

Phytochemical Screening, Antioxidant and Cytotoxic Activities of Ethyl Acetate Extract of *Calotropis gigantea* Flower

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Abstract

The investigation of phytochemical constituents and biological activities of natural extracts holds considerable potential in pharmacological research. Bioactive components derived from plants are often utilized in the development of novel therapeutic agents. The present study aimed to evaluate the phytochemical profile, antioxidant activity, and cytotoxic potential of Calotropis gigantea flower extract. The extraction was carried out using ethyl acetate as the solvent through maceration for 72 hours. Antioxidant activity was assessed using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay, while cytotoxic activity was determined by the Brine Shrimp Lethality Test (BSLT). The ethyl acetate extract was found to contain alkaloids, flavonoids, tannins, and glycosides. It exhibited weak antioxidant activity (IC₅₀ = 329.74 mg/L) and moderate cytotoxic activity (LC₅₀ = 189.88 mg/L). These findings indicate that C. gigantea flowers possess diverse secondary metabolites with potential as antioxidant and anticancer properties.

Keywords: Antioxidant; BSLT; *Calotropis Gigantea*; Cytotoxic; DPPH.

Introduction

Indonesia is recognized as the third most biodiverse country in the world, with a wealth of flora and other biological resources. Plants have exhibited diverse medical advantages, one of that was the *Calotropis gigantea* plant (*C. gigantea*). *Calotropis gigantea* is an *Apocynaceae* family, commonly known as the giant milkweed or crown flower, is a tropical plant species contain phytoconstituents with therapeutic properties revered in traditional medicine systems across various regions of the world like cure boils, rheumatoid arthritis, gout, leprosy, infectious disorders and other inflammatory illnesses^{[1][2][3]}. This is supported by many bioactive compounds such as amyrin, alkaloid, phenol, sitosterol, giganterol, isogiganterol, flavonol glycosides, saponins, naphthalene, triterpenoids, tannins, sterols, and steroids^[4].

Calotropis gigantea has recently been scientifically confirmed for a variety of therapeutic qualities. it had shown antidiarrheal, anti-candida, antibacterial, and antioxidant properties of the plant leaves and aerial parts^{[5][6]}. The roots were reported to possess several activities like cytotoxic, antimicrobial, antipyretic, insecticidal, wound treatment, CNS, and load-blocking properties. Previous study reported that bioactive compound of *Calotropis gigantea* root bark from^[7]. Polyphenol chemicals have been reported to reduce the risk of emerging chronic diseases such as cancer, ageing, and cardiovascular diseases^[8]. Plants' antioxidant-rich bioactive components are particularly effective for cancer prevention by activating apoptosis of cancer cells^[9]. The lignan glycoside from *Calotropis gigantea* latex reported anticancer activity, and *Calotropis gigantea* L flowers were commonly used in antiquity medicine to treat diabetic mellitus, bronchial asthma, rheumatoid arthritis, and neurological diseases. The

extraction and characterization of bioactive components from *Calotropis gigantea* flowers revealed that they possess analgesic and antibacterial activities^[10]. However, all previous studies were reported the medical activity of *Calotropis gigantea* extracts with polar solvents. Herein, we will investigate with semi polar solvent (ethyl acetate). Ethyl acetate extract from the flower of *Calotropis gigantea* has never been reported for its antioxidant and cytotoxic activities.

This study aimed to screen the phytochemical constituents of *Calotropis gigantea* flower extract and to evaluate its antioxidant, cytotoxic, and therapeutic potential using ethyl acetate as the extraction solvent.

Experimental

Equipment

The instrument used in this research was UV-Vis Thermo Orion AquaMate 8000 spectrophotometer.

Material

This materials in this research are *Calotropis gigantea* flower that obtained from Benteng Inong Balee area, Lamreh Village, Masjid Raya District, Aceh Besar Regency. Chemicals are ethyl acetate (Merck), DPPH (2,2-diphenyl-1-picrylhydrazin), HCl, NaOH, distilled water, Alumunium Chloride AlCl₃, Iron (III) Chloride.

Methods

Extraction of *Calotropis.gigantea* flower

Dried powdered flowers of *Calotropis gigantea* (100 g) were macerated with ethyl acetate (1:5, w/v; 500 mL) in a glass container at room temperature for 72 h^[11]. The extract was filtered, and the solvent was evaporated at 40 °C using a rotary evaporator to obtain a concentrated extract. This extract was subsequently subjected to phytochemical screening, followed by evaluation of its antioxidant and cytotoxic activities.

Phytochemical Screening test

The ethyl acetate extract of *Calotropis. gigantea* flower was tested for alkaloids, saponin, steroids, terpenoids, flavonoids, tannins, glycosides, and phenols using a series of conventional phytochemical assays. All phytochemicals were systematically screened and identified using distinctive color changes in accordance with established procedures^[12].

Antioxidant activity test

The extracts' antioxidant activity was measured using the modified DPPH free radical scavenging test^[13]. A 100µM DPPH stock solution in methanol was produced. In methanol, the extract was produced at concentrations of 25, 50, 100, and 200 µg/ml. After adding 2.0 ml of 100µM DPPH to each extract, the mixture was incubated at 37 °C for 30 minutes in the dark. Absorbance at 517 nm was measured using a UV spectrophotometer, and the percentage of inhibition was determined using the equation: inhibition percentage = (Ao-At)/Ao x 100%. Ao = DPPH solution absorbance; At = test or reference sample absorbance. lastly, percentage inhibition was displayed against concentration, and IC₅₀ values were calculated using regression.

Cytotoxic activity test

Artemia salina eggs were hatched and allowed to grow for 48 hours prior to the brine shrimp lethality test (BSLT). For each assay, 10 larvae were transferred into test tubes containing saline water. The ethyl acetate extract was dissolved in DMSO to obtain concentrations of 100, 250, 500, 1000, and 2000 ppm. The test tubes containing larvae were maintained under continuous illumination from a tubular lamp. Control groups consisted of larvae without exposure to the ethyl acetate extract. After 24 hours of treatment, the numbers of surviving and dead larvae were recorded. Mortality percentage was determined as the average of three replicates, and the LC₅₀ value was calculated using a linear regression equation^[14].

Results and Discussion

Extraction and Phytochemical Screening

The initial step in assessing the therapeutic potential of any plant is the identification of its bioactive constituents, which provide insight into the natural compounds present^[15]. *Calotropis gigantea* is a notable source of pharmacologically active phytoconstituents with reported anticancer, antioxidant, anthelmintic, and other biological activities^{[3][16]}. Phytochemical screening of the ethyl acetate extract of its flowers revealed the

presence of alkaloids, flavonoids, tannins, and glycosides (Table 1). Flavonoids and other phenolic compounds of plant origin are widely recognized as valuable sources for pharmaceutical and medicinal applications, particularly for their antioxidant^{[17][18][19]} and anticancer properties^[20].

Antioxidant activity

The antioxidant activity of the extracts was determined by DPPH method and the results are presented in Table 2.

Table 1. Phytochemical screening result of *C. Gigantea* extract.

Secondary Metabolites	Result
Alkaloid	+
Flavonoids	+
Tanin	+
Triterpenoid	-
Steroid	-
Saponin	-
Phenol	-
Glycosides	+

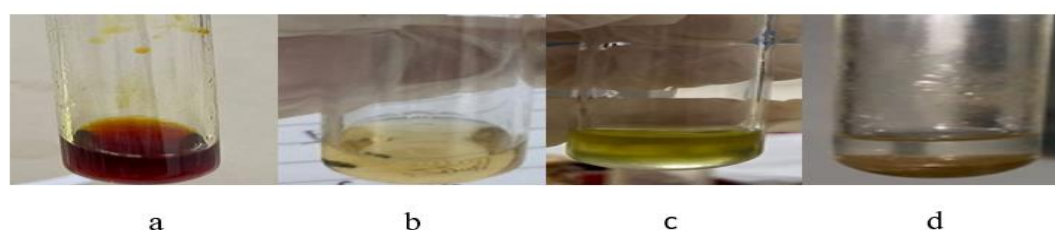


Figure 1. Phytochemical screening result of *Calotropis gigantea* extract (a) alkaloid (b) Flavonoids (c) Tanin (d) Glycosides.

Table 2. The percentage inhibition and the IC₅₀ Value of *Calotropis Gigantea* extract.

Sample	Concentration (mg/L)	% Inhibition	IC ₅₀ (mg/L)
Extract of <i>Calotropis gigantea</i>	200	32.80	329.74
	100	23.28	
	50	13.75	
	25	11.11	

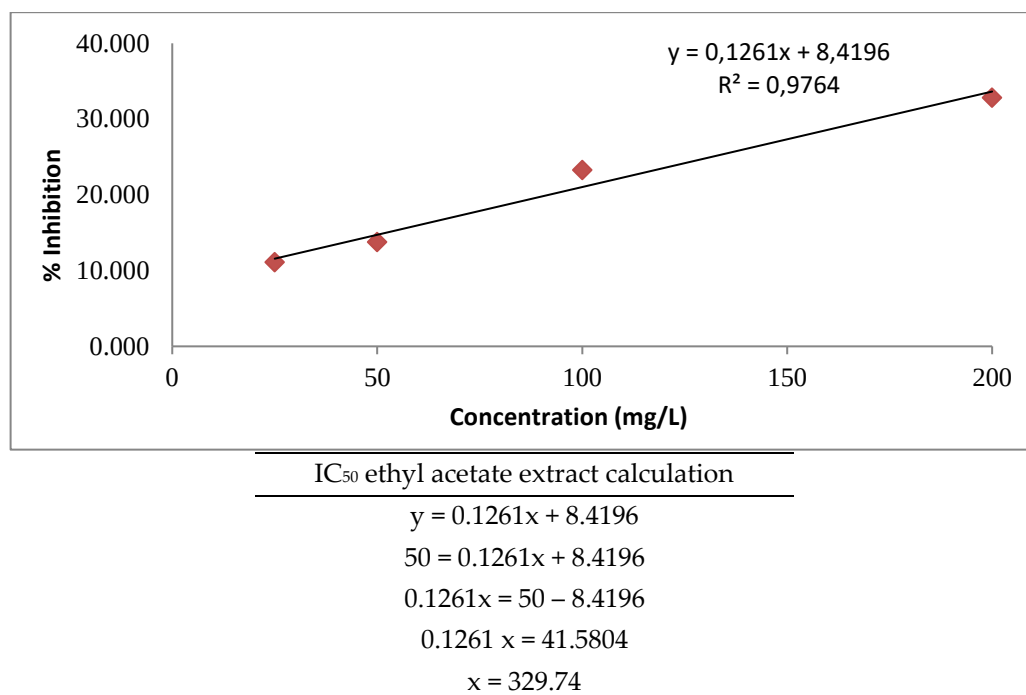


Figure 2. The percentage inhibition and the IC₅₀ value of *Calotropis Gigantea* extract.

The extract exhibited a concentration-dependent increase in inhibition percentage. The three paradigms employed are widely recognized for preliminary evaluation of antioxidant activity, as the radicals generated in these models are stable. Antioxidants interact with auto-generated DPPH radicals by donating protons, primarily through direct abstraction of phenolic hydrogen ions and electron transfer processes, thereby neutralizing the free radicals. This neutralization is visually confirmed by a color change from purple to yellow, indicating the scavenging potential of the antioxidants^[11]. Among reactive species, hydroxyl radicals are the most aggressive, as they initiate lipid peroxidation of cell membranes and can damage DNA and proteins through oxidative stress. Previous studies have shown their potential to cause lipid peroxidation and protein oxidation in living tissues^[21]. In the presence of the extract, the interaction with hydroxyl radicals produced a yellow coloration, further confirming its radical scavenging capacity. The ethyl acetate extract demonstrated a dose-dependent ability to reduce free radicals. Flavonoids and other phenolic compounds are key contributors to antioxidant activity, with their free radical scavenging potential primarily attributed to

their metal-chelating ability, which inhibits the conversion of hydroperoxides into reactive oxygen species^[22].

Calotropis Gigantea ethyl acetate extracts exhibited extremely weak antioxidant activity with IC₅₀ values of 329.74 mg/L. The Antioxidant activity is categorized as high (IC₅₀ < 50 ppm), moderate (50–100 ppm), weak (150–200 ppm), and extremely weak (>200 ppm)^[23]. The ability of the extract to function as a proton donor is lowly suggested by a high IC₅₀ value. The level of flavonoids and other phenolic compounds in plants vary among different part, tissue and age of the plant and is influenced by environmental factors^[24]. While ethyl acetate solvents may not always have strong antioxidant characteristics, this does not rule out the presence of antioxidant molecules in the extracts. Specific antioxidant substance may sometimes have a decreased solubility in ethyl acetate. In addition, the polarity of the solvent influences the extraction of antioxidant-rich phenolic compounds^{[25][26]}. The results of research by^[2] indicate that the methanol extract of *Calotropis gigantea* flowers has high antioxidant.

Cytotoxic activity

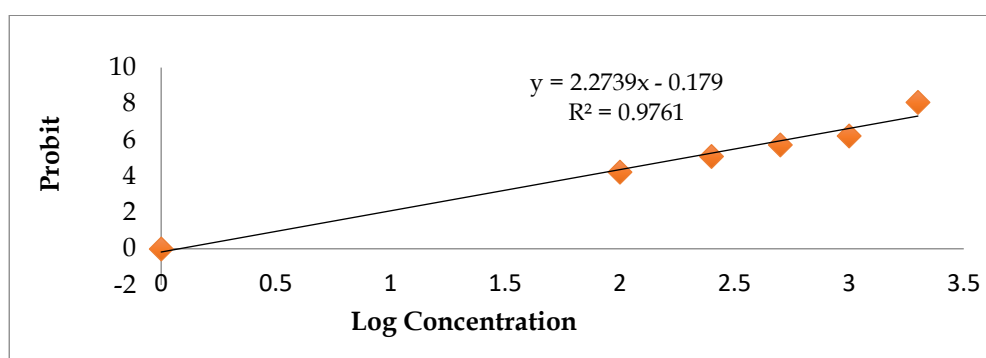
Anticancer drugs had the BSLT method cytotoxicity test, which is an important early screening assay for anticancer properties^[27]. The advantages of this method are simple, quick, reproducible, and shows an association with a particular anti-cancer agent^[28]. In this experiment, the effects on the death of *Artemia salina* nauplii are observed to vary with the content of the ethyl acetate extract of *Calotropis gigantea* flower.

Larval mortality was determined by recording the number of dead larvae in each vial at equal concentrations. Probit analysis using a

regression model estimated the LC₅₀ of the ethyl acetate extract of *Calotropis gigantea* at 189.45 mg/L. The LC₅₀ value was obtained through linear regression by plotting log concentrations against larval mortality probability, as illustrated in Figure 2.

The LC₅₀ values are categorized as moderate toxic to *Artemia salina* shrimp larvae, as shown in Table 3.

A substance's Lethal Concentration 50 (LC₅₀) is the concentration required to kill half of the examined organisms in a certain period. A lower toxicity level is associated with a greater LC₅₀.



LC₅₀ ethyl acetate extract calculation

$$y = 2.2739x - 0.179$$

$$5 = 2.2739x - 0.179$$

$$2.2739x = 5 + 0.179$$

$$2.2739x = 5.179$$

$$x = 2.2775$$

$$\text{LC}_{50} = \text{anti log } 2.2775$$

$$= 189.45 \text{ mg/L}$$

Figure 2. Probit Mortality Vs Log Concentration.

Table 3. The percentage mortality and the LC₅₀ Value of *C. Gigantea* extract.

Sample	Concentration (mg/L)	% Mortality	LC ₅₀ (mg/L)
Extract of <i>Calotropis gigantea</i>	2000	100	189.45
	1000	89.33	
	500	76.67	
	250	53.67	
	100	21.67	

According to the USA National Cancer Institute (NCI), LC₅₀ values ≤ 20 ppm are highly toxic, 21–200 ppm are moderate/moderately active, 201–500 ppm are weak/moderate, and ≥ 500 ppm are non-toxic^[29]. The results of the LC₅₀ value indicate that *Calotropis gigantea* flower extract has potential as an anticancer. This is supported by the presence of bioactive compounds contained. According to^[30] gigantini, a flavonol glycoside produced from *Calotropis gigantea*, has significant anticancer action. Furthermore, this bioactive compound has been shown to impede cancer cell migration and assault, indicating its potential as a treatment for invasive cancers^[31]. In addition, Calotropin, a cardenolide glycoside, was one of the most important bioactive chemicals discovered in *Calotropis gigantea*. Calotropin, a cardenolide glycoside, was one of the most important bioactive chemicals discovered in *Calotropis gigantea*. Calotropin has strong cytotoxic properties against a variety of cancer cell types, including breast, lung, and colorectal cancer cells. Calotropin causes apoptosis in cancer cells by altering a few signaling pathways, including the PI3K/AKT and MAPK pathways^{[32][33]}. *C. gigantea* compounds' anticancer efficacy is due to their capacity to alter multiple molecular pathways involved in carcinogenesis and tumor growth^[30].

Conclusions

The ethyl acetate extract of *Calotropis gigantea* flowers demonstrated the presence of tannins, flavonoids, alkaloids, and glycosides. The extract exhibited weak antioxidant activity (IC₅₀ = 329.74 mg/L) and moderate cytotoxicity (LC₅₀ = 189.45 mg/L) against *Artemia salina* nauplii. These results indicate that *C. gigantea* may serve as a potential source of bioactive compounds with pharmaceutical relevance, particularly as antioxidant and cytotoxic agents.

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None.

Declaration of Generative AI and AI-Assisted Technologies

The authors declare that no generative AI or AI-assisted technologies were used in the preparation of this manuscript.

Author Contributions

I.I.: Conceptualization, supervision, and corresponding author responsibilities. M.: Methodology and investigation. Y.A.L.: Data collection, formal analysis, and data curation. N.A.P.: Visualization, writing —original draft preparation, validation, and writing —review and editing.

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Declaration of Conflicting Interests

The authors declare no conflict of interest.

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