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Research Article

Study of flavanoid determination of uwi sap extract (*Dioscorea alata.*L)

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ABSTRACT
Purple Uwi (Dioscorea alata L.) is a type of tuber which is a prospective local food plant and can be used as a source of functional food. The purpose of this study was to analysis the phenol and flavanoid content of uwi sap (Dioscorea alata.L) extract. This research was carried out by extracting the sap of Uwi and then screening thin layer chromatography (TLC) phytochemicals. The results of this study showed that the uwi sap ethanol extract and the total rendamen of uwi sap ethanol extract at a concentration of 96% gave the rendamen amount of 39%. The ethanol extract of uwi sap based on phytochemical screening thin layer chromatography produced flavonoids. By ethanol extract, uwi sap can be used as a candidate for antioxidants and antibacterial properties.

Keywords: uwi sap, flavonoids, thin layer chromatography, antioxidants

INTRODUCTION
Indonesia is a country that has natural wealth with various types of plants that can act as traditional medicines. Traditional medicine is increasingly in demand by the community because its vegetable ingredients are easy to obtain, easy to mix and the price is affordable, so that the ingredients used must be upgraded in quality and in accordance with community needs.
Uwi plants (Dioscorea alata L) can produce tubers which are classified by air bulbs and tubers found in the soil. Plant genus from *Dioscorea* has 600 species spread throughout the world [1]. Uwi tubers grow by means of vines and include monocot plants. Purple uwi tuber contains anthocyanins, and antioxidants besides that it has a solid gel / sap. The gel / sap in these tubers was identified to contain secondary metabolic components. Secondary metabolite compounds found in plants have antibacterial activity, which can inhibit bacterial growth with a mechanism that works in synergy. Many parts of plants that contain secondary metabolites have been reported, including the presence of phytochemicals phenols and flavonoids in the fruit of harendong (Melastoma af ne D. Don) [12] (Novalia et al, 2014). Phenolic components in the ethanol extract of akway bark (Drymis piperita Hook. F) [2]. Flavonoids in akway (Drymis piperita Hook. F) (Pladio & Villasenor, 2004). Flavanoid and phenolic phytochemicals are found in the tubers and leaves of Dioscorea alata. [1]. Phenolic components, flavonoids in teak leaves (Tectona grandis) as antimicrobials and antioxidants [3]. The secondary metabolite components in plants act as antimicrobials [4]. Flavonoids and phenols are one of the secondary metabolic components found in green plants and on generally found in the leaves, stems and fruit [5]. The mechanism of flavonoids and phenols as antimicrobials is that metabolic phenols have the ability to denaturate proteins and damage cell membranes. The content of flavonoids inhibits DNA synthesis and energy metabolism from bacteria [6]. The sap from the uwi tuber has not been widely used. So this study aims to identify the activity of the total phenol and flavanoid content of the uwi sap extract.

METHODS
Extraction
The extraction method for uwi tuber sap uses the maceration method [13] (Harbrone, 1996). Uwi tubers (Dioscorea alata. L) were obtained randomly from several areas in South Sulawesi. Sample preparation by cleaning the tubers from the soil, separating the tubers from the epidermis, the tuber meat then grated, soaking as much as 500 grams is carried out with ethanol based solvent. each treatment (96%, 80%, 65%, 50%) and maceration for 3 days. Filtrate filtering with Whatman 01 paper, concentrated ethanol filtrate with *rotary evaporator* at a temperature of 60-75

° C (± 2 hours). After evaporation the extract was weighed to determine the extract yield. Then stored in the refrigerator (± 4 ° C) in a light-tight bottle until used. The extract obtained was used for the test qualitative phytochemicals and antimicrobial activity tests contained in the uwi tuber sap / mucus extract.

Thin layer chromatography (TLC) phytochemical screening

The mobile phases used are butanol, aquadest, glacial acetic acid with a ratio (4: 1: 0.5) to separate the components. A total of 10 mg of the extract were dissolved in 1 ml of ethanol and then put in a dot capillary tube in the stationary phase with a distance of 2.5 cm from the bottom edge of the silica gel plate, then eluted as far as 5 cm in the chamber and dried. After drying, several reagents were sprayed for component identification [7]. Identification of the standard standard flavonoid compound by spraying the cytoborate reagent, a positive reaction was shown to form a yellow stain. Observations with visible light and blue color at UV 365 nm confirm the presence of flavonoids [8]. Identification of gallic acid standard phenol by spraying with FeCL3 5%. A positive reaction is indicated by the formation of black stains after heating [9].

Measurement of the total activity of phenol and flavonoid substances in the sap extract

Measurement of total phenol in uwi sap extract used the method [10]. A total of 1 mg / ml of uwi sap extract (10 mg in 10 mL of methanol). A total of 0.5 mL of extract that has been dissolved with methanol is taken, supplemented with 2.5 mL of 10% Folin Ciocalteu reagent dissolved in water, and added with 2.5 mL 7.5% NaHCO3. The blank used was a mixture of 0.5 mL of methanol, 2.5 mL of Folin Ciocalteu reagent dissolved in water, and 2.5 mL NaHCO3 7.5%. The samples were then incubated at 450C for 45 minutes. The repetition was done three times and the absorbance measurement was done at a wavelength of 765 nm. The same procedure is performed to construct a standard curve of gallic acid. Based on the absorbance measurement, the total phenol can be read from a standard curve, then the total phenol extract is shown in gallic acid equivalent (GAE) (mg / g). Total fenol GAE = c (V/m). Where: c = total phenol concentration from the standard curve of gallic acid (mg / L), V = volume of extract (L), m = weight of extract (g). Measurement of total flavonoids [11].

A total of 10 mg of extract was dissolved in 10 mL of distilled water, then as much as 5 mL of extract solution was taken and added with 0.3 mL of 5% NaNO2. The next step, the extract mixture was added with 0.3 mL of 10% AlCl3 which was dissolved with methanol and incubated at room temperature for 5 minutes. After incubation, 2 mL of 1 M NaOH were added and the volume was sufficient to 10 mL with distilled water. The absorbance measurement was carried out at a wavelength of 510 nm. Measurements were carried out 3 times and the determination of the total avonoid was expressed in catechin equivalent (CE) (mg / g) using the formula below. Total avonoid CE = c (V/m). Where: c = total avonoid concentration from the standard catechin curve (mg / L). V = volume of extract (L). m = weight of extract (g)

RESULTS AND DISCUSSION

Extract the purple uwi tuber

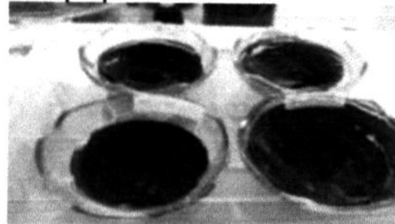

Fig.1: ethanol extract of uwi sap

The use of ethanol as a solvent is a compound that is polar and semi-polar. The nature of the solvent is dissolvent-like in that polar compounds dissolve in polar solvents, semi-polar with semi-polar solvents, as well as non-polar substances that dissolve in non-polar solvents. The ethanol solvent has a polarity index of 5.2 with soluble properties so that in extraction it can increase the permeability of the cell wall of simplicia and the extraction process becomes more efficient in attracting polar to semi-polar components [12]. Ethanol solvent is a solvent that has high polarity. Ethanol has two groups with different levels of polarity, namely a hydroxyl group which is polar and an alkyl group which is nonpolar. the two groups in ethanol causes ethanol to be used to extract compounds with different levels of polarity [13]. The use of water as an extracting solution combined with ethanol causes the ability The ethanol mixture of water in extracting is maximized, where water is a polar compound [14].

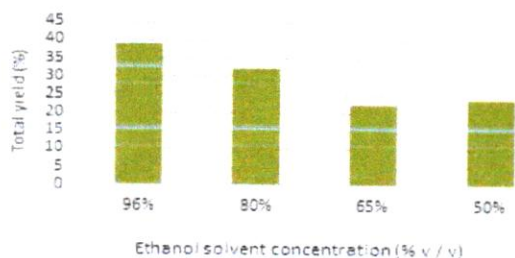

Fig.2: Histogram of yield of Uwi sap ethanol extract

The treatment of ethanol solvent concentration of 65% (k3) produced the lowest yield, namely (22.37). The results of the BNJ α 0.05 in Figure 3 show that the highest average yield was produced in the 3-day maceration treatment. (m3) namely (39.28). Based on the results of variance, it is known that the yield of maceration for 3 days shows the highest yield in 96% ethanol extract. Maceration time affects the yield rate of the extract, because the longer the material is in contact with the solvent, the greater the component that is attracted to the material. The yield is the effectiveness of the method maceration. explained that the longer the extraction time, the longer the material contact with the solvent will last up to both of which will occur by diffusion of mass deposition until equilibrium occurs, the concentration of the solution inside and outside the extract material. During the maceration process of molecules The solvent will first diffuse into the matrix of the extracted sample and then contact the compound and withdraw the compound according to the polarity of the solvent. The active substance components of flavonoids are polar, containing sugar group bonds. Phenols and tannins are polar, including the phenolic group and can dissolve in solvents and water [13].

Identify phenols and flavonoids

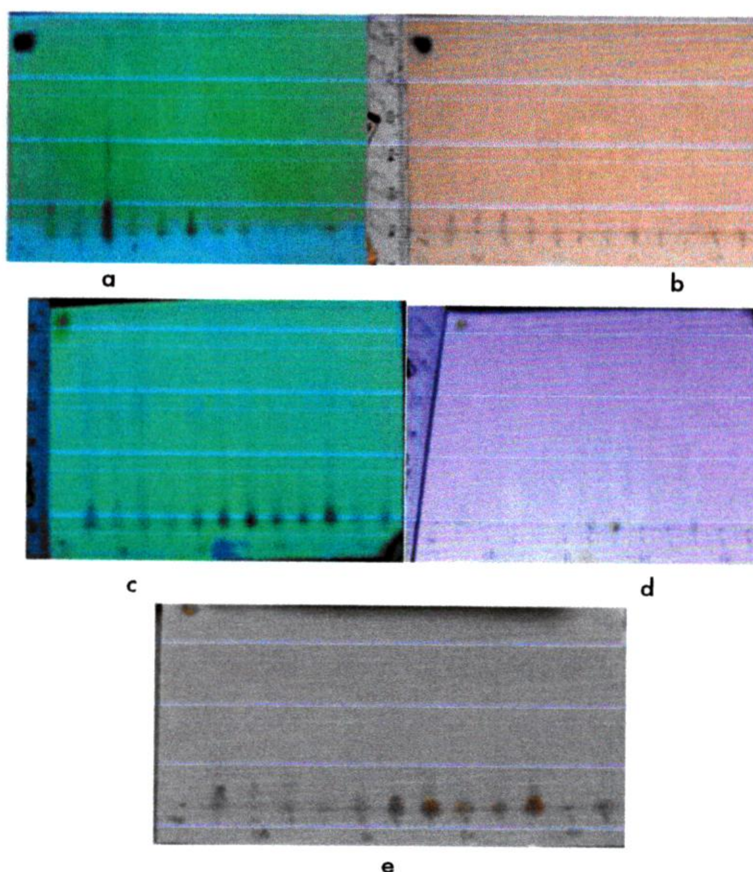

Fig 3: (a) identification of phenolic substances at UV 254 after spraying of FeCl3 reagent

(b) identification of phenol substances on UV 365 after spraying the FeCl3 reagent
(c) identification of flavonoids at UV 254 after spraying the sitobaorate reagent
(d) identification of the flavonoids at UV 365 after spraying the sitobaorate reagent
(e) identification of flavonoids after spraying of the sitobaorate reagent

Identification of flavonoids using a cytoborate reagent on the TLC plate, the results of the observations showed that at 256 nm UV and 365 nm UV there were several points that glow green under the UV light. After spraying the TLC plate with the cytoborate reagent several yellow dots appeared. The Rf value of 0.96 was found in extract 96% (1-3 days), extract 65% (1 day). Flavonoids can be detected with cytoborate reagents, a greenish yellow discoloration is formed on 366/365 nm UV observatio. explained that the addition of citroborate in detecting the presence of flavonoids will show a yellow to orange color change after spraying. Flavonoids and tannins are part of phenolic compounds. Flavonoids which have a hydroxy group which will give an intensive yellow fluorescence at UV 366, if reacts with boric acid. Flavonoids have various types and exist in free form (aglycone) or bound as glycosides. Polymethoxy aglycones are non-polar, polyhydroxy aglycones are semi-polar, while glycosides flavonoid is polar because it contains a number of hydroxyl groups and sugars [13].

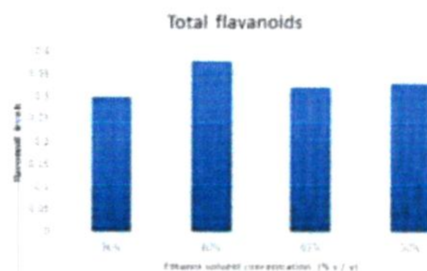

Fig.4:

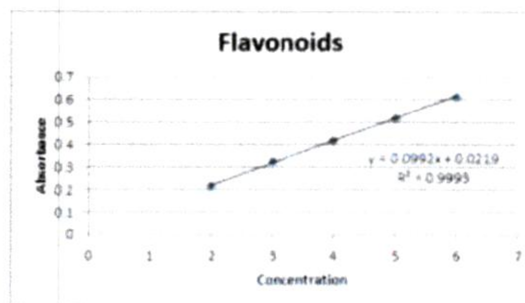

Fig.5:

Total flavonoids of Uwi sap ethanol extract

The total flavonoids in the sample used quartzetin as a standard solution with a concentration series. Concentration series is used because the method used in determining the content is a method that uses standard curve equations, to make a standard curve several concentration series are first made to get a linear equation that can be used to calculate the percent of content. Quartzetin is used as a standard solution because quercetin is a flavonoid in the flavonol group which has a keto group at C-4 and has a hydroxyl group on the C-3 or C-5 atom which is the neighbor of flavones and flavonols. The maximum wavelength absorption measurement was carried out running from a wavelength of 400 ± 450 nm. The result of running shows that the maximum wavelength of quartzetin standard is at a wavelength of 435 nm. The maximum wavelength used to measure the absorption of the uwi sap ethanol extract sample can be seen in Figures 4 and 5

From these measurements, it can be concluded that each concentration has a high absorbance obtained. The concentration of quercetin standard results obtained is plotted between the levels and the absorbance, in order to obtain a linear regression equation, namely $y = 0.0992x + 0.0219$ with the R2 value obtained is 0.9943. Quercetin calibration curve equation can be used as a comparison to determine the concentration of total flavonoid compounds in the sample extract.

CONCLUSION

Based on the results of the research that has been done, it can be concluded that the uwi sap contains flavonoids, therefore the uwi sap extract can be used as a candidate for medicine for health.

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Competing Interests: The authors declare no competing interests.

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