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Research Article

ANTIOXIDANT ACTIVITY OF EXTRACT AND FRACTION OF YELLOW PASSION FRUIT (*PASSIFLORA FLAVICARPA*) LEAVES

***NYI MEKAR SAPTARINI, YULIA WARDATI, AND RESTI JULIAWATI**

Faculty of Pharmacy, Padjadjaran University, Jl Raya Bandung Sumedang Km 21 West Java Indonesia. Email: n_mekars@yahoo.com

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ABSTRACT

Antioxidants are compounds that can inhibit free radicals. Natural antioxidants found in many plants, such as leaves of yellow passion fruit (*Passiflora flavicarpa*). The purpose of this study was to determine the antioxidant activity of extract and fractions of yellow passion fruit leaves against 1,1-diphenyl-2-picrilhydrazyl. The step of this study consist of extraction, fractionation with n-hexane, ethyl acetate and water, phytochemical screening and antioxidant activity determination. The results showed that the IC_{50} value for the ethanolic extract, n-hexane fraction, ethyl acetate fraction, and water fraction were 66.7; 264.3; 49.3, and 52.7 μg/mL, respectively.

Keywords: *Passiflora flavicarpa*, Leaves, Extract, Fraction, Antioxidant

INTRODUCTION

Free radicals defined as chemical species possessing unpaired electrons, which are formed by homolytic cleavage of a covalent bond of a molecule by loss of a single electron from a normal molecule or by the addition of a single electron to a normal molecule. These free radicals have very short half-life, high reactivity, and damaging activity towards macromolecules like proteins DNA and lipids. Oxidative stress is among the major causative factors in the induction of many chronic and degenerative diseases including atherosclerosis, ischemic heart disease, ageing, diabetes mellitus, immunosuppression, neurodegenerative diseases, cancer, and others[1,2,3]. The most effective compound to eliminate this free radicals is antioxidants. Antioxidants effective in preventing free radical formation by scavenging them or promoting their decomposition and suppressing such disorders[4,5,6].

Natural antioxidant in plant resources can protect biology systems from oxidative stress[7,8,9], such as yellow passion fruit (*Passiflora flavicarpa*). Yellow passion fruit leaves contain flavonoids, alkaloids, saponins, riboflavine, and theobromine. The antioxidant activity of this plant came from flavonoid and riboflavine[10]. The most important property of flavonoids and their derivatives is their capacity to act as antioxidants protecting the body against reactive oxygen species and may have an additive effect to the endogenous antioxidants[11]. The purpose of this study was to determine the antioxidant activity of yellow passion fruit leaves with 1,1-diphenyl-2-picrilhydrazyl (DPPH) method.

MATERIALS

Yellow passion fruit (*Passiflora flavicarpa*) were grown in the field of Manoko, West Java, 1,1-diphenyl-2-picrilhydrazyl (Sigma), vitamin C (Merck), Butylated Hydroxy Toluene (BHT, Merck), and 95% ethanol (Merck).

METHODS

Samples Preparation

Simplicia macerated with 70% ethanol for 3 days. Each day, the solvent changed with the fresh one. All macerat collected and evapored. Dissolve 10 g of ethanolic extract with aquadest to obtain 100 mL solution, then done liquid liquid extraction with ethyl acetate and n-hexane, threetimes for each solvent. All fraction collected and evapored. Phytochemical screening to simplicia, extract, and fractions were conducted with Fransworth methods[12].

Antioxidant Activity Determination

Dissolve 3 mg of DPPH with 96% ethanol in 100 mL volumetric flask (30 μg/mL). Dissolve 5 mg of vitamin C and BHT, each compounds, with 96% ethanol in 100 mL volumetric flask, then diluted the solution until 2.5, 5, 10, 15, and 20 μg/mL. Dissolve 50 mg of samples with 96% ethanol in 100 mL volumetric flask (500 μg/mL). Then diluted the solution to 40, 80, 100, 120, and 160 μg/mL for ethanolic extract and ethyl acetate fraction; 200, 300, 400, 500, and 600 μg/mL for n-hexane fraction; and 80, 100, 120, 160, and 200 μg/mL for water fraction.

The stable DPPH radical was used for the determination of free radical-scavenging activity of the extracts and fractions. The modified method of Okada and Okada (1998) was employed[13]. A portion (1.5 mL) each of the different concentrations of extracts, fractions or positive control in test tubes was added 3 mL of 30 μg/mL DPPH. The mixtures were vortexed and incubated in a dark chamber for 30 min, then the absorbancies were measured at 517 nm using spectrophotometer (Shimadzu) against DPPH control containing only 1.5 mL of ethanol in place of samples. Percentage scavenging activity was calculated using this formula:

$$\% \text{ of DPPH inhibition} = [(Ab-Aa)/Ab] \times 100$$

Where Aa and Ab are the absorbance values of the test and of the blank, respectively. A percent inhibition versus concentration curve was plotted and the concentration of sample required for 50% inhibition was determined and expressed as IC_{50} value.

RESULTS AND DISSCUSSION

Maceration of 250 g simplicia was produced 104.92 g ethanolic extract with 20.98% rendement. The color of extract was dark reddish green. Liquid liquid extraction was conducted to seperate the secondary metabolites based on its polarity, thus obtained 2.8% n-hexane fraction, 52.5% ethyl acetate fraction, and 44.7% water fraction. Phytochemical screening showed that extract has the same constituents as simplicia. It's mean that maceration can extracted all secondary metabolites in simplicia. Secondary metabolites in simplicia and extract are alkaloids, phenolic compounds, saponins, quinones, and flavonoids. All fraction still contain alkaloids, phenolic compounds, and flavonoids. While, liquid liquid extraction successfully separated saponin compounds and quinones in ethyl acetate fraction and quinone in water fraction.

The antioxidant capacity of the plant is mainly dependent on phenolic compounds. Phenolic compounds are an important group of secondary metabolites, which are synthesized by plants due to plant adaptation in response to biotic and abiotic stresses (infection, water stress, cold stress, and high visible light)[14]. The antioxidant activity of phenolic compounds depend on their molecular structure, based on the availability of phenolic hydrogens, which result in the formation of phenoxyl radicals due to hydrogen donation[15].

The DPPH molecule contains stable free radical which has been widely used to evaluate the radical scavenging ability of antioxidants. The colour of DPPH radicals solution was purple, because the unpaired nitrogen electrons. Reaction of DPPH radicals with oxygen electrons from antioxidants made DPPH-H (2,2-diphenyl-1-picrilhidrazyn), i.e DPPH that less reactivity, that showed with colour alteration from purple to yellow (Fig 1). It is possible to determine the antiradical potential of antioxidants by measurement of the decrease in the absorbance of DPPH at 517 nm[16]. The results of the DPPH free radical scavenging assay suggest that components within the extracts are capable of scavenging free radicals via electron or hydrogen donating mechanisms and thus should be potent enough to prevent the initiation of deleterious free radical mediated chain reactions in susceptible matrices e.g. biological membranes. The expression of antioxidant activity is thought to be concomitant with the development of reductones, as these species are known to be free radical chain terminators[17]. The absorbance of 30 µg/mL DPPH radical solution was 0.818. The mixing of sample or positive control solutions with DPPH solution measured, then % of DPPH inhibition were counted (Table 1).

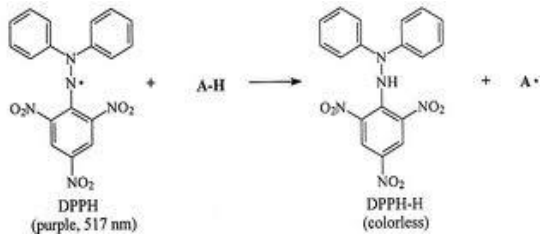

Fig. 1: Structure of DPPH before and after reaction with antioxidant[16]

Table 1: Antioxidant Activity of Yellow Passion Fruit Leaves to 30 µg/mL DPPH Solution

Sample	Concentration (ppm)	Absorbance	% inhibition	IC_{50} (µg/mL)
Ethanolic extract	40	0.525	35.8	66.7
	80	0.341	58.3	
	100	0.289	64.7	
	120	0.195	76.2	
	160	0.088	89.2	
n-hexane fraction	200	0.493	39.7	
	300	0.351	57.1	264.3
	400	0.286	65.0	
	500	0.224	72.6	
	600	0.178	78.2	
Ethyl acetate	40	0.483	41.0	49.3
fraction	80	0.229	63.5	
	100	0.186	77.3	
	120	0.127	84.5	
	160	0.086	89.5	
Water fraction	80	0.356	56.5	52.7
	100	0.301	63.2	
	120	0.248	69.7	
	160	0.102	87.5	
	200	0.089	89.1	
Vitamin C	2.5	0.514	37.2	13.7
	5	0.451	44.9	
	10	0.433	47.7	
	15	0.397	51.5	
	20	0.368	55.0	
BHT	2.5	0.571	30.2	16.0
	5	0.506	38.1	
	10	0.483	41.0	
	15	0.409	49.9	
	20	0.372	54.5	

Smaller IC_{50} mean higher antioxidant activity. Ethanolic extract, ethyl acetate fraction, and water fraction had similar IC_{50} value, i.e 66.7, 49.3, and 52.7 µg/mL, respectively. While n-hexane fraction had the biggest IC_{50} value, because this fraction had no saponins and quinones content as compared with the other samples. Saponins and quinones are phenolic compounds derivatives that can donating hydrogen atom and increases the antioxidant activity of the other samples. None of the samples was as effective DPPH scavengers as the positive control, vitamin C (natural antioxidant) and BHT(synthetic antioxidant), because the sample is not a pure compound, but contains other compounds that do not have antioxidant activity. Vitamin C had higher antioxidant activity than BHT, because vitamin C had more hydroxyl groups than BHT, so vitamin C can donating more hydrogen atoms to reacted with DPPH radicals than BHT. The antioxidant activity of samples were compared to positive controls. The aim of this comparison is to see the reactivity of samples to DPPH radicals (Table 2).

Table 2: Comparison of Antioxidant Activity of Yellow Passion Fruit Leaves to Positive Control

	IC_{50}	Vitamin C (%)	BHT (%)
Ethanolic extract	66.7	20.8	23.8
n-hexane fraction	264.3	5.2	6.1
Ethyl acetat fraction	49.3	27.8	32.3
Water fraction	52.7	26.3	30.3

A compound said as very powerful, strong, middle, and weak antioxidant if the IC_{50} values is less than 0.05 mg/ml, 0.05 to 0.10 mg/ml, 0.10 to 0.15 mg/ml, and 0.15 to 0.20 mg/ml, respectively[16]. Ethyl acetate fraction had the best antioxidant activity.

CONCLUSION

The IC_{50} value for ethanolic extract, n-hexane fraction, ethyl acetate fraction, and water fraction were 66.7, 264.3, 49.3, and 52.7 μg/mL, respectively. The ethyl acetate fraction had the best antioxidant activity.

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