IN VITRO ANTIOXIDANT ACTIVITY OF LAMPES (*Ocimum sanctum*) LEAVES AND SEEDS ETHANOL EXTRACT USING DPPH METHOD

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Abstract

Background. *Recent study reported that polyphenol compounds have a large contributionin antioxidant activity. Polyphenol compound was reported as one of the content of Ocimum sanctum.L The aim of this study was to observe the antioxidant activity of lampes (Ocimum sanctum) leaves and seeds ethanol extract using DPPH method.*

Method. *The extraction was done using maceration method. The antioxidant activity was measured byDPPH method and gallic acid was used as comparative substance. Total phenolic content of ethanol extract was determinated by spectrophotometric method with Folin-Ciocalteu reagent quantitatively. Furthermore, the effective Scavenging 50 (ES50) value was calculated using linear regression between ethanol extract or galic acid concentration versus % of free radical scavenging. The ES50 values were statistically analysis with anova and LSDTest.*

Result*.This study showed that ethanolextract of Ocimum sanctum seeds has antioxidant potency with* ES_{50} *value was 131.81 ìg/ml> ES50 of leaves (91.94 ìg/ml) > ES 50of of gallic acid (3.54 ìg/ml)(* $p<0.05$*).The total phenolic content of ethanolextract of Ocimum sanctumleaves was 41.33 mg/gram, while the phenolic content of etanolic extract of seeds was 26.81 mg/gram wich was expressed as gallic acid equivalent (GAE).*

Key words : *Ocimum sanctum, leaves, seeds, ethanolextract, antioxidant activity, DPPH method.*

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INTRODUCTION

The role of free radicals in many disease coditions has been well established. Several biochemical reaction in our body generated reactive oxygen species and these are capable of damaging crucial bio-molecules. If they are not effectively scavenged by cellular constituent, they lead to disease conditions such as cancer, atherosclerosis, aging, inflammatory, diabetic, hair fall, and neuro-degenerative disease like Alzheimer and Parkinson (Surveswaran, *et.al.,* 2007) (Halliwell &Gutteridge, 1999). The harmful action of free radicals can, however, could be blocked by antioxidant substances, which scavenge free radicals and detoxify the organism.

Synthetic antioxidants, such as butylated hydroxyanisol, butylated hydroxytoluene and tertiery butylhidro-quinone, have been widely used in food for preventing lipid peroxidation. However, the use of these syinthetic antioxidants in food is discouraged because of their toxicity and carcinogenicity (Soong dan Barlow, 2004). A few natural antioxidants have attracted special interest because of their ability to scavenge free radicals. Natural antioxidants such as phenolics, flavonoids, tannins, etc are found in various plant products such as leaves, seeds, fruits, etc.

Lampes (*Ocimum sanctum)* , in India was widely used in ayurvedha. It's used in many medicated of many disease , such as bronchitis, diarhea, dysentry, skin disease etc. In some region of Indonesia, lampes was used as spices. The ther studies reported that Lampes has a protective effect agains genotoxicity, chemical-carcinogen, miocardial damage induced isoproterenol. Lampes also has hepatoprotector , aintiinflamatory, antidiabetic, antiulcer effect. Muthuraman *et al* (2008) reported that Lampes's leaves consist of flavonoid (luteolin, orientin, vicenin), triterpenoid (ursolic acid); fixed oil (palmitic , stearic, linoleic, linolenic), essensial oil (eugenol, champhor, carvakol, caryopelen, desyaldehide, nerol, cirsilineol, cirsimartin), tannin, etc.

The aims of this studi were to determine the radical scavenger activity and the Total phenolic content of Lampes's leaves and seeds extract compared with gallic acid as standart

METHODS

Materials

Lampes (Ocimum sanctum) leaves and seeds were obtained from Karanganyar, Central Java; DPPH (Sigma), Folin-ciocalteu reagent, Na_2CO_3,quercetine p.a. (Merck), 96% ethanol (Brataco Chem.), gallic acid p.a (sigma) dan methanol p.a (Merck), aquadest (Brataco Chem.).

Method

Preparation of Lampe's leaves and seeds extract

The Lampes's leaves and seeds were washed thoroughly in tap water to remove adhering mud particles, rinsed in distilled water, drained, and dried in hot air oven at 50±2^{o}C. The dried leaves and seeds were finely powdered. About 20 grams of each dried powder were extracted in 300 ml portions of ethanol, shaked with ultrasonic for 1 hour. The extract were decanted or filtered after 24 hours and the extraction was`repeated with fresh ethanol until the extract was colorless. The extracts were pooled and evaporated in a rotary evaporator.

DPPH radical scavenging activity assay

The Free radical scavenging activity against 2,2-diphenyil-1-picrylhydrazyl (DPPH) radicals was measured spectrophotometrically. The radical scavenging activity assay was done by DPPH methd because of it's a stable substance, commercially available, and does not have to be generated before assay Therefore, it is considered an easy and useful spectrophoto metric method . This method based on the decrease intensity or absorbance at 517 nm of DPPH in the presence of the extract .

Different dilution of extract were prepared (5 times replicated). Then 1.0 ml of each dilution was added to 1.0 ml of 0.15 mM

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DPPH. The mixture was allowed to stand about 30 min before measuring the absorbance at 517 nm. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity. The scavenging of DPPH radical in percent was calculated by equation :

Scavenging Activity (%)= $(1-A_1)/A_0)$ x 100%. A_0 was the absorbance of control reaction and A_1 was the absorbance in the presence of extracts, gallic acid was used as positive standart.

The Effective Scavenging 50% (ES 50) was calculated by interpolated the 50 as Y value into the regresioon linear equation between concentration vs. % of radical scavenging activity. The lower ES_{50} value showed the higher scavenging activity of the solution test (Pokorny, *et.al*., 2001).

The ES_{50} values were statistically analysed. The Kolmogorof-smirnof test result showed that the ES_{50} datas were normal, the Levene test showed a homogenityy of data. The statistical analysis was followed by anova analysis. It's indicate that there were significantly differences among the ES_{50} values. The posthoc (LSD) test result showed that each ES_{50} value was significantly different.

Determination of polyphenol

The present of polyphenol in the extracts was determined by the formation of greenblue colour after added $FeCl_3$ solution into the diluted extract.

The Total polyphenol content (TPC) of leaf and seed extracts were determined spectrohotometrically using Folin-Ciocalteau's reagent. Three hundred microlitre of samples were added into test tubes followed by 1.5 ml of Folin-Ciocalteau reagent (diluted 10 times) and 1.2 m of sodium carbonat 7.5%. The content of the tubes were mixed thoroughly and stored in the dark for 36 min before the absorbance was measured at 743.5 nm using Pharmaspec UV-1700 Shimadzu. TPC was expressed as mg gallis acid equivalents (GAE) per g of extract.

RESULT AND DISCUSSION

This study showed that the lampes's leaves and seeds ethanol extract have potency as free radicals scavenger.

The reaction between DPPH radical phenolic compound is presented in figure 1.

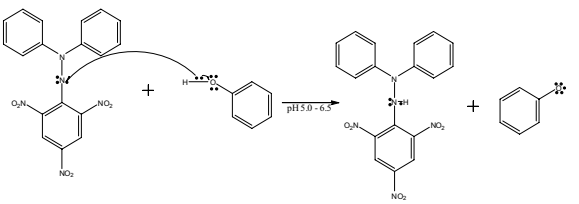

Figure 1. Free Radical scavenging reaction by phenolic compound (Son and Lewis, 2002)

The scavenging of DPPH radical activity of gallic acid and the ethanol extract of leaf and seed of Lampes are presented in table I, table II and table III.

Table I. Scavenging activity of gallic acid

No.	% Scavengig of DPPH radical Gallic acid level (g/ml)					Linear Regression equation,;	ES50 (μg/ml)	X ± SD (μg/ml)
	0.2	0.6	1.0	1.4	1.8			
1	22.33	30.36	44.67	49.06	62.86	Y=24.94x-16.916; r=0.9905*	1.33	1.32* ±0.053 CV=4.01%
2	8.41	23.21	40.90	49.69	62.48	Y=23.18x+3366; r=0.9953*	1.39	
3	26.98	34.38	42.41	49.18	61.73	Y=21.08x+21.86; r=0.9933*	1.33	
4	17.57	24.22	45.27	53.07	65.24	Y=31.05x+10.03; r=0.9873*	1.29	
5	18.32	24.09	45.79	59.71	67.50	Y=9.83x+32.25; r=0.9870*	1.25	

*p < 0,05

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Table .II. Scavenging activity of Lampes's leaves ethanol extract

No.	% Scavengig of DPPH radical Lampes' leavess Ethanol extract level (g/ml)						Linear Regression equation,; r	ES50 (μg/ml)	X ± SD (μg/ml)
	90	**100**	**110**	**120**	**130**	**140**			
1	49,26	50.89	55.36	58.04	60.42	62.35	Y=0.276x+24.27; r=0.9915*	93.12	91.94*±1.7 CV=1.95%
2	47.47	54.17	55.21	58.63	59.97	63.10	y=0.283x+23.91 ; r=0.9699*	92.26	
3	47.47	52.83	55.80	60.86	62.35	66.07	Y=0.362x+15.96; r=0.9903*	94.09	
4	49.55	52.83	57.14	61.61	62.95	65.92	Y=0.333x+19.996; =0.9899*	89.99	
5	49.40	51.49	59.52	61.76	63.24	66.22	Y=0.347x+18.654; =0.9675*	90.23	

- $p < 0.05$

Table III. Scavenging activity of Lampes's seeds ethanol extract

No.	**% Scavenging of DPPH radical Lampes'seed ethanol extract level (g/ml)**					**EμS50 (g/ml)**	**X ± SD (μg/ml)**
	100	**120**	**140**	**160**	**180**		
1	43.80	47.06	49.81	53.44	58.70	Y=0.181x+25.24; r=0.9918*	136.87
2	43.43	48.06	50.69	54.07	56.95	Y=0.165x+27.505; =0.9955*	136.09
3	43.30	49.47	52.19	59.70	62.20	Y=0.225x+21.251; =0.9939*	127.66
4	44.43	48.06	52.07	53.82	59.99	Y=0.134x+31.918; =0.9723*	135.04
5	46.31	50.56	51.94	54.32	58.82	Y=0.144x+32.244; =0.9838*	123.39

- $p < 0.05$

The presence of Hydroxy phenolic groups in Gallic acid has contributed to the higher activity as radical scavenger. In the other hand, the ethanol extract has lower radial scavenger activity. This fact can be explained that the gallic acid was a single compound, while the extract was a mixture of many compound that has posibility eiher have or no radical scavenger activity.

Figure 2 presented the phytochemical constituent of ethanol extract ie. flavonoid (luteolin, orientin, vicenin), tannin, etc (Muthuraman,et al, 2008) were predicted as the main role in radical scavenging activity.

It can be understood that a number of hydrxyphenolic groups in those compounds have a wide contribution to the radical scavenger activity.

In the other hand, there were significantly differences of ES_{50} value between leaves extract and seeds extract. The ES_{50} value of leaves extract was (91.94±1.7) mg/m) less than (131.81±05.968) mg/ml (ES_{50} of seeds extract) ($p<0,05$). This phenomenon was in line with the total phenolic content of each extract. The

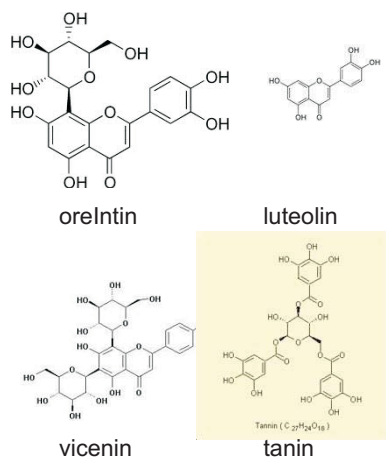

Figure 2.Some flavonoid compound in Lampes's extract

leaves's extract has higher content of TPC (41.33 ± 1.36) mg/g GAE > seed's extract (26.81±0.73) mg/g GAE.

The other studies reported that there was a positive correlation between the level of TPC vs radical scavenging ctivity.The higher level of TPC has higher radical scavenger activity.

This result recognized to be combined by others method of antioxidants assay to represent as antioxidant capacity ie. Thiobarbiruric acid method, Ferric reducing antioxidant power, deoxyribose method, etc.

CONCLUSION

Ethanol extract of Lampes's leaves and seeds have potencial activity as radical scavengers. The radical scavenging activity of extracts were lower than gallic acid's. It was explained by the ES50 value of gallic acid < leaf extract (91.94±1.7) mg/ml <seed extract (131.81±05.968) mg/ml. The Leaves's extract has higher content of TPC (41.33 ± 1.36) mg/g GAE > seed's extract (26.81±0.73) mg/g GAE.Furthermore, it's need to research to determined the real correlation between TPC of extract vs free radical scavenger activity.

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