

PHYTOCHEMICAL SCREENING, TOTAL FLAVONOID, ANTIOXIDANT ACTIVITY, AND TOXICITY OF ETHANOL EXTRACT *Cleome gynandra* L. HERB

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ABSTRACT

Cleome gynandra L. is one of the plants that the people of Palu, Central Sulawesi use as food ingredients. This plant is empirically used in traditional medicine. This study aims to determine phytochemical constituents, total flavonoid, antioxidant activity, and toxicity of 96% ethanol extract of *C. gynandra* herb. *C. gynandra* herb extract was obtained by maceration. Phytochemical screening of the ethanol extract was carried out qualitatively according to the standard methods. Determination of total flavonoid using $AlCl_3$ then determined by Spectrophotometric UV-Vis. Antioxidant activity using the DPPH method and determined IC_{50} value. Toxicity test was assessed using shrimp lethality as an indicator of toxicity. Phytochemical screening showed 96% ethanol extract containing alkaloid compounds, flavonoids, saponins, steroids, and tannins. Total flavonoid of the 96% ethanol extract was $4,778 \pm 0,522$ mg QE/g extract. Antioxidant activity (IC_{50}) of the ethanol 96% extract was 189,455 μ g/ml. Lethal concentration 50% of the 96% ethanol extract was 472,648 mg/L (toxic). The results of this study indicate that 96% ethanol extract of *C. gynandra* herb has antioxidant activity and has the potential to be further tested as an anticancer activity.

Keywords: *Cleome gynandra* L., total flavonoid, antioxidant, toxicity

INTRODUCTION

Free radicals are reactive molecules produced from various processes in the body, such as metabolism, cell respiration, and inflammatory reactions. Free radicals not only come from the body, but can also be produced from environmental pollution, excessive exposure to ultraviolet light, gamma ray radiation, X-ray radiation, and cigarette smoke. At high concentrations, free radicals can oxidize cell components such as nucleic acids, proteins, fats, and DNA, so that they can initiate the onset of various diseases such as hypertension, atherosclerosis, neurological disorders, diabetes, asthma, aging and even cancer [1-2].

Antioxidants are compounds that can counteract and prevent damage caused by free radical compounds [3]. The source of antioxidants can be synthetic antioxidants and natural antioxidants. Synthetic antioxidants such as synthetic antioxidants such as Butylated hydroxyanisole (BHA) and Butylated hydroxytoluene (BHT) are limited because the results of animal studies indicate that these compounds can cause liver and kidney dysfunction, allergic reactions, and are carcinogenic. Therefore, now more and more research is conducted on natural ingredients that have antioxidant activity and are not toxic [4-5].

Flavonoids are the most common phenolic compounds, because they are widely distributed in plant tissues, and are responsible for giving color to plants. Flavonoids have a 15-carbon frame consisting of two substituted benzene rings connected by a three-carbon aliphatic chain [6]. Phenolic compounds can provide protection as antioxidants because phenolic compounds can react with reactive oxygen species (ROS) and eliminate their radical activity so that they are no longer harmful to human body cells [7]. These phenolic compounds can prevent heart disease, reduce inflammation, reduce the incidence of cancer and diabetes, and reduce the level of mutagenesis in human cells. The protection gained from consuming plant products such as fruits, vegetables and nuts is mostly related to the presence of phenolic compounds in these plants [8].

Cleome gynandra L. is known as *Bavo*a (Kaili tribe) by most of the people of Central Sulawesi, especially the Palu, Donggala, and Sigi areas using it as food (vegetables). This plant is known to contain beta carotene, vitamin C, and levels of calcium, magnesium, and iron. *C. gynandra* in Africa, empirically used to induce labor, increase lactation, increase blood, reduce migraines, vomiting, diphtheria, vertigo, headache, pneumonia, ear infections, abdominal pain, eyewash, and consumption of boys after circumcision [9]. This study aims to determine the antioxidant activity and toxicity of the ethanol extract of *C. gynandra* herb so that scientific information is obtained for the development of this plant in the field of health, especially pharmacy.

MATERIAL AND METHOD

Materials

C. gynandra herb; 96% Ethanol; Methanol p.a; Quercetin (Sigma); DPPH (2,2-Diphenyl-1-picrylhydrazyl) (Sigma), 10% Aluminum chloride; Potassium acetate; distilled water; sea water; and shrimp eggs (*Artemia salina*).

Instrumentation

UV-Vis Spectrophotometer (Cecil CE 7410); rotary evaporator (Eyela); micropipette; analytical balance; water bath, aerator; hatching lights; vial; and laboratory glassware.

Procedure

Plant Materials and Extraction Preparation

C. gynandra herb were obtained from a cultivated crop in Sigi, Central Sulawesi, Indonesia. This plant was identified by the Department of Biology, Faculty of Pharmacy, Airlangga University under the voucher number voucher number 80/UN28.1.28/BIO/2018. Simplicia powder of *C. gynandra* herb was macerated using 96% ethanol with a ratio (1:10 w/v) for 3x24 hours to obtain the filtrate. The filtrate is then concentrated by a rotary vacuum evaporator at 50°C and a water bath to get a dried extract.

Phytochemical Screening

Secondary metabolite phytochemical screening uses the standard method for qualitative testing of the content of alkaloids, flavonoids, saponins, steroids, and tannins [10-11].

Total Flavonoid Content

Total flavonoid content was determined using Aluminum chloride assay [12]. 10 mg of the extract was dissolved in 10 ml of 96% ethanol to obtain a concentration of 1000 ppm, as much as 0.5 ml of the test sample was added 1.5 mL of 96% ethanol, 0.1 ml of Aluminum chloride 10%, 0.1 ml of 1 M Potassium acetate, and 2.8 ml of distilled water. Incubated for 30 minutes, absorbance was measured using a UV-Vis spectrophotometer at maximum wavelength. Total flavonoid content was expressed as milligrams of quercetin equivalent per gram of dry extract (mg QE/g DE) via a quercetin calibration curve with a linearity range of 5-40 $\mu\text{g/mL}$ and $R^2=0.9677$ (Figure 1).

Antioxidant Activity

The antioxidant activity of the extract was measured based on the reducing activity of free radicals 2,2-Diphenyl-1-picrylhydrazyl (DPPH). The extract was dissolved in methanol at five different concentration variations (100-300 $\mu\text{g/mL}$). 1 mL extract was mixed with 1 mL 0.1 mM DPPH, incubated for 30 minutes at room temperature and dark area, then the

absorbance of the solution was measured using a UV-Vis spectrophotometer at maximum wavelength. Pure methanol is used as a blank solution and DPPH is used as a control solution [13]. The percentage of inhibition of DPPH radical from each sample solution concentration was calculated by the formula:

$$\text{Inhibition (\%)} = [(\text{Abs. control} - \text{Abs. sample}) / \text{Abs. control}] \times 100$$

The 50% inhibitory concentration (IC_{50}) was calculated by plotting the percentage of inhibition to the sample concentration (Figure 2). Quercetin is used as a standard (Figure 3).

Toxicity Test

A. salina eggs are placed in a container filled with filtered sea water. The eggs, continue to be given light for 48 hours until the hatch is perfect. Ethanol extract was dissolved in sea water to obtain concentrations of 50, 100, 200, 500, and 1000 ppm. The extract solution was then put into a vial and added 10 shrimp larvae, left at room temperature and the number of live larvae counted after 24 hours [14]. The test was carried out using three replications. The lethal concentration of 50% (LC_{50}) was determined using a correlation curve between the extract concentration of \log_{10} (x-axis) and the probit value (y-axis) (Figure 4).

RESULTS AND DISCUSSION

Phytochemical Screening

Phytochemical screening showed *C. gynandra* herb containing alkaloid compounds, flavonoids, saponins, steroids, and tannins (Table 1). These compounds are known to show medical and physiological activity. Alkaloids are reported to have antispasmodic and antibacterial activity [15]. Flavonoids are known to have antiviral activity [16-17], antioxidants, antiarterosclerosis, anti-inflammatory, prevent neurodegenerative diseases, and anticancer [18-20]. Saponins are known to have anti-inflammatory activity [21]. Steroids are reported to have antibacterial activity and are associated with sex hormones [22-23]. Tannins are reported to have antifungal, antibacterial, and antiviral activity [24].

Total Flavonoid Content

The results of the determination of total flavonoid of 96% ethanol extract of *C. gynandra* herb obtained levels of 4.778 ± 0.522 mg QE/g extract (Table 2). Flavonoids are natural antioxidant compounds, which have solubility in polar solvents such as water,

methanol, and ethanol. Flavonoid glycosides are easily soluble in water, methanol, and ethanol, whereas flavonoids of aglycones only dissolve in methanol and ethanol. This study uses 96% ethanol solvent. Based on the type of solvent used, flavonoid compounds extracted are flavonoid glycosides and aglycone flavonoids [25-26]. Research conducted by Do et al. [27] reported variations in concentration and type of solvents affecting total flavonoids and antioxidant activity. In the future it is necessary to do research on variations in concentration or type of solvent, to determine its effect on the total flavonoids of *C. gynandra* herb.

Antioxidant Activity

Antioxidant activity (IC_{50}) of 96% ethanol extract of *C. gynandra* herb was lower than quercetin. The antioxidant activity of *C. gynandra* herb was about 7 times lower than quercetin (Table 2). Research conducted by Ira, S. et al. [28] reported that extract from each part of the plant showed different antioxidant activities. The samples used in this study are aerial parts of the plant (stems, leaves, flowers, and fruit), so that it is necessary to study the antioxidant activity of each plant organ, including the roots, to determine the potential of each part of the *C. gynandra*.

Toxicity Test

Lethal concentration 50% (LC_{50}) of 96% ethanol extract of *C. gynandra* herb is 472,648 mg/L (Table 2). LC_{50} value <1000 mg/L, indicating that 96% ethanol extract of *C. gynandra* herb is toxic to *A. salina* larvae [29]. These results indicate that 96% ethanol extract of *C. gynandra* herb has the potential to be further tested as an anticancer activity. The activity was suspected due to the content of alkaloid [30] and flavonoid compounds [18,20] in the extract of *C. gynandra* herb.

CONCLUSION

C. gynandra has antioxidant activity and has the potential as an anticancer, so further testing is needed to develop this plant, especially in herbal medicine.

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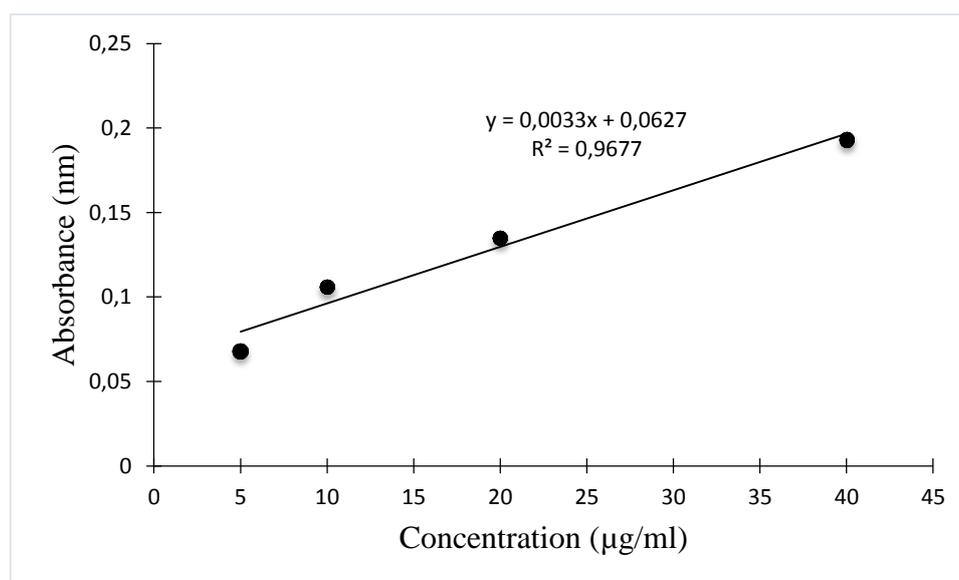
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Table 1. Phytochemical screening of 96% ethanol extract of *C. gynandra* herb

Phytochemicals	Test Results
Alkaloids	+
Flavonoids	+
Saponins	+
Steroids	+
Tannins	+

Table 2. Antioxidant activity, total flavonoid, and toxicity of 96% ethanol extract of *C. gynandra* herb

Sample	Antioxidant IC ₅₀ (%)	Total Flavonoid (mg QE/g ekstrak)	Toxicity LC ₅₀ (mg/L)
<i>C. gynandra</i> herb extract	189,455	4,778 ± 0,522	472,648
Quercetin	25,071		

**Figure 1.** Calibration curve for standard quercetin

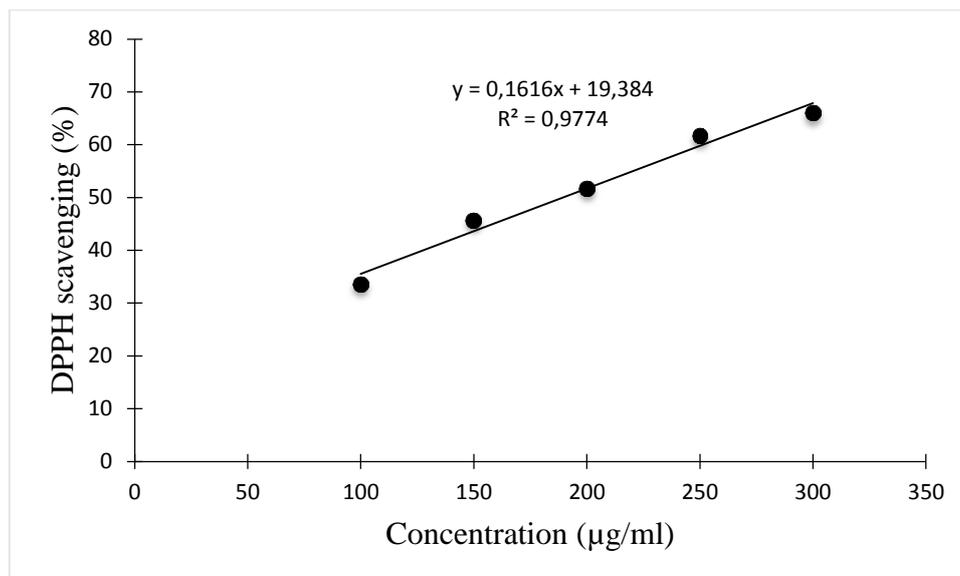


Figure 2. The DPPH free radical scavenging activity (%) of 96% ethanol extract of *C. gynandra* herb

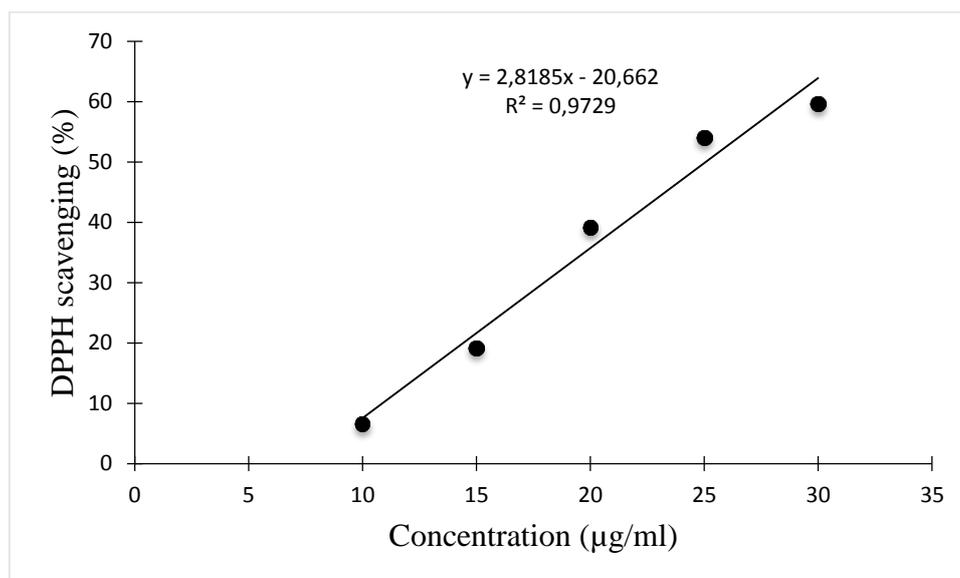


Figure 3. The DPPH free radical scavenging activity (%) of quercetin

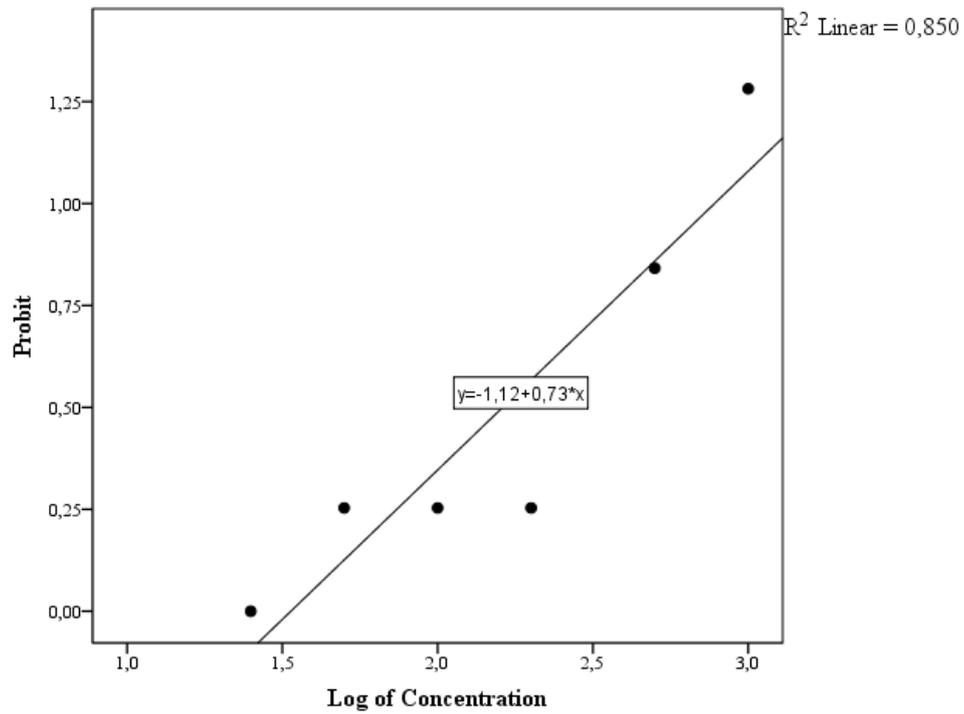


Figure 4. Relationship of log concentration of 96% ethanol extract of *C. gynandra* herb and the mortality of *A. salina* larvae