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TOTAL PHENOLIC CONTENTS, ANTIOXIDANT, ANTICANCER AND ANTIDIABETIC PROPERTIES OF *Myrmecodia tuberosa* (RUBIACEAE)

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ABSTRACT

Myrmecodia tuberosa, locally known as "Sarang Semut", belongs to the Rubiaceae family. The aim of the study is to investigate the total phenolic contents and bioactivity of the crude extract of *M. tuberosa*. The tuber, bark and leaves of the plant were cut, washed and air-dried. The plant was extracted with ethyl acetate and ethanol to yield EtOAc and EtOH crudes. The crudes were then tested for antioxidant 2,2-diphenyl-1-(2,4,6-trinitrophenyl)hydrazyl (DPPH) assay, *α*-glucosidase assay for anti-diabetic activity and [3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium] MTT Assay for cytotoxicity test. The results showed that bark ethanolic extract demonstrated the highest DPPH scavenging of 95.16% while other crudes were ranging from 92.77% to 94.55% scavenging of DPPH compared to ascorbic acid. The tuber ethyl acetate extract demonstrated the highest inhibition of *α* -glucosidase enzyme with 72.58% while other crudes were ranging from 63.27% to 72.46% inhibition. In addition, the ethanolic tuber extract exhibited the strongest cytotoxicity against Human colorectal Cancer (HT-29) and Cervical Cancer (HeLa) cell lines with the IC_{50} value of 16 µg/mL and 14 µg/mL respectively. Meanwhile, the ethanolic bark extract exhibited the strongest cytotoxicity against Human Breast Cancer (MCF-7) with IC_{50} value of 6.0µg/mL. Furthurmore, the tuber extracts contained the highest phenolic content with 1087mg GAE/ g extract compared to the leaves and barks of the plants. The findings suggest that the whole part of *M. tuberosa* is a potential natural source for anti-oxidative, anti-diabetic and anti-cancer agent.

Indexing terms/Keywords

Rubiaceae; *Myrmecodia tuberosa*; Antioxidant, Anticancer; Antidiabetic.

Academic Discipline And Sub-Disciplines

Chemistry; Natural Products

SUBJECT CLASSIFICATION

Natural Drugs Discovery

TYPE (METHOD/APPROACH)

Experimental

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INTRODUCTION

Myrmecodia tuberosa locally known as “Sarang Semut”, belongs to Rubiaceae family. Myrmecodia, together with *Anthorrhiza, Hydnophytum, Myrmephytum* and *Squamellaria* are the five known genera belong to the family of Rubiaceae that has tuber and epiphytic myrmecophytes. *Myrmecodia* genus has the second-widest distribution in the world [1] after *Hydnophytum* genus. *Myrmecodia sp*. can be found in Malaysia, Philippines, south to the Cape York Peninsula in Queensland, southern Thailand, Cambodia, Vietnam and New Guinea [2]. Usually, the genera of *Hydnophytum* and *Myrmecodia* species are associated with *Iridomyrmex* ant species. However, other ant species of the genera like *Anaplolepis, Camponotus, Crematogaster, Pedomyrma, Pheidole, Polyrachis, Monomorium, Technomyrex, Turneria, and Vollenhovia* also reported live in the tuber of this plants [3]. The ant-plants are saturated by ant colonies, and they are aggressive towards enemies of the host plant and are important for plant defense [4]. Plant parts produce sweet secretions consumed by the ants, [5] and the plant utilizes directly nutrients derived from animals [6].

Pack of *M.tuberosa* selling at night market, Sabah

Natural habitat of *Myrmecodia tuberosa*

The plant is traditionally used in Malaysia and Indonesia as an alternative treatment for cancer and tumor especially, in breast, liver, lung, ovarian and brain cancer. This plant is also used to lowering the glucose level in the blood. Therefore, the aim of this study was to investigate the total phenolic contents and the potential of *M. tuberosa* as an antioxidant, anticancer and antidiabetic agent.

METHODOLOGY

a) Preparation of EtOAc and EtOH crude

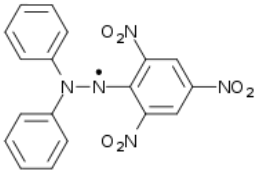
The purchased plants sample from the local market were cleaned and cut into different parts; the tuber, bark and leaves of the plant. They were air-dried for three weeks before the plants were extracted with ethyl acetate (3 X 4 days) and ethanol (3 X 4 days) to yield EtOAc and EtOH crude.

b) Total Phenolic Content

The modifications of *Folin Ciocalteu assay* by Kahkonen *et al* (1999)[7] were used to determine the total phenolic compounds (TPC) of plant crude extract. A 1.5 mL of *Folin-Ciocalteu* reagent was diluted ten times and 1.2 mL of sodium carbonate (7.5% w/v) was added to 300 µL of crude extracts in three replicate. The tubes were vortexed and stood for 30 minutes at 40 ºC for colour development. UV-VS spectrophotometer was used to monitor the absorbance of sample at 765 nm. Total phenolic content were expressed as mg Gallic acid equivalents/g dry weight (GAE).

c) DPPH Scavenging Activity

Antioxidant reducing activity on DPPH radical is estimated according to the method of Blois (1958)[8] with slightly modifications. The plant stock solution (1 mg/mL) was prepared by adding 1.0 mg crude plant extract into 1.0 mL ethanol. The DPPH stock solution (1 mM, 0.4 mg/mL) prepared by using 2.0 mg of DPPH in 5.0 mL of ethanol and used as negative control. The positive control was prepared by dissolving Ascorbic acid (A.A) 1.0 mg with 1.0 mL ethanol to make solution concentration of 1 mg/mL.



$$(DPPH^{\bullet}) + (H{-}A) \rightarrow DPPH{-}H + (A^{\bullet})$$

Purple Yellow

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d) MTT assay

Cell seeding for MTT assay was prepared to get 10,000 cells/well for each of HELA, HT-29 and MCF-7 cells and incubated overnight. The stock solution of samples with concentration 10 mg/mL was prepared as a cell treatment for MTT assay. Sample serial dilutions was performed starting with highest concentration and working down the well plate to get 30, 15, 7.5, 3.75, 1.875, 0.375 and 0.46875 µg/mL in each rows. The samples incubated for 24, 48 and 72 hours for the samples to take effect. The optical density was monitored using UV Spectrophotometer at 570 nm and 630 nm.

e) Antidiabetic activities test (α-glucosidase inhibitors assay)

Each well of 96 microliter plate was filled with 140 µL phosphate buffers (50 mM). In control wells, 15 µL dimethysulphade (DMSO) was added. 15 µL sample solution (1 mg/mL) was added in triplicate to the well. The enzyme solution (20 µL) previously prepared by dissolving 0.2mg enzyme in 27.5 mL phosphate buffer was added to each well. The plate was incubated for 15 minutes at 37 ºC. 25 µL of substrate solution (7 mM) of *p*-nitrophenyl-α-*D*-glucopyranosidase is quickly added to each well.The absorbance of the reaction mixture was taken within 30 minutes at 400 nm

The calculation of inhibition percentage is as below:

$$\%\, inhibition = \frac{(Absorbance\, of\, control - absorbance\, of\, sample)}{Absorbance\, of\, control}\, x\, 100\,\%$$

RESULTS AND DISCUSSION

a) Total Phenolic Contents

The results showed that the tuber extracts contained the highest phenolic content with 1087±0.06 mgGAE/g extract compared to the leaves and barks of the plants which only contain 669± mgGAE/g and 523 mg±0.02 GAE/g extract respectively.

Table 1: Total phenolic contents in *M.tuberosa*

Sample Extracts	Total Phenolic Content (mg GAE/ g extract)
MTB-E	523 ± 0.02
MTL-E	669 ± 0.04
MTT-E	1087 ± 0.06

Note: MTB-E: Ethanol Bark; MTL-E: Ethanol Leaves; MTT-E: Ethanol Tuber

b) DPPH Scavenging Activity

The results showed that the ethanolic bark extract demonstrated the highest DPPH scavenging of 95.16% while other part of extracts were ranging from 92.77% to 94.55% scavenging of DPPH compared to ascorbic acid (97.61%). The highest percentage scavenging activity of *Myrmecodia tuberosa* against free radical DPPH shows that this plant contains very high antioxidant properties.

Table 2: DPPH scavenging activity

Sample	ƛ	Concentration	Mean Absorbtion	Scavenging of DPPH (%)
Negative control	520nm	1000µg/mL	1.4184	-
Positive control			0.0338	97.6170
MTT-E			0.0933	93.4222
MTT-EA-RT			0.0807	94.3105
MTB-E			0.0686	95.1636
MTL-E			0.0773	94.5502
MTL-EA			0.1025	92.7735

Note: MTT-E: Ethanol Tuber; MTT-EA-RT: Ethyl acitate Tuber (remove tannins); MTB-E: Ethanol Bark; MTL-E: Ethanol Leaves; MTL-EA: Ethyl acetate Leaves.

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c) MTT Assays

The ethanolic tuber extract exhibited the strongest cytotoxicity against HT-29 and Hela cell lines with the IC_{50} value of 16 µg/mL and 14 µg/mL respectively. Meanwhile, the ethanolic bark extract exhibited the strongest cytotoxicity against MCF-7 with IC_{50} value of 6.0 µg/mL as shown in the graph.

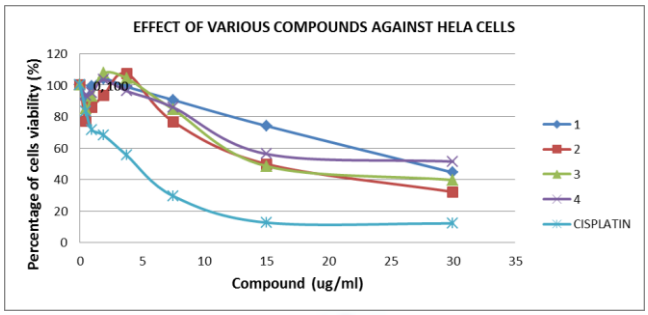

Figure 1: Effect of various extracts against HELA cells

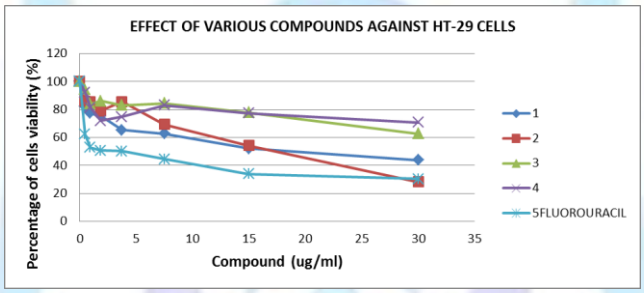

Figure 2: Effect of various extracts against HT-29 cells

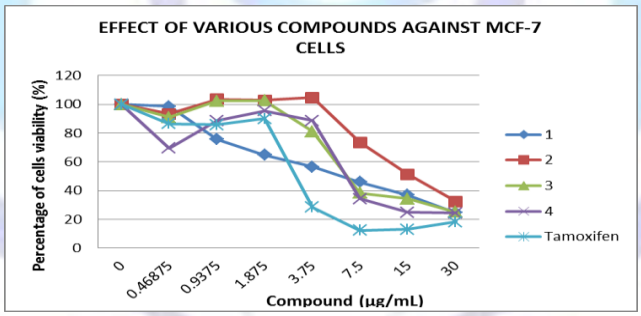

Figure 3: Effect of various extracts against MCF-7 cells

Note for Figure 1, Figure 2 and Figure 3: 1: Ethyl Acetate Tuber; 2: Ethanol Tuber; 3:Ethanol Leaves; 4: Ethanol Bark

d) α-Glucosidase InhibitoryActivity

An *in-Vitro* *α*-glucosidase assay (Anti-diabetic assay) of *Myrmecodia tuberosa* shows an average of 69.30% inhibitions of *α*-glucosidase enzyme as shown in table 3.

The ethyl acetate extract of tuber *M. tuberosa* demonstrated the highest (72.58%) inhibitions of *α*-glucosidase enzyme while the others ranging from 72.46% to 63.27%. This plant was considered has moderate activity of lowering the sugar level in blood for diabetic patients.

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Table 3: Percentage inhibitions of α-glucosidase enzyme

Sample	ƛ	Concentration	Mean Absorbtion	Scavenging of DPPH (%)
Control	400nm	1000µg/mL	0.395	-
MTT-EA			0.1451	72.5823
MTT-E			0.1083	63.2658
MTB-E			0.1088	72.4557
MTB-EA			0.1250	68.3544
MTL-E			0.1185	70.1266
MTL-EA			0.1225	68.9873

Note: MTT-EA: Ethyl acetate Tuber; MTT-E: Ethanol Tuber; MTB-E: Ethanol Bark; MTB-EA: Ethyl Acetate Bark; MTL-E: Ethanol Leaves; MTL-EA: Ethyl acetate Leaves;

CONCLUSION

The findings indicated that *M.tuberosa* is rich with phenolic contents. It also contains high antioxidant activity, strong cytotoxicity against MCF-7, HT-29 and HELA cell lines, and moderate antidiabetic activity.

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