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## Antioxidant Activity and Total Phenol Content of Ethanol Extract Takokak Fruit (*Solanum torvum*)

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**Abstract:** Takokak is a potential source of antioxidants for scavenger free radical. This study carried out to examines the affect of ethanol concentration to the yield extract, total phenol and antioxidants activity in variety maturity stage takokak (*Solanum torvum*). Research design was experiment laboratorium. The yield was calculated by comparing the weight of extract with baseline weight, phenol assay using the Folin-Ciocalteu reagent and antioxidants activity assay using DPPH method. The result showed that ethanol concentration ethanol 70 and 96% was significantly affected the yield extract ( $p = 0.000$ ) and ( $p = 0.000$ ). Concentration 70 and 96% did not significantly affect on level phenol ( $p = 0.056$ ) and ( $p = 0.342$ ). Concentration 70% was significantly affected antioxidant activity ( $p = 0.014$ ), but concentration 96% was not significanlyt affected ( $p = 0.114$ ) on the variety maturity stage of *Solanum torvum*. The best extracts was ethanol extract 70% old dried mature fruits.

**Key words:** *Solanum torvum*, antioxidant activity, phenol content

### INTRODUCTION

Takokak (*Solanum torvum*) is commonly known as Turkey berry. Takokak originated from African and western India (Kamble *et al.*, 2009). Some studies show that takokak were come various countries, among others Mexico, Peru and Venezuela. Additionally takokak distributed in several countries among others India, West India, Malaysia, China, Philipina and Indonesia. Takokak is one group of solanaceae that contains components of natural medicine (Yogananth *et al.*, 2009).

Phenol and flavonoid components are secondary metabolites produced by plants. Phenols and flavonoids act as antioxidants, anti-inflammatory, lowering blood pressure and cholesterol (Arif, 2011). Ethanol extract fruits contain alkaloids, glycosides, terpenoids, carbohydrates, saponins, tannins, flavonoids, phytosterols, amino acids and some phenols (Archana *et al.*, 2012). Likewise takokak a group of plants that also contain chemicals such as steroid lactones, glycosides, alkaloids, and flavonoids.

Polyphenols are one of the most abundant antioxidant in foods (Scalbert *et al.*, 2013). Polyphenols are the result of plant secondary metabolism. Polyphenols are aromatic ring structure containing one or more hydroxyl molecules. Distinguished two polyphenols; flavonoid (flavanoen, flavones, flavonols, isoflavones, proanthocianine) and non-flavonoids (phenols, benzoate acid, tannin, acetophenon, phenilasetat acid, siamik acid, coumarin, benzophenones, xanthonenes, stilbenes, chalcone, ligana and secoiridoid) (Michalak 2006).

Phenol has functions such as antioxidant, antimutagenic, anti-carcinogenic (Satheesh *et al.*, 2013).

Antioxidants are compounds that useful against cancer and other reactions that could potentially cause disease. Examples of such diseases are caused by atherosclerosis, Alzheimer's, Parkinson's, diabetes, heart disease, cancer, etc. Polyphenol components commonly found in plants and is reported to have the ability of biological activities including antioxidant (Enein *et al.*, 2009).

Fruit maturity is a process of biochemical, physiological and structural changes such as hydrolysis of starch, chlorophyll degradation, production of carotene, anthocyanin, phenol components, accumulation of sugars and organic acids, modification of the structure and composition of cell wall polysaccharides, color, flavor and texture changes (Asthma *et al.*, 2013). Total phenol decreases with increasing level of maturity, but some plants total phenol have increased with increasing levels of maturity (Fox *et al.*, 2005). Crude extract antioxidant capacity of different fruit ripening stages, raw fruit contains more antioxidant capacity greater than fruit or old ripe fruit (Boonyuen *et al.*, 2009).

Takokak plants as potential sources of natural antioxidants. Carotene, flavonoids, cinnamic acid, benzoic acid, folic acid, ascorbic acid, tokoperol, tocotrienol, etc. is an antioxidant produced by plants as long as they produce food. The high material benefits contained in takokak and fruit maturity stages in producing secondary metabolites, as well as the ability of ethanol to extract chemical components. The aim of

this study to assess the affect of ethanol concentration at various stages of maturity age takokak (*Solanum torvum*) both dry and fresh to the yield, phenol content and antioxidant activity.

## MATERIALS AND METHODS

**Research design:** This study examined the affect of ethanol concentration on yield extracts, total phenols, antioxidant activity at different stages of fruit maturity age takokak both dry and fresh. Treatment ethanol Concentration were 70 and 90% at various stages of maturity age takokak fruit. Takokak fruit age were dried fruit old age, dry fruit enough age, Fresh fruit old age, fresh fruit Old enough, Fruit fresh young age. Design research is a laboratory experiment with a Randomized Block Design (RBD).

**Materials and equipment:** Takokak fruit (*Solanum torvum*) obtained from the Village of Mount Leutih toga gardens around the city of Bogor. Young fruit was the fruit that is harvested before the age of 30 days, enough fruit is a fruit that is harvested between harvest 30 to 60 days and the old fruit was a fruit that is harvested after the age of 60 days. Fresh fruit was a fruit that without having drying, while the dried fruit pieces are experiencing drying temperature of 50°C. Technical ethanol 96 and 70% obtained from Panadia store, filter paper. The tools used include rotary evaporator, mill, oven, erlenmeyer, vacuum pumps, analytical balance KERN brand ALJ 220-4nm Germani and UV-VIS spectrophotometer VARIAN Cory 50 Conc.

**Flour fruit:** A total of 1-3 kg of fruit takokak weighed and oven-dried at 50°C for 9 days. After takokak dry milled and sieved with a grinding machine with a mesh size of 20. Furthermore takokak flour extracted with technical ethanol (Wetwitayaklung, Phaechamud, 2011).

**Ethanol extraction method:** A total of 50 g of flour that is finely takokak fruit weighed, then extracted (macerated) with ethanol 7x dry matter (50 g), with stirring for  $\pm 2$  h and allowed to stand for 1 night. The solution was then filtered with a filter paper, the solvent is removed or evaporated by the evaporator at a temperature of 50°C. Further results are weighed viscous extract (Alakilli, 2010; Amador *et al.*, 2007).

**Yield extract:** Yield extract determination was done by weighing the results of extraction with analytical balance, then the yield is calculated by the formula:

$$\text{Yield (\%)} = \frac{\text{Weight extract}}{\text{sample weight}} \times 100$$

**Determination of total phenols:** Total phenol was determined by Folin-Ciocalteu reagent. Gallic acid was used as a standard. The content of total phenols

expressed as mg gallic acid equivalents (GAE)/100 g. Concentration of gallic acid were made by weighing as much as 10.1 mg gallic acid, dissolved in 10 ml of methanol, then the solution is taken as 25, 50, 75, 100, 125 and 150  $\mu\text{g/mL}$  in methanol. Concentration extract was prepared 10 mg/10 mL extracts in methanol and 0.05 ml was taken. Then each sample is introduced in a test tube and mixed with 0.25 mL of Folin-Ciocalteu reagent and 0.75 mL of 7.5% sodium carbonate. Then incubated for 30 min at room temperature, then read the absorbance at a wavelength of 655 nm (Charanjit, Harish, 2002). Readings were repeated 2 times. Standard curve equation:

$$y = 0.000190x + 0.039257, R^2 = 0.997$$

**Determination of antioxidant activity:** A total of 10 mg of sample was weighed and dissolved in 10 mL of deionized water. Taken as much as 3 ml sample solution and add 1 ml of 0.2 mM DPPH in methanol and shaken, then incubation at 37°C for 30 min. Decreasing of free radical DPPH absorbance was read at 517 nm. A total of 100 mL of pure methanol is used as a control. The results were compared with the antioxidant activity expressed as vitamin C equivalent antioxidant capacity (AEAC/Ascorbit acid equivalent antioxidant capacity) in mg AEAC/100 g (Abdullah, 2009). Standard curve prepared from standard vitamin C, 10 mg weighed, then diluted with 10 mL of methanol. The standard solution were taken respectively by 10, 25, 50, 75, 100 (mL) and dissolved in 5 mL of methanol. Subsequently the absorbance was read at 517 nm. Readings were repeated 3 times. Obtained curve equation:

$$y = 6.70x + 16.08, R^2 = 0.777$$

**Statistical analysis:** The data were analyzed using analysis of variance with SPSS 20, with a confidence level of 0.05. Furthermore, to determine the difference in Duncan test Duncan.

## RESULTS

**Yield:** Yield ranged ethanol extract was 4.1% up to 32.7%, the highest yield was ethanol extract old-dried fruit with concentration 70% and lowest was ethanol extract young fresh fruit age with concentration 96%. The mean yield ethanol extract dried fruit enough age 96% ethanol was 13.9%. Analysis of variance showed that concentration ethanol 7% was significantly affected the yield ( $p = 0.004$ ) and the concentration of ethanol 96% ( $p = 0.000$ ) on various types of fruit takokak age. Takokak extract yield based on the type of fruit and ethanol concentration can be seen in Table 1.

**Phenol levels:** Phenol levels extract was 2112.0 mg GAE/100 g up to 50576.3 mg GAE/100 g. Highest levels of phenols was ethanol extract type fresh fruit that old

Table 1: Yield ethanol extract takokak\*

Variety takokak	Concentration ethanol (%)		Average	p-value
	70	96		
Dried fruit old age	32.7±3.1	17.7±1.6	25.2±2.4 <sup>a</sup>	0.000
Fruit dry enough age	22.2±2.2	13.9±0.3	18.0±1.2 <sup>b</sup>	
Fresh fruit old age	7.4±2.8	9.1±0.2	8.3±1.5 <sup>c</sup>	
Fruit fresh young age	5.1±0.5	4.5±0.6	4.8±0.5 <sup>c</sup>	
Average	4.9±0.7	4.1±0.4	44.5±0.6 <sup>c</sup>	
Average	13.5±1.9	9.0±.6	11.3±1.2	
p-value	0.021			

\*(mg AEAC/100 g)

Table 2: Total phenol ethanol extract takokak\*

Variety takokak	Concentration ethanol (%)		Average	p-value
	70	96		
Fresh fruit old age	505.76±237.4	374.24±115.8	440.00±166.6 <sup>a</sup>	0.002
Fruit fresh young age	358.25±44.9	237.47±303.7	297.86±174.3 <sup>ab</sup>	
Old enough fresh fruit	186.81±150.4	133.32±82.0	160.07±116.2 <sup>bc</sup>	
Fruit dry enough age	58.39±65.6	74.11±73.4	66.25±69.5 <sup>c</sup>	
Dried fruit old age	21.12±2.7	47.15±19.1	34.138±10.9 <sup>c</sup>	
Average	226.07±100.24 <sup>a</sup>	173.26±118.8 <sup>a</sup>	170.72±109.5	
p-value	0.368			

\*(mg AEAC/100 g)

Table 3: Antioxidants activity ethanol extract takokak\*

Variety takokak	Concentration ethanol (%)		Average	p-value
	70	96		
Fruit dry enough age	1,383.7±8.6 <sup>a</sup>	1,216.5±27.8 <sup>ab</sup>	1,364.3±16.8	0.009
Dried fruit old age	1,334.7±2.1 <sup>a</sup>	1,393.9±25.1 <sup>a</sup>	1,300.0±15.0	
Fresh fruit old age	586.8±291.2 <sup>b</sup>	918.0±107.0 <sup>b</sup>	752.4±199.1	
Fruit fresh young age	303.8±510.9 <sup>b</sup>	1,142.9±190.6 <sup>ab</sup>	723.4±350.8	
Old enough fresh fruit	98.0±90.1 <sup>b</sup>	1,066.9±216.0 <sup>ab</sup>	582.5±153.0	
Average	741.4±180.6 <sup>b</sup>	1,147.6±113.3 <sup>a</sup>	944.5±146.9	
p-value	0.012			

\*(mg AEAC/100 g)

age concentration 70% and lowest was ethanol extract dried fruit old age concentration 70%. Phenol content ethanol extract dried fruit enough age 96% ethanol was 7,411.1mg GAE/100 g more higher than phenol content ethanol extract dried fruit enough age concentration 70% was 5,339 mg GAE/100 g. Analysis of variance showed that concentration ethanol 70% does not significantly affect the levels of phenol ( $p = 0.056$ ) and the concentration ethanol 96% ( $p = 0.342$ ). Phenol levels ethanol extract based on the type of fruit takokak and ethanol concentration can be seen in Table 2.

**Antioxidants activity:** Antioxidant activity was 98.0 mg g AEAC/100 up to 1393.9 mg AEAC/100 g. The highest antioxidant activity ethanol extract was dried fruit old age with ethanol 96% and lowest ethanol extract was fresh fruit age enough with 70% ethanol. Antioxidant activity of 96% ethanol extract dried fruit enough was 1216.5 mg/100 g AEAC, while the antioxidant activity of 70% ethanol extract Fruit dry enough age was 1383.5 mg AEAC/100 g. Analysis of variance showed that the 70%

ethanol concentration was significantly affected the antioxidant activity ( $p = 0.014$ ), but concentration 96% ethanol was not significant ( $p = 0.114$ ). Antioxidant activity ethanol extract based on the type of fruit takokak and ethanol concentration can be seen in Table 3.

## DISCUSSION

**Yield extract:** The average yield ethanol extract concentration 96% of the dried fruit enough aged similarity studied Waghulde *et al.* (2011) who showed that ethanol extract takokak yield was 12%, but the yield of ethanol extract concentration 70 and 96% old fresh fruit, fresh fruit enough age, young fresh fruit age and dried fruit old age were different from that study of Waghulde *et al.* (2011). The mean yield ethanol extract dried fruit is different from the findings of Loganayaki (2010) who showed that the chloroform extract fruit yield was 68.2%, the yield ethanol chloroform extract was 34.4%, the yield acetone extract was 3.8%, the yield methanol extract was 2%, the yield acetone extract was 7.8% and the yield methanol extract was 4.1%. The

yield ethanol extract dried fruit more than fresh fruit, because the dry matter content of the water reduced, but fresh fruit had high water content. Yield also depends on the variety of solvent used. Takokak fruit harvest will affect the resulting yield levels, there was a tendency to produce more fresh fruit than old fruit of the yield. Concentration ethanol affect the yield produced, concentration ethanol extract 70% tends to produce more higher than concentration ethanol extract 96%.

**Phenol content:** The studies was contrast with research Waghulde *et al.* (2011) who showed that ethanol extract takokak phenol content was 9,952 mg GAE/100 g and with methanol extract was 7,890 mg GAE/100 g. Phenol content ethanol extract 96% of fruit age fresh enough was 133.32 mg GAE/100 g, while phenol content ethanol extract 70% of fruit age fresh enough was 186.81 mg GAE/100 g. This studies was contrast with Kusirisin (2009) who showed that the phenol content ethanol extract was 16,030 mg GAE/100g. Phenol content ethanol extract 70% of dried fruit old age was 21.12 mg GAE/100 g and 96% ethanol extract was 47.15 mg GAE/100 g. The studies was in line research Arif (2011) who showed that methanol extract of phenol was 1960.7 mg GAE/100 g. And the studies Wetwitayaklung and Phaechamud (2011) showed that the phenol content of dry methanol extracts takokak was 2,330 mg GAE/100 g as galic acid. Phenol content of *Solanum aculeatissimum* was 2.540 mgGAE/100 g, phenol content *Solanum melongena* was 3.200 mg GAE/100 g, phenol content *Solanum mammosum* was 3.080 mg GAE/100 g, phenol content *Solanum wrightii* was 4.070 mg GAE/100 g, phenol content *Solanum stramonifolium* was 1.550 mg GAE/100 g and phenol content *Solanum trilobatum* was 3.250 mg GAE/100 g.

The mean levels phenol content of the ethanol extract *Solanum torvum* was not different from other *Solanum* species, the average levels of phenol content was 170.72 mg GAE/100 g.

Phenol content ethanol extract concentration 70% fresh fruit old age was 505.76 mg GAE/100 g and ethanol extract concentration 96% was 374.24 mg GAE/100 g. Phenol content ethanol extract concentration 70% fresh fruit young age concentration ethanol extract was 358.25 mg GAE/100 g and ethanol extract concentration 96% was 237.47 mg GAE/100 g. The results were in line with studies of Waghulde *et al.* (2011), Kusirisin (2009), Arif (2011), Wetwitayaklung and Phaechamud (2011). Likewise Nithiyanantham research (2012) who showed that the phenol content of fresh fruit takokak was 580 mg GAE/100g and phenol content 80% methanol extract was 500 mg GAE/100 g.

Phenol content ethanol extract dried fruit more lower than ethanol extract fresh fruit, because the ethanol extract dried fruit had a warming so that it caused decreasing levels of phenol. However, there was a tendency ethanol extract fresh fruit total phenol content

more higher than ethanol extract dried fruit. Likewise harvesting will affect the levels of total phenols. Phenol content of ethanol extract old fresh fruit more higher than total phenol of ethanol extract old enough fresh fruit and ethanol extract young fruit fresh age. Ethanol extract dried fruit harvest quite phenol content more higher than ethanol extract dried fruit old age.

**Antioxidant activity:** The average antioxidant activity of ethanol extracts concentration 70% was 741.4 mg AEAC/100 g and average antioxidant activity of ethanol extracts ethanol concentration 96% was 1.147,6 mg AEAC/100 g different extract fruits takokak from Kusirisin research (2009) who showed that the antioxidant activity was 368 mg TEAC/100 g or 360,000 mg AEAC/100 g. Antioxidant activity ethanol extract concentration 70% fruit dried old age and dried fruit old enough more higher than antioxidant activity ethanol extract young age fresh fruit, age enough fresh fruit and old age fresh fruit. In Table 3 showed that antioxidant activities ethanol extract concentration 96% more higher than antioxidant activity ethanol extract concentration 70%. In conclusion, ethanol extract concentration were significantly influenced the yield, total phenol and antioxidant activity.

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