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By ANY GUNTARTI

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Any Guntar[1], Nurdiansyah[2]

[1]Departmen of Pharmacy, Faculty of Pharmacy, Universitas Ahmad Dahlan, Yogyakarta, Indonesia
[2]Student of Pharmacy, Faculty of Pharmacy, Universitas Ahmad Dahlan, Yogyakarta, Indonesia

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***Corresponding author:**
any_guntarti@yahoo.co.id

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ABSTRACT

Background: Salam leaves (*Eugenia polyantha* Wight) contains antioxidant compounds, flavonoids and polyphenols. These compounds are reported to inhibit oxidation activity by binding with free radicals. The unlimited amount of free radicals in the body will increase the risk of contracting degenerative diseases.

Objective: The purpose of this study was to identify compounds from the ethanol extract of salam leaves and to test the antioxidant activity of its compounds using DPPH method and also determine the IC_{50} (Inhibition Concentration $_{50}$) of between the ethanol extract of salam leaves and quercetin standard.

Methods: Ethanol concentration extract of salam leaves were obtained by maceration method with the ethanol solution. The compounds were identified by phytochemical screening. The concentration used for the extraction of salam leaves were 30%, 40% and 50%. The variations of each extract concentration were 50, 75, 100, 125 and 150 mg/mL. The variations of standard quercetin concentration were 1, 2, 3, 4 and 5 mg/mL. Antioxidant activity was tested by visible spectrophotometry using the DPPH method. The antioxidant activity is represented as the per cent of free radical scavenging and IC_{50}.

Results: The 30%, 40% and 50% ethanol extract of salam leaves contained flavonoid and polyphenol compounds, and also had free radical binding activity. The average value of IC50 quercetin, 30%, 40% and 50% ethanolic extract of salam leaves were 1.68, 102.48, 149.88 and 197.19 mg/mL.

Conclusion: Statistical analysis showed that the antioxidant activity between quercetin, 30%, 40% and 50% ethanol extract of salam leaves were significantly different (P=95%).

Latar Belakang: *Daun salam (Eugenia polyantha Wight) mengandung golongan senyawa yang bersifat antioksidan yaitu flavonoid dan polifenol. Senyawa tersebut dilaporkan memiliki aktivitas untuk menghambat reaksi oksidasi dengan mengikat radikal bebas. Jumlah radikal bebas dalam tubuh yang tidak terkendali beresiko terkena penyakit degeneratif.*

Tujuan: *Penelitian ini bertujuan untuk mengidentifikasi senyawa dari ekstrak etanol daun salam dan menguji aktivitas antioksidannya dengan metode DPPH serta mengetahui perbandingan nilai IC_{50} (Inhibition Concentration $_{50}$) antara ekstrak etanol daun salam dengan standar kuersetin.*

Metode: *Ekstrak etanol daun salam diperoleh metode maserasi menggunakan pelarut etanol. Identifikasi dilakukan dengan skrining fitokimia. Kadar etanol yang digunakan untuk ekstraksi daun salam adalah*

30%, 40% dan 50%. Variasi kadar masing-masing ekstrak adalah 50, 75, 100, 125 dan 150 µg/mL. Variasi kadar yang digunakan untuk standar kuersetin adalah 1, 2, 3, 4 dan 5,0 µg/mL. Uji aktivitas antioksidan dilakukan secara spektrofotometri visibel dengan menggunakan metode DPPH. Aktivitas antioksidan dinyatakan sebagai persen penangkapan radikal bebas dan IC_{50}.

Hasil: *Ekstrak daun salam dengan etanol 30%, 40%, 50% daun salam memiliki kandungan senyawa flavonoid dan polifenol, serta mempunyai aktivitas menangkap radikal bebas. Nilai rata-rata IC50 kuersetin, ekstrak etanol daun salam 30%, 40% dan 50% secara berturut-turut 1,68; 102,48; 149,88 dan 197,19 µg/mL.*

Kesimpulan: *Hasil analisis statistik menunjukkan bahwa antara kuersetin, ekstrak daun salam dengan etanol 30%, 40% dan 50% daun salam memiliki aktivitas antioksidan yang berbeda bermakna (P=95%).*

INTRODUCTION

Free radicals are defined as an atom or molecule that has one or more free electrons in its structure, and able to damage cell membranes or susceptible cell DNA.[1,2] However, this molecule can be inhibited by an antioxidant system that complements the immune system.[3,4] Antioxidants are defined as substances that can delay or prevent the occurrence of free radicals auto-acylation reactions in lipid oxidation.[5] Adequate amounts of antioxidant consumption are reported to reduce the incidence of degenerative diseases, such as cardiovascular, cancer, atherosclerosis, and osteoporosis.[6,7] Consumption of foods containing antioxidants can also improve the immunological status and inhibit the emergence of degenerative diseases due to the ageing process.[1,8,9]

Salam plants (Eugenia polyantha Wight) are herbal plants that can be useful in addition to spices; they can also be used as medicinal plants. According to Taiz and Zeiger (2007) and Zhou et al. (2011) the chemical content of Eugenia polyantha leaves and bark contains saponins and flavonoids.[10,11] Besides, that part also contain alkaloids and polyphenols, while the stem includes tannins. Ismiyati (2013) stated that the total phenol of salam leaf ethanol extract calculated as gallic acid was 11.6 ± 0.3 % and the total flavonoids calculated as quercetin was 43.9 ± 0.9 %.[12]

One method used in testing antioxidant activity is the DPPH (1,1-diphenyl-2-picrylhydrazyl) method. The DPPH method is easy to use, fast, accurate and suitable for use in organic solvents.[13,14] In the extraction process, ethanol is the best solvent compared to methanol, n-hexane and acetone solvents for the extraction of polar compounds such as polyphenols and flavonoids.[15]

METHODS

Sample

The sample used was bay leaf (Eugenia polyantha Wight) with dark green colour (taken in January 2015) obtained from a community garden in Kotagede District, Yogyakarta, Indonesia. Plant determination was carried out at the Biology Laboratory of the Faculty of Mathematics and Natural Sciences, Ahmad Dahlan University, Yogyakarta.

Materials

The materials used in this study were bay leaves (Eugenia polyantha Wight), DPPH powder (1,1-diphenyl-2-picrylhydrazyl), quercetin powder, ethanol pa, toluene P, Phosphomolybdate reagent, 1% $AlCl_3$ solution and FeCl3 solution 1%.

Equipment

The equipment used in this study is spectrophotometer, cuvette, rotary evaporator, water bath, glassware, Buchner funnel.

Research Procedure

Determination of Powder Drying Loss

Determination of drying loss of bay leaf simplicia powder was carried out using a Halogen Moisturizer Analyzer.

Production of Ethanol Extract of Salam Leaves (*Eugenia polyantha* Wight)

250 g of simplicia powder was extracted by maceration with an ethanol content of 30%, 40%,

and 50% as much as 1000 mL, then left for 24 hours. Remaceration was carried out to obtain an ethanolic extract of bay leaves.[16]

Determination of Water Content of Extracts

Determination of water content was carried out using toluene distillation by weighing carefully 5 g of extract and put into a flask, then about 200 ml of toluene P was added which was saturated 18-24 hours.

Flavonoids and Polyphenols Test

Test for Flavonoid Compounds: with ammonia vapor (NH_4OH), $AlCl_3$ reagent, and $FeCl_3$ reagent.

Test for Antioxidant Activity

The antioxidant activity test used was DPPH 0.15 mM reagent, and Phosphomolibdate 0.5% b/v.

DPPH Free Radical Scavenging Activity Test

a. Determination of Operating Time
The standard solution of quercetin and sample were taken 1 mL each and added with 1 mL of DPPH 0.15 mM solution. Measured at a wavelength of 517 nm, a stable Operating Time on absorbance is sought.[4]

b. Determination of the Maximum Absorption Wavelength DPPH Solution.
As much as ethanol p.a. 1 mL, 1 mL of DPPH 0.15 mM solution was added, the absorption was measured at a wavelength of 450-550 nm.

c. DPPH Radical Scavenging Absorbance Measurement
Sample solution, negative, and positive control solution with various levels were taken as much as 1 mL each and 1.0 mL DPPH 0.15 mM solution was added. Each mixture of the solution was stored in a dark place during the operating time, then measured its absorbance at the maximum absorption wavelength of DPPH 0.15 mM with a UV-Vis spectrophotometer.[17]

RESULTS

Determination of drying losses

The test results of drying shrinkage of bay leaf simplicia powder are presented in Table I.

Table I. Results of the determination of shrinkage of simplicia powder of bay leaves

Replication	Powder Weight (Gram)	Powder drying losses (%)	CV	x ± LE
1	1,004	8,07		
2	1,005	7,38	5,68	7,89 ± 0,61
3	1,001	8,22		

LE = limit of error, CV= coefisien variasi

Determination of moisture content

Determination of moisture content carried out utilising toluene distillation. The results of water content determination are presented in Table II.

Table II. The results of water content determination from bay leaf extract

Salam Leaves Extract	Replication	Powder Weight (gram)	Water Volume (mL)	Water content (%)b/v	$\bar{x}$ ± LE
Etanol extract 30%	1	5,0014	0,28	5,598	5,30 ±0,89
	2	5,0051	0,25	4,994	
Etanol extract 40%	1	5,0093	0,23	4,591	4,69 ±0,29
	2	5,0070	0,24	4,793	
Etanol extract 50%	1	5,0012	0,23	4,598	4,40 ± 0,58
	2	5,0030	0,21	4,197	

LE= limit of error

Qualitative Test of Flavonoid Compounds

The flavonoid test aims to ascertain the presence of flavonoids which are part of the polyphenol compound in bay leaves. The reactions and examples of flavonoid test results on bay leaf ethanol extract can be seen in Table III and Figure 1.

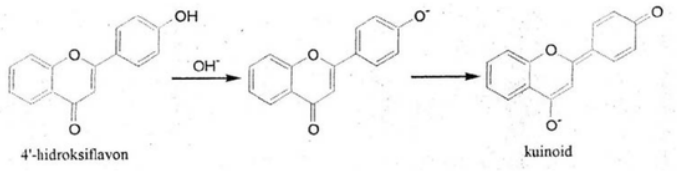

Figure 1. Formation of the cinoid structure in flavonoids with ammonia vapour.[18]

Qualitative Test for the presence of Polyphenol Compounds

The phenolic test results are characterised by the occurrence of reactions between polyphenol compounds and ferric chloride to form coloured complexes.[18]

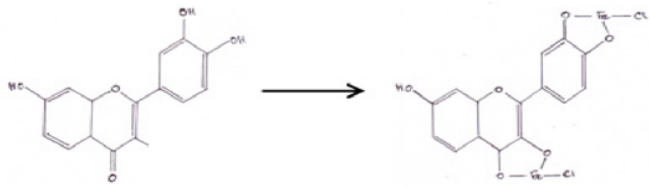

Figure 2. The reaction between $FeCl_3$ and polyphenolic compounds.[18]

Qualitative Test of Antioxidant Activity

The reagents used for the qualitative test are DPPH (1,1-diphenyl-2- picrylhydrazyl) reagent and phosphomolybdatereagent. The quercetin test and ethanol extract showed bay leaves had positive antioxidant activity. Test results of antioxidant activity are presented in Table IV.

Quantitative Test of DPPH Free Radical Scavenging Activities

The DPPH method is one of the most commonly used methods to evaluate antioxidant activity, specifically phenol or polyphenol compounds.[19,20]

Operating time measurement results for quercetin at 14-40 minutes, meanwhile, bay leaf extract with 30%, 40%, and 50% ethanol was at 15-33 minutes, 20-33 minutes and 20-33 minutes respectively. The maximum absorption wavelength of quercetin was 515.10 nm. Meanwhile, bay leaf extract with 30%, 40%, and 50% ethanol was 515.40 nm, 515.90 nm, and 515.50 nm respectively.

The quercetin antioxidant activity and sample as DPPH radical scavenger were expressed in per cent free radical scavenging.[21] The graph of the relationship between levels and percent of free radical scavenging is presented in Figure 3. The results of% free radical scavenging are used to look for IC_{50}.[22] IC_{50} results are presented in Table V.

In Table V, the higher the bay leaf extract level, the greater the IC_{50}. The potential for antioxidant activity is higher if the IC_{50} value is getting smaller. Bay leaf extract with 30% ethanol has the highest possible as an antioxidant.

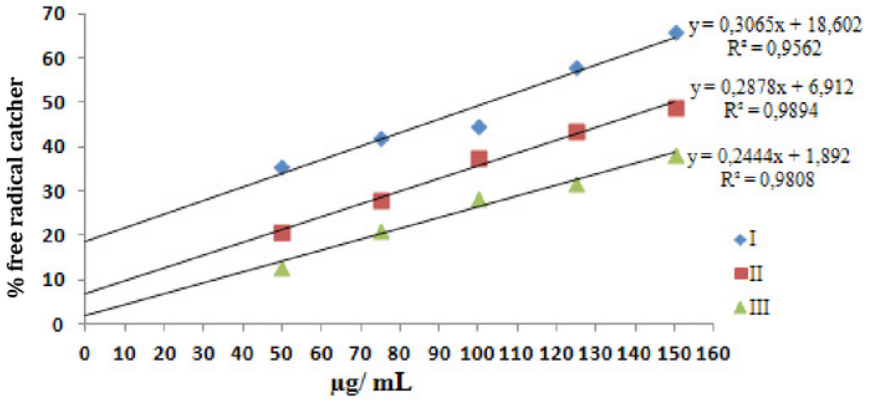

Figure 3. Graph of the relationship between bay leaf content and per cent free radical extract with 30% ethanol (I), extract with 40% ethanol (II) and extract with 50% ethanol (III).

Table V. IC_{50} Quercetin Value, 30% Ethanol Extract, 40% Ethanol Extract, and 50% Ethanol Extract

No	Sample			
	Kuersetin	Ekstrak Etanol 30%	Ekstrak Etanol 40%	Ekstrak Etanol 50%
IC50 (µg/ml)	1,68	102,48	149,88	197,19
CV (%)	`4,48	1,57	3,71	4,60
x± LE	1,68 ± 0,07	102,48 ± 1,45	149,88 ± 5,01	197,19 ± 8,18

LE=limit of error, CV= coefficient of variation

DISCUSSION

The calculation of powder drying losses was averaged (7.89 ± 0.61) %, indicating the presence of compounds that can evaporate at a temperature of 105°C, this might include water and alcohol derivatives. Determination of water content by the toluene distillation method obtained an average level in all concentrations of salam leaf ethanol extract below 10%. This fulfils the requirements for water content provisions because high water content will cause the growth of fungi and moulds, and accelerate the decay process.

In terms of flavonoids presence, a test using $AlCl_3$ reagent showed a yellow colour. This was due to the salt formation and the formation of the cinoid structure on ring B.[18] Besides $AlCl_3$ reaction, an intense yellow color will also be formed in the presence of flavonoids with NH_4OH. Also, the reaction on the presence of polyphenols with reagent $FeCl_3$ will form a complex yellow reaction.

Meanwhile, antioxidant activity test using DPPH reagent gave a positive reaction if the purple colour of DPPH became yellow/lost and green for phosphomolybdate test. Measurements of antioxidant activity were measured by decreasing the absorbance of DPPH solution which had been added to the sample solution. The measured absorbance is the absorbance of the remaining DPPH which does not react with antioxidant compounds. The smaller the absorbance produced shows that the sample has activity as an increasingly free radical scavenger.

Antioxidant activity of bay leaf extract with 30% ethanol was higher than 40% and 50%

ethanol extract. This is because 30% of ethanol solvent can dissolve more flavonoid glycosides. The results of this study are consistent with Safriani et al. (2011) which stated that antioxidant activity in bay leaf water extract was higher than bay leaf extract with 50% ethanol and bay leaf extract with n-hexane.[23] This shows that the more water in the solvent mixture, the higher the antioxidant activity in bay leaves. However, the content of flavonoids and polyphenols in bay leaves is more abundant in water solvents.

CONCLUSION

Bay leaf extract with 30% ethanol, 40% ethanol, and 50% ethanol containing flavonoids and polyphenols. Bay leaf extract with 30% ethanol has the most considerable activity.

CONFLICT OF INTEREST

None declare.

Acknowledgement

None declare.

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