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Identification of Alkaloid Compound and Antioxidant Activity of *Rafflesia cantleyi* and its Host, *Tetrastigma tuberculatum*

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Abstract: Alkaloid compounds in *Rafflesia cantleyi* and host, *Tetrastigma tuberculatum* were identified using the phytochemical screening method and HPLC technique. The results showed positive test for the host, *T. tuberculatum* which indicates the presence of alkaloid whereas the screening test for *R. cantleyi* showed that alkaloid is absenced. Both extract were then subjected to HPLC for alkaloid identification and caffeine was used as standard. The spectroscopic data from HPLC revealed the presence of caffeine in both methanol extracts. The methanol extract of both samples were also tested for antioxidant activity by using DPPH free radical scavenging activity. Result showed that the radical scavenging activity of *R. cantleyi* and *T. tuberculatum* are comparable to the standard reference, BHT but higher than the standard alpha tocopherol.

Keywords: HPLC, phytochemical screening, *Rafflesia cantleyi*.

INTRODUCTION

Rafflesia sp. is a small genus of parasite from the family *Rafflesiaceae* whereby the flower and fruit are the only parts that come outside the tissues of the host plant [1]. As an endophyte holoparasitic plant, it grows completely embedded within its host and relies entirely on their host for all nutrients [2]. Locally known as bunga pakma and patma (Java), it can be found in Sumatra and Peninsular Malaysia (hills of Perak and northern Pahang). The aborigines Sakai believe that it can accelerate delivery in childbirth [3] and in Java, women used it as an aphrodisiac [1]. *Tetrastigma sp*., belongs to the grape family *Vitaceae* and it was known as Lipoi by the local communities in Sabah [4]. The species are found in subtropical and tropical regions of Asia, Malaysia, and Australia which they grow in primary rainforest. Species of this genus are famous as being the single hosts of parasitic plants in the family *Rafflesiaceae* which is *Rafflesia arnoldii* and also *Rafflesia cantleyi* [4]. This plant species is considered to have medicinal properties that is used to treat swelling pancreas, fever, cough and beside that it also been used for improving blood circulation, anti-inflammatory, analgesic, treatment of high fever, pneumonia and for anti-cancer [4]. The genus *Rafflesia* has lack of basic biology and related science study especially its chemical compounds. Previous study on genus *Rafflesia* revealed the presence of two alkaloid compounds (nicotine and caffeine) together with three phenolic compounds (catechin, proanthocyanidin and phenolic acid) which were firstly detected in *Rafflesia hasseltii* and its host, *Tetrastigma leucostaphylum* [5]. Earlier study on *Rafflesia* species detected four tannin compounds along with phenilpropanoid glucoside in *Rafflesia kerrii* [6]. Up to now, there is no publish report on phytochemical study of *Rafflesia cantleyi* except for its antimicrobial activity [7]. This study involves the identification of caffeine by combination of phytochemical screening and HPLC detection and evaluation of antioxidant activity on the crude methanol extract of *Rafflesia cantleyi* and host, *Tetrastigma tuberculatum.*

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MATERIALS AND METHODS

General Experimental Procedure

TLC: Precoated Silica gel 60 F_{254} (0.25 m thick, Merck). HPLC: Shimadzu LC Solution HPLC System (pump, UV/Vis detector SPD-20A, auto-sampler SIL-20A, controller CBM-20A) equipped with analytical column (Zorbax eclipse XDB-C18, 4.6 mm i.d x 250 mm ODS, 5μm). Reagents and solvents used are all of analytical and HPLC grade.

Plant Material

Buds of *Rafflesia cantleyi* and host, *Tetrastigma tuberculatum* was collected at Royal Bellum, Tasik Banding, Grik, Perak, Malaysia in 2012.

Extraction and Preparation of Plant Extracts

The buds of *Rafflesia cantleyi* (966.80g) and host, *Tetrastigma tuberculatum* (16.45g) was dried for three days before it was chopped and milled into dried powder form. Both samples were extracted by being macerated with petroleum ether (2x24 hours) and filtered using Buchner funnel. The samples were macerated again with methanol (3x24 hours). The samples were filtered and the solvent was evaporated to get the crude methanol extract of *R. cantleyi* and *T. tuberculatum*.

Alkaloid Detection by Alkaloid Screening Test

The crude methanol extract of *R. cantleyi* and host, *T. tuberculatum* were screened qualitatively for alkaloid

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Table 1. Alkaloid screening test of *R. cantleyi.*

No	Constituents	Test	Inference
1	Alkaloids	Dragendorff's (Sofowora, 1993)	-
		Mayer's (Sofowora, 1993)	-

Table 2. Alkaloid screening test of *T. tuberculatum.*

No	Constituents	Test	Inference
1	Alkaloids	Dragendorff's (Sofowora, 1993)	+
		Mayer's (Sofowora, 1993)	+

Key: + = present; - = absent

Table 3. Results of HPLC.

Samples	Retention Time (min)	Area (%)
Standard caffeine	2.319	100.0
Rafflesia cantleyi	2.396	14.45
Tetrastigma tuberculatum	2.358	39.85

constituents utilizing standard methods of analysis. Few quantity of the each portion of crude methanol extracts were stirred with 5 ml of 1% aqueous HCl on water bath and filtered. Of the filtrate, 1 ml was taken individually into 2 test tubes. To the first portion, few drops of Dragendorff's reagent was added; occurrence of orange-red precipitate was taken as positive. To the second 1 ml, Mayer's reagent was added and appearance of buff-coloured precipitate will be an indication for the presence of alkaloids [8].

Alkaloid Detection by HPLC

The crude methanol extracts solutions were then subjected to HPLC (High Performance Liquid Chromatography) for identification of caffeine using caffeine solution as standard. The condition of HPLC was based on the method of Miean and Mohamed (2001) [9] with slight modification. The HPLC condition is, column: Nova-Pak C18 (3.9 x 150 mm, 4µm); mobile phase: methanol/water (30:70 v/v, pH 2.5 with trifluoroacetate acid; flow rate: 1mL/min.; detector: PDA 254 nm.

Antioxidant Activity Test by DPPH Radical Scavenging Activity [10]

Five different concentrations of samples solutions which were 20 µg/mL, 40 µg/mL, 60 µg/mL, 80 µg/mL and 100 µg/mL were prepared. DPPH solution was prepared by dissolving 7.9 mg of DPPH into 100 mL methanol to produced 20 µM of DPPH solution. Then, 1 mL of 20 µg/mL sample solution was added with 3 mL of DPPH solution and this mixture was allowed to stand for 30 minutes before the absorbance can be measured. The color of the solution changed from dark purple to yellow. The absorbances of the samples were measured using Spectronic 20 at 517 nm. Butylated hyroxytoluene (BHT) and α-tocopherol were prepared and served as standard antioxidant). The formula used to calculate the percentage of free radical scavenging is shown below:

RESULTS

The results of the alkaloid screening of *Rafflesia cantleyi* and host, *Tetrastigma tuberculatum* is as presented in Table **1** and Table **2** respectively.

DISCUSSION

The alkaloid screening test of the crude methanolic extracts of *R. cantleyi* and *T. tuberculatum* showed positive test for the host, *T. tuberculatum* which indicates the presence of alkaloid whereas the screening test for *R.cantleyi* showed that alkaloid is absenced. Spectroscopic analysis by HPLC showed that both *R. cantleyi* and *T. tuberculatum* contain caffeine but the concentration of caffeine is higher in *T. tuberculatum*. The evidence is constructed based on the value of retention time of both samples as compared to the retention time of standard caffeine. DPPH test is based on the ability of DPPH, a stable free radical to decolourize in the presence of antioxidants. In this study, butylated Hydroxy Toluene (BHT) and α-tocopherol were used as standard reference antioxidant. The characteristics of the antioxidants and the inhibitory effects of plant extracts was expressed as IC_{50}, as presented in Table **4**. Highest scavenging activity was observed with butylated hydroxytoluene (BHT) which IC_{50} value is 11.15µg/mL. The scavenging activity of crude methanol extract of *R. cantleyi* and *T. tuberculatum* were comparable to the standard (BHT) with IC_{50} value of 14.58µg/mL and 19.12 µg/mL respectively. However the scavenging activity of both extracts were higher than the standard α-tocopherol which IC_{50} value is 36.07 µg/mL. This study demonstrate that *R. cantleyi* and host, *T. tuberculatum* possess antioxidant activity and showed that both contained caffeine.

Table 4. Results of antioxidant activity by DPPH radical scavenging activity.

Samples	IC_{50} (μg/ml)
Butylated hydroxytoluene	11.15
α-tocopherol	36.07
Rafflesia cantleyi	14.58
Tetrastigma tuberculatum	19.12

CONCLUSION

Screening of alkaloid on *R. cantleyi* and *T. tuberculatum* showed that alkaloid is presence in *T. tuberculatum* while in *R. cantleyi* the alkaloid was not detected. However, spectroscopic analysis by HPLC showed that both *R. cantleyi* and *T. tuberculatum* contain caffeine. Methanol extracts of *R. cantleyi* and *T. tuberculatum* showed high antioxidant property based on radical scavenging activity by DPPH.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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REFERENCES

[1] Burkill, I.H. A Dictionary of the Economic Product of the Malay Peninsular. Volumes I & II. Ministry of Agriculture and Cooperatives, Kuala Lumpur, **1966**.

[2] Barkman, T.J.; Seok-Hong, L.K.; Mat-Salleh, J.N. Mitocondrial DNA sequences reveal the photosyntetic relatives of Rafflesia, the world's largest flower. *Proc. Nat. Acad. Sci. USA*, **2004**, *101*(3), 787-792.

[3] Burkill, I.H.; Haniff, M. Malay village medicine. *Gard. Bull. Straits Settlem.*, **1930**, *6(2)*: 167-332.

[4] Hossain, A.M.; Shah, D.M.; Gnanaraj, C.; Iqbal, M. *In vitro* total phenolics, flavanoid contents and antioxidant activity of essential oil, various organic extracts from the leaves of tropical medicinal plant *Tetrastigma* from Sabah. *Asian Pac. J. Trop. Med.,* **2011**, 717-721.

[5] Sofiyanti, N.; Wahibab, N.N.; Purwanto, D.; Syahputra, E.; Salleh, M.K. Alkaloid and Phenolic *Rafflesia hasseltii* Suringar and its host *Tetrastigma leucostaphylum* (Dennst.) Alston ex Mabb. In Bukit Tigapuluh National Park, Riau: A Preliminary Study, **2008**, *9*, 17-20.

[6] Kanchanapoom, T.; Kamel, M.S.; Picheansoonthon, C.; Luecha, P.; Kasaiand, R.; Yamasaki, K. Hydrolyzable tannins and phenylpropanoid from *Rafflesia kerrii* Meijer (Rafflesiaceae). *J. Nat. Med.*, **2007**, *61*: 478-479.

[7] Azizan, N.; Mohamad, N.; Sahalan, Z.A. Antibacterial activity *Rafflesia cantleyi* Solms-Laubach against Gram positive and negative. *Jurnal Sains Kesihatan Malaysia*, **2011**, *9*(2), 51-54.

[8] Sofowora, A. Screening Plants for Bioactive Agents. In: Medicinal Plants and Traditional Medicinal in Africa. 2nd Ed. Spectrum Books Ltd., Sunshine House, Ibadan, Nigeria, **1993**, pp. 134-156.

[9] Miean, K.H., Mohammed, S. Flavonoid (myricetin, quercetin, kaempferol, luteolin and apigenin) content of edible tropical plants. *J. Agric. Food Chem.,* **2001**, *49*: 3106-3112.

[10] Pyo, Y.H.; Lee, T.C.; Logendra, L.; Rosen, R.T. Antioxidant activity and phenolic compounds of Swiss chard extracts. *Food Chem.*, **2004**, 19-26.

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