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Short Communication: The potential of secondary metabolites of *Myrmecodia tuberosa* from different host trees

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Abstract. *Sari YP, Kustiawan W, Sukartiningsih, Ruchaemi A. 2017. Short Communication: The potential of secondary metabolites of* Myrmecodia tuberosa *from different host trees. Nusantara Bioscience 9: 170-174.* Ant-plants (*Myrmecodia tuberosa* Jack.) is a medicinal plant that could potentially inhibit cancer cell growth. Ant-plants is epiphytic plants whose commonly life was attached to the host tree. Several information from local people stated that ant-plants attaching to different host trees possesses different active compounds. The purpose of this study was to determine the secondary metabolites of each parts of ant-plants including leaves, stems and tubers from different tree hosts i.e mango and durian. Result from phytochemical analysis showed that ant-plants living in mango and durian trees positively contained the metabolic compounds including phenolics, flavonoids, alkaloids, saponins and steroid/triterpenoid. The Total of Phenolic Content (TPC) and the Total of Flavonoids Content (TFC) on the leaves of ant-plants was higher than that in tubers or stems of ant-plants derived from both host trees i.e mango and durian. The value of TPC and TFC of the ant-plants leaves derived from the durian host tree (319.33 ± 0.06 mg GAE/g and 272.33 ± 0.02 mg CE/g) was higher than those from mango host trees (172.80 ± 0.02 mg GAE/g and 162,83 ± 0.01 mg CE/g). Ant-plants, whose life was attached to a different host tree, had the same content of secondary metabolites although those quantities were different in each host trees. Therefore, ant-plants from different host trees could be used as a medicinal plant by concerning the amount of used extract for effectiveness medicinal purposes.

Keywords: Ant-plants, durian, mango, *Myrmecodia tuberosa*, secondary metabolites

INTRODUCTION

Ant-plants or anthill plants or *sarang semut* belonging to the *Hydnophytinae* tribe (Rubiaceae) is categoryzed as epiphytes. Ant-plants consists of 5 genus i.e *Hydnophytum, Myrmecodia, Anthorrhiza* (in mainland of New Guinea), *Myrmephytum* (in mainland of New Guinea and the Philippines) and *Squamellaria* (a local plant on the island of Fiji), but the closest genus associated with ants is only genus *Hydnophytum* and *Myrmecodia*. *Hydnophytum* genus comprised of 45 species, while *Myrmecodia* has 26 species. All species of this genus possess a bloated stem and hollow-cavity. Of all those species, the only type of species i.e., *H. formicarum*, *M. tuberosa*, *M. pendens* could potentially be used as medicine (Soeksmanto et al. 2010). In Indonesia, ant-plants was traditionally used by local people as a medicine for several diseases for a century. Ant-plants contain important compounds such as glycosides, vitamins, minerals, flavonoids, tocopherols, polyphenols and tannins (Sudiono et al. 2015), which is very useful as an antioxidant and anti-cancer.

From in vitro analysis, it is highly evidenced that ant-plants had potent to inhibit the cancer cells growth. The extract of ant-plants has an antiproliferation which could effectively use to against three different types of cancer i.e., cervical cancer, lung cancer and colon cancer (Manoi 2008). In a study conducted by Soeksmanto et al. (2010), it was reported that the extract of ant-plants (*M. pendens*) could inhibit the growth of cervical and breast cancer cells (HeLa and MCM-B2 cells). Ahmad (2010) also stated that a total of high phenolic contents and antioxidant activity (AOA) of *Hydnophytum formicarum* could be used as an anti-oxidant and anti-microbial agent (Prachayasittikul et al. 2008).

The ant-plants is highly demanded due to its benefits for medicinal plants. Of that reason, the number of hunting activities in nature was high causing the dangerous threat of ant-plants existence in nature. Moreover, the massive exploitation of ant-plants in their habitat in Borneo forest without effective cultivation and rejuvenation managements cause the loss genetic of ant-plants which lead to the status of ant-plants as endangered plants. The information gathered from local people in East Kalimantan showed that ant-plants from different host trees have different medicinal benefits. Another information mentioned that ant-plants from mango host tree had no medicinal effect compared to that from another host tree such as the banyan tree, durian trees, olive trees, jackfruit trees and others. That information spread sporadically to the local society without the scientific proof regarding the truth. In addition, the part of ant-plants used as medicine is tubers of plants which could further interfere the survival rate of the plant in nature. Therefore, objectives of this study were to determine different contents of the secondary

metabolites in ant-plants (*Myrmecodia tuberosa* Jack.) from different host trees (mango and durian trees) and also to find out the part of ant-plants with the highest content of secondary metabolites.

MATERIALS AND METHODS

Materials

The research material used in this study was ant-plants including roots, stems and leaves and the tree hosts including stems and leaves of mango tree and durian. The samples were taken from the village Antutan and Mount White, District of Tanjung Palas, Bulungan, North Borneo, located at 02º15'.43,8"- 02º84'.9,78" North Latitude and 117º29'.60,9 "- 117º34'.75,3" East Longitude. The tuber of ant-plants were taken with a diameter of ± 15 cm and the color of leaves was from green to dark green. Of the tree hosts, trunk/branches with a diameter of ± 5 cm and green leaf were used as samples.

Sample preparation

Ant-plants used in this study were ant-plants taken from the tree hosts i.e., a mango tree and durian. All sample including tubers, stems, leaves and stems of ant-plants and also leaves of the host plant (mango and durian) were washed and then were cut into pieces. Afterward, samples were dried at room temperature until the moisture content was not more than 10%. All samples were then crushed using a blender.

The extraction of secondary metabolites

Crushed samples of ant-plants and the host tree were extracted around 1 kg using 10 L of ethanol 96%. The extraction process was done until the extract was colorless (± 48 hours), followed by the filtration process using filter paper (Whatman 2; Sigma-Aldrich, Germany). After samples had been filtered, the solvent was evaporated with a rotary evaporator to obtain the ethanol extract (leaf around 132, .65 g, 18.45 g stems and tubers 30.33 g of ant-plants from mango host); (134.40 g leaf, stem 27.35 g, 28.90 g tuber plants of ant-plants from the durian host tree); (leaves and stems were 119.85 g and 27.33 g of mango host); and (53.05 g leaves and 22.04 g of durian host), followed by the phytochemical analysis in order to know the content of secondary metabolites in the extract of samples.

Phytochemical analysis

The phytochemical analysis was done by testing the extract samples to determine the active compound using the description published by Pal et al. 2012 such as:

Alkaloid test (Mayer's test). One mL of Wagner's reagent was added. In this test, the white cloud will be formed if or it contains alkaloid compound.

Flavonoids test (Shinoda's test). The filtrate sample of 5 mL was added with 0.05 mg of Mg powder and 1 mL of concentrated HCl, which were then shaken. The positive test was indicated by the formation of red, yellow or orange.

Phenolic test. few drops of 1% FeCl3 was added. The positive extract contained phenols if it produced green, red, purple, blue or black.

Saponin test (Foam test). Extract samples were inserted into a test tube and added with 10 mL of hot water and then were cooled. Samples were then shaken vigorously for 10 seconds. If there were saponin contents in the tested extracts will form bubbles.

Steroids/triterpenoids test (Libermann Burchard test). The extract solution was added with glacial CH_3COOH and concentrated H_2SO_4 with a ratio of 20: 1. The positive test of extract samples containing steroid will form in blue or green, while for triterpenoids, it will give a red or purple color.

The determination of total phenols (Total Phenolic Content/TPC)

Gallic acid standard solution was made with several serial concentrations from 0 to 250 mg/L in methanol: water with ratio of 50: 50 v/v. 0.5 mL of ant-plants extract was put into a test tube. Samples were then added to 5 mL of reagent Folin-Ciocalteau and 4 mL of 2% Na_2CO_3. Furthermore, samples were incubated at room temperature for 15 minutes. The absorbance was measured at a wavelength of $\pm$750 nm using a UV-Vis spectrophotometer (Shimadzu UV mini-1240) (Orak 2007)

The determination of total flavonoids (Total Flavonoid Content/TFC)

Determination of total flavonoids was done using aluminum chloride colorimetric technique (ACCT). Catechins as a standard solution were made in several series of concentrations from12.5 to 100 ug/mL dissolved in ethanol. A total of 0.5 mL of methanol extracts (crude extracts of Ant-plants) was put in a test tube and was added to 1.5 mL of ethanol, $AlCl_3$ with a concentration of 10% around 0.1 mL, 1 M CH_3COOH around 0.1 mL and 2.8 mL of distilled water. The solution was then incubated at room temperature for 30 minutes. The absorbance was measured at a wavelength of $\pm$500 nm using a spectrophotometer. Data were then measured by creating a calibration curve of the relationship between the concentration of the sample (mg/L) and the absorbance from the obtained results (Imran et al. 2011).

The total value of phenolic and flavonoid contents was calculated using the formula used by Kumari and Sharma (2015).

$$T = \frac{(C \times V)}{M}$$

Note :

T = Total phenolic content (mg GAE/g sample)/ flavonoids (mg CE/g sample)

C = The concentration of gallic acid/catechins (mg L^{-1})

V = volume of extract (mL)

M = weight of the extract (g)

Statistical analysis

The obtained results were presented by exposing the result of a mean ± standard error with three replicates. Statistical analysis was then performed using SPSS version 21 and Excel 2008 with the significant level of differences at $p < 0.05$.

RESULTS AND DISCUSSION

Phytochemical tests

Results from phytochemical test showed that in all parts of ant-plants including roots, stems and leaves from different hosts (mango and durian) contained the same metabolites compounds regarding flavonoids, phenolics, and alkaloids, while another metabolite compounds such as saponins, steroids and triterpenoids varied on any part of the plant. Saponins compounds were only found in the stem of Ant-plants, steroids were on stem and tuber, while triterpenoids were only found in leaf organs (Table 1). Similar results were also seen in the content of secondary metabolites from the host tree, which also positively contained flavonoids, phenolic, alkaloids in the leaves and stem organs. Steroidal compounds were only found in leaf organs and triterpenoids were found on the stem organ (Table 2). This indicated that some secondary metabolite content of ant-plants was similar to those in the tree hosts. The different host trees as an attached place of ant-plants had no different result in the production of secondary metabolites of ant-plants themselves.

Of some secondary metabolites possessed by ant-plants and their tree hosts, flavonoids had a function as an antioxidant activity. According to Pourmorad et al. (2006), flavonoids are a group of compounds playing an important role in counteracting free radicals. In addition, Kandaswarni and Middleton (1997) suggested that flavonoids have antioxidant capabilities enabling to transfer an electron to a free radical compound. This mechanism made flavonoids could press tissue damages caused by free radicals and inhibit the action of several enzymes. This means that the plants containing flavonoid compound could act as effective antioxidants. The intensity of the color produced on phytochemical test also affects the amount of secondary metabolites of the plant organ. In this study, the leaf organ produced a more concentrated color intensity compared to other organs (stems and tubers), indicated the high production of secondary metabolites in the leaf organ.

Some plants of the Rubiaceae family had been known containing the same secondary metabolites in almost all parts of the plant. According to a study conducted by Yuliastuti (2011), the leaves and stems of ant-plants (*Hydnophytum formicarum* Jack.) contained the secondary metabolites i.e., alkaloids and flavonoid.

Total of Phenolic Content (TPC)

The total of phenolic content varied in all part of ant-plants including tuber, stem, and leaf from different tree hosts (mango and durian). Based on the source of explants, the highest number of phenolic contents was obtained from leaf explants compared to that from tuber and stem of both ant-plants and their host trees (mango and durian). The standard curve was used with the linear regression equation $y = 0.020 + 0{,}001x$ and $R2 = 0.9997$. This gave evidence that the spread information from local people who taught that the powerful medicinal material of ant-plants lies in tubers was less true. The total of phenolic content was highly achieved from the leaf explants of Ant-plants. Medicinal plants usually contain secondary metabolites which differ in every part of plants. Kumari and Sharma (2015) who examined the leaves and stems of *Oxalis corniculata* extracts using methanol solvents showed that the highest content of total phenolic compound was found in leaves.

The total of the phenolic content of ant-plants tubers from durian host was 61.80 ± 0.006 mg Gallic Acid Equivalent (GAE)/g and ant-plants tubers from mango host was 55.06 ± 0.021 mg GAE/g. This result was smaller than the total phenolic content in *Myrmecodia pendens* tubers conducted by Engida et al. (2013). The total phenolic content of the *M. pendens* tubers using the ethanol solvent was 330.61 ± 2.13 mg GAE/g. In addition, Ahmad et al. (2010) reported that the total phenolic content in 22 kinds of medicinal herbs from the Malaysian Rubiaceae family (subfamily Rubioideae) showed that *Hydnophytum formicarum* which had a close relative of *Myrmecodia* (but a different genus) possessed the highest total of phenolic contents compared to other plants (120.63 ± 3.09 mg GAE/g).

Table 1. Results of phytochemical test from different part of ant-plants (*Myrmecodia tuberosa* Jack.) from different tree hosts (Durian and Mango) using ethanol solvents

Explant source from Ant-plants	Tree hosts	Secondary metabolite compounds					
		Flavonoid	Phenolic	Alkaloid	Saponin	Steroid	Triterpenoid
Tuber (D)	Durian	+	+	+	-	+	-
Stem (D)	Durian	+	+	+	+	+	-
Leaf (D)	Durian	+	+	+	-	-	+
Tuber (M)	Mangga	+	+	+	-	+	-
Stem (M)	Mangga	+	+	+	+	+	-
Leaf (M)	Mangga	+	+	+	-	-	+

Note : + = contain secondary metabolites, - = had no secondary metabolites, D = Durian, M = Mango

Table 2. Result of phytochemical test from stem and leaf of tree hosts (Durian and Mango) using ethanol solvents

Explant source	Secondary metabolite compounds					
	Flavonoid	Phenolic	Alkaloid	Saponin	Steroid	Triterpenoid
Stem (D)	+	+	+	-	-	+
Leaf (D)	+	+	+	-	+	-
Stem (M)	+	+	+	-	-	+
Leaf (M)	+	+	+	-	+	-

Note : : + = contain secondary metabolites, - = had no secondary metabolites, D = Durian, M = Mango

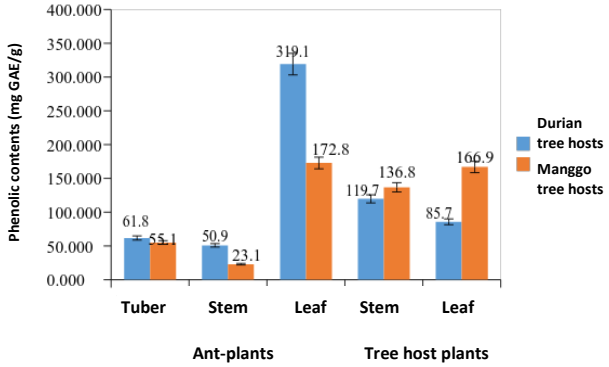

Figure 1. The total number of phenolic contents in tubers, stem and leaves of ant-plants (*Myrmecodia tuberosa*) from different tree hosts

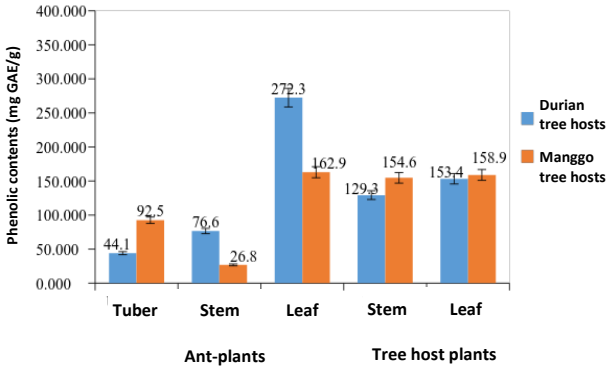

Figure 2. The total of flavonoid contents in tubers, stems and leaves of ant-plants (*Myrmecodia tuberosa*) from different tree host

The result also proved the previous false folk spread in a society that the ant-plants attached in mango tree host did not contain secondary metabolites. Ant-plants growing in a different tree host had no effects on the loss content of ant-plants metabolites, but they only had a different quantity of metabolite contents. The tree host of ant-plants also had the same secondary metabolites as ant-plants but it was different in the concentration of compounds. The total of phenolic content from mango host was larger than that from durian tree in both of leaves and trunk (Figure 1).

Total of Flavonoid Content (TFC)

The content of total flavonoids obtained from ant-plants and different tree hosts was not significantly different with the total of phenolic contents. The total of flavonoid contained in the ant-plants leaf from the durian host was 272.33 ± 0.02 mg Catechins Equivalent (CE)/g, while those from the mango host, the largest flavonoid contents were also found on leaf explants (162.83 ± 0.01 mg CE/g). Total flavonoid of mango and durian leaf was not different with 158.83 ± 0.00 mg CE/g and 153.33 ± 0.01 mg CE/g (Figure 2), with the linear regression equation y = 0.006 + 0,002x and R2 = 0.9996.

The total of flavonoid contents in ant-plants tubers from durian host was 44.10 ± 0.02 mg/g CE, while that from the mango host was 92.48 ± 0.02 mg CE/g. These results were almost in line with the result from Engida et al. (2013) in *M. pendens* bulbs. Where the total flavonoids in *M. pendens* tubers were 63.28 ± 1.75 mg QE/g using catechin as a metabolite comparison. Quercetin and catechin are a group of flavonoids which could be used as a reference solution for calculating the total of flavonoids contents. In addition, Ahmad et al. (2014) reported that the use of various fraction (ethanol, ethyl acetate, hexane, and water) for testing flavonoid function of *M.pendens* could inhibit the development of oral cancer cells (SP-C1). Furthermore, the fraction of ethanol and ethyl acetate was the highest potential fraction for inhibiting the development of cancer cells.

Antioxidants have a function to protect the body from oxidative stress by neutralizing free radicals. Exploration of antioxidant compounds from natural plant sources has been carried out intensively. Plants containing a high amount of polyphenols such as TPC and TFC potent to be used a source of natural antioxidants. Gan et al. (2010) have tested the content of TPC and antioxidant activity from 40 kinds of medicinal plants and other plants as a potential source of natural antioxidants because it has a high value of antioxidant activity and TPC i.e., *Sanguisorba officinalis*, *Rosa sinensis*, *Millettia dielsiana*, *Polygonum cuspidatum*, *Caesalpinia sappan* and *Sophora japonica*. Sharma et al. (2009) have studied *Trichosenthes dioica* leaf extract, *Moringa olifera* and *Ficus bengalensis* fruits, and seeds of *Emblica officinalis* using water solvent for analysing the TPC contents. Results showed that the TPC level of *T. dioica* leaves had two times larger than those from fruits and seeds of *M. olifera* and *E. officinalis*, while the high TFC level and the highest antioxidant activity was found in *E. officinalis* seed. It can be concluded that the seed extract of *E. officinalis* could be used as a potential material for antioxidant agents.

The conclusion of this study were the leaves of ant-plants possessed the highest value of TPC and TFC compared to those in other parts of ant-plants (roots and stems) so that ant-plants leaves could be potentially used as a source of antioxidants. In addition, this study gave a high contribution to the conservation of Borneo's indigenous germplasm where most of the ant-plants tubers were sporadically exploited for medicinal purposes leading to the increased status of ant-plants as endangered plants.

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