B I O D I V E R S I T A S
Volume 9, Nomor 1
Halaman: 17-20

ISSN: 1412-033X
Januari 2008
DOI: 10.13057/biodiv/d090105

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

NERY SOFIYANTI[1,2,♥], NINIK NIHAYATUL WAHIBAH[1], DEDEK PURWANTO[1], EDY SYAHPUTRA[1], KAMARUDIN MAT-SALLEH[2]

[1]Department of Biology, Faculty of Mathematics and Natural Sciences, University of Riau, Pekanbaru, Riau, Indonesia, 28293
[2]School of Environmental and Natural Resource Science, Biology Building 4th floor, Room 4117 Faculty of Science and Technology, National University of Malaysia, Bangi 46300, Selangor, Malaysia

Received: 27th December 2008. Accepted: 31st January 2008.

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. Meiyer (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,

♥ **Alamat korespondensi:**
Jl. Raya Bangkinang Km 12,5, Pekanbaru 28293
Tel. & Fax.: +62-761-63273
e-mail: sophie_yanti@yahoo.com

hopefully, may help in this effort. This study is proposed to report the preliminary result in detecting the alkaloid and phenolic compounds of *R. hasseltii* and *T. leucostaphylum* from BTNP Riau.

MATERIALS AND METHODS

Materials. *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 *Rafflesia* bud or flower). This part was used in order to maintain its life cycle. *T. leucostaphylum*. The infected tree by *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.

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 90^0C. 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 *R. hasseltii* and *T. leucostaphylum*, is given in Table 1. Based on the data presented in this table, all alkaloid and phenolic compounds that found in *R. hasseltii*, were also found in its host, *T. leucostaphylum*, but the contents is higher in *R. hasseltii* for all compounds. The formula and chemical name of each compound presented in Table 2, while their structure in Figure 1.

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 *Tetrastigma* species, and all of them gave negative reaction on alkaloid. They used different species from this study. While in *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 1. Alkaloid and phenolic compounds of *R. hasseltii* and *T. leucostaphylum.*

Compounds		*R. hasseltii*		*T. leucostaphylum*			
		Scale (ppm)		Root (ppm)		Stem (ppm)	
		P1	P2	P1	P2	P1	P2
Alkaloid compounds	1. Nicotine (Pyrrolidine)	146.24	143.49	29.68	32.37	71.75	73.12
	2. Caffeine (Purine)	275.49	274.25	91.88	86.70	137.13	137.75
Phenolic compounds	1. Leucoanthocyanin (Tannin)	60.87	64.87	12.63	12.25	24.52	25.50
	2. Catechin (Flavonoid)	368.26	364.00	113.75	115.62	182.00	184.13
	3. Phenolic acid	610.27	572.25	154.49	149.62	277.38	280.99

Note: ppm = mg/L

Table 2. The formula and chemical name of each compound

Compounds	Formula	Synonym
Nicotine	$C_{10}H_{14}N_2$	(-)-3-(1-Methyl-2-pyrrolidyl)pyridine
Caffeine	$C_8H_{10}N_4O_2$	1,3,7-Trimethyl-2,6-dioxopurine
Leucoanthocyanin (proanthocyanidin)	C_{31}-H_{28}-O_{12}	Polyhydroxyflavan-3-ol
Catechin	$C_{15}H_{14}O_6$	()-2-(3,4-Dihydroxyphenyl)-3,5,7-chromantriol
Phenolic acid	C_7-H_6-O_3	Hydroxybenzoic acid

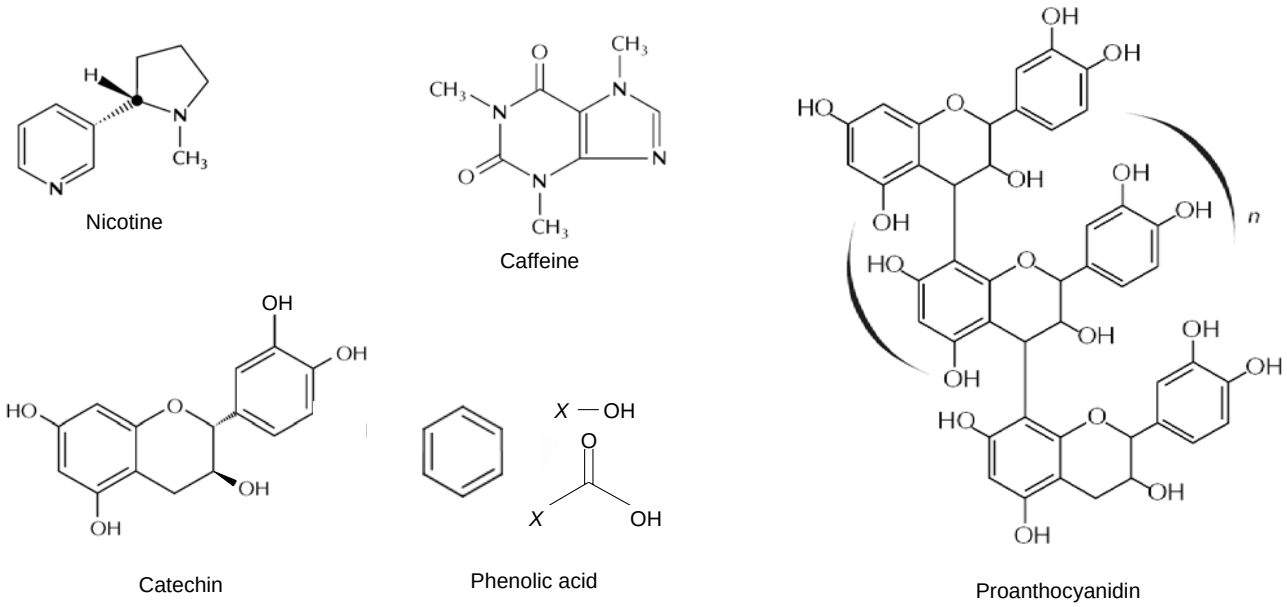

Figure 1. The structure of alkaloid and phenolic compounds found in *R. hasseltii* and *T. leucostaphylum* (Croteau *et al.*, 2000).

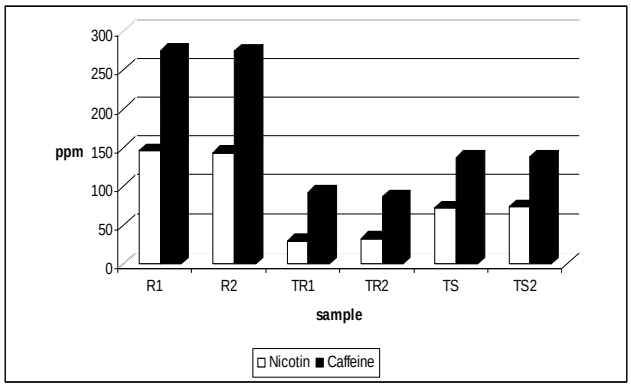

Figure 2. Nicotine and caffeine content in *R. hasseltii* and *T. leucostaphylum* (R = *R. hasseltii*, TR = root bark of *T. leucostaphylum*, TS = stem bark of *T. leucostaphylum* 1 = population 1, 2 = population 2).

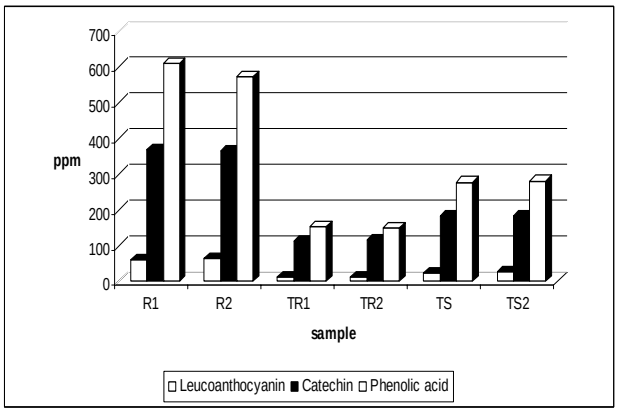

Figure 3. Phenolic compounds in *R. hasseltii* and *T. leucostaphylum* (R = *R. hasseltii*, TR = root bark of *T. leucostaphylum*, TS = stem bark of *T. leucostaphylum* 1 = population 1, 2 = population 2).

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-proanthocyanidiol OPC, pycnogenol, leukocyanidin (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 *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 it 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.

ACKNOWLEDGEMENTS

We thank to Berbagai Bidang Ilmu (BBI-DIKTI) grant for its financial support, Laboratory of Biotechnology (Bogor Agricultural University) for the assistance in preparing HPLC, Head of Forestry Department (Indonesia) for the permit to conduct this research, Haryono and his staff from Bukit Tigapuluh National Park Riau for their collaboration. Special thanks to Lancar (our guide) and Katak (head of Datai Bawah Village). We also thanks to Prof. Dato. Dr. Abdul Latiff Mohamed (UKM Malaysia) for the identification of *Tetrastigma*, Dr. Dong Liu (Louisiana State University) and Prof. Dr. Tripetch Kanchanapoom (Khon Kaen University, Thailand).

REFERENCES

Anonym. 2006. Tobacco Addiction. Research report serie 3. *National Institute on Drugs Abuse*. 1-8

Anonym. 2007a. *Caffeine*. http://en.wikipedia.org/wiki/Caffeine

Anonym. 2007b. *Proanthocyanidin*. http://en.wikipedia.org/wiki/Proanthocyanidin

Anonym. 2007c. *Biological_pigment*. http://en.wikipedia.org/wiki/Biological_pigment).

Barkman, T.J. Seok-Hong L., K. Mat-Salleh, J. Nais. 2004. Mitocondrial DNA sequences reveal the photosyntetic relatives of **Rafflesia**, the world's largest flower. *Proceeding of the National Academic of Sciences of the United State of America* 101 (3): 787-792.

Bolton, S. and G. Null. 1981. Caffeine: psychological effects, use and abuse. *Arthomolecular Psychiatry* 10 (3): 202-211.

Croteau, R., T.M. Kutchan, and N.G. Lewis. 2000. Natural products (secondary metabolites). *In:* Buchanan, B., W. Gruissem, and R. Jones (eds). *Biochemistry and Molecular Biology of Plants*. New York: American Society of Plant Physiologists.

Din, L. bin, N.I. Yusoff, M.W. Samsudin, U. Suki, K. Mat-Salleh, A.Z. Ibrahim, A. Latiff, and I.M. Said. 2002. A preliminary phytochemical survey on plants in Croker range, Sabah, Malaysia. A*SEAN Review of Biodiversity and Environmental Conservation (ARBEC)*. www.arbec.com.my/pdf/art7julysep02.pdf

Hakkinen, S. 2000. *Flavonols and Phenolic Acids in Berries and Berry Product.* [Dissertation]. Kuopio: Faculty of Medicine, Kuopio University.

Kanchanapoom T., M.S. Kamel, C. Picheansoonthon, P. Luecha, R. Kasai and K. Yamasaki. 2007. Hydrolyzable tannins and phenylpropanoid from **Rafflesia kerrii** Meijer (Rafflesiaceae). *Journal of Natural Medicines* 61: 478-479.

Khairunadwa, J. 2006. *Penilaian Ketoksikan* **Rafflesia azlanii** *(Rafflesiaceae) secara In-Vitro dan In-Vivo.* [Tesis]. Selangor: Fakulti Sains dan Teknologi, Universiti Kebangsaan Malaysia.

Latiff, A. and M. Wong. 2004. A New species of **Rafflesia** from Peninsular Malaysia. *Folia Malaysia* 4: 135-146.

Liu, D., J.-H. Ju, and J.-S. Yang. 2003. Studies on Chemical Constituent on **Tetrastigma hypoglaucum**. *Chinese Traditional and Herbal Drugs* 34 (1): 4-6.

Liu, D. and J.-S. Yang. 1999. A study on chemical components of **Tetrastigma hemsleyanum** Diels et Gilg. native to China. *Zhongguo-Zhong-Yao-Za-Zhi* 24 (10): 611-612, 638.

Liu, D., J.-H. Ju, G. Lin, X.-D. Xu, J.-S. Yang, and G.-Z. Tu. 2002. New C-glycosylflavones from **Tetrastigma hemsleyanum** (Vitaceae). *Acta Botanica Sinica* 44 (2): 227-229.

Meiyer, W. 1997. Rafflesiaceae. *Flora Malesiana* Series I. 13: 1-42

Miean, K.H. and S. Mohammed. 2001. Flavonoid (myricetin, quercetin, kaempferol, luteolin and apigenin) content of edible tropical plants. *Jurnal of Agricultural and Food Chemistry* 49: 3106-3112.

Modun, D., I. Musiæ, V. Kataliniæ, I. Salamuniæ and M. Boban. 2003. Comparison of protective effects of catechin applied *in vitro* and *in vivo* on ischemia-reperfusion injury in the isolated rat hearts. *Croatian Medical Journal* 44 (6): 690-696.

Nakagawa, K., S. Fujii, A. Ohgi, and S. Uesato. 2005. Antioxidative activity of 3-O-octanoyl-(+)-catechin, a newly synthesized catechin, in vitro. *Journal of Health Science* 51 (4): 492-296.

Noor, S.M., A.A Mahmood, I. Salmah, and K. Phillip. 2006. Prevention of acute gastric mucosal lession by **Rafflesia hasseltii** in rats. *Journal of Animal and Veterinary Advances* 5 (2): 161-164.

Ritchie, K., I. Carrière, A. de Mendona, F. Portet, J.F. Dartigues, O. Rouaud, P. Barberger-Gateau, and M.L. Ancelin. 2007. The neuroprotective effects of caffeine-A prospective population study (the Three City Study). *Neurology* 69: 536-545.

Shao, Z.-H.. L.B. Becker, T.L. Vanden Hoek. P.T. Schumacker. C.-Q. Li, D. Zhao, K. Wojcik, T. Anderson, Y. Qin, L. Dey, and C.-S. Yuan. 2003. Grape seed proanthocyanidin extract attenuates oxidant injury in cardiomyocytes. *Pharmacological Research* 47: 463-469.

Sun, B. and M.I. Spranger. 2005. Review: quantitative extraction and analysis of grape bg wine proanthocyanidin and stilbenes. *Ciência Téc. Vitiv* 20 (2): 59-89.

Suzuki, J., M. Ogawa, Y. Maejima, K. Isobe, H. Tanaka, Y. M. Sagesaka, and M. Isobe. 2007. Tea catechins attenuate chronic ventricular remodeling after myocardial ischemia in rats. *Journal of Molecular and Celluler Ardiology* 42 (2): 432-440

Tang, J.L., M. Law, N. Wald. 1994. How effective is nicotine replacement therapy in helping people to stop smoking? *BioMedical Journal* 308: 21-26.

Wiart, C., S. Mogana, S. Khalifah, I. Salmah, A.K. Narayana, M. Sulaiman and M. Buckle. 2004. Antimicrobial screening of plants used for traditional medicine in the State of Perak, Peninsular Malaysia. *Fitoterapia* 75: 68-73

Yang, D., H. Liu, X. Li, X. Huang, and J. Qin. 1998. Chemical constituents of **Tetrastigma hemsleyanum** Diels. et Gilg. *Zhongguo Zhong Yao Za Zhi* 23 (7): 419-421,447-448.