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Antioxidant-Free Radical Scavenging of Some Euphorbiaceae Herbs

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Abstract

In order to screen natural antioxidant, the research about antioxidant of some Euphobiaceae herbs, have been conducted. The air-dried herbs of *Euphorbia heterophylla* L, *Phyllanthus acidus* (L.) Skeels, and *Phyllanthus buxifolius* Muell Arg were extracted with metanol. The obtained extract was concentrated and then suspended to produce *n*-hexane, ethyl acetat and aqueous fractions. Free radical scavenger activity against DPPH (1,1-diphenyl-2-pycrylhydrazyl) measured by spectrophotometric method and the IC50 value was determined. The compounds of active fraction had been identified by TLC method. All of the herbs showed activity as DPPH scavenger. Among these herbs, *Euphorbia heterophylla* L. and *Phyllanthus buxifolius* Muell, Arg. exhibited a strong free radical scavenging of ethyl acetat fraction with IC_{50} value 5,88 µg/ml and 4,64 µg/ml. The result of TLC by mobile phase *n*-buthanol-acetic acid-water (4:1:5) and acetic acid 15% showed flavonoid compound.

Keywords: Euphorbiaceae herbs, antioxidant, DPPH, flavonoid

INTRODUCTION

Phyllanthus acidus (L.) Skeels, *Phyllanthus buxifolius* Muell, Arg., *Euphorbia heterophylla L.* belongs to family Euphorbiaceae, also known as ceremai, seligi and katemas, repectively. The herbs heve been used in Indonesian folk medicine for treatment of anti-inflammatory, analgetic, laxative and cough. However, the potential of higher plants as sources for new drugs is still largely unexplored. Recent interest in the study of antioxidant may be connected with efficacy of these compounds to cure the most diseases.

Antioxidants are radical scavenging which protect the human body against free radical that may cause phathological conditions such as ischemia, anemia, asthma, arthritis, inflamation, degenerative syndromes and ageing process (Oke and Hambuger, 2002; Bartosikova *et al.,* 2003). The oxygen comsumption inherent in cell growth leads to the generation of a series of reactive oxygen species (ROS). These ROS are molecules such as a superoxide anion radicals ($O^{2\cdot}$) and hydroxyl radicals ($OH^{\cdot}$). However, non-free radical species such as hydrogen peroxide (H_2O_2) and singled oxygen ($^1O_2$) are formed *in vivo*. Both oxygen species play a positive role in energy production, phagocytosis, regulation of cell growth intercellular signaling, and synthesis of biologically important compounds (Gulcin *et al.,* 2004). However, ROS may also be very damaging; they can attack the lipids of cell membranes and DNA. The oxidation induced by ROS can result in cell membrane disintegration, membrane protein damage and DNA mutation, which can further initiate or propagate the development of many diseases (Gulcin *et al.,* 2004; Cos *et al.,* 2000 and Bergman *et al.,* 2001).

Flavonoid are groups of naturally occuring compounds widely distributed, as secondary metabolites in the plant kingdom. These flavonoid have also been report to possess antioxidant and antiradical properties. The scavenging properties of antioxidants are often associated with their ability to form stable radicals.

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Also, it is well known that aromatic compounds containing hydroxyl groups, especially those having ortho-di- or trihydroxyl functions can give rise to radical stable enough to be detected by spectroscopy (Gulcin *et al.*, 2004 and Pokorny *et al.*, 2001).

The DPPH test provided information on the reactivity of test compounds with a stable free radical. Because of its odd electron, DPPH (1,1 diphenyl-2-pycrylhydrazyl) give a strong absorption band at 517 nm in visible spectroscopy (deep violet colour). As the electron become paired off in the presence of a free radical scavenger, the absorption varnishes, thus the resulting decolorization is stoichiometric with respect to the number of electrons taken up (Oke dan Hamburger, 2002; Yagi *et al.*, 2002).

METHODS

Instruments and reagents

Shimadzu UV-Vis Spectrophotometry, vacuum rotary evaporator, Thin-layer chromatography plates coated with celulose from Merck, DPPH (1,1-diphenyl-2-picrylhydrazyl from Sigma Co. (St. Louis, USA). All solvents were routinely distilled prior to use. Other reagent were analytical grade.

Plant materials

The all plant material, were collected and classified by *B2P2TO2T* (Center for Research and Development of Medicinal Plants and Traditional Medicine) Tawangmangu, Karanganyar, Central of Java.

Extracts preparation and fractination of plant materials

Plant material was dried, before being powdered and sieved. Plant material was macerated with methanol at room temperatur during 5 days. The extract was concentrated under reduced pressure to produce methanolic extract. This Methanolic extract was suspended in hot water and then partitioned successively with *n*-hexane, EtOAc. Each fraction was evaporated in vacuum to yield the residues of *n*-hexane fraction, ethyl acetate fraction, aquous fraction. Experimental procedure for *P. acidus*, *P. buxifolius* was leaving a residual water insoluble fraction.

DPPH radical scavenging activity

The scavenging activity was corresponded to the intensity quenching DPPH radical (Kwon and Kim, 2003) Four mL of methanol solution of test fractions at various concentration was added to a solution of DPPH 0,45 mM in MeOH (1 mL), and the reaction mixture (total volume, 5 mL) was shaken vigorously. After storage at room temperature for 30 min, the remaining DPPH was determined by spectrophotometry at 517 nm, and the radical scavenging activity of each sample was expressed by the ratio of the reduction in DPPH absorption (%), relative to the absorption (100%) of DPPH solution in the absence of test sample (control). The mean values were obtained by triplicate experiments.

Identification of flavonoid compounds

Thin layer chromatography were used for analysis of flavonoid. The all fractions was doing identification on celulose with solvent *n*-butanol:acetic acid: water (4:1:5) and acetic acid 15%. The spots was detected by UV 366 nm, ammonia vapor and citroborat spray reagent.

RESULTS

This work reports the antioxidants activity of the leaves of *Phyllanthus buxifolius* Muell, Arg, *Phyllanthus acidus* (L.) Skeels and herbs of *Euphorbia heterophylla* L.The result of the experiment extraction and fractination is shown in Table 1 and DPPH radical scavenging activities is shown in Table 2.

Table 1. The rendemen extraction of plant material and fractionation of extracts

No	Material	Yields (%)				
		methanolic Extract	*n*-hexane fraction	ethyl acetate fraction	aquous fraction	Insoluble fraction
1	*P. acidus* (400 mg)	11.29	20.84	9.22	47.40	6.13
2	*P. buxifolius* (400 mg)	10.08	21.78	11.61	21.85	13.48
3	*E.heterophylla* (400 mg)	10.13	60.14	7.46	45.56	-

Table 2. DPPH radical scavenging activities

No	Sample	IC_{50} (ppm)*)		
		P. acidus	*P.buxifolius*	*E.heterophylla*
1	Rutin	5.11	5.11	5.11
2	methanolic extract	113.30	11.56	44.43
3	*n*-hexane fraction	306.03	115.53	212.81
4	ethyl acetate fraction	26.61	4.64	5.88
5	aquous fraction	162.03	23.83	130.63
6	insoluble fraction	169.30	7.75	-

*) Concentration giving a 50% decrease of DPPH radical. The values are the means of triplicate experiment

The methanolic extract of the *P. acidus*, and *E. heterophylla* showed weak activity as DPPH scavenger. However, when this extract was partitioned between *n*-hexane and aquadest then ethyl acetate and aquadest. The ethyl acetate fraction of the *P. acidus P. buxifolius* and *E. heterophylla* L showed a strong free radical scavenging activity with IC_{50} value 4,64 ppm and 5.88 ppm, respectively. The *n*-hexane fraction of all herbs showed a very weak activity.

Table 3. TLC data for the ethyl acetate fraction by stationary phase celulose

Mobile Phase	Plant	Fraction	hRf	Color of spot TLC		
				UV 366nm	NH3	Citro Boric
BAW*) (4:1:5)	*P. acidus*	EtOAc	82	Yellow	Yellow	Yellow
	P. buxifolius	EtOAc	92	Yellow	Yellow	Yellow
	E. heterophylla	EtOAc	68	Brown	Yellow	Yellow
	Rutin	EtOAc	61	Brown	Yellow	Yellow
Acetic acid 15%	*P. acidus*	EtOAc	48	Yellow	Yellow	Yellow
	P. buxifolius	EtOAc	35	Yellow	Yellow	Yellow
	E. heterophylla	EtOAc	35	Brown	Yellow	Yellow
	Rutin	EtOAc	50	Brown	Yellow	Yellow

*) *n*-buthanol : acetic acid : water (4:1:5)

Using two solvent systems, the TLC analysis of ethyl acetat (EtOAc) fraction that as active fraction showed flavonoid compound. The result identification of spot on UV 366 showed yellow and brown color.

DISCUSSION

In present study, the antioxidant activities of *P.acidus, P. buxifolius* and *E. heterophylla* were determined by extraction with methanol and fractination with *n*-hexane and aquadest then ethyl acetate and aquadest. The methods can isolated based-polarity natural compound. As can be seen in Table 3, the studies showed that ethyl acetat fraction include polyphenol as flavonoid. These natural antioxidative substances usually have a phenolic moiety in their molecular structure.

Phenolic antioxidants are potent free radical terminators. Phenolic compounds, biological active components, are the main agents that can donate hydrogen to free radicals and thus break the chain reaction of lipid oxidation at the first inisiation step. This high potential of phenolic compounds to scavenge radical may be explained by their phenolic hydroxyl groups.

The antioxidant activites were obtained from extract methanolic and fractions from methanolic extract. The IC_{50} values obtained are presentated in Table 2. All of the herbs showed activity as DPPH scavenger. The antioxidant activities of ethyl acetate fraction increased, and greater than that of the same herbs of methanolic extract. Among these herbs, *Euphorbia heterophylla* L. and *Phyllanthus buxifolius* Muell, Arg. exhibited a strong free radical scavenging of ethyl acetat fraction with EC_{50} value 5.88 µg/ml and 4.64 µg/ml.

Antioxidant activities of *n*-hexane, ethyl acetat, and aquous fraction were determined by DPPH radical. DPPH is a stable free radical in aquous or methanol solution and aceppts an electron or hydrogen radical to become a stable diamagnetic molecule (Gulcin *et al.,* 2004). In order to evaluate antioxidant potency through free radical scavenging with the test sample, the change in the optical density of DPPH radicals was monitored. Hence, DPPH is usually used as a substrate to evaluate the antioxidative activity of antioxidants (Duh et.al., 1999).

CONCLUSION

In conclusion, *P.acidus, P. buxifolius* and *E. heterophylla* have antioxidant. All of the herbs showed activity as DPPH scavenger. Among these herbs, *Euphorbia heterophylla* L. and *Phyllanthus buxifolius* Muell, Arg. exhibited a strong free radical scavenging of ethyl acetat fraction with IC_{50} value 5,88 µg/ml and 4,64 µg/ml. The result of TLC by mobile phase *n*-buthanol-acetic acid-water (4:1:5) and acetic acid 15% indicated present of flavonoid compound. Therefore, it is suggested that further work to be performed on the isolation and identification of antioxidative component of ethyl acetat fraction.

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