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STILBINOID COMPOUND FROM ETHANOL EXTRACT OF THE BARK "RARU", *VATICA PAUCIFLORA* BLUME (DIPTEROCARPACEAE)

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ABSTRACT

Vatica pauciflora Blume (local name : Raru) is known by Tapanuli's people widely as additional ingredients mixed to make toddy beverage as *tuak* which made from nira (sugar palm juice). This addition is intended to match the sweet taste and alcohol. The local people also know raru bark is used as diabetic treatment. In this research, isolation of chemical compound based on cytotoxic treatment with Brine Shrimp Lethality Assay (BSLA) of ethanol extract raru's bark. Separation process was conducted by column chromatography [(SiO_2; *i*. *n*-hexane-ethylacetate = 1 : 1; *ii*. $CHCl_3$-MeOH = 10 : 1 ~ 1 : 1; *iii*. $CHCl_3$-MeOH-H_2O = 5 : 5 : 1; *iv*. $CHCl_3$-MeOH = 1 : 1 and further purification with preparative TLC (SiO_2; $CHCl_3$-MeOH: H_2O = 7: 3: 1 and gave one pure isolate with LC_{50} of 34.20 ppm. Based on the interpretation of Ultra Violet (UV), FT-Infra Red (FT-IR) and Nuclear magnetic Resonance (^{1}H & ^{13}C-NMR) spectra shows that the compound is a stilbinoid, piceid acid.

Keywords: brine shrimp lethality assay; Indonesian medicinal plant; raru; stilbinoid; *Vatica pauciflora*

INTRODUCTION

Indonesia is a country rich in biodiversity that can be utilized in all aspects of human life. Traditional medicine is one of the tangible form of utilization of these resources. So far, the people of Indonesia, especially rural communities have been using plants in their environment as traditional medicine. However, at this time in urban communities also tend to choose traditional treatment, because it has been used for generations and made with a simple process known as traditional medicine or herbal medicine.[1] This is also supported by the economic situation where the price of modern medicines is increasingly expensive and the side effects caused by traditional medicines are relatively smaller compared to synthetic drugs. Therefore, the Indonesian people are even more advanced in developing medicinal plants to be used as traditional medicine as an alternative treatment.[2] One of the Indonesia medicinal plants that can be used as traditional medicine is "Raru" (local name), *Vatica pauciflora* Blume (Dipterocarpaceae) that needs to be researched and developed. This raru plant grows a lot in the forest, including Sumatra and Kalimantan, Indonesia. Part of the bark of this plant in Tapanuli community used as a mixture in a wine drink widely. The addition of raru bark on palm wine will make the palm wine taste sweet and the alcohol is suitable. Most people in Tapanuli also know skin bark raru as a cure for diabetes.[3] Several studies of chemical

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compounds of the *Vatica* spp. plants have been carried out, such as Latip, J. *et al* (2011)[4]; Kamarozaman. *et al*. (20013)[5] has successfully isolated five oligostilbinoids, (-)-*trans*-resveratrol, (-)-e-viniferin, (-)-laevifonol, (-)-hopeaphenol, (-)-vaticanol B, G, ε-viniferin and together with a gallic acid derivative, (-)-bergenin from *Vatica oodorata*. Keylor *et al* (2015)[6], reported that *Vatica pauciflora* contains many active compounds resveratrol oligomers which useful as anticancer, antioxidants such as pauciflorols A, B and C; isovaticanols B and C; pauciflosides A, B, and C.

In previous studies it was found that some bark extracts from *Vatica pauciflora* such as *n*-hexane, ethylacetate, ethanol and water extracts have been researched for antioxidant, α-glucosidase enzyme inhibitor[7]. The purpose of this research is to know the chemical structure that has cytotoxic properties in ethanol extracts from raru bark (*Vatica pauciflora* Blume) collected from the Tapanuli region, Indonesia.

EXPERIMENITAL

METODOLOGY

Research material : extract of raru's bark (*Vatica pauciflora* Blume)

Chemicals: ethylacetate, chloroform, methanol, tlc silica gel GF_{254} plate, silica gel (60 mesh), sea sand, celite 545, cerium sulfate, DMSO, NaCl (NaCl 38 g/L aquadest), *Artemia salina* eggs, KBr discs

Instruments : UV-Vis spectrophotometer, Fourier Transform-Infrared (FT-IR), Nuclear Magnetic resonance (^{1}H- & ^{13}C-NMR) (Jeol NMR, 500 MHz and 125 MHz) and column chromatography and some glassware.

RESEARCH METHOD

Isolation and purification of ethanol extract

Ethanol extracts (25.0 g) was carried out by column chromatography (SiO_2; *i*. *n*-hexane-ethylacetate = 1 : 1; *ii*. $CHCl_3$-MeOH = 10: 1 ~ 1: 1; *iii*. $CHCl_3$-MeOH-H_2O = 5 : 5 : 1) giving nine fractions (fr. EtOH-1 ~ fr. EtOH-9) . The Fr. EtOH-7 had the highest cytotoxic properties (5.76 ppm), so it continued second column chromatography (SiO_2; $CHCl_3$-MeOH = 1 : 1) gave six fractions (fr. EtOH-7-1 ~ fr. EtOH-7-6) and fractions 7-4 give the highest cytotoxic among six fractions, with LC_{50} of 11.94 ppm. The fr. EtOH 7-4 was purified with preparative TLC ($CHCl_3$-MeOH: water = 7: 3: 1) giving one pure isolate with LC_{50} of 34.20 ppm.

Identification of pure isolate

Identification of pure isolates based on interpretation of Ultra Violet (UV), Fourier-Transform Infrared (FTIR); ^{1}H-& ^{13}C-NMR (Nuclear Magnetic Resonance) and Mass Specta (LC-MS).

Brine Shrimp Lethality ASSAY (BSLA)

The cytotoxic activites of extracts and fractions was performed using Brine Shrimp Lethality Assay (BSLA) method described by Sarah *et al*. (2017)[8] with slight modification.

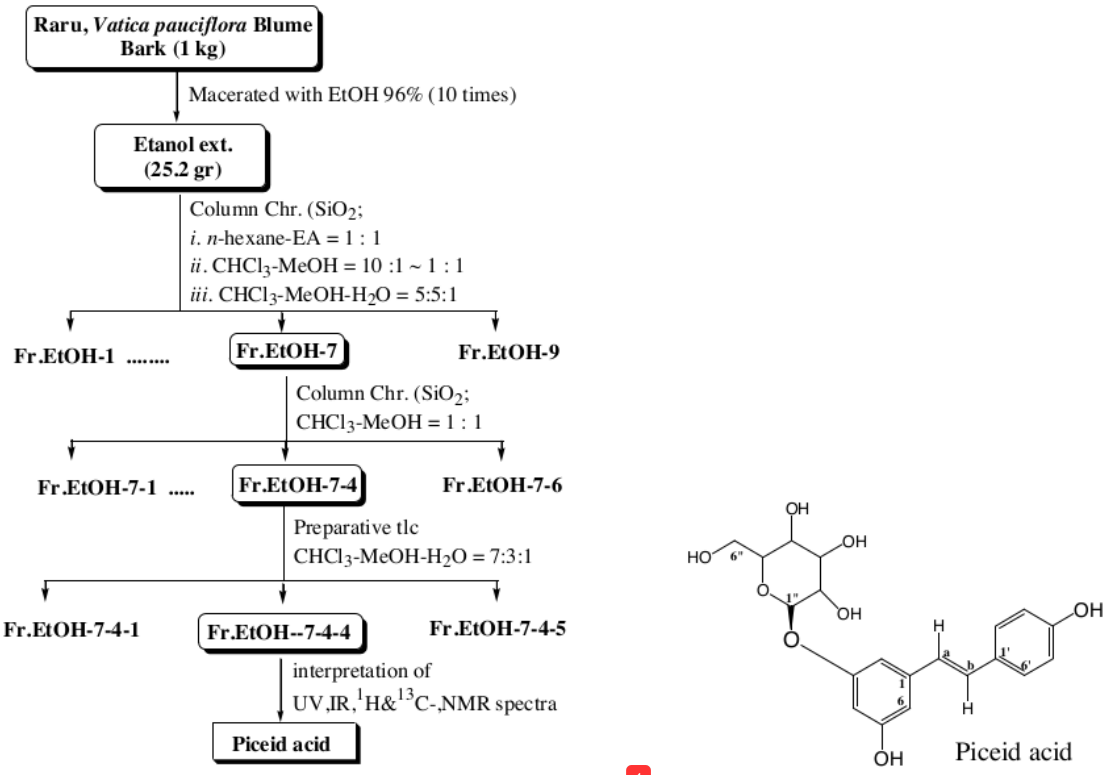

Fig 1. Scheme of isolation and purification piceid acid from ethanol extract of bark "Raru", *Vatica pauciflora* Blume)

RESULTS AND DISCUSSION

Characterization of pure compound

Pure compound (Fr.EtOH-7-4-4) is obtained in the form of a white solid which shows at *m/z* 390 in it's mass spectra by LC-MS, which is related to a molecular formula, $C_{20}H_{22}O_8$, which can be predicted that the compound is a glycoside derivative of resveratrol. Ultra-violet (UV) spectra indicates that the compound has maximum absorption at λ 267.0 and 307 nm indicating the presence of aromatic group. Infrared (FT-IR) spectra shows the presence of hydroxyl, alkene, ether groups in wave numbers (υ) at 3420.79 ; 1916.21; 1608.25; 1518.24; 1420.11; 968.01; 863.09 cm^{-1}. Investigation of ^{1}H-NMR spectra for pure compound in the high magnetic field region showed the protons at δH 3.26 ~ 3.88 ppm which showed the presence of oxygenated hydrogen (the presence of hydroxyl groups). There is an anomeric proton signal found at δH 3.70 (m). While in the low magnetic field region there are protons indicate the presence of aromatic and alkene of a pair of doublets at δH 7.02 and 7.13 (d, J = 16.5 Hz) which can be confirmed by the presence of monosaccharide of glucose moiety of *trans*-resveratrol.[4] Investigation of ^{13}C-NMR spectra gave twenty two carbons, and specifically for carbon glycosides at δC 102.33 ppm (See Table 1). By comparison the chemical shifts (δH and δC) isolated compound with by research of Chu *et al*., (2005)[9] the chemical structure of isolation and purification from ethanol extract of raru (*Vatica pauciflora*) was determined as piceid acid (Fig. 2)

Table 1. Comparison of chemical shift ^{1}H- & ^{13}C-NMR (δH, δC) for Fr. EtOH.7-4-4 and isolated comp. by Chu *et al*.,(2005)[9].

No	^{13}C-NMR pure comp.	^{13}C-NMR [9)]	^{1}H-NMR Pure comp. (*J* in Hz)	^{1}H-NMR [9)] (*J* in Hz)
1	139.46 (s)	139.4 (s)	-	-
2	107.03 (d)	107.2 (d)	6.75 (s)	6.70 (s)
3	159.00 (s)	158.9 (s)	-	-
4	105.01 (d)	104.8 (d)	6.39 (s)	6.31 (s)
5	156.92 (s)	158.3 (s)	-	-
6	109.61 (d)	109.3 (d)	6.58 (s)	6.54 (s)
A	129.12 (d)	128.0 (d)	7.02(d,*J*= 16.5)	6.84 (d, *J*=16.4)
B	126.20 (d)	125.2 (d)	7.13(d,*J*= 16.5)	7,28 (d, *J*=16.4)
1’	130.02 (s)	128.6 (s)	-	-
2’	128.09 (d)	127.9 (d)	7.41 (d)	7.38 (d)
3’	116.52 (d)	115.5 (d)	6.76 (d)	6.74 (d)
4’	157.67 (s)	157.3 (s)	-	-
5’	116.52 (d)	115.5 (d)	6.76 (d)	6.74 (d)
6’	128.09 (d)	127.9 (d)	7.42 (d)	7.38 (d)
1”	103.22 (d)	102.8 (d)	3.70 (m)	3.70 (m)
2”	73.49 (d)	73.3 (d)	3.26~3.88 (m)	3.16~3.70 (m)
3”	77.24 (d)	77.1 (d)	3.26~3.88 (m)	3.16~3.70 (m)
4”	70.02 (d)	69.8 (d)	3.26~3.88 (m)	3.16~3.70 (m)
5”	77.00 (d)	76.7 (d)	3.26~3.88 (m)	3.16~3.70 (m)
6”	61.12 (t)	60.8 (t)	3.26~3.88 (m)	3.16~3.70 (m)

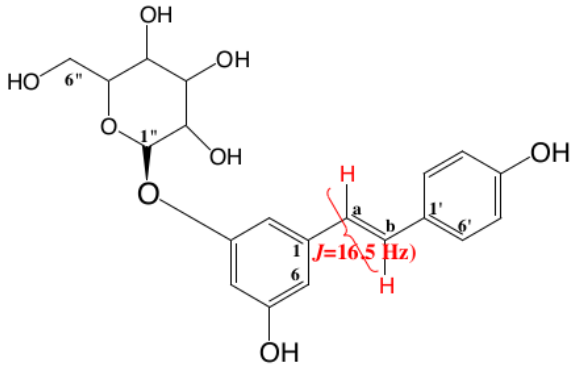

Figure 2. Chemical structure for isolates of Fr. EtOH-7-4-4 (Piceid acid)

Brine Shrimp Lethality Test (BSLT)

Cytotoxic test by BSLT method for all fractions of the first column chromatography (nine fractions), second column (six fractions) and preparative tlc (two fractions) can be seen in Table 2.

Table 2. Cytotoxicity for all fractions and isolated compound by the BSLT method

No	Fraction	LC $_{50}$ (ppm)
1	EtOH-1	299.50
2	EtOH-2	159.15
3	EtOH-3	595.85
4	EtOH-4	475,67
5	EtOH-5	27.77
6	EtOH-6	17.97
7	EtOH-7	**5.76**
8	EtOH-8	86.09
9	EtOH-9	110.15

No.	Fraksi	LC $_{50}$ (ppm)
1	EtOH-7-1	233.61
2	EtOH-7-2	269.00
3	EtOH-7-3	334.52
4	EtOH-7-4	**11.94**
5	EtOH-7-5	40.08
6	EtOH-7-6	19.61

No	Fraction	LC_{50} (ppm)
1	EtOH-7-4-1	38.93
2	**EtOH-7-4-2**	**13.95**

CONCLUSION

One stilbinoid compound, piceid acid from Indonesian medicinal plant "Raru", *Vatica pauciflora* Blume has been isolated and identified which has a cytotoxicity against *Artemia salina* of 13.95 ppm.

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