Alkaloid and Phenolic Compounds of Rafflesia hasseltii Suringar and its host Tetrastigma leucostaphylum (Dennst.) Alston ex Mabb. in Bukit Tigapuluh National Park, Riau: A Preliminary Study

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ABSTRACT

Two alkaloid compounds (nicotine and caffeine) together with three phenolic compounds (catechin, proanthocyanidin and phenolic acid) were firstly detected in Rafflesia hasseltii and its host, Tetrastigma leucostaphylum in Bukit Tigapuluh National Park, Riau. The content of all compounds is higher in R. hasseltii than its host.

Key words: Rafflesia hasseltii, Tetrastigma leucostaphylum, alkaloids, phenolic compounds, HPLC.

INTRODUCTION

Rafflesia hasseltii Suringar belongs to a parasitic plant family, Rafflesiaceae. As well as other Rafflesia species, it becomes a public interest due to its unique and extraordinary flower. This plant is notable for its white blotches on reddish perigone lobes. The look of its flower is more akin to tiger face, and due to this pattern it’s known as tiger face mushroom (cendawan muka rimau). As an endophyte holoparasitic plant, it grows completely embedded within its host and relies entirely on their host for all nutrients (Barkman et al., 2004). Tetrastigma leucostaphylum (Dennst.) Alston ex Mabb., a member grapevine family (Vitaceae), is the host of R. hasseltii.

The genus Rafflesia, including R. hasseltii in Bukit Tigapuluh National Park, Riau (BTNP), lack of basic biology and related science study especially its chemical compounds. Meijer (1997) stated that the family of Rafflesiaceae is rich of tannin. Currently, only three out of 26 Rafflesia species had been studied on its chemical compounds. R. hasseltii from Peninsular Malaysia had been screened its antimicrobial by Wiart et al. (2004), while study on its cimetedine that may prevent the acute gastric mucosal lesion on rat conducted by Noor et al. (2006). Based on the current taxonomic treatment, this species is probably R. cantleyi Solm. or R. azlanii Latiff et Wong, as the name of R. hasseltii in Peninsular Malaysia has been revised (Latiff and Wong, 2004). In 2006, Khairunadwa studied the toxicology of R. azlanii. The most current study by Kanchanapoom et al. (2007), detected four tannin compounds along with phenilpropanoid glucoside in R. kerrii from Thailand. Furthermore, it was explained that R. kerrii is used for Thai traditional medicine to help restore the female uterus after giving birth, as well as for treatment for fever. The use of Rafflesia for traditional treatment also reported in Perak, Peninsular Malaysia. Nevertheless, the phytochemistry study on this genus is poorly known.

In the genus Tetrastigma, currently two species in China had been investigated the chemical constituent. T. hemsleyanum Diels et Gilg and T. hypoglaucum are well-known as Chinese folk medicine (Liu et al. 2002; 2003). Furthermore he stated that T. hemsleyanum possesses the function of antipyretic, detoxification, anti-inflammatory, improving blood circulation and relieving pain. While T. hypoglaucum used for the treatment of fracture, traumatic injury and swelling pain. Yang et al. (1998), Liu and Yang (1999), and Liu et al. (2002) had observed the chemical constituent of T. hemsleyanum. On T. hypoglaucum, 10 chemical compounds had been isolated by Liu et al. (2003). A preliminary survey on phytochemistry of five Tetrastigma species from Sabah, East Malaysia, showed that T. dubium Planch., T. hookeri Planch. and T. pedunculare Planch. gave a positive reaction of saponin, while T. diepenhorstii (Miq.) Latiff and T. glabratum Planch. gave a negative reaction. All of them had a negative reaction of alkaloid and steroid (Din et al., 2002).

Currently, no study on chemical compound in both Rafflesia and its host (Tetrastigma) even though Rafflesia is an obligate parasitic plant that depends on its life from its host. In Indonesia, that well-known with its Rafflesia species, many scientists have tried to make ex-situ conservation of Rafflesia by use of tissue culture method. Unfortunately, no tissue can develop to a bud. Therefore, the information of chemical compounds on both plants,
hopefully, may help in this effort. This study is proposed to report the preliminary result in detecting the alkaloid and phenolic compounds of \textit{R. hasseltii} and \textit{T. leucostaphylum} from BTNP Riau.

**MATERIALS AND METHODS**

\textbf{Materials.} \textit{R. hasseltii}. We examined two populations in Datai Atas, BTNP Riau. Due to its rarity in this park, only one individual per population had been extracted. We used bract (the outer black layers that cover \textit{Rafflesia} bud or flower). This part was used in order to maintain its life cycle. \textit{T. leucostaphylum}. The infected tree by \textit{R. hasseltii} had been studied from two populations. The root and stem barks were extracted from one tree per population. Population 1 will be further mentioned as P1, and population 2 as P2. In order to detect the alkaloid and phenolic compounds in those species, we used the flavonoid standard compound.

\textbf{Methods.} A 4 g sample were blended to make powder form. Sample were added with 62.5\% aqueous methanol, and refluxed for two hours in 90°C. Let the sample cool down before filtering with Buchner filter. Methanol liquid was added to obtain 50 mL solution. This solution was dried and diluted with 10 mL methanol. Filtering was done by the use of Whatman 0.45 μm filter paper. And 2 μl of solution were injected to HPLC (High Performance Liquid Chromatography). The analysis of alkaloid and phenolic compounds had been conducted in Laboratory of Biotechnology, Bogor Agricultural Institute, by use of HPLC based on the method of Miean and Mohamed (2001). The HPLC condition is, column: Nova-Pak C18 (3.9 x 150 mm, 4 μm); mobile phase: methanol/water (50:50 v/v, pH 2.5 with trifluoroacetate acid; flow rate: 1mL/min.; detector: UV 280 nm.

**RESULT AND DISCUSSION**

The list of alkaloid and phenolic compounds of \textit{R. hasseltii} and \textit{T. leucostaphylum}, is given in Table 1. Based on the data presented in this table, all alkaloid and phenolic compounds that found in \textit{R. hasseltii}, were also found in its host, \textit{T. leucostaphylum}, but the contents is higher in \textit{R. hasseltii} for all compounds. The formula and chemical name of each compound presented in Table 2, while their structure in Figure 1.

\textbf{Alkaloid.} Alkaloid is one of major chemical compound in plant and animal, and found as secondary metabolites. These compounds are usually derivates of amino acid and many alkaloids have pharmacological effects on human and animal. It had been previously mentioned that Din et al. (2002) had reported the phytochemistry survey on five \textit{Tetrastigma} species, and all of them gave negative reaction on alkaloid. They used different species from this study. While in \textit{Rafflesia}, currently the report of this compound is not available. There many groups that belongs to alkaloid. Nicotine (pyrrolidine group) and caffeine (purine group) are the alkaloid found in the taxa studied. Nicotine was firstly found in tobacco in 1800s and known as an additive drug. It explains why the smokers are difficult to stop smoking because they are addicted to this compound (Anonym, 2006). National Institute on Drugs Abuse reported that nicotine withdrawal symptoms include irritability, craving, cognitive and attention deficits, sleep disturbances, and increased appetite. In 1994, Tang et al. use the nicotine replacement therapy in helping people to stop smoking.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>\textit{R. hasseltii}</th>
<th>\textit{T. leucostaphylum}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloid compounds</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. Nicotine (Pyrrolidine)</td>
<td>P1 146.24</td>
<td>P2 143.49</td>
</tr>
<tr>
<td>2. Caffeine (Purine)</td>
<td>P1 275.49</td>
<td>P2 274.25</td>
</tr>
<tr>
<td>Phenolic compounds</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. Leucoanthocyanin (Tannin)</td>
<td>P1 60.87</td>
<td>P2 64.87</td>
</tr>
<tr>
<td>2. Catechin (Flavonoid)</td>
<td>P1 368.26</td>
<td>P2 364.00</td>
</tr>
<tr>
<td>3. Phenolic acid</td>
<td>P1 610.27</td>
<td>P2 572.25</td>
</tr>
</tbody>
</table>

Note: ppm = mg/L

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Formula</th>
<th>Synonym</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nicotine</td>
<td>C_{10}H_{14}N_{2}</td>
<td>(-)-3-(1-Methyl-2-pyrrolidyl)pyridine</td>
</tr>
<tr>
<td>Caffeine</td>
<td>C_{3}H_{10}N_{2}O_{2}</td>
<td>1,3,7-Trimethyl-2,6-dioxopurine</td>
</tr>
<tr>
<td>Leucoanthocyanin (proanthocyanidin)</td>
<td>C_{31}H_{26}O_{12}</td>
<td>Polyhydroxyflavan-3-ol</td>
</tr>
<tr>
<td>Catechin</td>
<td>C_{9}H_{14}O_{6}</td>
<td>(-)-2-(3,4-Dihydroxycinnamoyl)-3,5,7-chromanol</td>
</tr>
<tr>
<td>Phenolic acid</td>
<td>C_{7}H_{6}O_{3}</td>
<td>Hydroxybenzoic acid</td>
</tr>
</tbody>
</table>
Caffeine is widely occurred in our consumed beverage and acts as a psychoactive stimulant and diuretic in humans (Anonym, 2007a). Bolton and Null (1981) reported that caffeine affects the psychological state of those who consume it. The effect of caffeine on cognitive decline was studied by Ritchie et al. (2007). In this study, the content of caffeine in *R. hasseltii* is about two times higher than nicotine (Figure 2.). While in *T. leucostaphylum*, the different part of bark (root and stem) gave the different result, for both compounds the content is higher in stem bark. The means of caffeine and nicotine content from two populations are, 144.87, 274.87 ppm (*R. hasseltii*), 31.06, 89.29 ppm (root bark *T. leucostaphylum*), 72.44, 137.44 ppm (stem bark of *T. leucostaphylum*), respectively. As well as nicotine, caffeine content in *R. hasseltii* is higher than *T. leucostaphylum*.

**Phenolic compounds.** Phenolic compounds that detected in *R. hasseltii* and *T. leucostaphylum*, are catechin (flavonoid), leucoanthocyanin (tannin) along with phenolic acid. To our knowledge, this is the first report on those constituent on both species. Many scientists had studied that flavonoid and phenolic acid have antioxidative and anticarcinogenic effects (Hakkinen, 2000). Catechin belongs to flavonoid group, commonly known as bioflavonoid, a secondary metabolites in plant. It is also well-known as tea extract, that have been recognized as health-promoting factors due to its antimutagenic, antioxidative and antibacterial activity (Nakagawa et al. 2005). Modun et al. (2003) and Suzuki et al. (2007) had proved that this compound may support human health. This compound belongs to tannin group. In *Tetrastigma*, catechin had been firstly isolated by Liu et al. (2003) from *T. hyglaucoum*. They had obtained 10 compounds from this species including catechin. While in *Rafflesia*, the first isolation of four hydrolysable tannins (1,2,4,6-tetra-O-galloyl-b-D-glucopyranoside, 1,2,6-tri-O-galloyl-b-D-glucopyranoside, 1,4,6-tri-O-galloyl-b-D-glucopyranoside; and 1,2,4-tri-O-galloyl-b-D-glucopyranoside) was conducted by Kanchanapoom et al. (2007).
Leucoanthocyanin is also known as oligomeric-proanthocyanidin (OPC), pycnogenol, leucocyanidin (Anonym, 2007b) and proanthocyanidin (a condensed tannin) (Sun and Sprangler, 2005). The term of proanthocyanidin thus defined because these colorless compounds release colored anthocyanidin, that known to possess broad pharmacological activity and therapeutic potential (Shao et al., 2003). This compound is water soluble vacuolar flavonoid pigments that reflect the red to blue (Anonym, 2007c). It explains why all Rafflesia flowers are reddish in color.

Phenolic acids are plant metabolites that widely spread throughout the plant kingdom. Its content is the highest in both taxa studied. For all phenolic compounds, as well as alkaloids, R. hasseltii has higher content than its host (Figure 3). The means of leucoanthocyanin, catechin, and phenolic acid content from two populations are, 62.87, 366.13, 591.26 ppm (R. hasseltii), 12.44, 114.69, 152.055 ppm (root bark of T. leucostaphylum), 25.01, 183.07, 297.19 ppm (stem bark of T. leucostaphylum), respectively.

CONCLUSION

In this study, R. hasseltii and its host, T. leucostaphylum, showed the same alkaloid and phenolic compounds. Five compounds found in both taxa are nicotine, caffeine, catechin, leucoanthocyanin and phenolic acid. The content of all compounds is higher in R. hasseltii than its host. Eventhough the compound that support health were detected in R. hasseltii, the use of this species for traditional treatment is not recomended, due to the presence of nicotine and caffeine. Moreover it is an endangered species that protected by law. A further study is necessary to detected the detail chemical compounds of both species, especially to support conservation efforts.

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REFERENCES

Hakkiren, S. 2000. Flavonol and Phenolic Acids in Berries and Berry Juice. [Dissertation], Kuala Lumpur. Faculty of Medicine, Kuoelp University.
Neurology 69: 536-545.