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has participated in

43rd Meeting of National Working Group on Indonesia Medicinal Plant
INTERNATIONAL CONFERENCE ON MEDICINAL PLANTS

Scientification of Jamu (Evidence-based Jamu Development): A Breakthrough
Program from Plant to Medicine for Health Care

Purwokerto, Indonesia
October 11 – 13, 2012

as

Presenter

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Jalan Prof. Dr. H.R. Boenyamin 708 Purwokerto
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Telepon 635292 (Hunting) 638337, 638795
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Contents

Antioxidant Activity of Extracts and Fractions of Sambiloto Herbs and Salam Leaves

Lannie Hadisoewignyo*, Martha Ervina, Lisa Soegianto, Hendra Kurniawan

**Faculty of Pharmacy, Widya Mandala Catholic University Surabaya*
Dinoyo 42-44 Surabaya, Indonesia, telp 6231-5678478 ext. 179,
fax. 6231-5630169, email: lanhadi@yahoo.co

Abstract
Antioxidants are substance which able to scavenge free radicals. Sambiloto herbs (Andrographis paniculata Nees) and salam leaves (Syzygium polyanthum) extracts have potential of antioxidant activity, so the combination is expected not only reduce blood sugar levels, but also prevent further complications of chronic diseases. In the case of chronic hyperglycemia will increase oxidative stress which is resulting in a reduced number of glucose transporters. This mechanism will increase insulin resistance, lack of insulin signaling and impair insulin secretion by pancreatic β cells. The aims of this research was to determine antioxidant activity of extracts and fractions of sambiloto herbs and salam leaves. As a screen by TLC autography method of extracts and fractions were shown that aqueous fraction of the leaves and herbs ethanol extract of sambiloto and salam have strongest scavenger capacity of DPPH on Rf 0.58; 0.73 and 0.84 for sambiloto aqueous fraction; while salam aqueous fraction on Rf 0.21; 0.34; 0.40; 0.44 and quercetin as antioxidant reference on Rf 0.86. Quantitative antioxidant capacity of aqueous fraction of the extracts were assessed by inhibition of DPPH (1,1-diphenyl-2-picryl-hydrazyl) free radical scavenging method which assay by spectrophotometry at wavelenght 515 nm. Antioxidant capacity of the fractions was showed by IC_{50}. As result was measured that IC_{50} sambiloto herbs aqueous fraction was 46.15 µg/mL; while IC50 salam leaves aqueous fraction was 18.74 µg/mL. These results was suggested that the antioxidant capacity of aqueous fraction of sambiloto herbs and salam leaves higher than its extracts and it might be have therapeutic value in prevent advanced complication in chronic diseases.
Keywords: sambiloto herbs, salam leaves, antioxidant capacity, DPPH

Background

There have been many studies conducted to determine the effects of antidiabetic and antioxidant extracts of sambiloto herbs. Infusion of sambiloto herbs 20% were given a dose of 12.5 mL/kg and 37.5 mL/kg orally in rabbits, hypoglycemic effect at both doses, for comparison used tolbutamide 50 mg /kg (Puslitbang, Jakarta). Research conducted by Soetarno et al, 1999, stating that the use of sambiloto herbs in traditional form, with crude brew sambiloto herbs with water, is appropriate, as the sambiloto herb extracts obtained with organic solvents showed no antidiabetic effects.

Dandu and Inamdar, 2009, conducted a study to determine the antioxidant properties of water extract of sambiloto herbs on mice made diabetic with streptozotocin induced. His study states that a dose of 400 mg /kg, administered by mouth, significantly lowers blood glucose levels and increases the activity of superoxide dismutase (SOD) and catalase enzymes. Hypothetically sambiloto herb can lower blood glucose levels through antioxidant properties. Borhanuddin et al, 1994, noted that there is a significant hypoglycemic effect of aqueous extract of sambiloto herb at a dose of 10 mg /kg, in a study conducted in rabbits. Verma and Vinayak, 2007, stating that the water extract of sambiloto herbs have antioxidant defense system in lymphoma in the liver of mice. Tripathi and Kamat, 2007, stating that the water extract of sambiloto herbs showed the potent of effects anti-radical, conducted on rat liver.

While research on salam leaf extract states that salam leaf extract has antidiabetic and antioxidant effects. Studiawan,and Santoso, 2005, stated in his research that extract ethanol salam leaves can lower blood glucose alloxan induced mice, at doses of 2.62 and 5.24 mg/20 g body weigh. Lelono et al, 2009, stating that the methanol-water extract of salam leaf has free radical scavenging activity was high, with ED50 0.18 mg /mL, and protection from the effects of β-carotene was 85.7% at a concentration of 100 ug /mL.

Sambiloto herbs have a working mechanism accelerates the release of glucose by increasing metabolism, while the salam leaves can serve as an astringent that can inhibit glucose intake. Seeing both the mechanism of action of these two natural ingredients, it is possible to combine of to obtain a synergistic effect. According to research conducted by Widjajakusuma et al, 2009, the specific combinations and doses of water extract of sambiloto and salam leaves can provide a synergistic effect as antidiabetic, and has a better effect in lowering blood sugar than the use of a single extract, as well as to metformin.

Based on research conducted by Widjajakusuma et al, 2009, the purpose of this study was to investigate the antioxidant activity of extract combination of sambiloto herbs and salam leaf that have been shown to have antidiabetic effects.

Methods

Equipments: percolator, rotavab, UV-VIS spectrophotometer, UV lamps, TLC plates, and filter paper.

Materials: sambiloto herbs and salam leaves obtained from Materia Medika Indonesia, DPPH (1,1-diphenyl-2-picrylhidrazyl), methanol, n-hexane, ethyl acetate, 96% ethanol, chloroform, and quersetin.

Standardization of simplisia including the determination of ash content, the assay of water extrateable, and assay of ethanol extractable (Anonim, 1980). Preparation of extract were done by maceration. At the end of the extraction of total extract obtained from sambiloto herbs and salam leaves. Standardization of extracts had been conducted were moisture content, ash content, extract yield, and chromatogram profiles of active substances (Anonim, 1980).

Preparation of crude plant extract

The dried plant materials were powdered using a grinder. The extraction was done at room temperature. About 5 kg of dried, ground plant materials were soaked in methanol (37.5 L) for 24 h while stirring occasionally. The final extracts were passed through Whatman filter paper (maserat I) and the residue were soaked with 96% ethanol (20 L), and after 24 hours, the ethanol extract filtered (maserat II). Maserat I and II combined and concentrated by using a evaporator, followed by heating in a porcelain dish on a water bath until a thick extract. The viscous extract weighed and then diluted with 90% methanol-water as much as 100 mL. After that, a gradual fractionation in a separating funnel with 100 mL of n-hexane in a way shaken for 5 minutes and separated fractions of n-hexane. Fractionation done several times until the n-hexane fraction colorless again. All the n-hexane fraction obtained put together and concentrated in the evaporator. Methanol-water fraction was evaporated in a porcelain dish over a water bath until methanol evaporated and the fraction of water. Do fractionation of water by using ethyl acetate in a separating funnel as the fraction of n-hexane. All ethyl acetate fraction obtained and collected into one concentrated in the evaporator. Water fraction derived from the fractionation of the ethyl acetate remainder concentrated in porcelain dish over a water bath.

At the end of the extract obtained by extraction of the total, the fraction of hexane, ethyl acetate fraction and water fraction of sambiloto herbs and salam leaves.

In-Vitro determination of antioxidant capacity with DPPH (1,1-diphenyl-2-picrylhidrazyl) (Cavin, dkk., 1988)

a. TLC Autography technique

Each fractions were impregnated with the mobile phase n-hexane: ethyl acetate (9: 1); methanol: chloroform (1:2), and butanol: acetic acid: water (3:1:1) , which is then saturated in each chamber. Further stages as made stock solution of each fractions test extract: 20 mg dissolved in 5 ml methanol pa. After spotting the extracts on the TLC plates, the spot sprayed with DPPH 1% and observed the formation of yellow on spot stain solution is then compared with the benchmark being used.

b. Determination of Inhibition Concentration (IC_{50})

On the quantitative determination of antioxidant activity, determined IC_{50} values, with the following stages of work: preparing the test solution of the water fraction of extract with a concentration of the mother liquor (10000 ppm) and 0.13% DPPH solution. Created 5 different concentration of the mother liquor, pipetted 85 µL DPPH solution and add 3 mL of methanol pa 0.13% and then shaken and allowed to stand for 30 minutes; then added with methanol, and determined the maximum absorption wavelength and absorbance examined. Each test preparation was taken as much as 3 ml, placed in vials, 85 µL so lution of 0.13%, DPPH mixed and allowed to stand for 30 minutes, after which the preparation was observed absorbance at a wavelength of maximum absorption. IC_{50} values, calculated from a linear regression curve between% inhibition of uptake versus ln concentration of the extract, as in equation (1).

% inhibition DPPH activity = (A DPPH – A sampel)/A DPPH x 100% ..... (1)

IC_{50} is a number that indicates the concentration of extract (mg /mL) were able to inhibit 50% oxidation. The smaller the IC_{50} values higher antioxidant activity. An antioxidant compound is said to be very powerful if IC_{50} values less than 50, a strong (50-100), moderate (100-150), and weak (151-200).

Results

The results for the determination of ash content of sambiloto herbs and salam leaves in the form of crude extract, can be seen in Table 1.

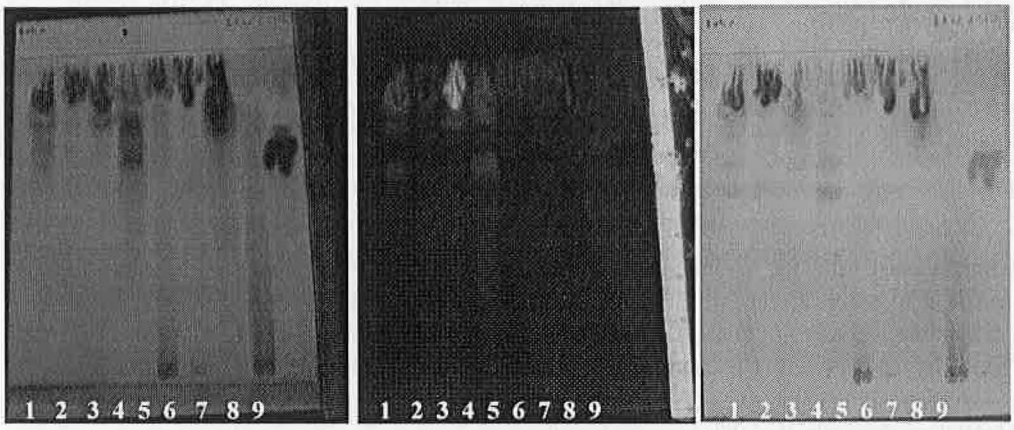
Table 1. Standarization of Simplisia of Sambiloto Herbs and Salam Leaves (Anonim, 1978)

No.	Parameter test	Requirements	Test Result	
			Sambiloto Herbs	Salam Leaves
1	Ash content	< 5%	11,13 ± 0,35	5,69 ± 0,09
2	Assay of water extractable	> 15,6%	17,03 ± 0,38	5,45 ± 0,24
3	Assay of ethanol extractable	> 4,3%	13,53 ± 0,10	11,55 ± 0,03

Preparation of extract of sambiloto herbs and salam leaves using maceration method. The final result is obtained that the sambiloto herbs extract yield as much as 245 g (24.50%), while the salam leaves extract as much as 141.3 g (14.15%) yield. Extract and fractions were screened using silica gel stationary phase and mobile phase butanol: acetic acid: water = 3: 1: 1, to determine the class of active compounds from the extracts and the fractions.

TLC Autography technique

Antioxidant activity assays carried to all factions, with KLT autography, using a quersetin as a standard. The TLC results contained in Figure 2 and Figure 3. Based on test free radical activity in TLC autography, it is known that which has the greatest antioxidant activity, both sambiloto herbs and salam leaves is the fraction of water.



(a) (b) (c)

Figure 1. TLC profile of extract and fraction of sambiloto herbs and salam leaves with stationary phase is silica gel and mobile phase is Diam butanol :acetic acid : water = 3 : 1 : 1, (a) UV 254 nm, (b) UV 366 nm, (c) observations with spot spray vaniline-sulfuric acid

Description: 1= crude extract of sambiloto herbs
2 = n-hexane fraction of sambiloto
3 = ethyl acetate fraction of sambiloto
4 = water fraction of sambiloto
5 = crude extract of salam leaves
6 = n-hexane fraction of salam leaves
7 = ethyl acetate fraction of salam leaves
8 = water extract of salam leaves
9 = rutine

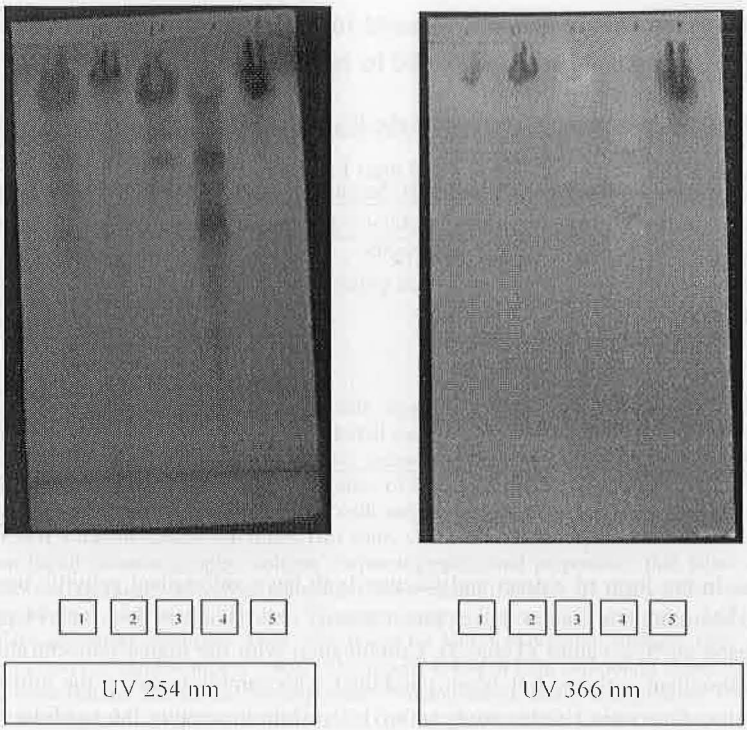


Figure 2. TLC profile of antioxidant of sambiloto herbs with the stationary phase is silica gel and mobile phase is butanol: acetic acid: water = 3: 1: 1
Description: (1) crude extract, (2) the fraction of non-polar n-hexane, (3) semi-polar ethyl acetate fraction, (4) the fraction of water, (5) quersetin

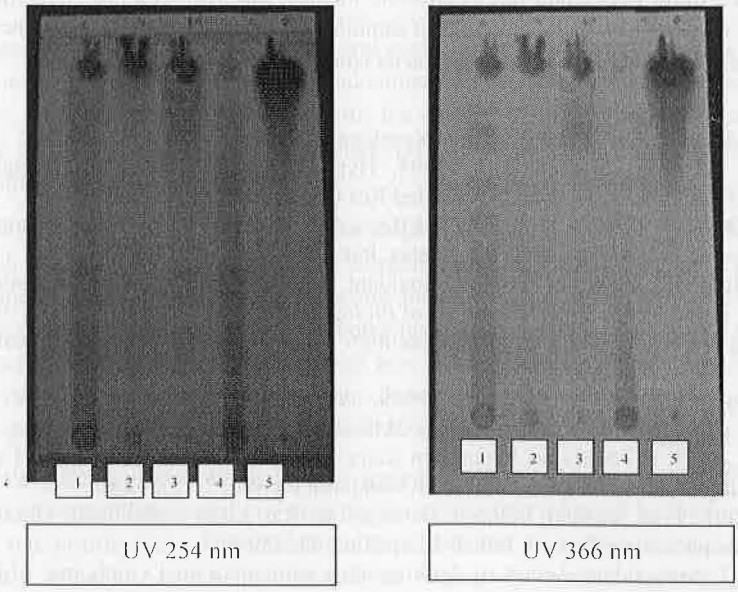


Figure 3. TLC profile of antioxidant of salam leaves with the stationary phase is silica gel and mobile phase is butanol: acetic acid: water = 3: 1: 1
Description: (1) crude extract, (2) the fraction of non-polar n-hexane, (3) semi-polar ethyl acetate fraction, (4) the fraction of water, (5) quersetin

Determination of Inhibition Concentration (IC_{50})

The qualitative antioxidants activities had been test by determining the IC_{50} values (Table 3).

Table 3. IC_{50} values of Extracts and their Fractions

Sample	IC_{50} (µg/mL)
DPPH 0,13% + metanol p.a	-
Water fraction of Sambiloto herbs	46.15 ± 10.46
Water fraction of Salam leaves	18.74 ± 0.25
Water fraction of salam leaves : sambiloto herbs = 1 : 2	15.78 ± 1.14
Water fraction of salam leaves : sambiloto herbs = 2 : 1	30.02 ± 3.22
Water fraction of salam leaves : sambiloto herbs = 1 : 6	37.60 ± 1.37
Water fraction of salam leaves : sambiloto herbs = 6 : 1	29.09 ± 3.08
Quercetin	7.00 ± 0.12

Discussion

Sambiloto herbs and salam leaves in the form of extract and fraction both have antioxidant activity, based on TLC autography assay and IC_{50} determination. Salam leaves has a greater antioxidant activity (18.74 µg/mL) than sambiloto (46.15 µg/mL) based on IC_{50} values (Table 3). Combination with the higher concentrations of salam leaves indicates greater antioxidant activity, but when combined with sambiloto herbs the antioxidant activity will increase up to a certain value ratio (in this study up to 1:2), while increasing the sambiloto herbs concentrations up to six times compared to the salam leaves showed a decrease in antioxidant activity.

Conclusion

Antioxidant capacity of the fractions was showed by IC_{50}. As result was measured that IC_{50} sambiloto herbs aqueous fraction was 46.15 µg/mL; while IC_{50} salam leaves aqueous fraction was 18.74 µg/mL. These results was suggested that the antioxidant capacity of aqueous fraction of sambiloto herbs and salam leaves higher than its extracts and it might be have therapeutic value in prevent advanced complication in chronic diseases.

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