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Herbal Medicines : Indigenous, Molecular Aspects, and Clinical Application

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Foreword from Dean of Faculty of Pharmacy Universitas Padjadjaran

Dear Delegates,

On behalf of the Conference Committees, I would like to thanks for your participation in The International Seminar and Expo 2010, which take place from November 5th-6th 2010, at Bandung, Indonesia. This seminar of "Herbal Medicines: Indigenous, Molecular Aspects, and Clinical Application" offer a comprehensive understanding of utilization of herbal medicines from various aspects, including those associated with their regulation, traditional use, chemical analysis, biological activity, mechanism of action, and clinical application.

This proceeding is consist of approximately 34 papers. We thanks to The President of Universitas Padjadjaran, Prof. Dr. Ganjar Kurnia, DEA.; The Dean of Faculty of Pharmacy of Universitas Padjadjaran, Prof. Dr. Anas Subarnas, M.Sc., Apt.; and all the authors that participated in this conference for all their support and contribution in publishing this proceeding.

As the organizing committee, we greatly appreciate your participation in The International Seminar and Expo 2010. We look forward to meeting you and welcoming you again in our next meeting.

Bandung, November 5th 2010.
Sincerely,

Prof. Dr. Anas Subarnas, M.Sc., Apt.
Dean of Faculty of Pharmacy
Universitas Padjadjaran

Content

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IN VITRO DETERMINATION OF ANTIOXIDANT ACTIVITY OF EXTRACTS OF *MAHKOTA DEWA, TEMU PUTIH, SAMBILOTO* AND *KELADI TIKUS*

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Abstract

Mahkota dewa (Phaleria macrocarpa, Boerl), temu putih (Curcuma zeodaria), sambiloto (Andrographis paniculata, Nees), and *keladi tikus (Typhonium flagelliforme)* are medicinal plants that have potency as antioxidant and anticancer. The objective of this study was to determine the antioxidant capability of extract of *mahkota dewa, temu putih, sambiloto*, and *keladi tikus* using tiobarbituric acid method. In this method, linoleic acid was oxidized by oxygen on 40° C for 8 days and produced malondialdehyde. The corresponding malondialdehyde have reacted with tiobarbituric acid and produced red product, the absorbance was measured on wavelength of 532 nm. The antioxidant potency of all plant extracts was monitored through its capability on inhibiting oxidation. Inhibition capability of the respective extracts on concentration of 200 ppm for aquademineralized, hot water, and ethanol extract were 83.44, 70.86, and 81.84% (*mahkota dewa*), 60.21, 82.74, and 69.28% (*temu putih*), 81.45, 81.45, and 67.96% (*sambiloto*), 68.60, 63.53, and 72.17% (*keladi tikus*), plus 87.01% (vitamin E), respectively. Based on analysis of variance and Duncan's test, antioxidant potency of all of extracts at concentration of 200 ppm, if compared with vitamin E, were significantly different on confidence level of 95%.

Keywords: Water and ethanol extracts, *Mahkota dewa (Phaleria macrocarpa, Boerl), temu putih (Curcuma zeodaria), sambiloto (Andrographis paniculata, Nees), and keladi tikus (Typhonium flagelliforme)*, antioxidant.

INTRODUCTION

Cancer is a class of diseases in which a cell, or a group of cells display *uncontrolled growth, invasion* (intrusion on and destruction of adjacent tissues), and sometimes metastasis (spread to other locations in the body via lymph or blood). Cancer prevention is defined as active measures to decrease the incidence of cancer. Greater than 30% of cancer is preventable via avoiding risk factors including: tobacco, overweight or obesity, low fruit and vegetable intake, physical inactivity, alcohol, sexually transmitted infection, air pollution. This can be accomplished by avoiding carcinogens or altering their metabolism, pursuing a lifestyle or diet that modifies cancer-causing factors and/or medical intervention (chemoprevention, treatment of pre-malignant lesions). Antioxidants are widely used as ingredients in dietary supplements in the hope of maintaining health and preventing diseases such as cancer, coronary heart disease and even altitude sickness. Although initial studies suggested that antioxidant supplements might promote health, later large clinical trials did not detect any benefit and suggested instead that excess supplementation may be harmful. In addition to these uses of natural antioxidants in medicine, these compounds have many

industrial uses, such as preservatives in food and cosmetics and preventing the degradation of rubber and gasoline. Natural antioxidant agent can be found from plants such as: *mahkota dewa* (*Phaleria macrocarpa*, Boerl), *temu putih* (*Curcuma zeodaria*), *sambiloto* (*Andrographis paniculata* Nees), and *keladi tikus* (*Typhonium flagelliforme*) (Wijayakusuma1994). Those predictions are based on the active compounds contained in those plants. According to various researches recently, *mahkota dewa* flesh contains flavonoids, alkaloids, terpenoids, saponinns, and resins (Harmanto 2002; Winarto 2003).

The utilization of above plants mostly is empirical evidence based on users' experiences and the scientific evidence. Thus, the scientific research especially for the antioxidant potency of these plants is important to perform.

The purpose of the research is to determine the antioxidant activity of *mahkota dewa*, *temu putih*, *sambiloto*, and *keladi tikus in vitro* by thiobarbituric acid (TBA) method. This study also is expected to give scientific informations about the antioxidant potent of these plants.

METHOD

Analysis of hydroperoxide from linoleic acid by thiocyanate method (Chen *et al.* 1996)

Before the measurement of the antioxidant potency from each extract, it was measured the hydroperoxide as primary product of oxidized linoleic acid by thiocyanate method. This method measures the peroxide through color complex of $Fe[Fe(SCN)_6]$.
Amount of 2 mL of phosphate buffer 0.1 M pH 7.2 mL of linoleic acid 50 mM in ethanol 99.8% and 1 mL of deionized water were placed into dark bottle, the the mixture was incubated at 40°C. The period of incubation time was until reached the maximum absorbance.
After the maximum absorbance was reached, into 50 µL of incubated mixture, it was added 6 mL of ethanol 75%, 50 µL of ammonium thiocyanate 30%, and 50 µL of $FeCl_2$ 0.02 M in HCl 3.5%. After left for 3 minutes, the absorbance was measure at a wavelength 482 nm. The measurement of hydroperoxide was carried out every day until the maximum absorbance was reached.

Analysis of antioxidant potency by TBA method (Kiuzaki & Nakatani 1993)

Analysis of antioxidant capability from each extract was carried out using serial concentration of sample test solution, namely 50, 200, and 1000 ppm. Standard solution of 1,1,3,3-tetramethoxy propane (TMP) 6 M was diluted into serial concentration of 0.15, 0.3, 0.6, 0.75, 1.5, and 3.0 µM. Each solution was taken 1 mL and added 2 mL TCA 20% and 2 mL TBA 1% (b/v) in acetate solvent 50% (v/v). Next, all of test tubes were incubated at 100°C for 10 minutes and cooled at room temperature. Once cool, the mixtures were centrifuged at speed 300 rpm then the absorbance was measure using spectrophotometer at a wavelength 532 nm (Yagi 1968).

Sample solution was prepared from mixture containing of 2mL of phosphate buffer pH 7.2 mL of linoleic acid 50 mM in ethanol 99.8% containing tocoferol (Vitamin E 200 ppm), and 1 mL of deionized water.

All of the reaction mixtures were incubated in water bath 40°C with the period of incubation time was based on the measurement of hydroperoxide from linoleic acid. Reaction mixtures were assayed for antioxidant potency after 1 or several days from the maximum absorbance of linoleic acid. Each reaction mixture was taken 1 mL, then added 2 mL of TCA 20%, and 2 mL of TBA 1% solution in acetic acid 50% solvent. Next, those reaction mixtures were placed in waterbath 100°C for 10 minutes. After cool, centrifuged at 300 rpm for 15 minutes, then the absorbance was measured using spectrophotometer at a wavelength of 532 nm.

RESULTS AND DISCUSSION

Linoleic acid oxidation

Antioxidant activity assay by TBA method is based on measurement of malondialdehida (MDA), level which is the end product of lipid peroxide reaction. MDA is a reactive three-carbon dialdehyde compounds. MDA generated can be measured by TBA test. MDA can react with TBA to form a product for which fluorescence and can be measured at a

wavelength of 532 nm. As a standard, it is used 1,1,3,3 tetrametoksipropana (TMP) that can measure MDA formed level. TMP is an MDA derivative compounds which is quite stable. Measurement of antioxidant activity with this TBA method were carried out after the level of linoleic acid oxidation was maximum because at that time also established the maximum MDA generated from lipid oxidation reactions.

Antioxidant activity of *mahkota dewa*, *temu putih*, *sambiloto*, and *keladi tikus* can be determined from its ability to inhibit oxidation of linoleic acid. The presence of secondary metabolites such as alkaloids, flavonoids, tannins, and saponins suspected to inhibit the oxidation of linoleic acid. Hydroperoxide measurement result which was the result of oxidation of linoleic acid showed peak absorbance at 6^{th} day.

Oxidized Linoleic acid by oxygen in the early stages will form the hydroperoxide. This hydroperoxide levels increased and after reaching the maximum level, hydroperoxides will decompose to form malondialdehyde which is the end product of lipid peroxide reaction. Malondialdehyde formation was occurred on 7th day.

Antioxidant activity analysis

Measurement of antioxidant activity was carried out on the 8th day in the hope that all hydroperoxide formed from the oxidation of linoleic acid had undergone decomposition into malondialdehyde (MDA). Antioxidant activity of all types of plants can be seen by comparing the absorbance value that describes the concentration of MDA. Absorbance value is proportional to the concentration of MDA and inversely proportional to the antioxidant potency. Low absorbance values indicate that plants have a high antioxidant potential. This indicates that the sample may inhibit the oxidation process (means to reduce the amount of MDA which reacts with the thiobarbituric acid to form red product).

This study used vitamin E as standard with a concentration of 200 ppm. The reason for the selection of vitamin E as a standard was at a concentration of 200 ppm inhibition percentage was near 100%. The results of Satria (2005), inhibition of vitamin E (200 ppm) by TBA method amounted to 93.0%, while the results of research Indariani (2005) the antioxidant potency of vitamin E (200 ppm) in inhibiting the oxidation of lipids up to 92.11%. In this study, vitamin E with a concentration of 200 ppm had inhibitory amounted to 87.01%. The value of the inhibition was greater than aquademineral, hot water, and ethanol extract. This is because the content of antioxidant compounds in the vitamin E is more pure. From the results of standard measurements of 1,1,3,3- tetrametoxy propane (TMP), obtained the linear regression $y = 0.0240\ X + 0.0269$, $R = 99.82\%$. The regression equation used to calculate the MDA level of TBA test results for each extract.

Based on Salim (2006) research, obtained three extracts of *mahkota dewa*, that were extracted by solvent aquademineral, hot water, and ethanol. After that, the three extracts were tested antioxidant activity. Inhibition of plant extracts tested, using a solvent aquademineral, hot water and ethanol at concentrations of 50, 200, and 1000 ppm can be seen in Table 1.

Table 1 Inhibition percentage obtained from plants extracts with various solvent

Plant	Extract (%)								
	Aquademineral (ppm)			Hot Water (ppm)			Ethanol (ppm)		
	50	200	1000	50	200	1000	50	200	1000
Mahkota Dewa	69.70	83.44	84.34	61.09	70.80	76.24	69.28	81.73	86.05
Temu Putih	44.75	60.21	72.24	71.74	82.74	86.27	54.33	69.28	79.44
Sambiloto	74.56	81.45	86.17	69.46	81.45	76.91	54.50	67.96	74.05
KeladiTikus	64.05	68.60	78.66	24.32	63.53	78.66	47.72	72.17	75.99

Antioxidant activity of mahkota dewa extract.

The inhibition of *mahkota dewa* at a concentration of 50 ppm for all three types of solvent was quite large (more than 50%) in inhibiting the formation of MDA. The concentration of MDA in *mahkota dewa* extract can be seen in Figure 1.

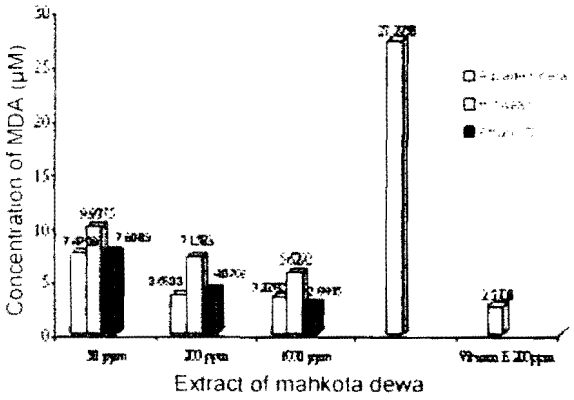

Figure 1 Antioxidant activity of *mahkota dewa* flesh extract.

Almost all of secondary metabolites are polar compounds. Aquademineral have a level higher polarity than ethanol. Aquademineral extract (50 and 200 ppm) of *mahkota dewa* had a higher antioxidant activity than the hot water and ethanol extract. This is due to the ability of solvent extraction is directly proportional to the level of purity of the solvents. In this case aquademineral is more pure than hot water. While the ethanol extract (1000 ppm) had higher potency compared with aquademineral extract and hot water. This is due to the concentration of extract concentration had reached maximum standard limit (Vitamin E 200 ppm), so it is believed that there were other compounds been extracted.

Aquademineral extracts of *mahkota dewa* had a good antioxidant activity at concentration of 200 ppm because it was quite close to the value of the inhibition of vitamin E as a positive control. Based on research Salim (2006), the phytochemical test: alkaloids, flavonoids, tannins, and saponins showed positive test results. These compounds act as a substance that can inhibit lipid oxidation reactions. In Figure 1, shows that the negative control/treatment which not given the extract (without antioxidants) has a high concentration of MDA, namely 27.2958 µM. This was due to absence of compounds that could inhibit the oxidation process.

Antioxidant activity of temu putih extract. Antioxidant activity of *temu putih* extract also showed the value of the inhibition is quite high (Figure 2). Hot water has a higher level of polarity of the ethanol so that there are allegedly many secondary metabolites are extracted. Compounds that have a high level of polarity will be more distributed in hot water. In addition, this may also be caused by the influence of temperature and chemical compounds content in each extract tested were different. These factors cause the type of solvent that is able to extract the maximum yield is not the same for each type of plant extracts tested, so the results of *temu putih* and *mahkota dewa* inhibition was different. Based on Pratiwi (2006) research, the phytochemical test: alkaloids and flavonoids showed positive test results. These compounds are believed to act as antioxidants to inhibit lipid peroxide reaction.

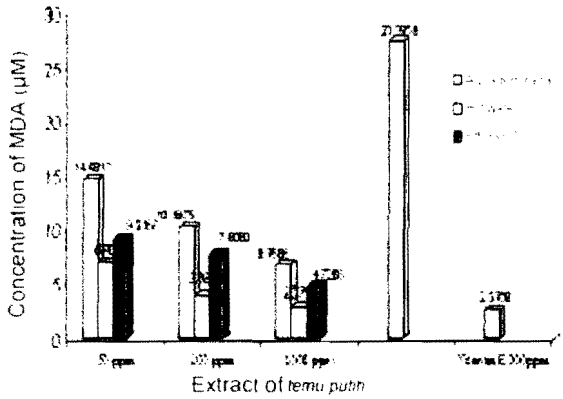

Figure 2 Antioxidant activity of *temu putih* extract.

Antioxidant activity of sambiloto extract. aquademineral extract of *sambiloto* (concentration 50, 200 and 1000 ppm) had the greatest inhibition when compared with hot water ethanol and extract (Figure 3). The content of secondary metabolites such as alkaloids, tannins, saponins, and flavonoids contained in the aquademineral extract was quite large. Based on Puspitasari (2006) research, the phytochemical test: alkaloids and terpenoids showed positive results for all three types of solvent. While flavonoids showed negative results only on ethanol and tannin only in the solvent of aquademineral. Inhibition at a concentration of 200 ppm for aquademineral and hot water extract had the same value. This showed that aquademineral and hot water extract had the same inhibitory ability despite the purity level of solvent was different.

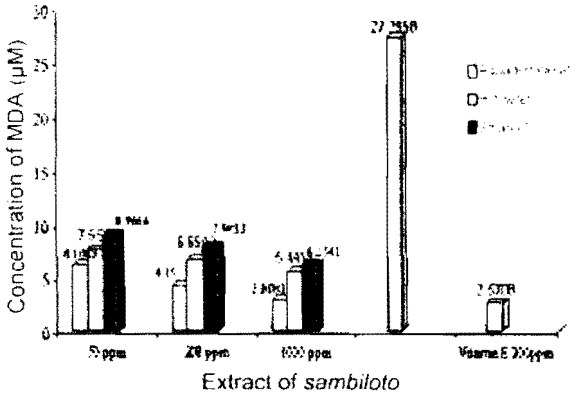

Figure 3 Antioxidant activity of *sambiloto* extract.

Antioxidant activity of each of these extracts at a concentration of 200 ppm when compared with vitamin E (200 ppm) still looked much lower (Figure 1). Despite this difference between the antioxidant potency of each extract at a concentration 0f 50, 200, and 1000 ppm was not so visible. This was because the concentration of the extract was too large and not proportional to the concentration of linoleic acid substrate.

Antioxidant activity of keladi tikus extract. Aquademineral extract of *keladi tikus* (50 ppm) had higher antioxidant activity than the hot water and ethanol extract (Figure 4). This is because aquademineral has a high level of polarity than ethanol and more pure than hot water, so that alleged some secondary metabolites such as alkaloids and flavonoids that have a high level of polarity are also distributed in it. Based on Affandi (2006) research, the phytochemical test: alkaloids, flavonoids, and tannins showed positive results. These compounds are an antidote compound which can inhibit free radical oxidation reactions.

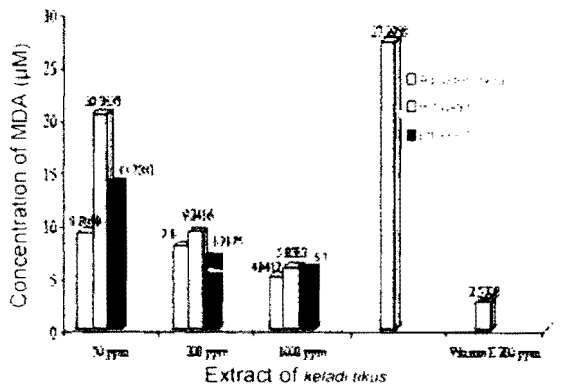

Figure 4 Antioxidant activity of *keladi tikus* extract.

At a concentration of 200 ppm ethanol extraction had a higher inhibitory This was due to the compounds contained in extracts of *keladi tikus* distributed more on ethanol, although the level of polarity lower than aquademineral and hot water. Based on these results we can conclude that the inhibition of antioxidants is not always proportional to the level of purity and polarity of the solvent used.

Meanwhile, at a concentration of 1000 ppm, the inhibition of aquademineral and hot water had the same value but higher than ethanol. This was due at concentrations above the maximum limit (200 ppm) the extract had different antioxidant potential which was less visible, making it difficult to distinguish the influence of the purity and polarity of a solvent.

Based on the results obtained, it was concluded that *mahkota dewa*, *temu putih*, *sambiloto*, and *keladi tikus* with three kinds of extracts, namely aquademineral, hot water and ethanol were very potent antioxidants. This can be seen at a concentration of 200 ppm, the value of the inhibition of each extract was close to the value of the inhibition of vitamin E.

Alkaloids and flavonoids gave positive results on all types of plants tested (Salim 2006). Flavonoids and alkaloids is a good reductor compound. Flavonoids act as a good container for hydroxyl and superoxide free radicals (Robinson 1995). According to Mangan (2003), flavonoid substance served as an antidote to free radicals that could disrupt the body's balance system and could lead to cancer.

Tannins are compounds that contained in the tea plant. Based on the Yen (1995) research, it reported that various types of tea had antioxidant activity. Based on the results of the study also, tannins could inhibit the process of mutation and cancer, and free radicals and induced enzymes that act as antioxidants.

Saponins in the plant have been known to be used for treatment. Saponin contained in samples plants (*Physalis angulota* Linn.) had efficacy as an antitumor and inhibits the growth of cancer especially colon cancer (Mangan 2003). In addition, saponins

contained in *kunyit*, *tapak dara*, *sabung nyawa*, *mengkudu*, *kitolod*, and *pegagan* had efficacy as anticancer.

ANOVA and Duncan's Test

The statistical test used for the antioxidant potency were ANOVA and DUNCAN. The tests were only done at a concentration of 200 ppm for each extract of akuademineral, hot water, and ethanol extract, which were directly compared with vitamin E (200 ppm).

ANOVA test of *mahkota dewa*, *temu putih*, *sambiloto*, and *keladi tikus* extract showed significantly different results. Therefore it was necessary to do further test using DUNCAN test to see whether there were differences in antioxidant potency in aquademineral, hot water, ethanol extract and vitamin E on each plant. DUNCAN test showed different results from each plant extract. This was because the content of secondary metabolites contained in extracts were different that the inhibition power against MDA formation was also different.

CONCLUSION

The extract of *mahkota dewa*, *temu putih*, *sambiloto*, and *keladi tikus* had potency as antioxidants based on their ability to inhibit the formation of MDA. Data results showed that the smallest inhibition was found in *keladi tikus* extract of 24.32% at concentrations of 50 ppm with hot water solvent. While the greatest inhibition found in *temu putih* extracts at 1000 ppm , namely 86.27% with the same solvent.

ANOVA of extract at a concentration of 200 ppm for aquademineral, hot water, ethanol extract, and vitamin E showed significantly different results. DUNCAN statistical test showed that the antioxidant activity was different.

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