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by St Ratnah

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DETERMINATION OF TOTAL PHENOLIC CONTENT (TPC) AND ANTIOXIDANT ACTIVITY OF LONGAN *(Euphoria Longana)* SEED AND PEEL EXTRACTS

Alfrida Monica Salasa[1], St. Ratnah[1a]
[1]Pharmacy Department, Health Polytecnic of Makassar
[a]Corresponding author : mansjurratnah@yahoo.com

ABSTRACT

Longan (Euphoria longana) is a widely consumed fruit in Indonesia. The flesh is consumed, while the seed and the peel of Longan remain as waste despite of its chemical contents such as phenolic compounds (phenolic acids and flavonoids) with antioxidant properties. This study aimed to determine the total phenolic contents (TPC) and antioxidant activity of Longan seed and peel extract and to find out the relationship between total phenolic content and antioxidant activity. The seed and peel of Longan was separated from the flesh, then mashed and then dried. Furthermore, Longan seed was extracted by soxhletation method and fruit peel was extracted by maceration method using 96% ethanol as a solvent. The diluted extract was evaporated until a concentrated extract was obtained. The TPC was determined using Folin ciocalteu reagent and the absorption was measured using a UV-VIS spectrophotometer at a wavelength of 745 nm. Antioxidant activity was determined using 1,1-diphenyl-2-picryl-hydrazil (DPPH) reagent and the absorption was measured using a UV-VIS spectrophotometer at a wavelength of 516 nm. The TPC of Longan seed extract was 17.24% while fruit peel extract was 9.67%. Antioxidant activity expressed as IC_{50} for seed extracts was 57.24 ppm(strong antioxidant) and fruit peel extracts was 120.82 ppm (moderate antioxidant). These results indicate that Longan seed extract has a higher TPC and stronger antioxidant activity compared to Longan fruit peel extract. It can be concluded that the higher the TPC the more active the antioxidant activity

Keywords : Longan Seed Extract, Longan Peel Extract, TPC, Antioxidant Activity

INTRODUCTION

Natural plants contain phytochemical compounds with antioxidants and antibacterial properties. Longan ((Euphoria longana) Stend (Harborne, 1987)) is a widely consumed fruit in Indonesia with aforementioned properties. The most edible part of the fruit is the flesh, while the seed and the peel remain as organic waste. Despite as organic waste, both contain phytochemical compounds that can be used both as antioxidants and antibacterial. Based on the results of several scientific studies, Longan seed and peel have various chemical compounds. Jaitrong et al. (2006) reported that the chemical content in the fruit peel is gallic acid, flavone glycoside, and hydroxynamic with the main content of flavone in the form of quercetin and kaemferol while Longan Seed Components are in the form of Phenolic and Flavonoids. Some of chemical compounds that actively act as antioxidants include polyphenol compounds (phenolic acids and flavonoids), alkaloids, steroids or triterpenoids (saponins), and anthraquinones (Bai et al, 2013 in Perwiratami et al, 2014).

METHOD

1. Sample Preparation
   Longan fruit were collected from the fruit store. The fruit was washed and left to dry. The flesh was separated from the peel and the seed. The seed was washed to remove the remaining flesh. After that, the seed was roughly grown and dried. The peel was cut into small pieces and left to dry.
2. Extract Preparation
   Longan seed was extracted by soxhletation method and fruit peel was extracted by maceration method using 96% ethanol as a solvent. The diluted extract was evaporated until a concentrated extract was obtained.
3. Determination of TPC
   a. Qualitative Evaluation
      The concentrated extract was dissolved in 2 ml of ethanol 96%, added 1 ml of 10% Ferric Chloride. The formation of dark blue, blackish blue or greenish black color indicates the presence of phenolic compounds.
   b. Quantitative Evaluation
      Standard solution of gallic acid series 10, 20, 30, 40, 50 and 60 ppm was prepared. 25 mg of each extract was weighed and transferred into 25,0 ml volumetric flask, then it was diluted by ethanol 96% to make a stock solution of 1 mg/ml. 2,0 ml of stock solution was diluted in a 10 ml volumetric flask

to make a final concentration of 0,2 mg/ml. Each standard solution and diluted extracts was piped as much of 0.3 mL and then transferred into a vial. 1,5 ml of Folin (1:10) was added and left to stand for 3 minutes. After 3 minutes, 1,2 mL of 7,5% Sodium carbonate was added and let it sit for 30 minutes. The absorption was measured using a UV-VIS spectrophotometer at a wavelength of 745 nm.

The antioxidant activity was measured based on the method developed by Mensor et al with some modifications, using 2,2-diphenyl-1-picryl-hydrazyl (DPPH). Stock solution of 1 mg/ml of the extract were diluted in ethanol to final concentration of 15, 30, 45, 60 and 75 µg/ml (ppm) for the Longan seed extract and a final series concentration of 30, 60, 90, 120 and 150 for the Longan peel extract. DPPH solution were added to the diluted sample and allowed to react at room temperature for 30 minutes. Each concentration was replicated three times. The absorbance values were measured at 516 nm

4. Antioxidant Activity Assay

RESULT AND DISCUSSION

1. TPC

a. Qualitative Test

Type of Extract	Reagent	Observation	Conclusion
Seed	$FeCl_3$	Blackish green	+
Peel	$FeCl_3$	Blackish Green	+

b. Quantitative Test

The TPC was determined using Folin ciocalteu reagent and the absorption was measured using a UV-VIS spectrophotometer at a wavelength of 745 nm, the result was shown at the table below :

Sample	Weight (gram)	TPC (mgGAE/g extract)	TPC (%)	TPC Average (%)
Seed Extract	0,0251	177,04	17,70	17,24
	0,0255	171,67	17,17	
	0,0258	168,45	16,85	
Peel Extract	0,0258	89,19	8,92	9,67
	0,0254	103,05	10,31	
	0,0253	97,83	9,78	

2. Antioxidant Activity Assay

Antioxidant activity was determined using 1,1-diphenyl-2-picryl-hydrazil (DPPH) reagent and the absorption was measured using a UV-VIS spectrophotometer at a wavelength of 516 nm. The result was presented in the table below :

Table 3. Antioxidant Activity of Longan Seed Extract

Replication	Concentration (ppm)	Absorption	% Inhibition	IC50 (ppm)	IC50 average (ppm)
I	15	0,74264	16,5469	56,61	57,24
	30	0,64901	27,0685		
	45	0,53496	39,8847		
	60	0,42482	52,2615		
	75	0,30145	66,1250		
II	15	0,73623	17,2673	57,09	
	30	0,64431	27,5966		
	45	0,53947	39,3779		
	60	0,41494	53,3717		
	75	0,31886	64,1686		
III	15	0,75146	15,5558	58,02	
	30	0,65104	26,8404		
	45	0,54008	39,3094		
	60	0,42017	52,7841		
	75	0,32888	63,0426		
	DPPH	0,88989			

Table 4.Antioxidant Activity of Longan Peel Extract

Replication	Concentration (ppm)	Absorption	% Inhibition	IC50 (ppm)	IC50 average (ppm)
I	30	0,76442	14,0995	120,21	120,82
	60	0,64870	27,1033		
	90	0,53514	39,8645		
	120	0,44054	50,4950		
	150	0,35386	60,2355		
II	30	0,76225	14,3433	119,99	
	60	0,64683	27,3135		
	90	0,54045	39,2678		
	120	0,44108	50,4343		
	150	0,34946	60,7299		
III	30	0,76089	12,9000	122,25	
	60	0,67917	23,6793		
	90	0,54519	38,7351		
	120	0,45645	48,7071		
	150	0,34788	60,9075		
	DPPH	0,88989			

DISCUSSION

This study aims to determine the TPC and antioxidant activity of Longan Seed and Peel Extract. The most edible part of Longan fruit is the flesh, while the seed and the peel remain as organic waste. However the remaining organic waste contains chemical compounds in the form of phenolic compounds act as antioxidants. Antioxidant activity of phenolic compound is based on their ability to form phenoxide ions as a donor electron to free radicals to form non-radical compounds.

The seed and peel of Longan was separated from the flesh, then mashed and then dried. Furthermore, Longan seed was extracted by soxhlet ion method and fruit peel was extracted by maceration method using 96% ethanol as a solvent. The

selection of extraction methods was adjusted to the texture of each simplicia, where the longan peel had a soft texture so that the extraction method used was maceration. While longan seed had a rather hard texture so that the soxhletation method was used. Ethanol 96% which is a semipolar solvent dissolves all polar and nonpolar compounds so that all the natural compounds can be extracted perfectly. The diluted extract was evaporated until a concentrated extract was obtained and tested for TPC and antioxidant activity.

The TPC testing began with a qualitative test using $FeCl_3$ reagent to determine whether the seed and the peel contain phenolic compounds. Both extract reacted with $FeCl_3$ forming a blackish green color which confirm the presence of phenolic compounds. The test was continued to determine the TPC quantitatively using Folin ciocalteu reagent and sodium carbonate. This method is based on the strength of reducing the hydroxyl groups of phenolic compounds. Folin ciocalteu reacts with phenolic compounds to form yellow color and after addition of sodium carbonate, the solution will be in blue color. Sodium carbonate provides an alkaline atmosphere for the overall reaction which allow folin ciocalteu to react with phenolic compounds, which cannot be done in an acidic atmosphere. The presence of aromatic nuclei in phenol compounds reduce phosphotungstate phosphomolibdate to blue molybdenum. The absorption was measured by using UV-VIS spectrophotometry at a wavelength of 745 nm. The standard solution for TPC was gallic acid. The results showed that the TPC equivalent to gallic acid for seed extract was amounted to 17.24% while for the peel extract was 9.67% indicating that the TPC of the seed extract was greater than that of the peel extract.

The next assay was the antioxidant activity assay using DPPH as a reagent. DPPH is a stable free radical with purple color(absorbed at 516nm). If free radicals have been scavenged, DPPH will generated it's color to yellow. This assay uses this character to show herbs free radical scavenging activity. Antioxidant activity is expressed in Inhibition Concentration 50% (IC_{50}). IC_{50} represents the concentration in which the inhibition of free radical DPPH by 50%. Antioxidant activity expressed as IC_{50} for seed extracts was 57.24 ppm (strong antioxidant (50-100 ppm)) and fruit peel extracts was 120.82 ppm (moderate antioxidant (101-250 ppm)). This result indicated that the longan seed extract has a stronger antioxidant activity than the longan peel extract.

Based on the result on TPC and antioxidant activity, there was a correlation between the TPC and the antioxidant activity. The higher the TPC the stronger the antioxidant activity.

CONCLUSION

Based on the result it can be concluded that:

1. TPC of Longan seed and peel extract was 17,24% and 9,67% respectively.
2. Antioxidant activity expressed as IC_{50} was 57,24 ppm for the Longan seed extract and 120,82 ppm for the Longan peel extact.
3. There was a correlation between the TPC and the antioxidant activity. The higher the TPC the stronger the antioxidant activity

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