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Antioxidant Potential of Tempuyung (Shoncus Arvensis L.) Leaf Extract in Two Drying Methods

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ABSTRACT

Drying is an important step in maintaining the stability of compounds, so that proper treatment of extracts is needed to get metabolite compounds to have efficacy. This study aims to determine the effect of extract drying method on the antioxidant activity of tempuyung leaves (Sonchus arvensis L.). Extract drying method used in conducting this research there are two methods, using waterbath and the combination of waterbath and oven is set at a temperature of 40 ° C. Antioxidant activity was carried out using the 1.1 diphenyl-2-picrilhydrazil (DPPH) method. Phytochemical screening result showed that the two samples using the waterbath extract drying method and the combination of waterbath and oven contained flavonoids. The average IC50 value of the tempuyung extract drying method was obtained (72,37±37,78) extract by dryng method using a combination of waterbath and oven (79,6±8,39) vitamin C as a positive control (5,46±7,874). The result of the analysis using one way ANOVA obtained a significance p<0,05,so there is a not difference in the IC50 value between the group of the extract drying method. In conclusiom, there was a not significant difference in the value of antoksidan activity for each sample dryied by different method.

INTRODUCTION

Immunity is a reaction in the body against foreign materials that enter the body molecularly or cellularly. Molecules that can enter the body can occur through oxidation reactions this reaction can cause the formation of free radicals. Free radicals are molecules that do not have a pair in their outer electrons. Free radicals in normal amounts can be useful for inflammation and killing bacteria, whereas if they accumulate

excessively in the body, they can cause oxidative stress.

This state of oxidative stress can cause oxidative damage at the cell, tissue and organ levels. So that antioxidants are needed as an antidote to the antioxidation that occurs in these tissues. Antioxidants are compounds that can reduce the negative effects of free radicals.

Various plants in Indonesia have compounds that contain antioxidants. Antioxidants can be produced from compounds that have secondary metabolites. One of the plants that has secondary metabolites is the Tempuyung plant (Shoncus arvensis L.). Tempuyung (Shoncus arvensis L.) including the Asteraceae family ranks seventh as a potential medicinal plant in Indonesia which is used as an ingredient in traditional and modern medicine. Tempuyung is a gummy plant, grows wild in the open protected from sunlight and its reproduction is spread. Secondary metabolites contained in tempuyung are flavonoids, phenols, alkaloids and saponins (Hapsaria et al., 2018).

According to research (Harun, Fitria, et al., 2019) compounds in tempuyung leaves that act as immunomodulators and diuretics are secondary metabolite compounds contained therein, besides that pharmacologically in research (Harun, Indriastuti, et al., 2019) Tempuyung leaves have the efficacy to treat appendicitis, hypertension, gout, hemorrhoids and facilitate urinary tract.

Flavonoid group compounds contained in tempuyung are in the form of glycosides, namely apigenin-7-glycoside and luteolin-7-glycoside (Manoi, 2015). according to research (Kusumawati et al., 2014) shows that phenolic compounds such as tempuyung flavonoids have very high antioxidant activity as free radical catchers by freeing hydrogen atoms from their hydroxyl groups. These phenolic compounds can be influenced by various factors, one of which is the drying method. The drying method can have an impact on the activity of certain herbal extracts (Bernard et al., 2014).

METHOD

This research was conducted at the Pharmaceutical Chemistry Laboratory of STIKes Muhammadiyah Ciamis.

Tools and Materials

The tools used are Uv-Vis Spectrophotometry, Waterbath, Oven, Analytical Scales, Beakerglass, Measuring Flask, Volume Pipette, Spuit, Measuring Cup, Stirring Rod, Drop Pipette, Thermometer, Blender. Aluminum Foil. The materials used in this study are Tempuyung Leaf Simplisia, vitamin C as a positive control, tempuyung leaf extract, Magnesium Powder, HCl, FeCl3, Mayer Reagent.

Research procedure

Includes sample preparation, extract preparation, phytochemical screening including flavonoid test, alkaloid test, tannin test, saponin test, and antioxidant activity test using waterbath and waterbath and oven combination samples of 1000 ppm each.

RESULTS AND DISCUSSION

Extract Quality Analysis

The results of the quality analysis of tempuyung (Shoncus Arvensis L.) leaf

extract using 2 drying methods (table 1) showed a yield of 7.8%.

Table 1. *Differences in extraction results*

No	Method	Simplisia Weight (g)	Weight of Viscous Extract (g)	Yield	FHI Standard
1	*W*	300 g	26,7 g	8,9 %	≥ 7,5%
2	*W* + O	300 g	23,4 g	7,8 %	

Table 2. *Organoleptic testing*

Organoleptic	Inspection Result	
	Waterbath	***Waterbath* + Oven**
form	Viscous Extract	Viscous Extract
smell	Typical Extract	Typical Extract
color	Brownish Black	Greenish Black
taste	Bitter	Bitter

According to the Indonesian Herbal Pharmacopoeia, the standard yield of the extract is not less than 7.5%. has met the standard of a good extract. Furthermore, organoleptic examination, this examination is carried out as one of the specific parameters determined using the five senses and aims to provide an initial introduction to the identification of extracts that indicate the characteristics of the extract (table 2).

In organoleptic testing of the two extracts there is a slight difference in the color of the extracts produced. For extracts with drying methods using waterbath, a brownish black color is produced, while for extracts using a combination of drying methods between waterbath and oven, greenish black extracts are obtained. This can occur because the drying of the extract using the waterbath method is left in open conditions so that the heat generated is unstable and the resulting temperature is not constant so that it affects the color of the extract, while for the condition of the extract made by the combination of waterbath and oven conditions are not too long open and when in the oven the conditions are closed so that the temperature is maintained and stable. These factors can affect the color difference in the extract.

Phytochemical Screening

In this study (table 3) phytochemical screening was carried out to test alkaloids, flavonoids, tannins and saponins. Phytochemical screening was carried out on each extract marked by the presence of sediment, foam and color change as a hint of a positive reaction occurred.

The results of the examination on the alkaloid test in tempuyung leaves were not identified because no reaction occurred when reacted with mayer reagent. Phytochemical tests obtained showed that

tempuyung leaf extract contained flavonoids.

Tabel 3. *Skrining fitokimia*

Test Compound	Reagents	Result		Description
		W	**W+O**	
Alkaloid	Mayer	-	-	No white/yellow precipitate
Flavonoid	Serbuk Mg ,HCl	+	+	putih/kuning W = Red orange W+O = Yellow
Tanin	Fe_3Cl	+	+	Blackish Green
Saponin	HCl	+	+	foam

Ket. (+) : Teridentifikasi W = *Waterbath*

(-) : Tidak Teridentifikasi W+O = *Waterbath* dan Oven

The extract added with magnesium powder and hydrochloric acid will cause the reduction of flavonoid compounds present in the tempuyung leaf extract (Shoncus arvensis L.) so that it will give a color change reaction to a red color which is characteristic of the formation of flavilium salts. The color change that occurs in the tempuyung leaf extract with the waterbath drying method produces a red color change so that the possibility of flavonoid groups is flavanone, flavanonol and flavanol, while for extracts with drying methods using a combination of waterbath and oven, a yellow color change is obtained, according to (Malik et al., 2018; Harun, Indriastuti, et al., 2019) the flavonoid compound complex contained in tempuyung leaves is a flavononol, flavonol, flanon and xanthone compound. In addition to flavonoids, tempuyung leaves are also identified as containing tannins, tannins as polyphenolic compounds that have a fairly high molecular weight and can form complex compounds with proteins, tannins have a compound structure consisting of a benzene ring (C6) which binds to a hydroxyl group (-OH) which has a biological role as a protein precipitator and metal chelator, following the reaction of tannin compounds with FeCl3. The following reaction occurs between tannin and FeCl3.

Phytochemical test using the addition of FeCl3 solution which will bind to one of the phenol groups present in tempuyung leaf extract (Shoncus arvensis L.). Tannins are polyphenol compounds, the change in blackish green color after the addition of FeCl3 indicates the presence of polyphenol compounds so that if the phytochemical test with FeCl3 then the possibility of polyphenol compounds is tannins. The formation of a blackish green color after FeCl3 is added is because tannins will form complexes with Fe3+ ions. In line with research (Desinta, 2015) that the presence of FeCl3 will bind to the phenol group and form a complex compound characterized by a blackish green color change.

Furthermore, tempuyung leaves (Sonchus arvensis L.) were also identified as having saponin content.

The positive reaction of saponin is characterized by the presence of foam after the addition of HCl. Saponins have glycosyl as polar groups and steroid groups or terpenoid groups as non-polar groups so

that they are surface active and form micelles when shaken with water in the micelle structure of polar groups facing out while non-polar groups face in and this situation is what causes the tempuyung leaf extract to look like foam where this foam shows that the extract contains saponin compounds (Simaremare, 2014).

Antioxidant Activity Testing

Determination of the maximum wavelength was chosen based on the determination of the wavelength that can give the maximum DPPH absorbance. Determination of this wavelength is done to get the most optimum absorbance value, when the compound gives optimum absorbance, the measurement has high sensitivity and linear so that a slight change in the concentration of the compound will give a big change in the resulting absorbance and the change in concentration is proportional to the change in absorbance of the resulting compound. The maximum wavelength of DPPH obtained from the measurement results obtained a wavelength of 507 nm.

From the results obtained Operating time obtained stable absorbance results at minute 15 to minute 30. Thus it can be concluded that DPPH can already react perfectly from minute 15 to minute 30. This is in line with research (Kusumawati et al., 2014) that the operating time obtained in DPPH testing to be able to bind to the sample is at minute 30.

Antioxidant activity testing was tested quantitatively using the DPPH method, which was measured using UV-Vis spectrophotometry. The maximum wavelength of DPPH obtained was 507 nm. The amount of antioxidant activity obtained was characterized by the IC50 value, which is the concentration of sample solution needed to inhibit 50% of DPPH free radicals. The IC50 value of each extract was determined using the linear equation of the relationship curve of sample concentration to percent inhibition using the equation Y = ax + b, sample concentration as the axis (X) and the percent inhibition value as the axis (Y). Antioxidant activity is expressed by the IC50 value, which is the concentration needed to produce a 50% decrease in DPPH activity, the smaller the IC50 value, the greater the antioxidant activity.

DPPH solution was added with test samples made in dilutions with concentrations of 10 ppm, 20 ppm, 30 ppm, 40 ppm 50 ppm and 60 ppm. The test samples with various concentrations were compared with the DPPH control (without the addition of samples) and analyzed based on the maximum wavelength obtained which was 507 and measured at the operational time at the 30th minute.

Based on the results of the study, it can be seen that the greater the concentration of tempuyung leaves, the greater the inhibition to counteract DPPH free radicals, this is indicated by the smaller absorbance value when the concentration increases. So there is a relationship between the concentration of the test sample and the increase in free radical silencing. The decrease in absorbance value indicates the antioxidant activity of tempuyung leaf extract.

Tabel 4. *Difference in IC_{50} values of tempuyung leaves using waterbath extract drying method, combination of waterbath and oven, and positive control*

Extract Drying Method	Value IC_{50} (ppm) $\bar{X}$±SD	P.*Value*
Waterbath Extract	72,37±37.78	
Waterbath +Oven Extract	79,6±8,39	0,013
Positive Control	5.46±7,874	

Based on the results obtained, the average IC50 value of Vitamin C as a Positive Control (5.46 ± 7.874). The IC50 value of tempuyung leaf extract samples using the waterbath method obtained an average (72.37 ± 37.78) and the IC50 value of tempuyung leaf extract samples using the combination method of waterbath and oven obtained an average value (79.6 ± 8.39).

The data in Table 4 shows that the drying method of extracts using waterbath and a combination of waterbath and oven has no significant difference in IC50 value. So for production needs, drying methods using waterbath or oven can be done as a good choice of drying method as long as the temperature used is measurable and stable against secondary metabolite compounds contained in herbal plants.

The antioxidant activity of tempuyung leaves is due to the high content of flavonoid compounds that have the ability to donate hydrogen atoms to produce free radical neutralization reactions caused by DPPH. In addition, the tannin content contained in tempuyung leaf extract also has antioxidant inhibitory activity against free radicals according to research (Sekarini, 2011) epigallocatechingallate in tannins is a flavonoid constituent compound that acts as the largest antioxidant in flavanol compounds. The saponin content in tempuyung leaf extract also has the ability to reduce superperoxide through the formation of hydroperoxide intermediates so as to prevent biomolecular damage by DPPH free radicals (Yuhernita & Yuniarti, 2011).

CONCLUSIONS

From the results obtained, tempuyung leaf extracts using the waterbath drying method and a combination of waterbath and oven contain flavonoids, and there is no difference in the effect of drying methods on the antioxidant activity of tempuyung leaves (Shoncus arvensis L.).

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