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Phytochemical Screening, Total Phenolics, Total Flavonoids and Antioxidant Activity of Talas Steam Ethanol Extract *(Colocasia esculenta* (L.) Schott)

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Abstract: ***Talas stems (Colocasia esculenta (L.) Schott) are known to have antioxidant activity. Extraction of talas stems used maceration method with 96% ethanol. Determination of total phenolic content used the Folin-Ciocalteu method with a gallic acid comparison compound. Whereas the colorimetric method was used to determine total flavonoid content with 10% $AlCl_3$ reagent and 5% acetic acid. The antioxidant activity test applied DPPH (1,1-diphenyl-2-picrylhydrazyl) method with a quercetin comparison compound. The series of ethanol extract of talas stem were 20, 40, 60, 80 and 100 ppm. The comparison of quercetin was 2, 4, 6, 8 and 10 ppm. The results of total phenolic levels obtained were 78.98 mg GAE / g extract, total flavonoid levels were 97.38 mg QE / g extract and IC50 results of talas stem extract were 74.75 ppm, quercetin was 5.96 ppm. Based on the level of antioxidant strength, quercetin had very strong antioxidant activity (IC50 <50 ppm), because quercetin was a pure compound. While talas stem extract had strong antioxidant activity (IC50 50-100 ppm).***

Keywords: Talas, Total phenolic, Total flavonoids, Antioxidants

1. Introduction

Damage to cells and tissues which are the root of most diseases was caused by free radicals. Antioxidants are compounds that can inhibit free radicals in the human body, so cell damage caused by free radicals can be prevented. Compounds that can neutralize free radicals are antioxidants [1].

Phenolic compounds are compounds that are quite widely used. Its ability provides as a biological compound on human interests. One of the examples is as an antioxidant in preventing and treating degenerative diseases, cancer, premature aging and immune system disorders [2].

Largest phenol groups in nature. Flavonoids act as antioxidants by donating the hydrogen atoms or through their ability to chew metal as glucoside (containing glucose side chains) (Redha, 2010). Talas plants *Colocasia esculenta* (L.) Schott proved positive containing flavonoids and phenols which act as antioxidants [3]

2. Materials and Methods

The materials were talas stems obtained from Sumbaga Village, Bumijawa District, Tegal Regency, 96% ethanol, aquadest, toluene, ethyl acetate, 10% $AlCl_3$, 5% acetic acid, gallic acid, reagents Folin-Ciocalteau, 7.5% Na_2CO_3, quercetin p.a, DPPH p.a (1,1-diphenyl-2-picrylhidrazyl).

The tools used in the study were maceration jars, blenders (Maspion), buchner funnels (Desaga), micropipettes 50-200 µL (Socorex), centrifuges (Hettich), magnetic stirrers (Thermo Scientific), vacuum rotary evaporators (Butchi), waterbath (Memmert), UV-Vis spectrophotometer (Shimadzu UV mini-1240), analytical balance 0.1 mg (Mettler Toledo), test tube rack, and tools glassware (Pyrex-Germany).

2.1 Extraction

The making of talas stem ethanol extract was done by talas stem powder macerated with 96% ethanol at room temperature, then allowed to stand for 2 x 24 hours. The maceration process was carried out under tightly closed container at room temperature, after 2 x 24 hours the solution was filtered then the filtrate obtained was evaporated with a 40ºC vacuum rotary evaporator at a speed of 100 rpm. Viscous extract obtained was weighed to calculate its yield [4].

2.2 Phenolic Test (TLC)

The ethanol extract solution of talas stems was bottled on TLC plates and eluted using toluene: ethyl acetate (1: 4), then spotting was observed on a UV lamp and sprayed with iron (III) chloride ($FeCl_3$) reagent which showed black color [5].

2.3 Flavonoid Test (TLC)

The ethanol extract of talas stem was dissolved with ethanol, then it was bottled on the TLC plate, then the TLC plate was put in a chamber containing the toluene: ethyl acetate: ethanol (3: 3: 0.5) phase eluent, let it run completely. After that the spots were observed under UV light and with $AlCl_3$ showing a yellow color [5].

2.4 Determination of Total Phenolic Content

2.4.1. Determination of maximum wavelength

From the 1000 ppm gallic acid main solution, it was pipetted as much as 1 mL and diluted with ethanol 96% to a volume

of 10 mL resulting in a concentration of 100 ppm. From this solution, it was taken 0.5 mL, added 5 mL folin ciocalteau reagent (1:10), then vortexed and allowed to stand for 3 minutes. Then the solution was added with 4 mL of 7.5% Na_2CO_3 solution, vortexed and allowed to stand for 30 minutes. Then the absorbance was measured at a wavelength of 600-800 nm [6].

2.4.2. Determination of total phenolic of talas stem extract

Weighed 3 mg of talas stem extract dissolved with 10 mL 96% ethanol, pipetted as much as 0.5 mL then the sample was added with 5 mL of folin-ciocalteau reagent (1:10), thereafter added 4 mL of 7.5% Na_2CO_3 solution. The contents were mixed and incubated for 30 minute as operating time. Measure uptake at maximum absorption wavelengths [6]. Galic acid standard is used as a comparison made with concentration of 10; 15; 20; 25; 30 ppm [7].

2.5 Determination of Total Flavonoid Content

2.5.1. Determination of maximum wavelength

Quercetin solution was taken as much as 1 mL and put into a 10 mL volumetric flask, then 96% ethanol was added to the mark so that a concentration of 100 ppm was obtained. The solution was pipetted 1 mL, added with 1 mL of 10% $AlCl_3$ and 8 mL of 5% acetic acid, then homogenized and allowed to stand for 30 minutes. Perform reading with UV-Vis spectrophotometry at wavelengths of 350-450 nm.

2.5.2. Determination of total flavonoid of talas stem extract

Weighed 3 mg of talas stem extract dissolved with 10 mL 96% ethanol, pipetted as much as 1 mL then the sample was added with 1 mL 10% $AlCl_3$, added 8 mL of 5% acetic acid, mixed to homogeneous and let stand during 16 minute as a operating time (Bakti, et al., 2017). Absorbance readings were made at the maximum wavelength [6].

2.6 Antioxidant Activity Test

2.6.1. Determination of the antioxidant activity of the test solution

Determination of antioxidant activity was carried out by adding 1 mL of each concentration test solution of 20; 40; 60; 80; 100 ppm and comparator solution with concentration of 2; 4; 6; 8; 10 ppm into 4 mL of 0.05 mM DPPH solution. The contents were mixed and incubated for 30 minute as operating time [8]. The solution was then measured its absorbance at the specified maximum wavelength [9].

2.6.2. Determination of percent inhibition and IC_{50} values

The antioxidant activity was expressed in percent inhibition, described by the formula [10]:

$$\%\text{inhibition}=\frac{control.abs-sample.abs}{control.abs}x100\%$$

3. Result and Discussion

The samples used in this study were talas stems (*Colocasia Esculenta* (L.) Schott) obtained from Sumbaga Village, Bumijawa Subdistrict, Tegal Regency. Making talas stem extract was done by using the maceration method. Maceration method was chosen because it had the advantage of being able to attract active substances that were not heat-resistant, easy to do, and the tools used were simple [11].

Table 1: Yield Data of Talas Stem Extract (*Colocasia esculenta* (L.) Schott)

Sample	Extract Weight (grams)	Simplisia Weight (grams)	Yield (%)
Talas stem extract	9.66	500	1.93

Phenolic test used a comparator of gallic acid with eluent mobile phase, toluene: ethyl acetate (1: 4). TLC plates were eluted and stains were obtained which were then observed under UV light 254 and UV366, then sprayed with specific $FeCl_3$ reagents resulting from positive black stains. The observation results obtained Rf = 0.72 for gallic acid with black stain, and talas stem extract obtained two stains with Rf = 0.71 and Rf = 0.75 with black stain.

The flavonoid test used quercetin with eluent toluene: ethyl acetate: ethanol (3: 3: 3.5). TLC plates were eluted and stains were obtained which were then observed under UV light 254 and UV 366, then sprayed with specific $AlCl_3$ reagents. Based on the observation, it was obtained Rf = 0.87 for quercetin with yellow stain, and talas stem extract obtained Rf = 0.78 with yellow stain. These results indicated positively containing flavonoid compounds. Based on Ahmad's research (2015), Rf was 0.9. Separated components were good if the Rf was different at least 0.1 because each component had a typical Rf [12].

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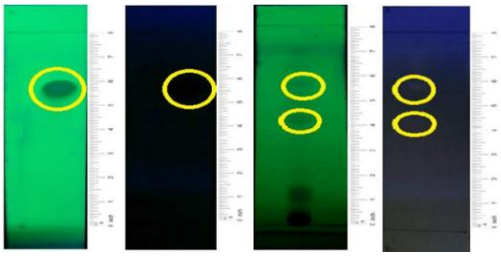
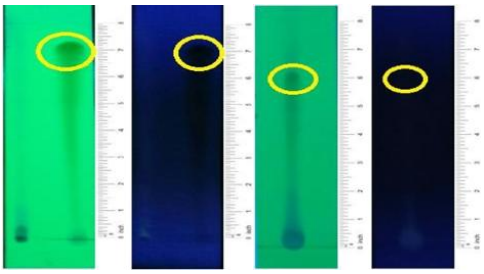


(a) (b) (c) (d)

Figure 1: Phenolic Identification Chromatogram
a. Detection of $FeCl_3$ spray reagents on UV_{254} galllic acid
b. Detection of FeCl3 spray reagents on UV_{366} galllic acid
c. Detection of $FeCl_3$ spray reagents on UV_{254} Talas stem extract
d. Detection of $FeCl_3$ spray reagents on UV_{366} Talas stem extract



(a) (b) (c) (d)

Figure 2: Flavanoids Identification Chromatogram
a. Detection of $FeCl_3$ spray reagents on UV_{254} quersetin
b. Detection of $FeCl_3$ spray reagents on UV_{366} quersetin
c. Detection of $FeCl_3$ spray reagents on UV254 Talas stem extract
d. Detection of $FeCl_3$ spray reagents on UV366 Talas stem extract

Determination of the maximum wavelength used gallic acid solution measured in the range of 600-800 nm it was obtained a maximum wavelength of 731.5 nm. Determination of total phenolic content was made in advance the standard curve of gallic acid standard solution with a concentration variation of 10: 15: 20: 25: 30 ppm. Based on the research results, total phenolic content of talas stem extract was 78.98 mg GAE/g extract.

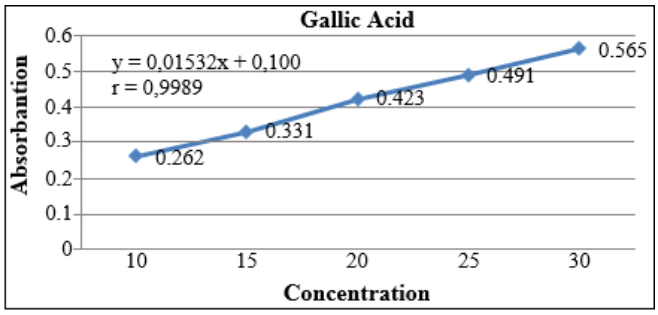


Figure 3: Gallic Acid Standard Curve

Determination of the maximum wavelength used quercetin, measured in the range of 350-450 nm (Das et al., 2014), the wavelength was 413.5 nm. Determination of total flavonoid levels was made in advance of a standard curve of quercetin standard solution with a concentration variation of 10: 20: 30: 40: 50 ppm. The standard solution was measured for its absorbance at a maximum wavelength of 413.5 nm and the operating time was 16 minutes. Based on the results of the study, an average level of total flavonoids of talas stem extract was 97.38 mg QE/g extract.

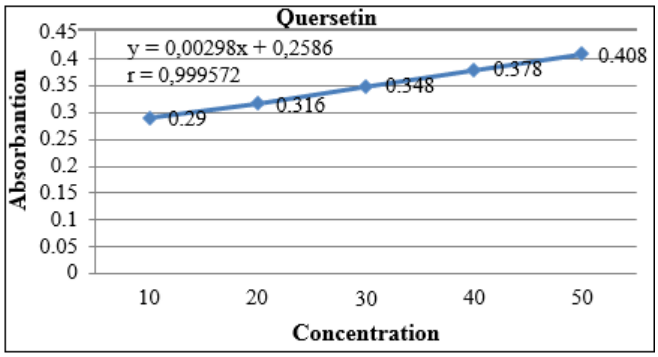
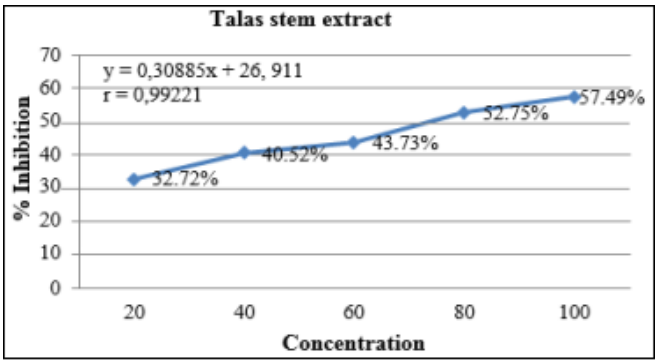
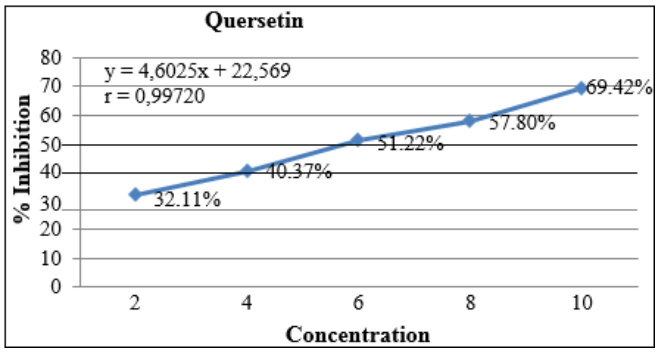


Figure 4: Quersetin Standard Curve

The antioxidant activity test of talas stem extract was carried out by DPPH method. DPPH color intensity can be measured at wavelengths of 400-600 nm [9]. The maximum wavelength obtained was 516.5 nm with an absorbance value of 0.611



(a)



(b)

Figure 5: (a) Graph showing correlation of quersetin with % inhibition, (b) Graph showing correlation of Talas stem extract with % inhibition

From the results obtained, the quercetin IC_{50} value was 5.960 ppm and IC_{50} talas stem extract was 74.75 ppm. Based on the level of antioxidant strength, quercetin had a very strong antioxidant activity (IC_{50} <50 ppm), while talas stem extract had strong antioxidant activity (IC_{50} 50-100 ppm). The high antioxidant activity of quercetin was caused by quercetin which was a pure compound, whereas talas stem extract consisted of various secondary metabolites interacting with each other to give rise to certain activities. One of the interactions among secondary metabolites was the reduction of certain activities [8].

Based on the test results, the strong antioxidant activity of talas stem ethanol extract was supported by high levels of phenolic compounds of 78.98 mg GAE/g extract and flavonoid levels of 97.38 mg QE/g extract. This was suitable with the theory by Al-Farsi et al (2007) that the higher the total phenolic and flavonoid values, the higher the antioxidant's ability to donate electrons in terms of suppressing the development of free radicals [13].

4. Conclusion

Based on the research results obtained, it can be concluded that:

1) Talas stem extract had a total phenolic content of 78.98 mg GAE/g extract, and a total flavonoid of 97.38 mg QE/g extract.
2) Talas stem extract had a strong antioxidant activity with IC_{50} value of 74.75 ppm.
3) The higher the total phenolic and flavonoid values, the higher the antioxidant's ability to donate electrons in terms of suppressing the development of free radicals.

References

[1] Pramiastuti, O. (2016). *Isolasi Dan Identifikasi Pinostrobin dan Pinocembrin dari Rimpang Temu Kunci (Bosenbergia pandurata (Roxb).(Schlecht) serta Uji Aktivitas Antioksidannya*. Yogyakarta: Universitas Gadjah Mada Press.

[2] Apsari, Pramudita Dwi., & Susanti, H. (2011). Penetapan kadar fenolik total ekstrak metanol kelopak bunga rosella merah (Hibiscus sabdariffa Linn) dengan variasi tempat tumbuh secara spektrofotometri. *Jurnal Ilmiah Kefarmasian*, 2(1), 73-80.

[3] Ogukwe, C.E., P.C. Amaechi, C.K. Enenebeaku. (2017). *Studies on the Flowers and Stems ofTwo Cocoyam Varieties: Xanthosoma sagittifolium and Colocasia esculenta. Nat Prod ChemRes*. DOI: 10.4172/2329-6836.1000263.

[4] Suryani., N.Y, Dewa G. M., Permana, A.A.G.N. Anom Jambe. (2015). *Pengaruh Jenis Pelarut Terhadap Kandungan Total Flavonoid Dan Aktivitas Antioksidan Ekstrak Daun Matoa (Pometia Pinnata).*Bali: Fakultas Teknologi Pertanian Universitas Udayana.

[5] Susanti, N. M. P., Dewi, L, Manurung, H. (2017). Identifikasi Senyawa Golongan Fenol Dari Ekstrak Etanol Daun Sirih Hijau (*Piper Betle* Linn.) Dengan Metode Klt-Spektrofotodensitometri.IV (1): 108-113.

[6] Sari A.K, dan Ayuchecaria, N. (2017). *Penetapan Kadar Fenolik Total danFlavonoid Total Ekstrak Beras hitam (Oryza sativa L) Dari Kalimantan Selatan*.Jurnal Ilmiah Ibnu Sina. 2 (2).327-335.

[7] Alfian, R., & Susanti, H. (2013). Penetapan kadar fenolik total ekstrak metanol kelopak bunga rosella merah (*Hibiscus sabdariffa* Linn) dengan variasi tempat tumbuh secara spektrofotometri.*Pharmaciana*, 2.

[8] Puspitasari, E., & Ningsih, I.Y. (2016). *Kapasitas Antioksidan Ekstrak Buah Salak (Salacca zalacca (Gaertn.) Voss) Varian Gula Pasier Menggunakan Metode Penangkapan Radikal DPPH*. 13(01), 1693-3591.

[9] Rastuti U dan Purwati. (2012). Uji Aktivitas Antioksidan Ekstrak Daun Kalba (*Albizia Falcataria*) Dengan Metode DPPH (*1,1-Difenil-2-Pikrilhidrazil*) dan Identifikasi Senyawa Metabolit Sekundernya. Program Studi Kimia, Jurusan Mipa, Fakultas Sains Dan Teknik, Universitas Jenderal Soedirman. *Molekul*, Vol. 7. No. 1: 33 – 42.

[10] Nurjanah, Laili Izzati, dan Asadatun Abdulla. (2011). *Aktivitas Antioksidan dan Komponen Bioaktif Kerang Pisau (Solen spp)*.Vol. 16.119-124ISSN 0853-7291.

[11] Wicaksono, I.B dan Ulfah, M.(2017).Uji Aktivitas Antioksidan Kombinasi Ekstrak Etanol Daun Sirsak *(Annona Muricata L.)* dan Daun Jambu Biji *(Psidium Guajava L.)* dengan Metode DPPH (2,2-Difenil-1-Pikrilhidrazil), Inovasi Teknik Kimia, Vol. 2, No. 1.

[12] Soeharsono, M. . (1989). Biokimia. Yogyakarta: Gadjah Mada University Press.

[13] Al-Farsi, M., Alasalvar, C., Al-Abid, M., Al-Shoaily, K., Al-Amry, M. & Al-Rawahy, F. (2007).*Compositional and functional characteristics of dates, syrups, and their by-products. Food Chemistry*, 104, 943-947.