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Antioxidant Activity of Polyherbal Syrup Extracted by the Boiling and Decoction Methods

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Abstract:

Polyherbal syrup is a pharmaceutical preparation widely used and developed in several countries. This syrup contains more than one type of plant part intended to obtain more effective and powerful health properties. One example of a polyherbal syrup used in Aceh-Indonesia is the Polyherbal Syrup which contains water extract from Moringa leaves, turmeric rhizome and ginger rhizome. This study aimed to examine the antioxidant activity of polyherbal syrup prepared by boiling extraction method (BPS) and polyherbal syrup prepared by decoction extraction method (DPS), as well as the determination of phytochemical compounds in syrup. The results showed that BPS contains saponins, tannins and alkaloids, and DPS contains saponins and tannins. The antioxidant activity of BPS is included in the very strong category with an IC50 value of 0.441 g/mL, and DPS is included in the strong category with an IC50 value of 52.53 g/mL. The IC50 value of BPS was significantly different from that of DPS ($P<0.05$). Polyherbal syrup prepared by boiling had higher antioxidant activity than polyherbal syrup prepared by decoction.

Keywords—**Antioxidant activity, Boiling extraction, Decoction extraction, DPPH, Polyherbal Syrup**

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I. INTRODUCTION

Herbal remedies have been used for many ailments. Herbal medicine has many active constituents for many diseases, but proper knowledge must be required to prepare herbal formulations. Otherwise, the active constituents will be damaged. The public's interest in traditional medicine has been increasing in recent years, but traditional herbal medicine must be converted into modern medicine to increase patient acceptance [1].

Several polyherbal formulas have been developed in several countries. One is a polyherbal syrup containing Tribulus Terrestris, Astercanthalongifolia, Lagenariasiceraria, Cucumissativus, Cuminumcyminum, Hemidesmusindicus, Aervalanata, which according to folklore, is useful for urolithiasis or which has diuretic properties and has been proven effective [2]. Strong antidiabetic activity has also been reported from a polyherbal syrup containing aqueous extracts of Emblica Officinalis, Trigonellafoenum-graecum, Azaridicta Indica, Ocimum Sanctum Syzygium Cumini[3]. In addition to oral preparations, topical polyherbalof Tinosporacordifolia, Acoruscalamus, Coscinium fenestrated, Smilax china, and Terminalia chebulahave been developed into preparations for allergies, and give satisfactory results [4].

Indonesia is famous for its rich natural resources, both flora and fauna. Indonesia's natural wealth mainly comes from plants that are useful as ingredients for medicine, food, fruits, spices, and

others. Traditional Indonesian drinks are commonly produced by konventional manufacture and the herbal medicine industry. Most of these drinks are syrup, which is easy and efficient to use. These traditional drinks can empirically be used to treat various diseases, so they include functional foods [5].

In the last three years, one of the traditional drinks used by Banda Aceh, Aceh, Indonesia, is polyherbal syrup to maintain health and increase immunity. This polyherbal syrup is believed to maintain the body's vitality during the Covid-19 pandemic. The content in the syrup is moringa leaves, turmeric rhizome, and ginger rhizome. The ability of the polyherbal syrup to maintain a healthy body is suspected of coming from the phytochemical content that acts as an antioxidant.

Antioxidants are compounds that can help boost the immune system by binding to highly reactive molecules. Naturally, the human body has an antioxidant system to ward off free radicals sustainably. However, if the number of free radicals in the body is excessive, additional antioxidants are needed [6].

Moringa leaf extract contains flavonoids, tannins, terpenoids, alkaloids, and saponins[7]. Leaf extract with a concentration of 500 g/mL inhibited DPPH free radicals with a per cent inhibition of about 70%[8]. Turmeric and ginger rhizomes also contain high antioxidant compounds. Turmeric rhizome contains curcumin, the main active compound with a content of 7.798%. Ethanol extract of turmeric rhizome has antioxidant activity with an IC50 value of 41.95 g/mL[9].

The rhizome of zingiberofficinale contains gingerols, shogaols, and parasols. The total phenolic content in ethanolic and aqueous extract was 137.5 g/mg and 52.8 g/mg. The antioxidant activity of dry ginger methanol extract was 90.12%[10].

Drug syrup preparations can be made with aqueous extract, methanol, ethanol and other solvents. Water is the most readily available, inexpensive solvent with a higher level of safety. The extraction methods usually used to extract the extract are decoction, infusion, maceration, percolation, and soxhletation[11]. Decoction is an easy and fast method and is the standard method in the extraction method literature. Decoction uses temperatures up to 90oC but in a short time in order to avoid damage to the natural active substance[12]. The decoction method uses a special pot consisting of two pans. The first pot containing the plant parts is immersed in the second pot filled with water and then heated over the fire. This method prevents the plant powder from boiling and exposure to high temperatures.

In utilizing daily herbal plants, Indonesian people use the boiling method to extract plant extracts. The boiling method is done by boiling the plant parts in a pan and directly over the fire (100^{o}C), with a longer time of about 15 – 30 minutes.The extraction method will also affect the phytochemical compounds contained[2].

II. METHOD

The equipment used in this research is a hot plate, test tube, tube rack, measuring tube, volume pipette, funnel, filter paper, dropper pipette, watch glass, beaker glass, micropipette, and a set of UV-Vis spectrophotometry tools.

While the materials used in this study were Polyherbal syrup, DPPH (1,1-diphenyl-2-picrylhydrazil), aluminium foil, methanol p.a, ascorbic acid, n-hexane, ethyl acetate, ethanol, HCL (p), H2SO4 (p), magnesium powder, acetic anhydride, chloroform, FeCl3 5%, bismuth nitrate (p), KI, HgCl2, I2, amyl alcohol.

Table 1
Composition of Decoction and Decoction Polyherbal Syrup

Ingredient	Weight (%)
Moringa Leaf	0,2
Curcuma Rhizome	0,64
Ginger Rhizome	0,24
Palm Sugar	7
Sodium benzoate	0,05
Aquadest	100 mL

A. *Preparation of Boiling Method Extraction*

Weighed each ingredient according to the composition and worked separately. The plant

powder was extracted with 100 ml of distilled water, and boiled using a regular pan on the stove until the plant powder liquid boiled completely at 100°C temperature for 15 minutes. Then cooled and filtered with filter paper. Do the same for the three plant materials.

B. *Preparation of Decoction Method Extraction.*

Each ingredient was taken according to the composition (Table 1). The plant powder was mixed with 100 ml of distilled water and then put into the first pot. The first pot is put into the second pot of water. Both arrays were heated at 90□C for 30 minutes while stirring occasionally. Then cooled and filtered with filter paper. Do the same for the three plant materials.

C. *Preparation of Polyherbal Syrup*

Extract water from the decoction and boiling methods, add with other ingredients while stirring evenly and heat at a temperature of 50°C until well mixed.

D. *Phytochemical Identification*

1) Flavonoid Test

0.05 g of sample was added 0.10 mg Magnesium, 0.40 ml of amyl alcohol, and 4 ml of alcohol.A positive reaction for the presence of flavonoid is indicated by the red, yellow or orange colour formed on the amyl alcohol layer.

2) Saponin Test

Put 1 ml of the sample into a test tube, add 10 ml of water and heat for 2-3 minutes. Then refrigerate; once cool, shake vigorously for 10 seconds. The formation of a steady foam indicates the presence of saponins for not less than 10 minutes as high as 1-10 cm, and with the addition of 2 N HCl, the foam will disappear.

3) Tannin Test

Pipette 2 ml of aqueous extract from the sample into a test tube and add 2 ml of distilled water. Next, the extract solution was dripped with one or two drops of 1% FeCl3 solution. The presence of tannins is indicated by the appearance of a dark green or bluish-green colour.

4) Alkaloid Test

2 grams of sample wasadded 5 ml of 2 N HCl heated. Alkaloids analysis was carried out with three reagents, Mayer's reagent, Dragendrof and Wagner's. With Mayer's reagent, positive for alkaloids if it forms a white or yellow precipitate.With reagent of Dragendrof, positive for alkaloids if an orange precipitate is formed, and with reagent of Wagner's, positive for alkaloids if a brown precipitate is formed.

5) Steroid and Triterpenoid Test

Put 1 ml of the sample into a test tube, then add 2 ml of chloroform, ten drops of acetic anhydride and three drops of concentrated sulfuric acid (Liberman-Burchard reaction). The formation of a red-orange or purple colour for the positive presence of triterpenoids. A positive reaction for the presence of steroids is indicated by the formation of a blue or dark green solution.

E. *Polyherbal Syrup Antioxidant Activity Test*

Preparation of 50 ppm DPPH

A total of 50 mg of DPPH was dissolved with sufficient methanol, then put into a 50 mL volumetric flask, filled with methanol to the limit mark (1000 ppm). Next, 5 mL of a 1000 ppm solution was taken and then put in a 100 mL volumetric flask and filled with methanol to the limit mark (50 ppm).

F. *Absorbance Determination*

25 mg of BPS and DPS stock has weighed and dissolved in methanol, and then made up to 25 ml.. Further dilution was performed again by making five solution concentrations (50, 100, 150, 200, and 250 ppm). Each concentration was pipetted 1 mL with micropipette and put into a test tube. Added 4 mL of DPPH solution. The mixture was homogenized and left for 30 minutes in the dark and at room temperature. Absorption was measured with a UV-Vis spectrophotometer at 517 nm[13].

Measurements were carried out with three repetitions.

The per cent scavenging to DPPH was calculated using the following formula:

$$\%inhibition = \frac{(\text{Absorban blanko} - \text{Absorban sampel})}{\text{Absorban blanko}} \text{x} 100\% \quad \text{(Equation 1)}$$

Description:
Blank absorbance = DPPH radical absorption
Sample absorbance =Sample absorption in DPPH

Based on these calculations, a calibration curve was plotted between syrup concentration (µg/mL) versus the percentage of inhibition (%) to obtain the linear regression equation y = ax+ b. Determination of the IC50 value can be calculated using the following formula:

$$IC50 = \frac{(50-b)}{a} \quad \text{.....(Equation 2)}$$

Description:
Y = % Inhibition (50)
a = Intercept (the intersection of the lines on the Y axis)
b = Slope
X = concentration

III. RESULT

A. *Organoleptic Characteristics and Phytochemical Identification of Polyherbal Syrup*

A polyherbal syrup containing an aqueous extract of Moringa leaves, turmeric rhizome and ginger rhizome with the boiling method showed organoleptic properties with a yellow liquid dosage form, characteristic herbal odor, and sweet taste. Similarly, polyherbal syrup made by the decoction method has the same shape and taste. However, it is slightly different in colour, with a light yellow appearance than polyherbal syrup with boiling method (Table 2)

Table 2
Organoleptic Characteristics of Polyherbal Syrup

Organoleptic	**BPS**	**DPS**
Form	Liquid	Liquid
Colour	Yellow	light yellow
Odor	Herbal odor	Herbal odor
Taste	Sweet	Sweet

The phytochemical contained in the boiled polyherbal syrup and decoction have differences. BPS contains saponins, tannin and alkaloid. Only two phytochemicalwere contained in DPS, namely saponins and tannins (Table 3).

Table 3
Phytochemical Identification of Polyherbal Syrup.

Phytochemical	**BPS**	**DPS**
Flavonoid	-	-
Saponin	+	+
Tannin	+	+
Alkaloid	+	-
Steroid and Triterpenoid	-	-

Description:
+ = Contains a phytochemical
- = Does not contains a phytochemical

Based on the results of research conducted by Puspitasari et. al. (2018), it is stated that the boiling process at temperatures above 50□C can cause damage to flavonoid and terpenoid/steroid compounds[14]. This result supports the results of identifying flavonoid and terpenoid/steroid compounds in this study, where the results for boiled syrup and decoction were negative. These phytochemical compounds may still be present in the syrup but in very small amounts, so they are not visible during identification or have completely disappeared.

B. *Antioxidant Activity*

The concentration series used in this study aims to determine the level of colour reduction due to the presence of antioxidant compounds that cause DPPH radical colour changes from purple to faded

purple to yellow. The higher the sample concentration used, the lower the absorbance value. The decrease occurred because the test solution traps DPPH, and entrapment occurs due to compounds that react as radical scavengers that will reduce DPPH to form reduced DPPH-H [15].As shown in Fig. 1., it shows that the percentage of inhibition has increased with increasing the concentration of the syrup sample.

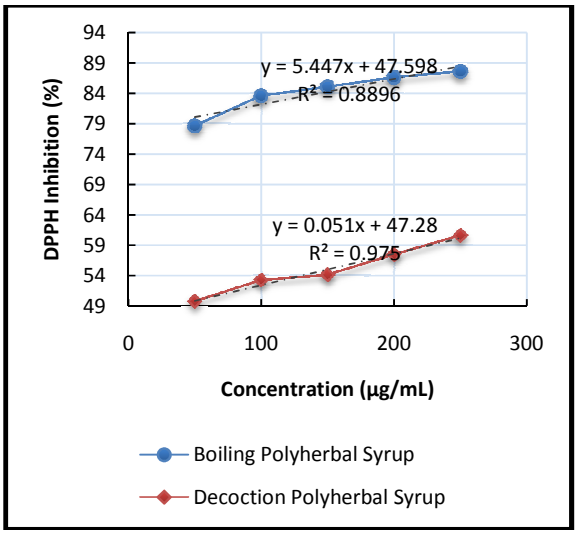

Fig. 1. Percentage Inhibition of BPS and DPS to Scavenging DPPH

The regression equation from BPS is Y= 5.4775x + 47.598 and R^2= 0.8896. Based on Equation 2, the IC50 value is 0.441 g/mL, which is included in the category of very strong antioxidant.

The regression equation of DPS is Y=0.0517x + 47.283 and R^2= 0.9757. Based on Equation 2, the IC50 value is 52.553 g/mL, which is included in the category of strong antioxidants (Table 4). Antioxidant activity is very strong if the IC50 value is <50 g/mL, strong if the value is 50-100 g/mL, while if the IC50 value is 100-150 g/mL, whereas if the IC50 value is 151-200 g/mL is said to be low in antioxidant and if IC50 is > 200 g/mL, the antioxidant activity is very low [16].

Table 4
Test results of antioxidant activity to reduce free radicals of Polyherbal syrup decoction and decoction (λ=517 nm, n=3)

Concentration (µg/mL)	Boiling Polyherbal Syrup			
	Mean Abs.	% Inhibition	IC_{50} (µg/ml)	Antioxidant activity
50	0,0742	78,62	0,441[a]	Very Strong (IC_{50} < 50 µg/ml)
100	0,0571	83,55		
150	0,0520	85,03		
200	0,0466	86,57		
250	0,0431	87,58		
Concentration (µg/mL)	**Decoction Polyherbal Syrup**			
50	0.1744	49.77	52,553[b]	Strong (IC_{50} =50-100 µg/ml)
100	0.1623	53.25		
150	0.1593	54.12		
200	0.1476	57.48		
250	0.1368	60.59		

The T-test results on IC50 values from BPS and DPS showed a significant difference in antioxidant activity ($P<0.05$).These results are influenced by the extraction method used. BPS was carried out at a temperature of 100°C, and the sample was boiled directly over the fire for 15 minutes. The possibility of attracting phytochemical compounds is more than that of DPS.

The efficacy of antioxidant compounds depends on structural properties, characteristics, temperature, concentration, the presence of synergistic and time. The efficiency of an antioxidant also depends on its concentration and localization in the system, for example, the distribution of the interface [15]. It was also reported that the antioxidant activity at extraction temperatures of 45, 60 and 100°C showed an increase of up to 68.06% of the inhibition per cent. The antioxidant activity decreased at the extraction temperature of 120°C to 53.42% [17].Likewise, as reported by other studies which state that extraction at a temperature of 60°C has no effect on DPPH radical scavenging[18].

IV. CONCLUSION

The polyherbal syrup prepared by the boiling extraction method (BPS) at 100°C had higher

antioxidant activity than the polyherbal syrup prepared by the decoction method at 90°C (DPS). The antioxidant activity of BPS was significantly different from that of DPS, where the content of phytochemical compounds in BPS was more than that of DPS. The antioxidant activity of BPS is in the very strong category, while the antioxidant activity of DPS is in a strong category.

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