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Research Article

DETERMINATION OF QUALITY PARAMETERS, TOXICITY TEST, ANTIOXIDANT ACTIVITY, AND α-GLUCOSIDASE INHIBITORY ACTIVITY OF 70% ETHANOL EXTRACT BUNGUR LEAVES (*LAGERSTROEMIA SPECIOSA* L. PERS.)

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ABSTRACT

Objectives: The objective of this study is to investigate quality of extract, toxicity test, antioxidant activity, and test the α-glucosidase enzyme inhibitory effects of extract of bungur (*Lagerstroemia speciosa* L. Pers.) leaves.

Methods: Extraction of leaves was conducted with maceration using 70% ethanol as solvent, followed by extract quality determination that involved extract-specific parameters. The toxicity test was performed by brine shrimp lethality test (BSLT) method, antioxidant activity by 1,1-diphenyl-2-picrylhydrazyl method, and the researchers used *in vitro* assay for α-glucosidase enzyme inhibitory effects.

Results: Organoleptic result of the extract showed that the extract has a thick consistency, greenish-brown color, spicy taste and has not a specific aromatic odor. Phytochemical screening result indicates the presence of the simplicia ethanol extract from bungur leaves containing flavonoids, saponins, tannins, steroids, and triterpenoids. The water-soluble content was 44.10%, ethanol-soluble content was 60.00%, loss on drying was 9.35%, water content was 7.30%, total ash content was 7.71%, acid-insoluble ash content was 0.64%, water-soluble ash content was 6.22%, ethanol residual content was 0.24%, Pb and Cd metals contaminant was 0.36 mg/kg and 0.10 mg/kg, microbial contamination of total plate number showed 4.32×102 colony/g, molds and yeasts number were ≤1×103 colony/g, BSLT toxicity test with IC50 value was 109.4 µg/mL, antioxidant activity with IC50 value was 26.5 µg/mL and had α-glucosidase enzyme inhibitory effects, with IC50 values of 78.6 µg/mL.

Conclusion: The standardized extract of bungur (*L. speciosa* L. Pers.) leaves are toxic, have antioxidant activity, and have α-glucosidase enzyme inhibitory activity.

Keywords: Determination of quality, Toxicity brine shrimp lethality test, Antioxidant, α-Glucosidase inhibition, Bungur leaves (*Lagerstroemia speciosa* L. Pers.).

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INTRODUCTION

Bungur leaves including Lythraceae family are purple flowers and are one of the nutritious plants as medicine in Indonesia. Bungur leaves contain chemicals, such as saponins, flavonoids, and tannins [1]. Empirically in the Philippines, tea made from bungur leaves (*Lagerstroemia speciosa* L. Press.) has been used as a beverage for the treatment and prevention of diabetes mellitus [2].

Lately often discussed the existence of foods or beverages labeled antioxidants, but around us many natural antioxidants that we get. Antioxidants are defined as inhibitors that work to inhibit oxidation by reacting with free radicals to form reactive free radicals that are not reactive and relatively stable. Meanwhile, the free radical is a compound or molecule containing one or more unpaired electrons in its outer orbitals. Hence, many possibilities that occur due to free radicals include cell function disorders, cell structure damage, autoimmune diseases, and degenerative diseases, to cancer [3].

One of the degenerative diseases is diabetes mellitus. Diabetes mellitus is a disease in which blood sugar levels increase. The approach is aimed at Type 2 diabetes mellitus by inhibiting the α-glucosidase enzyme. In addition, toxicity test conducted using brine shrimp lethality test (BSLT) method to determine the biological activity [4,5].

METHODS

Chemical and reagents

Simplicia bungur (*L. speciosa* L. Pers.), ethanol 70%, hydrochloric acid dilute P, aquadest, methanol, chloroform, silica gel, Karl Fischer reagents, ammonia 30%, hydrochloric acid 1:10, Dragendorff reagent, Meyer reagent, magnesium powder, hydrochloric acid (p), amyl alcohol, ferric chloride 1%, Stiasny reagent, sodium hydroxide 1 N, ether, anhydrous acetic acid, sulfuric acid (p), ammonia 10%, alcohol, sulfuric acid P, nitric acid 10%, potato dextrose agar (PDA), nutrient agar (NA), sodium chloride without iodine, egg shrimp *Artemia salina* Leach, 1,1-diphenyl-2-picrylhydrazyl (DPPH), Vitamin C, buffer phosphate pH 6,8, p-nitrophenyl-α-D-glucopyranoside, bovine serum albumin, dimethyl sulfoxide (DMSO), sodium carbonate 0.2 M, and acarbose were used [6].

Tools

Absorbance microplate reader EL×800, atomic absorption spectrophotometer, gas chromatography, ultraviolet-visible spectrophotometer, analytical scales, coarse scales, water bath, electric cooker, blender, desiccator, rotary evaporator, furnace, refrigerator, oven, brown bottle, pipette, micropipette, pipette volume, filler, stir bar, iron clamps, wood clamps, spatula, vials, Erlenmeyer, beaker glass, Petri dish, mortar and pestle, flask, weighing bottle, crucible, aluminum foil, ash-free filter paper, filter paper, and 18 watt TL lamp were used.

Sample collection

Sample of bungur leaves was collected from the Balitro, Bogor, West Java. The samples were identified in Research Center of Biology, Bogoriense Herbarium at Cibinong Bogor, West Java. Hot air-dried sample was processed for further analysis [7].

Preparation extract

Bungur leaves (500 g of simplicia powder) were extracted with ethanol 70% under maceration. The filtrate was concentrated with a rotary evaporator until obtained a thick extract then stored at 4°C for analysis.

Phytochemical screening [8]

The phytochemical analysis of bungur leaves' extract has been performed to find the presence of major secondary metabolites such as flavonoids, tannins, saponins, steroids, triterpenoids, glycosides, coumarins, anthraquinones, and alkaloids.

Specific parameters determination [9]

Organoleptics

Consistency and color of the ethanol extract were visually observed, while the smell was determined using sensory organs, such as nose and tongue.

Determination of water-soluble compound

1 g of extract was macerated for 24 h in 20 mL chloroform. Filter and evaporate 4 mL of the filtrate to dryness which has been preweighed, and heat the residue in an oven under a temperature of 105°C to reach a constant weight. Calculate the concentration in percent water-soluble compounds.

Determination of ethanol-soluble compound

1 g of extract was macerated for 24 h in 20 mL of ethanol (95%). Filter and evaporate 4 mL of the filtrate to dryness which has been preweighed, and heat the residue in an oven under a temperature of 105°C to reach a constant weight. Calculate the concentration in percent ethanol-soluble compounds.

Non-specific parameters determination [9]

Loss on drying

About 1 g of the extract was weighed in a careful way. Put the extract in a shallow capped weighing bottle, which has been heated under a temperature of 105°C for 30 min and dry the extract under a temperature of 105°C to reach a constant weight.

Water content

Water content was determined using Karl Fischer reactor.

Total ash content

About 2 g of extracts was weight in a careful way. Put the extract in the silicate crucible, which has been heated at 450°C until the weight is fixed and weighed. Calculate the ash content in comparison with the air dried extract.

Acid-insoluble ash content

Boil the ash that had been obtained in the measurement of total ash content by adding 25 mL chloride acid for 5 min. Collect the acid-insoluble ash. Filter using a ash-free paper filter, wash with hot water, and centrifuge the residue and paper filter under a temperature of 400–600°C to produce a constant weight. Acid-insoluble ash content was determined in comparison with the air-dried extract.

Water-soluble ash content

Boil the ash that had been obtained in the measurement of total ash content by adding 25 mL water for 5 min. Collect the water-soluble ash. Filter using a ash-free paper filter, wash with hot water, and centrifuge the residue and paper filter under a temperature of 400–600°C to produce a constant weight. Water-soluble ash content was determined in comparison with the air-dried extract.

Ethanol residual

Residual solvents determined with gas-liquid chromatography. Gas chromatography instrument equipped with a flame ionization detector and a glass column 30 cm×0.32 mm stationary phase flowed TR-WAX with a particle size of 100 mesh to 200 mesh. Used nitrogen P as a carrier gas.

Before used the column was conditioned ever night a temperature of 235°C, carrier stream with a slow flow rate. Set the carrier gas flow 20 mL/min and the injector and detector temperature, respectively, 200°C and 160°C.

Heavy metal contamination

Heavy metal contamination was determined using atomic absorption spectrophotometry method for assay of Pb and Cd.

Microbial contamination

Amount of 1.0 g of extract was weighed carefully examined or measured, put into 10 mL volumetric flask, add phosphate buffer (pH 7.2) up to 10 mL, mix. The obtained mixture was clear solution which was then analyzed for its total plate number.

a. Total plate number: After the procedure above is done (a 10^{-1} dilution) and then pipette 1 mL of sample that is mixed put 9 mL of phosphate buffer pH 7.2. It is a 10^{-2} dilution. Subsequent dilutions were made to 10^{-6}. From each dilution pipette 1 mL in to a sterile Petri dish and made triplo. Into each Petri dish poured NA 15–20 mL of seed medium (45±1°C) and then shaken and rotated until the suspension is spread evenly. Blanko made on the Petri dish filled with 1 mL of diluent and media in order. After the medium solidified, Petri dishes were incubated at 35–37°C for 24 h in the inverted position. The number of colonies that grew were observed and counted.
b. Molds and yeasts number: The procedure same with total plate number method using PDA as media.

Toxicity test by BSLT assay [10]

Filtered, artificial seawater was prepared by dissolving 38 g of sea salt in 1 L of distilled water for hatching the shrimp eggs. The seawater was put in a small plastic container (hatching chamber) with a partition for the dark (covered) and light areas. Shrimp eggs were added into the dark side of the chamber while the lamp above the other side (light) will attract the hatched shrimp. 2 days were allowed for the shrimp to hatch and mature as nauplii (larva). After 2 days, when the shrimp larvae are ready, 4 mL of the artificial seawater was added to each test tube and 10 brine shrimps were introduced into each tube. Thus, there were a total of 30 shrimps per dilution. Then, the volume was adjusted with artificial seawater up to 5 mL per test tube. The test tubes were left uncovered under the lamp. The number of surviving shrimps were counted and recorded after 24 h. Using Probit analysis, the lethality concentration (LC_{50}) was assessed at 95% confidence intervals. LC_{50} of <100 ppm was considered as potent (active). As mentioned by Meyer [6], LC_{50} value of <1000 µg/mL is toxic while LC_{50} value of >1000 µg/mL is non-toxic. The percentage mortality (% M) was also calculated by dividing the number of dead nauplii by the total number and then multiplied by 100%. This is to ensure that the death (mortality) of the nauplii is attributed to the bioactive compounds present in the plant extracts.

Antioxidant activity with DPPH (free radical scavenging activity determination) [3]

The stable DPPH was used for the determination of free radical scavenging activity of the extracts. Different concentrations of each herbal extract were added, at an equal volume, to methanolic solution of DPPH (100 µM). After 15 min at room temperature, the absorbance was recorded at 517 nm. The experiment was repeated for 3 times. Vitamin C was used as standard controls. IC_{50} values denote the concentration of sample, which is required to scavenge 50% of DPPH free radicals.

Assay for α-glucosidase inhibitory activity [10-12]

The reaction mixture consisting 250 µL of p-nitrophenyl-α-D-glucopyranoside 10 mM, 450 µL of buffer phosphate pH 7.0 adding to flask contain 50 µL sample dissolved in DMSO at various concentrations (25–225 ppm). The reaction mixture was pre-incubated for 5 min at 37°C, and the reaction was start by adding 250 µL for α-glucosidase 0.025 U/mL. Incubation was continued for 15 min. The reaction

stopped by adding 1000 µL of sodium carbonate 0.2 M. Activity of α-glucosidase was determined by measuring release of p-nitrophenol with absorbance microplate reader with EL×800 at λ 405 nm used positive control of acarbose.

α-glucosidase inhibitory activity assay was calculated using the equation:

$$\% \text{ inhibition } = \frac{c - s}{c} \times 100\%$$

C=Absorbance of the enzyme activity without inhibitors
S=Absorbance of the enzyme activity with the addition of the samples tested ($S_1 - S_0$)
S_1=Absorbance of p-nitrophenol as a result of the addition of enzyme first
S_2=Absorbance of p-nitrophenol as a result of the addition of sodium carbonate first.

RESULTS AND DISCUSSION

Yield of extracts

Extraction was conducted using 70% ethanol solvent, and it is a polar solvent. Therefore, it is expected that most of the chemical component are soluble in it. The yield of extract is presented in Table 1.

Phytochemical screening

Phytochemical screening in this research aims to determine the group of chemical compounds of the extract bungur leaves. Results of phytochemical screening showed that the extract bungur leaf secondary metabolites contain flavonoids, saponins, tannins, steroids, and triterpenoids. Table 2 presents the result of phytochemical screening of extract bungur leaves.

Specific parameters determination

Specific parameter is quality determination that involved extract specific parameters, such as organoleptic, ethanol soluble content, water soluble content.

Organoleptic result of the extract has a thick consistency, greenish-brown color, spicy taste and has not a specific aromatic odor. Assay of water-soluble content showed the amount of an organic compound in the extract, while the assay of ethanol-soluble content showed the amount of organic compounds in the extract. The result showed that ethanol-soluble content (60.00%) greater compared to water-soluble content (44.10%), suggesting that extract of bungur leaves contains more polar compounds. Table 3 presents the result of specific parameters determination.

Non-specific test of extract parameter

Non spesific parameter is quality determination that involved extract non-specific parameters, such as loss on drying, water content, total ash content, acid not soluble ash content, water soluble ash content, ethanol residual content, heavy metals contaminant, and microbial contaminant. Table 4 presents the result of non-specific parameters determination. Loss on drying was determined to find the water content and evaporating compound in the extract after a gravimetric drying process in an oven under a temperature of 105°C. The results showed that loss on drying was 9.35%. Water content measurement was conducted to find water contained in the extract. The lower water content is more stable and the extract will be for a longer term. The result showed that water content was 7.30%. Measurement of total ash content and acid=insoluble ash content in the extract aimed to find mineral elements in the extract, this is known as on organic substance or ash. In the heating process in an oven under a temperature of 450°C, it was found that organic matters in the extract could be burn, while the an organic matters, such as ash, could not. Measurement of total ash content aimed to find mineral compounds, both physiological compound such as K and Mg, and non-physiological compounds such as pollutant, ash, and soil in the extract.

Table 1: The present yield of extract

Simplicia powder (g)	Amount of extract (g)	Yield (%)
500	58.98	11.79

Table 2: Phytochemical screening of extract bungur leaves

Group of chemical compound	Extract bungur leaves
Alkaloids	–
Flavonoids	+
Saponins	+
Tannins	+
Quinone	–
Steroid/triterpenoids	+/+
Essential oils	–
Coumarins	–

Table 3: Specific parameters determination

Specific parameters organoleptic	Result
Form	Thick extract
Color	Greenish-brown
Smell	Has not specific aromatic odor
Taste	Bitter
Measurement of soluble compound content	
Ethanol-soluble compound content	60.00%
Water-soluble compound content	44.10%

Table 4: Non-specific parameters determination

Non-specific parameters	Result
Loss on drying (%)	9.35
Water content (%)	7.30
Total ash content (%)	7.71
Acid-insoluble ash content (%)	0.64
Water-soluble ash content (%)	6.22
Ethanol residual content (%)	0.24
Heavy metal contamination	
Pb metal contamination (mg/kg)	0.36
Cd metal contamination (mg/kg)	0.10
Microbial contamination	
Total plate number (colony/g)	4.32×102
Molds and yeasts number (colony/g)	≤10

The result of the determination of residual solvent ethanol with gas liquid chromatography in extract obtained 0.24% ethanol content. The result of the determination still fulfilling the requirements of maximum residual solvent in the extract is <1%. The result showed that the extract obtained can be used as raw material preparation because it contains low levels of ethanol. The content of Pb and Cd in the extracts can be derived from the environment the plants grow and the production process. The content of heavy metals such as Pb and Cd in the body should be limited in number because it is dangerous for health. The content of Pb excess in the body can cause nerve damage, urogenital, reproduction, and hemopoitic, while the content of Cd excess in the body can cause nerve poisoning and organ damage one, for example, in kidney damage. The result showed levels of Pb in the extract of 0.36 mg/kg whereas Cd levels of 0.10 mg/kg.

The result showed the total plate number and mold and yeasts number in extract are 4.32×10^2 coloni/g and $\leq1\times10^3$ coloni/g. The presence of microbes in the extract may result from processing the samples into extract, the air, and the storage of extract. The low of microbial growth in the extract caused the compound in the extract has efficacy as antibacterial. Testing total plate number was used to determine the growth of bacteria mesophyll, while the testing mold and yeasts number was used to determine the present of the fungus.

Toxicity test by BSLT

70% ethanol extract bungur leaves were tested for toxicity by BSLT method. The work begins with hatching an egg *A. salina* Leach. After 24 h, the egg that has been hatched into nauplii moved to another place, and 24 h after that, nauplii can already be used as a test animal. Prepared nine vials for three concentration levels, 1000, 100, and 10 ppm as well as three vials for control. Next calculated mortality rate or mortality by comparing the number of dead larvae divided by the total number of larvae to obtain the LC_{50} value. From the calculation of LC_{50} value it can be seen that the level of toxicity of bungur leaves extract based on % death was 109.4 ppm. Hence, in testing, the toxicity of BSLT shows that 70% ethanol extract bungur leaves is said toxic with LC_{50} value <1000 ppm Fig. 1.

Antioxidant activity with DPPH

The antioxidant activity of 70% ethanol extract of bungur leaves used DPPH method to obatain IC_{50} value which is the antioxidant concentration required to scavenge 50% of free radical activity. An antioxidant test is performed because the antioxidant properties of a compound are linked to various diseases caused by oxidative stress. From this result obtained, IC_{50} extract value of ethanol 70% by 26.5 ppm. Vitamin C served as standard showed IC50 value of 2.2 ppm. Based on the results, 70% ethanol extract bungur leaves is said to have very strong category antioxidant activity, because 70% ethanol extract bungur leaves have IC_{50} value <50 ppm as shown in Table 5 and Fig. 2.

α-Glucosidase inhibitory activity

In 70% ethanol extract bungur leaves as test and acarbose as standard, each with the same concentration was tested the activity of inhibition activity of α-glucosidase enzyme with p-nitrophenyl-α-D-glucopyranoside as substrate. The extract inhibition test against α-glucosidase indicates the presence of antihyperglycemic activity of the extract. In this study, enzyme α-glucosidase will hydrolyze p-nitrophenyl-α-D-glucopyranoside into p-nitrophenol yellow. The inhibitory activity of bungur extract on α-glucosidase enzyme was determined from the uptake of p-nitrophenol formed and measured using ELX 800 absorbance microplate reader at λ 405 nm. Result of inhibit activity test of α-glucosidase enzyme IC_{50} extract value of bungur leaves is 78.6 ppm with acarbose as positive control equal to 50.0 ppm. Table 6 shows the IC_{50} value of the extract in comparison with acarbose. The results showed that 70% ethanol extract bungur leaves are said to have antidiabetic activity because it can inhibit α-glucosidase enzyme as well as acarbose. Table 7 shows the IC_{50} value of the extract in comparison with acarbose and Fig. 3 shows the inhibitory of α-glucosidase enzyme.

Table 5: IC_{50} value of the extract in comparison with Vitamin C

Chemical/extract compound	IC_{50} (µg/mL)
Vitamin C	2.2
Extract bungur leaves	26.5

Table 6: LC_{50} value of the extract

Chemical/extract compound	LC_{50} (µg/mL)
Extract bungur leaves	109.4

Table 7: IC_{50} value of the extract in comparison with acarbose

Chemical/extract compound	IC_{50} (µg/mL)
Acarbose	50
Extract bungur leaves	78.6

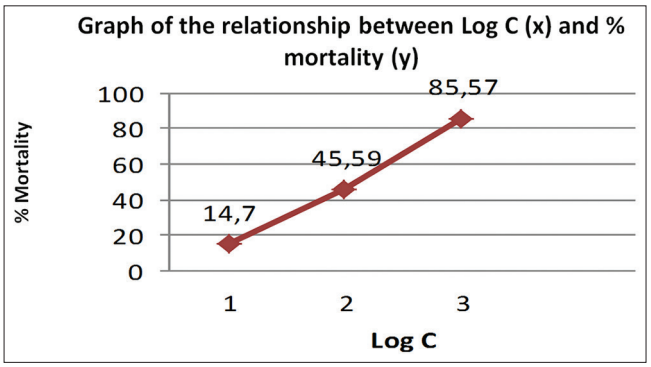

Fig. 1: Toxicity of extract Bungur leaves

CONCLUSION

1. Phytochemical screening result indicates the presence of the simplicia and ethanol extract from bungur leaves containing flavonoids, saponins, tannins, steroids, and triterpenoids.
2. Organoleptic results of the extract showed that the extract has a thick consistency, greenish-brown color, spicy taste and has not a specific aromatic odor. The water-soluble content showed 44.10%, ethanol-soluble content 60.00%, loss on drying 9.35%, water content 7.30%, total ash content 7.71%, acid-insoluble ash content 0.64%, water-soluble ash content 6.22%, ethanol residual content 0.24%, Pb and Cd metals contaminant are 0.36 mg/kg and 0.10 mg/kg, microbial contamination of total plate number showed 4.32×10^2 colony/g, and molds and yeasts number $\leq1\times10^3$ colony/g.

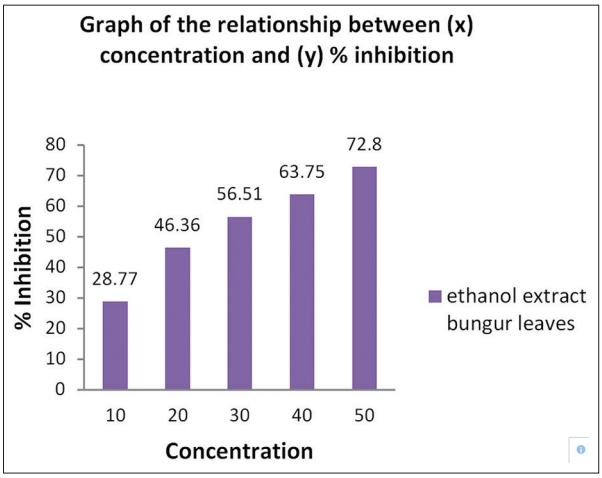

Fig. 2: Inhibitory of free radical

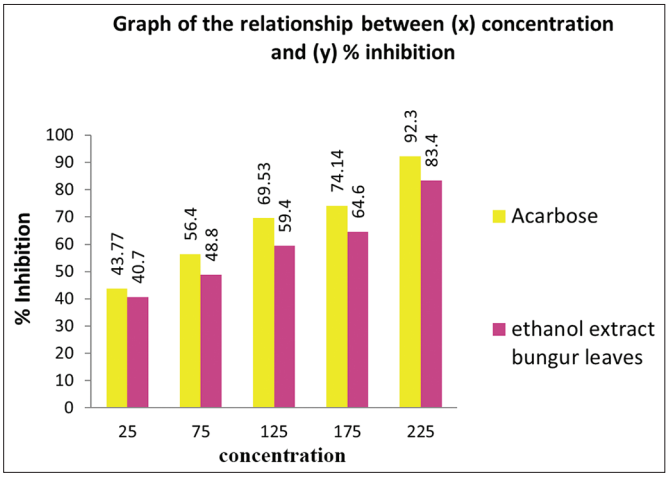

Fig. 3: Inhibitory of α-glocosidase enzyme

3. Extract bungur leaves had toxicity with LC_{50} value of 109.4 µg/mL, antioxidant activity IC_{50} value equal to 26.5 µg/mL, and α-glucosidase enzyme inhibitory effects, with IC_{50} values of 78.6 µg/mL.

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