ISBN: 978-602-7385-2-1

THE EFFECT OF THE WAY TO USE SARANG SEMUT (*Myrmecodia pendens)* IN CANCER TREATMENT AGAINST ATIOXIDANT ACTIVITY, TOCOPHEROL CONTENT, AND TOTAL FLAVONOIDS

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Abstract

Cancer is a major problem in the world. Cancer deaths reached 10 % of the total deaths. Chemotherapy provides a number of side effects which often encourage patients to stop the treatment. One of the potential plant as an alternative to chemotherapy is sarang semut. Sarang semut potential as anticancer associated with flavonoids and tocopherol content. Flavonoids work as an anticancer through free radical scavenging and inactivate carcinogens, while tocopherol is a powerful antioxidant. Measurement of antiokxidant activity and determination of total flavonoids content is done by Uv-vis spectrophotometry, whereas tocopherol assay performed by High Performance Liquid Chromatography. All means of use the sarang semut able to provide a very strong antioxidant activity with IC_{50} less than 50 ppm. Even the stew sample (IC_{50} 3,6086 ppm) and dry extract sample (IC_{50} 5,0563 ppm) more active than vitamin E (IC_{50} 7,1746 ppm), while the IC_{50} of steeping sample is 9,2971 ppm. Stew sample also produce a total flavonoid content with the highest levels that is 1,0750 than steeping sample 0,9141 and dry extract 0,7468 gram rutin equivalents of each 100 gram sarang semut dry simplicia. But in the stew dan steeping sample were not found any tocopherol, whereas tocopherol levels measured in the dry extract sample up to 0,1042 %. Therefore, the most appropriate way of sarang semut dry simplicia powder use to obtain the highest flavonoid content is stewed, while to get high tocopherol content better consumed in the form of dry extract. Both are able to provide a very strong antioxidant activity.

Keywords: *sarang semut, antioxidant, tocopherol, flavonoid*

INTRODUCTION

Cancer is the major problem in the world. Cancer deaths reached 10 % of the total deaths. This is important because half of which was attacked in developing countries, and 66 % died of cancer. Chemotheraphy will provide a number of side effects, which often encourage patients to stop their therapy. That reality ask for need a safety way with minimal side effects for cancer therapy, namely with natural ingredients. One of the plant as a potential alternative cancer chemotherapy is sarang semut (*Myrmecodia pendens*).

Several studies have been conducted to prove the pharmacological effects of sarang semut as anticancer. In-vitro experimental test for cytotoxicity effect of sarang semut extracts on some cancer cell has been done, among others by Suharyanto et.al (2013) on lung cancer cell, by Yessica, P (2012) on cell cultures of MCF-7 breast carcinoma, by Fatmawati et.al (2011) on HeLa cervical cancer cell line, by Soeksmanto at.al (2010) on HeLa cell and MCM B-2 which have been cultured. The anticancer potential of sarang semut related to the flavonoid.

Flavonoids work as an anticancer by inhibiting proliferation, induce apoptosis, inhibit the cell cycle, inactivate carcinogens, and free radical scavenging. Source of antioxidant compounds in the sarang semut in addition to flavonoids is tocopherol (vitamin E) which is a potent antioxidant group.

Until now, sarang semut was distributed on the market in the form of dry powder simplicia dan it's dry extract. There are two kinds of methods used for dry powder simplicia of sarang semut in medication, namely with steeping dan stewed. In order to make it easier to consumes steeping preparations, then sarang semut dry simplicia powder is also marketed in the form of teabags, while sarang semut dry extract marketed in capsule form. Due to flavonoids and tocopherol are the main source of antioxidant compounds contained in sarang semut, hence

effectiveness of sarang semut as an anticancer affected by the strength of the antioxidant activity with tocopherol level and total flavonoids in sarang semut consumed preparations.

The antioxidant activity test of sarang semut that has ever done is antioxidant activity test on fraction of sarang semut extract partition results (Noya dkk, 2013; Suharyanto dkk, 2013). Similarly, determination of sarang semut total flavonoids that has ever done is determination of total flavonoids in extract (Putri T.U., 2013). Determination of sarang semut tocopherol in the extract nor stew dan steeping sample preparation of its dry simplicia also has not been done. Therefore, this research has not been done in previous studies.

Through this research, can be known antioxidant activity, tocopherol content, and total flavonoids which is used in the form of steeping, stew, and its extract. Thus can be known the most appropriate way of sarang semut dry simplicia powder use to obtain the strongest antioxidant activity, with the highest total flavonoids and tocopherol content.

METHOD

A. Apparatus

The main instruments used were: spectrophotometre Uv-vis W-Mini-1240, 220-240 Shimadzu (serial number A 10934502629) equipped with kuvet (Helma); High Permormance Liquid Chromatography Shimadzu LC solution equipped with Shim-pack VP-ODS column (250 x 4,6 mmID x 5 μm), LC-20AT Prominence Liquid Chromatograph Pump, detector Uv-vis SPD-20A Prominence; rotary evaporator (IKA HV 10).

B. Material

Research materials used were: dry simplicia powder of sarang semut (*Myrmecodia pendens*, "Papua Herbal", Wamena Papua); DPPH (2,2-diphenyl-1-picrylhydrazyl) (pro-analysis, Sigma); tocopherol (pro-analysis, Sigma); rutin (pro-analysis, Sigma); ethanol 70 %, methanol, acetonitrile, $AlCl_3$, CH_3COOK all pro-analysis degree, (E. Merck); ethanol absolut, HCl, NaCl 10 %, $FeCl_3$, gelatin, dan akuades.

C. Sample preparation

Steeping sample

Weigh 2.0000 g (equal to 1 tablespoon) dry simplicia powder of sarang semut, then steeped with 200.0 mL (equal to 1 glass) boiling aquadest (temperature 100 ^oC). Let stand for 15 minutes in a closed condition, then filtered.

Stew sample

Weigh 2.0000 g (equal to 1 tablespoon) dry simplicia powder of sarang semut, then stewed with 400.0 mL (equal to 2 glass) aquadest until remaining 1 glass (200 mL). Let stand for 30 minutes in a closed condition, then filtered.

Extract sample

Weigh 250.0000 g dry simplicia powder of sarang semut then macerated with 2.5 L ethanol 70% for 5 days, with stirring frequently everyday. After 5 days macerated then filtered to obtain the first filtrrate. Dregs gained was dried in oven at 50 ^oC for 24 hours, then macerated again in the same way in order to obtain a second filtrate. The first and second filtrat then mixed and concentrated with rotary evaporator on 125 rpm and 60 ^oC to obtain the viscous extract. The viscous extract was then dried in an oven at 50 ^oC to obtain the dry extract.

D. Qualitative test

Flavonoid test

Each sample was dissolved with absolute ethanol and then divided into two tubes, the first tube as blank solution and the second tube as test solution. Add 2 drops of concentrated HCl into the second tube, then compare it with the blank solution. After that, the second tube warmed on a waterbath for 15 minutes. When formed red or violet, indicate the presence of flavonoids.

Tannin and polyphenol test

Each sample was dissolved with hot aquadest, then stired and cooled. Add 5 drops of NaCl 10 % then filtered. Filtrate divided into 3 tubes, the first tube as blank solution, the second and the third as test solution. Into

ISBN: 978-602-7385-2-1

the second tube add 3 drops of $FeCl_3$ and into the third tube add gelatin solution. If formed blackish green in the second tube, indicate the presence of hydrolyzed tannins, whereas if formed brownish green indicates the presence of condensed tannins. However, if formed a colour other than these colours it is showed the presence of polyphenol compounds. If a precipitate is formed in the third tube it is indicates the presence of tannins.

E. Measurement of antioxidant activity

Measurement of control absorbance

The absorbance of DPPH 50 µg/mL in ethanol 70 % was measured at a DPPH maximum wavelength which is scanned at 450 – 600 nm.

Measurement of the free radical scavenging capacity

An amount of sample was taken then reacted with 5.0 mL DPPH 100 µg/mL and diluted with ethanol 70 % to obtain a sample solution with a concentration series 5 – 11.5 µl/mL for steeping, 1 – 6 µl/mL for stew, and 0.5 – 6.5 µg/mL for extract. After homogenized and allowed to stand at room temperature in the dark for 30 minutes, then the absorbances were measured at DPPH maximum wavelength. Do the same way for vitamin E as a comparison at a concentration series 3 – 8 µg/mL. The DPPH free radial scavenging capacity of antioxidant compound is expressed with % inhibition which is calculated by the following equation:

$$\% \text{ inh} = \frac{\text{A control} - \text{A sample}}{\text{A control}} \times 100\% \quad (1)$$

A = Absorbance

Measurement of antioxidant activity

Calculate the linear regression equation which describes the relationship between the concentration of the solution to the % inhibition. Also determine the correlation coefficient. The antioxidant activity is expressed by IC_{50}. Calculate the IC_{50} by entering 50 as Y in the linear regression equation, hence will be obtain the value of X as IC_{50}.

F. Determination of Tocopherol

Determination of tocopherol was performed with HPLC, methanol : acetonitrile (8 : 2) as mobile phase at a flow rate 1 mL/menit, and Uv-vis detector was set at tocopherol maximum wavelength which is scanned at 250 – 350 nm. At this step methanol was used as a solvent. Linear regression equation was made from a series of standard solution concentration in the range of 0.5 – 0.9 µg/mL to chromatogram peak height of tocopherol. The steeping and stew sample was injected to the HPLC system at 400 µl/mL, while extract sampel solution was injected at 40 µg/mL and has been spiked by addition standard at 0.5 µg/mL. Observe the chromatogram peak height of tocopherol and determination the concentration with linear regression equation.

G. Determination of Total Flavonoids

Determination of total flavonoids was done with Chang method by Uv-vis spectrophotometry with rutin as reference standard at 10 – 28 µg/mL in methanol. The absorbance was measured at rutin maximum wavelength which is scanned at 300 – 450 nm. The steeping and stew sample was pipetted 1.5 mL respectively, then added with 0.2 mL $AlCl_3$ 10 % in methanol and 0.2 mL CH_3COOK 1 M in aquadest, then was diluted with aquadest up to 10.0 mL. For the extract sample, was weighed 25.0 mg then was diluted with methanol to obtain the concentration of 1000 µg/mL. The extract solution then was pipetted 2.5 mL and was prepared with the same way with the steeping and stew samples. After homogenized and allowed to stand for 30 minutes the absorbance was measured at rutin maximum wavelength. Determination of total flavonoids in sample by linear regression equation and expressed in gram equivalents for each 100 gram dry simplicia.

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H. Data collection technique

Data obtained by conducting a series of tests in the laboratory. Each group of samples, that is steeping, stew, and extract was carried out the antioxidant activity measurement, determination of tocopherol, and determination of total flavonoids with 3 times of replication. Each replication data was taken with three repetitions.

RESULT AND DISCUSSION

A. Qualitative Test

Before the measurement of antioxidant activity, determination of tocopherol and total flavonoids, at first each sample was tested qualitatively, namely the content of flavonoid, tannin, and polyphenol. It was done because flavonoid, tannin, and polyphenol were the main source of antioxidant compound in sarang semut. Based on the results of qualitative test was known that in the sarang semut steeping, stew, and extract sampels containing flavonoid, tannin dan polyphenol.

B. Measurement of Antioxidant Activity

In this study, the strength of antioxidant activity was measured from the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging capacity of antioxidant compound by spektrophotometry Uv-vis at 521,5 nm. As for the absorbance spectrum of DPPH standard solution is presented in figure 1.

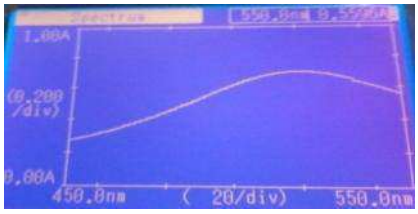

Figure 1. Absorbance spectrum of standard solution DPPH 175 µM

The antioxidant activity is expressed by IC_{50} (*Inhibitory Concentration* 50), namely the sample concentration required to inhibit free radical DPPH up to 50 %. The antioxidant activity is classified highly active when produced IC_{50} of less than 50 ppm, active if it has IC_{50} between 50 – 100 ppm, quite active if it has IC_{50} between 101 – 250 ppm, and less activity if it has IC_{50} between 250 – 500 ppm, and inactive when it has IC_{50} more than 500 ppm (Jun et al., 2003).

Antioxidant compound in sample will reduce free radical DPPH. Reduction capacity of antioxidant is expressed with % inhibition, that is the ratio in percent between the number of free radicals DPPH that has been reduce by antioxidants against amount of free radicals DPPH initially. The getting lower absorbance in measurement result indicates that DPP*Hydrazil* the ubstable compund is converted increasingly into DPP*Hydrazin* the stable one, so that the remaining of unstable DPP*Hydrazil* is getting a bit. This means % inhibition of sample against free radical DPP*Hydrazil* is more increasing, therefore IC_{50} of sample is getting lower and antioxidant activity is getting stronger.

Based on the result of antioxidant activity measurements in various ways the use of sarang semut and vitamin E as comparator, it can be arranged the order of samples that can provide antioxidant activity from the highest, that are IC_{50} 3,6087 ppm for stew sample, IC_{50} 5,0563 ppm for extract, IC_{50} 7,1746 ppm for vitamin E, and IC_{50} 9,2971 ppm for steeping sample. However, the whole preparation of sarang semut, among others steeping, stew, and extract can be expressed to have a very strong or very active of antioxidant activity with IC_{50} less than 50 ppm.

C. Determination of Tocopherol

Determination of tocopherol was performed with HPLC, methanol : acetonitrile (8 : 2) as mobile phase at a flow rate 1 mL/menit, and Uv-vis detector was set at 291 nm. As for the absorbtion spectrum of tocopherol standard solution is presented in figure 2.

ISBN: 978-602-7385-2-1

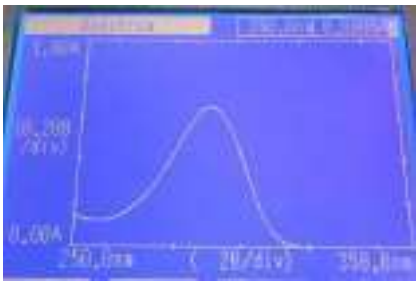

Figure 2. Absorbtion spectrum of tocopherol standard solution 120 µg/mL

At the HPLC system tocopherol perfectly capable eluted with a retention time of approximately 17 minutes, as can be seen in figure 3. While the peaks at minute 2.5 – 5 derived from components of mobile phase and solvent used. This is proved by injecting mobile phase and solvent into the HPLC system, with the results as can be seen in figures 4 and 5.

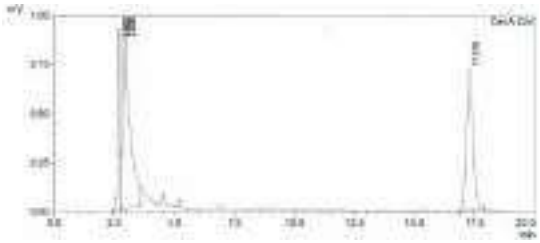

Figure 3. Chromatogram of tocopherol standard solution (T_R = 17.278 menit, H = 726) 0.9 µg/mL in methanol

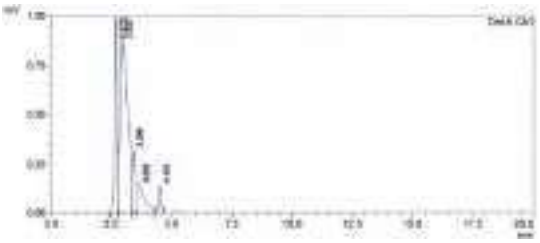

Figure 4. Chromatogram of mobile phase methanol : acetonitril (8 : 2)

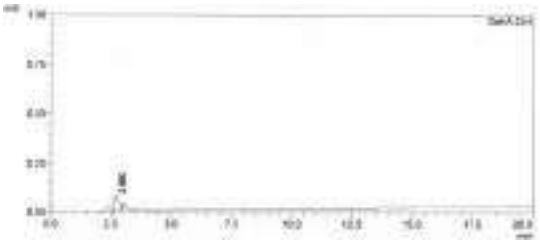

Figure 5. Chromatogram of methanol solvent

On the results of steeping and stew samples injection to HPLC system was not found tocopherol peak at the 17th minute, whereas the results of the extract sample injection into HPLC system showed a tocopherol peak at the 17th minute. Tocopherol is unable extracted when sarang semut consumed in steeping and stew preparation because tocopherol insoluble in water but soluble in ethanol 70%.

Due to the chromatogram of tocopherol peak which is narrow, sharp, and simetrical hence linear regression equation that is used to determinate of tocopherol is a correlation between concentration and peak high, it is Y = 679 X + 125.1 with coefficient of correlation (r) 0.9952, at range 0.5 – 0.9 µg/mL. Because of the high intensity of tocopherol peak has not meet the range of tocopherol peak high that make up the linear regression equation, then

determination of tocopherol in sarang semut extract is done by standard addition method. In addition to increasing the sensitivity and accuracy of the measurements, it is also done to avoid the increasing of peak intensity that derived from the sample matric because of the risk to column pressure and cleanliness. As for the chromatogram obtained can be seen in figure 6. In the extract sample tocopherol can be obtain at 0,1042%.

Figure 6. Chromatogram of tocopherol (T_R = 17.267 menit, H = 725) in extract sample solution which has spiked with addition standard 0.5 µg/mL in methanol

D. Determination of Total Flavonoids

Determination of total flavonoids was done with Chang method by Uv-vis spectrophotometry at 360.2 nm. The absorbtion spectrum of rutin standard solution is presented in figure 7.

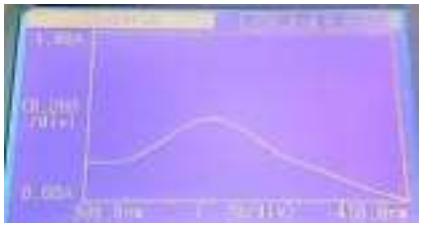

Figure 7. The absorbtion spectrum of rutin standard solution 15 µg/mL

Linear regression equations were used to determinate total flavonoids was Y = 0.0250 X + 0.0424, with coefficient of correlation (r) 0.9991, at range 10 – 28 µg/mL. Based on the determination of total flavonoids in various ways the use of sarang semut, it can be arranged the order of samples that can provide total flavonoids from the highest, that are: 1.0750 in stew sample, 0.9141 in steeping sample, and 0.7468 in extract sample in gram rutin equivalent of each 100 gram dry simplicia, respectively.

E. Analysis of The Results

Based on the results, it can be arranged recapitulation of such exposure in table 1. Based on the measurement of antioxidant activity, the whole preparation of sarang semut able to provide a very strong antioxidant activity, and even the stew and dry extract are stronger than vitamin E which has IC_{50} at 7.1746 ppm. The stew sample also can provide the higher total flavonoids than steeping and dry extract. That is what causes the stew sample has the highest antioxidant activity, even higher than vitamin E. However based on the determination of tocopherol, only dry extract that is able to take tocopherol contained in sarang semut up to 0.1042% atau 1042 ppm. That result is almost 3.5 times higher than tocopherol contained in the sarang semut plant itself, which is 313 ppm. Therefore to obtain a high tocopherol content, sarang semut better consumed in the form of dry extract.

Table 1. Test results in various ways to use sarang semut

Sample	Hasil Uji		
	Antioxidant Activity (IC_{50} in ppm)	**Tocopherol (%)**	**Total Flavonoids (g rutin equivalent / 100 g dry simplicia)**
Steeping	9.2971 very active	-	0.9141
Stew	**3.6087 very active**	-	**1.0750**
Extract	5.0563 very active	**0.1042**	0.7468

ISBN: 978-602-7385-2-1

CONCLUSION

The whole preparation of sarang semut, either on steeping, stew, and extract sample, able to provide a very strong of antioxidant activity with IC_{50} less than 50 ppm, even the stew and dry extract are stronger than vitamin E. The stew sample can also provide the highest total flavonoids than steeping and dry extract, but only by extraction which can take tocopherol with almost 3.5 times higher levels than tocopherol contained in the sarang semut plant itself. Therefore, the most appropriate way of sarang semut dry simplicia use to obtain the highest flavonoid content is by stew, while to get high tocopherol content better consumed in the form of dry extract. Both are able to provide a very strong antioxidant activity.

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