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Anticancer Activity Test for Extracts of Sarang Semut Plant (*Myrmecodya pendens*) to HeLa and MCM-B2 Cells

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Abstract: The aim of this study is to investigate anticancer activity of methanol extract (ethylacetate, n-buthanol and water partitions) and water extract from Sarang semut (local name), *Myrmecodya pendens* which is one of Rubiaceae family. Within Papua area (Indonesia), this medicinal plant has been used traditionally as alternative treatment for ulcer, tumor and cancer. In this study, the extracts of this plant were tested for their activities in some cancer cells (HeLa and MCM-B2 cell). The result showed that water extract of this plant has better anti cancer activity compared to other extracts. The IC_{50} value of water extract A is 27.61 ppm (HeLa) and 54.57 ppm (MCM-B2), while water extract B is 29.36 ppm (HeLa) and 74.20 ppm (MCM-B2). Our study concluded that polar extract (water) exhibited higher anticancer activity than non-polar extracts (ethylacetate and n-buthanol).

Key words: Sarang semut plant, anticancer, *Myrmecodya pendens*

INTRODUCTION

Indonesia, passed by the equator line, is a country with more than 17.000 islands. Sixty percent of its land is covered by 120.3 hectare of giant forest, making it one of the richest countries in biodiversity in the world. Indonesia possesses 30.000 plant species, nevertheless, only 940 of which have been identified (Pramono, 1999). One of the unidentified medicinal plants is Sarang semut (*Myrmecodia pendens*) which might be potential for new source of new therapeutic agents.

The first information about this plant originated from empirical knowledge of local society in Papua island which is located in Eastern Indonesia. They consider Sarang semut plant as remedy for ulcer, haemorrhoid, nosebleed, backache, allergy, uric acid disorder, stroke, coronary heart problem, TBC, tumor, cancer, lactagogum, etc. (Subroto and Saputro, 2006). Generally, part of plant used as remedy is its hypocotyl (caudex) by drinking its boiling water (decoction).

In Papua, Sarang semut plant can be found in central mountain, Jayawijaya, Tolikara, Puncak Jaya, Gunung Bintang and Paniai (Subroto and Saputro, 2006). This plant also scattered from Malay Peninsula to The Philippines, Cambodia, Sumatra, Java, Papua, Cape York as well as the Solomon Islands.

In Sumatra, Sarang semut plant is named rumah semut, in Jawa, ulek-ulek polo, Papua, lokon, suhendep, nongon, Malaysia, periok hantu, peruntak, sembuku, Vietnam, by ki nan, ki nam gai, ki nam kin.

Generally, Sarang semut plant lives as ephyphyte on Cajuput plant (*Melaleuca*), Cemara gunung (*Casuarina*), Kaha plant (*Castanopsis*) and Beech (*Nothophagus*). It is called Sarang semut since, the inner part of its hypocotyl is used as nest (sarang, Indonesian language) by ants from genus *Iridomyrmex*. Most of Sarang semut plants are usually colonized by 1 species of ants. Based on identification, plant *Myrmecodia pendens* is colonized by ant *Ochetellus* sp.

Sarang semut plant is a member of Rubiaceae family with 5 genus, however, only 2 of which have association with ants. They are *Myrmecodia* (45 species) and *Hypnophytum* (26 species). From those species, only *Hypnophytum formicarum*, *Myrmecodia pendens* and *Myrmecodia tuberosa* are considered to have medicinal value.

Scientific publication on Sarang semut plant is still difficult to obtain and generally only discussing about its ecology, taxonomy and ethnobotany. The toxicity test using BSLT method found that LC_{50} value of water extract, ethylacetate extract and n-buthanol extract of this plant is ranging from 37.03 to 55.58 $\mu L\ mL^{-1}$. This result is one of motivations to carry out anticancer activity test of this plant by using some cancer cells derived from both human cervic and canine breast, named HeLa cells and MCM-B2, respectively. This study has been carried out because

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cancer is still one of the primary causes of death in the world thus, far with the hope that this plant could be used as potential anticancer medicine.

Active compounds obtained from several plants such as *Angelica gigas*, *Catharanthus roseus*, *Taxus brevifolia*, *Podophyllum peltatum*, *Podophyllum emodii*, *Ocrosia elliptica* and *Campototheca acuminata* have been used as anticancer medicines (Park and Pezzuto, 2002). Moreover, many plant-natural compounds such as flavones, flavanols, isoflavones, catechin and taxanes exhibit preventive and curative activities to cure cancer (Lopez-Lazaro, 2002; Philip, 2005). Although, many anticancer compounds such as alkilating compounds, antimetabolites, radiomimetics, hormones and antagonist have been developed (Calabresi and Chabner, 1991; Hoppe *et al*., 1982) none of them gave satisfaction and no side effects (Green *et al*., 1982; Herzig *et al*., 1987). Therefore, it is very necessary to investigate the bioactive compounds especially from Indonesia's natural sources which might be potential to be used as new potential anticancer agents. Furthermore, as a country with abundant natural resources, Indonesia is full of potential in which to conduct research on anticancer drugs. This study has been carried out in order to reveal the anticancer potential of Sarang Semut plant (*Myrmecodia pendens*).

MATERIALS AND METHODS

This research was carried out from July 2008 to December 2008.

Sarang semut plant (*Myrmecodia pendens*) was collected from Wamena-Papua, Indonesia and identified at Herbarium Bogoriensis, Department of Botany, Research Center for Biology-Indonesian Institute of Sciences (LIPI), Cibinong, Indonesia. The HeLa and MCM-B2 cells were obtained from Laboratory of Tissue Culture, Department of Parasitology and Pathology, Faculty of Vetenary, Bogor Agricultural Institute, Indonesia.

Hypocotyl of Sarang semut plants was cleaned, cut, dried, ground and then refluxed with water and methanol. This method was chosen to yield thermostable compounds as similar with empirical experiences of local people who use it after boiling process. Water extract obtained from these processes were called water extract (A). Methanol extract was partitioned using ethylacetate: water (1:1) to yield ethylacetate extract. Subsequently, water extract was partitioned yet again using n-buthanol: water (1:1) to yield n-buthanol extract and water extract (B).

Subsequently, 100 μL culture of HeLa and MCM-B2 cells in 800 μL DMEM medium were mixed with 100 μL of each extracts (water, methanol, ethylacetate and n-buthanol) at doses 0, 30, 60, 90, 120 and 150 ppm, respectively while doxorubicine (5 ppm) was used as standard of comparison. Cells were incubated for 72 h and cell suspensions were then counted by using Haemocytometer Tiefe Neubauer (0.100 mm; 0.0025 mm^2). Inhibition percentage was calculated using this equation:

$$\text{Inhibition (\%)} = 1-\frac{A}{B}\times 100\%$$

Where:

A: No. of cells in medium with extracts
B: No. of cells in medium without extracts

RESULTS AND DISCUSSION

The result of this research revealed that the inhibitory activities of Sarang semut plant (*Myrmecodia pendens*) to the growth of Hela cells (showed in activity) are 27.61 ppm for water partition (B); 29.36 ppm for water extract (A); 42.33 ppm for n-buthanol and 48.3 ppm for ethylacetate. Meanwhile, the inhibitory activities to the growth of MCM-B2 cells are 64.57 ppm for water partition (B); 74.20 ppm for water extract (A); 87.13 ppm for n-buthanol and 111.06 ppm for ethylacetate partition. The complete inhibition activity of sarang semut extracts to the growth of both HeLa cells and MCM-B2 cells was shown in both Table 1 and 2.

The data showed that the extracts of Sarang semut plant have capability to inhibit the growth of HeLa and MCM-B2 cells. This result may come from flavonoid and tannin compound contained in tested extracts. Flavonoid is a class of natural compound which exhibits activity as reductor to hydroxyl, superoxide and peroxyl radicals (Harun and Syari, 2002). In addition, many polyphenols and flavonoids have been proved to inhibit proliferation and angiogenesis of tumor cells *in vitro* (Fotsis *et al*., 1997) as well as in experiment animals (Hertog *et al*., 1993). Other study that evaluated the cytotoxic activity of bioactive compounds isolated from *Hydnophytum formicarum* Jack (other species of this plant) has found that its compounds did not exhibit cytotoxic activity which was conducted using two cell lines HuCCA-1 and KB (Prachayasittikul *et al*., 2008).

According to Yi *et al*. (2005) flavonol and tannin fractions are capable of giving 50% cell growth inhibition at concentration of 70-100 and 50-100 μg mL^{-1}, respectively.

Table 1: Inhibition activity of some Sarang semut extracts to the growth of HeLa cells

	No. of HeLa cells (10^4)												
	1			2			3						
Concentration (ppm)	1	2	3	1	2	3	1	2	3	Σ	Mean	APP (%)	IC_{50} (ppm)
Ethyl acetate partition													
0	14	10	8	7	12	6	3	8	10	78	8.67	100.00	48.13
30	12	13	9	7	6	5	5	1	10	68	7.56	87.20	
60	6	6	3	6	3	12	9	8	5	58	6.44	74.28	
90	5	8	5	5	5	7	3	5	7	50	5.56	64.13	
120	5	1	9	6	1	7	3	2	3	37	4.11	47.40	
150	3	6	1	2	3	2	3	6	7	33	3.67	42.33	
n-buthanol partition													
0	6	9	12	7	7	10	10	7	12	80	8.89	100.00	42.33
30	9	2	3	12	8	4	6	12	9	65	7.22	81.21	
60	14	8	4	6	3	7	7	7	9	65	7.22	81.21	
90	1	3	7	6	0	4	3	3	8	35	3.89	43.76	
120	8	1	0	1	3	0	6	7	0	26	2.89	32.51	
150	7	0	0	2	1	1	4	2	8	25	2.78	31.27	
Water partition (B)													
0	6	9	7	12	7	10	10	7	12	80	8.89	100.00	27.61
30	6	7	8	6	7	7	6	8	7	62	6.89	77.50	
60	3	4	7	7	1	6	7	2	3	40	4.44	49.94	
90	4	3	6	2	4	0	4	6	3	32	3.56	40.04	
120	2	0	2	1	3	1	4	2	3	18	2.00	22.50	
150	0	2	0	2	2	0	1	1	1	9	1.00	11.25	
Water extract (A)													
0	14	8	11	6	11	11	7	6	6	80	8.89	100.00	29.36
30	8	14	8	4	3	8	3	6	4	58	6.44	72.44	
60	1	6	3	8	6	3	11	7	4	49	5.44	61.19	
90	0	8	0	8	3	5	5	4	4	37	4.11	46.23	
120	7	0	5	1	0	3	4	0	6	26	2.89	32.51	
150	0	0	5	4	0	0	3	6	0	18	2.00	22.50	
Doxorubicin													
5	9	5	6	5	4	4	3	4	2	42	4.67	52.86	

Table 2: Inhibitory activity of some Sarang semut extracts to MCM-B2 cells

	No. of MCM-B2 cells (10^4)												
	1			2			3						
Concentration (ppm)	1	2	3	1	2	3	1	2	3	Σ	Mean	APP (%)	IC_{50} (ppm)
Ethyl acetate partition													
0	14	15	15	15	12	11	11	11	10	114	12.67	100.00	111.06
30	13	13	11	14	12	10	12	10	11	106	11.78	92.98	
60	12	11	10	12	12	11	12	13	12	105	11.67	92.11	
90	10	12	13	11	11	12	11	10	12	102	11.33	89.42	
120	12	11	10	10	10	11	13	10	10	97	10.78	85.08	
150	11	12	10	11	11	11	12	10	9	97	10.78	85.08	
n-buthanol partition													
0	12	12	14	9	12	11	18	14	12	114	12.67	100.00	87.13
30	11	12	14	14	12	11	12	11	11	108	12.00	94.71	
60	16	12	11	10	10	12	11	11	11	104	11.56	91.24	
90	8	12	12	9	10	11	12	11	10	95	10.56	83.35	
120	12	11	12	9	10	9	9	11	12	95	10.56	83.35	
150	9	11	10	8	11	11	12	11	9	92	10.22	80.66	
Water partition (B)													
0	12	12	14	9	12	11	18	14	12	114	12.67	100.00	64.57
30	12	10	10	12	9	12	11	12	10	98	10.89	85.95	
60	9	9	12	14	11	11	11	10	10	97	10.78	85.08	
90	11	11	11	9	10	9	11	12	9	93	10.33	81.53	
120	11	9	11	9	10	9	10	12	11	92	10.22	80.66	
150	11	12	11	9	9	11	12	9	8	92	10.22	80.66	
Water extract (A)													
0	12	14	12	11	12	14	12	12	17	116	12.89	100.00	74.20
30	17	15	14	9	12	14	11	8	9	109	12.11	93.95	
60	8	15	9	9	11	14	18	8	6	98	10.89	84.48	
90	8	5	3	11	9	9	6	9	12	72	8.00	62.06	
120	6	3	6	9	6	8	6	6	6	56	6.22	48.25	
150	2	5	5	2	11	8	5	3	0	41	4.56	35.38	
Doxorubicin													
5	9	9	8	10	8	9	9	8	8	78	8.67	68.13	

The inhibition of cancer cells growth (HeLa and MCM-B2 cells) by water extract B began at dose 30 ppm with LC_{50} value = 27.61 ppm (HeLa cells) and 64.57 ppm (MCM-B2 cells). Water extracts A start their inhibition activity at dose 60-90 ppm with LC_{50} value = 29.36 ppm (HeLa cells) and 74.20 ppm (MCM-B2 cells).

Based on phytochemical test, the water extract B contains tannin and flavonoids as major compounds. Water extract A contains flavonoids, saponin, glycoside, tannin and carbohydrates. The presence of flavonoids in greater amounts was predicted to inhibit the growth of cancer cells more effectively compared to water extract A.

The n-buthanol extract at dose 90 ppm showed IC_{50} value = 42.33 ppm (HeLa cells) and 87.13 ppm (MCM-B2 cells). Based on phytochemical test, n-buthanol extract contains flavonoids, tannin and quinones. Ethylacetate extract at dose 90-120 ppm showed IC_{50} value = 48.13 ppm (HeLa cells) and 111.06 ppm (MCM-B2 cells). Ethyl acetate extract only contains flavonoids and tannin. It was estimated that the presence of quinone compound in n-buthanol extract increases the effectiveness of this extract in inhibiting cancer cells growth compared by ethyl acetate extract.

Comparison of each extracts to doxorubicin to HeLa cells showed that 30 ppm of sarang semut plant extract is equal to 5 ppm doxorubicin. For MCM-B2 cells, 5 ppm doxorubicin remains stronger than 150 ppm Sarang semut plant extract.

Other study that evaluated the cytotoxic activity of bioactive compounds isolated from *Hydnophytum formicarum* Jack (other species of this plant) has found that its compounds did not exhibit cytotoxic activity which was conducted using two cell lines HuCCA-1 and KB (Prachayasittikul *et al*., 2008)

Based on the above research results revealed that Sarang Semut plant (*Myrmecodia pendens*) is moderately active for the treatment of breast and uterus cancer. Further pharmacological research using other cancer cells is necessary in order to establish whether this plant can be used as a potential source for new anticancer medicine.

REFERENCES

Calabresi, P. and B. Chabner, 1991. The Pharmacological Basics of Therapeutis. 8th Edn., Pergamon Press, USA., pp: 1202-1290.

Fotsis, T., M.S. Pepper, E. Aktas, S. Breit and S. Rasku *et al*., 1997. Flavonoids, dietary inhibitors of cell proliferation and *in vitro* angiogenesis. Cancer Res., 57: 2916-2921.

Green, D., K.D. Tew, T. Hisamatsu and P.S. Schein, 1982. Correlation of nitrosoureamurine bone marrow toxicity with deoxyribonucleic acid alkylation and chromatin binding sites. Biochem. Pharmacol., 31: 1671-1679.

Harun, N. and W. Syari, 2002. Aktivitas antioksidan ekstrak daun dewa dalam menghambat sifat hepatotoksik halotan dengan dosis sub anastesi pada mencit. J. Sains Dan Teknol. Farmasi, 7: 63-70.

Hertog, M.G.L., P.C.H. Holmann, M.N. Katan and D. Kromhout, 1993. Intake of potentially anticarcinogenic flavonoids and their determinants in adults in the Netherlands. Nutr. Cancer, 20: 21-29.

Herzig, R.H., J.D. Hines and G.P. Herzin, 1987. Cerebellar toxicity with high-dose cytosine arabinoside. J. Clin. Oncol., 5: 927-932.

Hoppe, R.T., C.N. Coleman, R.S. Cox, S.A. Rosenberg and H.S Kaplan, 1982. The management of stage I--II Hodgkins disease with irradiation alone or combined modality therapy: The stanford experience. Blood, 59: 455-465.

Lopez-Lazaro, M., 2002. Flavonoids as anticancer agents: Structure-activity relationship study. Curr. Med. Chem. Anti-Cancer Agents, 2: 691-714.

Park, E.J. and J.M. Pezzuto, 2002. Botanicals in cancer chemoprevention. Cancer Metastasis Rev., 21: 221-255.

Philip, P.A., 2005. Experience with docetaxel in the treatment of gastric cancer. Semin Oncol., 32: S24-38.

Prachayasittikul, S., P. Buraparuangsang, A. Worachartcheewan and C. Isarankura-Na-Ayudhya, 2008. Antimicrobial and antioxidative activities of bioactive constituents from Hydnophytum formicarum Jack. Molecules, 13: 904-921.

Pramono, E., 1999. Pemberdayaan, Penggunaan Obat Asli Indonesia Dalam Menunjang Sistem Kesehatan Nasional. Indofarma Publishre, Jakarta.

Subroto, M.A. and H. Saputro, 2006. Gempur Penyakit Dengan Sarangsemut. Penebar Swadaya, Jakarta, pp: 11-22.

Yi, W., J. Fischer, G. Krewer and C.C. Akoh, 2005. Phenolic compounds from blueberries can inhibit colon cancer cell proliferation and induce apoptosis. Agric. Food Chem., 53: 7320-9.