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Antibacterial Activity Test, Evaluation of Pharmacognosy and Phytochemical Screening of Some Extract of Globe Amaranth (*Gomphrena globosa*)

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ABSTRACT

Indonesia is a rich biodiversity country where various medicinal plants have existed. One species of medicinal plants is Globe Amaranth (*Gomphrena globosa*, Amaranthaceae). This species is native to Central America and has been widely spread to the tropics. To date, the species can be easily found in the home gardens as an ornamental plant. Medicinal plants have been used for generations by traditional people. It was empirically proven that medicinal plants have the ability to cure certain diseases such as dysentery. All parts of this plant can be used as medicine. However, only the flower of the species was used in this study. The objective of the study was to identify the highest antimicrobial activity of *Gomphrena globosa* flower extract using ethanol, petroleum ether, ethyl acetate and n-butanol solvents. Gomphrena globosa flower was extracted using 96% ethanol and then was by partitioned using petroleum ether, ethyl acetate, and n-butanol respectively. The extracts were then evaporated using a rotapavor until condensed extract was obtained. Phytochemical screening was done on both of the flower powder and extract. The result of Pharmacognosy evaluation of the Globe Amaranth flower as follows: water content 8.17%, total ash content 9.11%, acid-insoluble ash 1.50%, acid-soluble ash 6.43%, water-soluble extract 10.79%, ethanol-soluble extract 3.51% and dry content 10.19%. The condensed extracts were tested for antimicrobial activity against *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Shigella dysenteriae*. Result of antibacterial activity test by agar diffusion method showed that the higher concentration of the extract led to higher bacterial inhibition zone. The highest antimicrobial activity was obtained from n-butanol extract as indicated by a significant inhibition zone around the paper disk.

Keywords: *Gomphrena globosa*, Pharmacognosy, Phytochemical, antimicrobial activity

INTRODUCTION

Indonesia is a tropical country which has a rich biodiversity of plants and animals that useful for health. Approximately 30,000 to 40,000 species of plants from Aceh to Papua, from lowland to highland can be used as medicine. The use of herbs as medicine has been carried out for generations even before modern medicine was found Empirical evidence showed that the plants have significant effect to prevent and cure the diseases.

Globe amaranth (*Gomphrena globosa* Amaranthaceae) is native to Central America and has been widely spread to the tropics. This species is a shrub with a single flower that came

out from the end of the stem, rounded shape like a ball, dark red-purple, white, or pink color. To date, the species can be easily found at the home gardens as an ornamental plant [1]. Another species of Gomphrena (*G. celosioides*) have been screened for the antimicrobial activity against microorganisms [2]. This plant has the expectorant effect to cure a cough, as anti-inflammatory and anti-dysentery agent. All parts of this plant can be used as medicine. According to Ferreres *et. al.* [3], this plant has a phenol content which acted as an antibacterial agent to cure dysentery. This disease caused by several bacteria species such as *Shigella dysenteriae.* Plants produce a multitude of organic compounds that have antimicrobial activity. The compounds are found in various plant parts such as stems, roots, leaves, bark, flowers or fruits and seeds and include alliin/allicins, isothiocyanates, plant pigments, hydrolytic enzymes, proteins, essential oils, and phytoalexins or phenolic compounds [4].

The objective of the study was to identify the highest antimicrobial activity of *Gomphrena globosa* flower extract against *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Shigella dysenteriae*.

EXPERIMENT

Source of plant

Mature flower of Globe Amaranth (*Gomphrena globosa* L.) was obtained from Cipanas, West Java- Indonesia

Sample preparation

A number of 200 grams of dried powder of Globe Amaranth was macerated at room temperature for 24 hours using ethanol 96% in the volume of 4000 ml. Macerate obtained was collected and filtered and then concentrated by rotary evaporator to obtain 11.43 grams of thick extract. The extract was then blended and then stored in sealed containers. A number of 9,500 g of thick extract was partitioned with petroleum ether, ethyl acetate, and n-butanol. The thick extracts were tested for the antimicrobial activity.

Total ash, acid insoluble ash, and water soluble ash were conducted to determine the purity level of Globe Amaranth flower. The symplicia was then burned in a furnace until the organic compounds were destructed and evaporated. The formed ash was calculated using the gravimetric method. To determine the level of organic and inorganic compounds of the Globe Amaranth, the symplicia was extracted using 96% ethanol and water-chloroform solvent.
Dry content of the symplicia was determined by gravimetric method using an oven with a temperature of 105ºC for four hours.

Phytochemical screening

Phytochemical screening was conducted on dry powder and condensed extracts of ethanol, petroleum ether, ethyl acetate and n-butanol phase of Globe Amaranth flower to determine the compound contained therein [5].

Antimicrobial activity test

Antimicrobial activity of Globe Amaranth flower was determined by agar diffusion method. The thick extract of the flower was tested against *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa,* and *Shigella dysenteriae* on the agar plate by measuring the formed inhibition [6,7,8]

RESULT AND DISCUSSION

Pharmacognosy parameter

Results of pharmacognosy parameter of Globe Amaranth flower is presented in Table 1. Determination of water content of the flower using distillation method showed that water content of the Globe Amaranth flower was 8.17%. The water content in the flower may affect the symplicia stability during storage or in the extract processing. Total ash content of the flower was 9.11%. Acid insoluble ash content of the extract showed that heavy metals level in the plant. Heavy metals occurrence in plants can be derived from inorganic fertilizer. A study conducted by Naser et. al. [9] on spinach and red amaranth indicated that the metal content increased at the early growing stage and fall during later stage of growth. Determination of dry content resulted in 10.19%. It means that the substance was a volatile compound.

Figure 1. Globe Amaranth (*Gomphrena globosa*) whole plant (A) and flower (B)

Tabel 1. Pharmacognosy of Globe Amaranth flower

No.	Pharmacognosy parameter	Value (%)
1	Water content	8.17
2	Total ash content	9.11
3	Acid-insoluble ash	1.50
4	Water-soluble ash	6.43
5	Water-soluble extract	10.79
6	Ethanol-soluble extract	3.51
7	Dry content	10.19

Extraction and partition of Globe Amaranth

The extraction process of 200 g Globe Amaranth flower resulted in 11.43 gr extract with the yield of 5.71%. Extraction process was done with ethanol because ethanol is a universal solvent where almost all compounds can be solved in ethanol.The result of ethanol extract partition of Globe Amaranth is presented in Table 2.

Table 2. Result of ethanol extract partition of Globe Amaranth

No	Solvent	Weight (g)	Color
1	Petroleum ether	1.7253	Green
2	Ethyl acetate	1.2267	Yellowish brown
3	*n*-butanol	1.3150	Reddish brown

The objective of extract partition process was to find target compounds in the samples based on the polarity level. An organic solvent used in the study was petroleum ether. Organic solvents have the ability to extract non-polar compounds. Meanwhile, ethyl acetate and *n*-butanol have ability to extract semipolar and polar compounds respectively.

Result of phytochemical screening test

Results of the phytochemical screening test of the dried powder and thick extract of Globe Amaranth flower is presented in Table 3. The objective of phytochemical screening test was to determine secondary metabolites content which has biological activity in these plants. Based on the phytochemical screening test there were several compounds such as steroids, triterpenoids, flavonoids, saponins, essential oils, and coumarin.

Table 3. Results of phytochemical screening of the dry powder and thick extract of Globe Amaranth flower partition.

No	Compounds	Dried powder	Ethanol extract	Petroleum ether phase	Ethyl acetate phase	*n*-butanol phase
1	Alkaloid	-	-	-	-	-
2	Flavonoid	+	+	-	+	-
3	Saponin	+	+	-	-	+
4	Tannin	-	-	-	-	-
5	Tannin galat	-	-	-	-	+
6	Quinon	-	-	-	-	-
7	Steroid/ triterpenoid	+/+	+/+	+/+	+/+	-/-
8	Essential oil	+	+	+	-	-
9	Coumarine	+	+	-	+	+

Note : + = positive, - = negative

The phytochemical screening test to ethanol, petroleum ether, ethyl acetate and n-butanol extract of Globe Amaranth resulted in steroid, triterpenoids, flavonoids, saponins, essential oils and coumarin compounds. The result of the phytochemical screening test of petroleum ether phase resulted in steroids, triterpenoids compounds, and essential oil. Ethyl acetate phase resulted in flavonoids. While n-buthanol thick extract contain flavonoids, saponins and coumarin compound. A study found that the species in Chinese herbal medicine was strongly inhibits tyrosinase activity which is important for skin pigmentation[10].

Antibacterial activity test

Test of antimicrobial activity of the extract Globe Amaranth flower (*Gomphrena globosa* L.) is performed with the agar diffusion method using paper discs by observing the inhibition zone is formed. Tests carried out on each viscous extract (ethanol, petroleum ether, n-butanol ethyl acetate). Microbes used (*Escherichia coli, Staphylococcus aureus,*

Pseudomonas aeruginosa, and Shigella dysenteriae). Anti-bacterial activity test result is presented in Table 4,5,6 and 7.

Table. 4 Results of the antimicrobial activity test of ethanol extract of Globe Amaranth flower

Concentration (ppm)	Inhibition zone in average (mm)			
	E. Coli	*S. dysenteriae*	*S. aureus*	*P. aeruginosa*
50,000	19.0	-	9.3	-
25,000	9.6	-	8.3	-
12,500	9.3	-	6.5	-
Positive control	16.2	28.8	13.4	7.2
Negative control	-	-	-	-

The experiment showed that all concentrations of ethanol extracts of Globe Amaranth flower used for antibacterial test results provide inhibition on *Escherichia coli* and *Staphylococcus aureus*. This was a partial inhibition zone, therefore, bacterial growth has remained within the inhibition zone. A larger inhibition zone of ethanol extract was obtained by *Escherichia coli*. It means that the ethanol extract was more active against *Escherichia coli* compared with *Staphylococcus aureus.* Meanwhile, the inhibition zone was not formed for bacteria *Shigella dysenteriae* and *Pseudomonas aeruginosa*. All compounds resulted from those ethanol extracts (alkaloids, flavonoids, saponins, steroids, triterpenoids, essential oils and coumarin) were also formed partial zones.

Table 5. Result of the antimicrobial activity test of petroleum ether phase of Globe Amaranth flower

Concentration (ppm)	Inhibition zone in average (mm)			
	E. coli	*S. dysenteriae*	*S. aureus*	*P. aeruginosa*
50,000	15.7	21.7	-	-
25,000	14.7	16.2	-	-
12,500	13.2	15.8	-	-
Positive control	16.2	28.8	13.4	7.2
Negative control	-	-	-	-

Petroleum ether phase provides inhibition for *Escherichia coli* and *Shigella dysenteriae* bacteria. The formed zone was a partial zone. The best inhibition zone was obtained for *Shigella dysenteriae* followed by *Escherichia coli*. *Staphylococcus aureus* and *Pseudomonas aeruginosa* bacteria provide no inhibition zone. All compounds resulted from that petroleum ether phase (steroids, triterpenoids and volatile oil) provides partially inhibition.

Table 6. Results of the antimicrobial activity test of ethyl acetate phase of Globe Amaranth flower

Concentration (ppm)	Inhibition zone in average (mm)			
	E. Coli	*S. dysenteriae*	*S. aureus*	*P. aeruginosa*
50,000	10.7	15.8	12.7	13.3
25,000	7.8	12.4	10.3	96
12,500	6.2	11.9	9.8	80
Positive control	13.3	29.2	1.42	6.7
Negative control	-	-	-	-

Ethyl acetate phase provides inhibition in all tested bacteria. However, the inhibition zone formed by *Shigella dysenteriae* was a partially zone. All compounds resulted from that ethyl acetate phase provides partially inhibition. In line with the study on *G. celosioides*, found that the ethyl acetate and methanol extracts of the plant displayed inhibition activities on *Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa, Escherichia coli* and *Salmonella typhi* [2].

Table 7. Results of the antimicrobial activity test of n-butanol phase of Globe Amaranth flower

Concentration (ppm)	Inhibition zone in average (mm)			
	E. Coli	*S. dysenteriae*	*S. aureus*	*P. aeruginosa*
50,000	14.2	14.8	14.6	13.7
25,000	13.6	12.8	13.3	12.7
12,500	10.3	11.8	8.1	10.6
Positive control	13.3	28.8	13.4	6.7
Negative control	-	-	-	-

The n-butanol phase provides inhibition zone in all tested bacteria (Table 7). All compounds resulted from that n-butanol phase formed clear zone around the paper disc on the agar plate. It means that n-butanol phase provides totally inhibition and can be used as an antibacterial.

CONCLUSION

Pharmacognosy parameter of Globe Amaranth (*Gomphrena globosa* L.) resulted in the water content of 8.17%, total ash of 9.11%, acid insoluble ash of 1.50%, water soluble ash of 6.43%, water soluble extract of 10.79%, ethanol soluble extract of 3.51%, and dry content of 10.19%.

Phytochemical screening test of Globe Amaranth flower extracts resulted in steroids, triterpenoids, flavonoids, saponins, essential oils and coumarin. Antibacterial activity test using agar diffusion method showed that the higher extract concentration resulted in a wider inhibition zone. N-butanol phase provides a total inhibition zone against the bacteria tested. It was evidenced by clearly visible zone around the paper disc. Antibacterial activity test needs to be done against other bacteria, molds, and yeasts to determine the inhibitory effect of Globe Amaranth flower extract against other microbes.

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