melanoxylon* is obtained from Riau. The extraction is done through maseration methods with ratio 1:10 w/v (*raru* barks : 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; and the barks of *Vatica pauciflora* contains triterpenoids, flavonoids, and β-sitosterol but no tannin.

Keywords: Raru, phytochemistry, *Vatica pauciflora*, *Cotylelobium melanoxylon*

BACKGROUND

Raru are the bark which are useb by the Batak ethnic for fermentation of the *Arenga pinnata* sap (*tuak*). The addition of the *raru* barks to *tuak* will change of its flavour and colour. Based on the review of the literature, the *raru* consist of: *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), and *Eurya* sp. (Anggraeni 2013; Silalahi et al. 2015).

Vatica pauciflora and *Cotylelobium melanoxylon* are the *raru* barks which are widely used for diabetes mellitus drugs (Silalahi 2014; Silalahi et al. 2015) and fermentation of *tuak*. So that it's easy found and traded in the traditional markets in North Sumatra (Silalahi 2016). Utilization of the *raru* barks as diabetes mellitus drugs is adopted from the tradition drinking *tuak* at night of the

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Batak ethnic (which is not prohibited by religion) in North Sumatra (Silalahi et al. 2015b). People whoregulary drinking of *tuak* doesn'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 used the medicinal plants that have bitter taste such as: *Andrographis paniculata, Eurycoma longifolia*, and *Lindernia viscose* (Silalahi 2014; Silalahi et al. 2015b). Wiryodidagdo et al. (2000) stated that the medicinal plants as medicine of diabetes mellitus are plants that produce compounds which can suppress or stimulate the endocrine glands. The current diabetic therapies include the 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 activities antidiabetic. Phenols and flavonoids are phenolic compounds biosynthesized by some plants (Taiz & Zeiger 2006). Flavonoids have activities as antidiabetic (Brahmachari 2011). Alkaloid is a class of alkaline compounds is, containing one or more nitrogen atoms (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 know the potential of *raru* barks as diabetes mellitus drugs, this research was conducted to analyzed phytochemical barks of *raru* (*Vatica pauciflo*ra and *Cotylelobium melanoxylon*).

MATERIALS AND METHODS

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

Extraction are 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 repeat 6 times to each species.

Profile of phytochemical of *raru* barks extract are screen through Thin Layer Chromatography (TLC). The eluent are used for each compounds (Table 1). If the colour of *raru* extract changes after added reagens are positive (Juarna 2016 modification). To determine the types of compounds are contained in the extract compared to the retention time of standard compounds. If retention time of the standart compouds similar with the *raru* extract, so the extracts contain compounds similar to standart.

Table1. The eluents and reagents are used 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 H_2SO_4.	Purple
Alkaloids	Toluene: etil acetate (7:3)	Dragendorff	Orange
Flavonoids	$CHCl_3$: Acetone: vanillin (10:2:1)	$AlCl_3$ 10%	Yellow
Tannins	Toluene : Acetone: Formic acid (5:4:1)	$FeCl_3$ 10%	Blackish Green

RESULT

The methanol extract of *raru* barks contain triterpenoids, β-sitosterol, flavonoids, saponins, and tannins (Table 1) but no alkaloids. The bark of

Cotylelobium melanoxylon contains triterpenoids, flavonoids, tannins and β-sitosterol; and bark of *Vatica pauciflora* doesn't contain tannin.

Table 2. The phytochemical profiles 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*)

Terpenoids

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

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 determined 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).

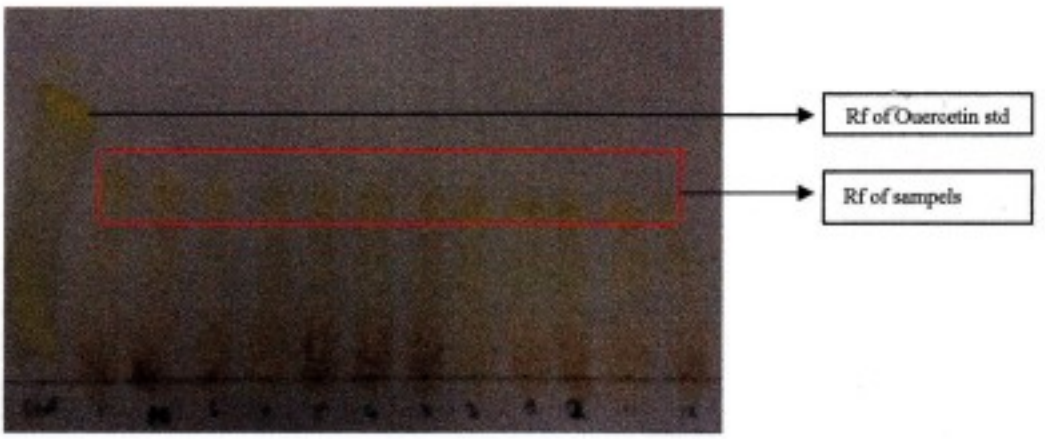

Figure 2. Retention time (Rf) of Quercetin and Rf of the samples (extract of *raru* barks)

Tannin (Phenolic)

One of the compound belonging to phenolic is tannin. The 7 samples of *raru* barks extract contain tannin (Figure 3), while 5 samples didn't contain tannin. The all of samples didn't contain tannin are *Vatica pauciflora*. Tannin in the *raru* extract implicates to the colour of *Cotylelobium melanoxylon* barks extract different from with *Vatica pauciflora* barks extract. The extract of *Cotylelobium melanoxylon* is darker than the *Vatica pauciflora* extract (Figure 4).

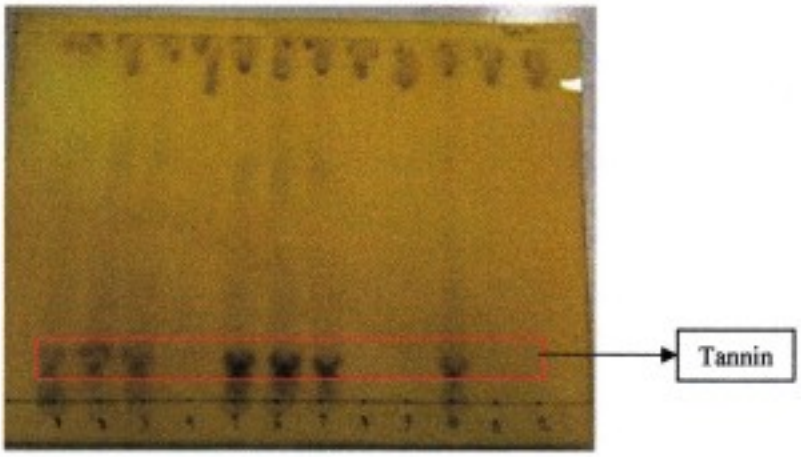

Figure 3. Profile of tannin in the *raru* barks extract (*Cotylelobium melanoxylon* and *Vatica pauciflora*).

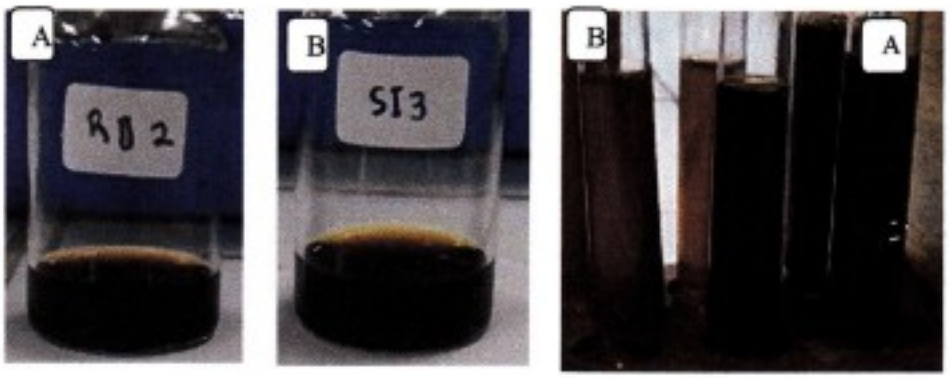

Figure 4. The colour of *raru* barks extract (A) *Cotylelobium melanoxylon* (B) *Vatica pauciflora*.

DISCUSSION

The use of medicinal plants as traditional or modern drugs related to the content of secondary metabolites. The *raru* barks 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 are more prevalent than diarrhea and malaria. The empirical data shows that diabetes mellitus requiring treatment in the long time. The man believe that uses medicine in long time is from natural products (plants extract) safer than medicine sintetic. This resulted in local people are always looking for alternative treatments that are considered safer or less side effects.

Efficacy *raru* barks as diabetes mellitus drugs associated with the content of secondary metabolites. In this study is found that the barks of *raru* contains terpenoids, alkaloids, tannins, saponins, β-sitosterol, and phenol. The compounds are believed associated directly or indirectly to regulator of levels of glucosa in the blood plasma. The barks of *raru* of *Cotylelobium melanoxylon* and *Vatica pauciflora* contain β-sitosterol but litte in its consentration. The β-sitosterol in the bark of *raru* related to the fact that the compounds are the main component in cells (Taiz and Zeiger 2006). β-sitosterol in the spermatophyta are distributed in organs (Gahlaut et al. 2015). Pasaribu et al. (2011) stated that terpenoids are secondary metabolites found in *Vatica* and *Cotylelobium*. Juarna (2016) reported that leaves, barks, roots of *Bischofia javanica* contain β-sitosterol, but various in concentration of organs.

Alkaloids group have 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 barks of *raru* in this study. The alkaloids in leaves related to its role in plants as anti herbivora. Alkaloids are role as a protective agent in the interaction of plants with other organisms (Aniszewski 2007).

Quercetin is one of six subclasses of a group of flavonoids, which is found in spermatophyta, but in this study wasn't find. 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 barks of. The content of tannin in the barks of *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).

CONCLUSION

The barks extract of *Cotylelobium melanoxylon* contains terpenoids, flavonoids, β- sitosterol, and tannins; and *Vatica pauciflora* contains terpenoids, flavonoids, β- sitosterol, and no tannins.

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