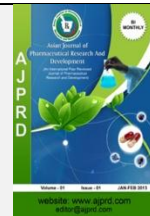


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Research Article

Phytochemical Screening and Assessment of Bioactivity of Pagoda Flower (*Clerodendrum paniculatum* L.) Using Brine Shrimp (*Artemia salina*) Lethality Assay

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ABSTRACT

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Objectives: The purpose of this study was to determine the bioactive content of the pagoda flower ethanol extract and measure its activity using the brine shrimp lethal dose method

Design: The design of this study was qualitative and quantitative experimental, where bioactive compounds of pagoda flower ethanol extract were tested by qualitative chemical analysis and brine shrimp lethal dose method was quantitatively calculated the number of deaths.

Interventions: The variable that was intervened in this study was the concentration of extract used.

Main outcome measure: The main measurement results in this study were to know the bioactive compounds contained in the extract and the value of Lethality Concentration 50% (LC₅₀).

Results: The results of phytochemical screening from the ethanol extract of pagoda flowers showed bioactive compounds such as alkaloids, flavonoids, steroids/triterpenoids, tannins, saponins, and glycosides. The value of LC₅₀ in the toxicity test for *artemia salina* was 49.415 ppm.

Conclusion: The pagoda flower ethanol extract contains alkaloid compounds, flavonoids, steroids/triterpenoids, tannins, saponins, and glycosides. Pagoda flower ethanol extract has strong potential activity.

Keywords: *Clerodendrum paniculatum* L., secondary metabolites, lethality concentration 50

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INTRODUCTION

The pagoda flower (*Clerodendrum paniculatum* L.) is a plant with the genus *Clerodendrum* which has 580 species variations spread across the continents of Asia, Africa, America and Australia. This plant is traditionally used as a medicine in Asia and Africa, including China, India, Korea, Thailand, Japan and Indonesia¹. The several studies that have been carried out on these types of plants include; anti-inflammatory activity², testing antibacterial activity in *C. paniculatum*³, anti-hepatotoxic activity in *C. inerme*⁴, hypotensive effects of *C. phlomidis*⁵, antidiabetic, antihypertensive and sedative *C. phlomidis* and *C. mandarinorum*¹.

The amount of research on pagoda flowers is the basis for bioactive screening and measurement of activity potential through toxicity testing using the brine shrimp bioassay method.

MATERIALS AND METHODS

Plant and Chemicals Materials

The pagoda used in this study was obtained from the Pancur Batu area in Deli Serdang Regency, North Sumatra, Indonesia. The part of the plant used is flowers. Pagoda flowers have been determined by the Herbarium Bogoriense Indonesian Institute of Science and it is known that the species is *Clerodendrum paniculatum* L. with Familia Lamiaceae.

The apparatus used in this study were mortar and pestle, sieves, maceration containers, analytical scales, aluminum foil, whatmann filter paper, spatulas, measuring cups, measuring flasks, beaker glass, test tubes, volume pipettes, rubber balls, water bath, and rotary evaporator.

The chemicals used in this study were ethanol, *n*-hexane, ether, H₂SO₄, HCl, amyl alcohol, isopropanol, CHCl₃, methanol, were obtained from Merck. Mayer, Dragendorff, Bouchardat, Liebermann-Burchard reagent,

Molisch reagent, Lead (II) acetate, Mg powder, FeCl₃ and dimethyl sulfoxide (DMSO).

Preparation of Ethanol Extract

Harvested pagodas are dried using a drying cupboard at 40°C for three days. The dried part of the plant is called simplicia. Simplicia which has been deemed dry and can be continued to be extracted is simplicia containing water content below 10%. Simplicia is then pollinated, then macerated using 96% ethanol for three days. The extract solution from the pagoda flower is separated from the pulp. The extract was then dried using a rotary evaporator until a thick extract was obtained⁶.

Phytochemical Screening

Identification of Alkaloids

The ethanol extract (500mg) was divided into 3 test tubes. Afterward, the tubes were added with one mL of hydrochloric acid 2N and 9mL of water, respectively. Next, heated over a water bath for 2 minutes, then cooled and strained. Then added to each tube 2 drops of Meyer, Bouchardat and Dragendorff reagents^{7,8}.

Identification of Flavonoids

The ethanol extract (500mg) was divided into 2 test tubes. After that, the tubes were added with 10mL of methanol, respectively. Next, refluxed using a cooler for 10 minutes. Filtered heat through multiple filter paper, diluted with 10mL of distilled water. After cold, 5mL of ether was added, stirring carefully, then let stand. The methanol layer is taken, evaporated at 40°C under pressure. the extract obtained was dissolved in 5mL of ethyl acetate^{7,8}.

The extract in the first tube was evaporated to dryness, then dissolved in 2mL of 96% ethanol, added 0.5 g of zinc powder and 2 mL of 2 N hydrochloric acid, left for 1 minute. Added 10 drops of concentrated hydrochloric acid, if within 2 to 5 minutes there is an intense red color indicating the presence of flavonoids (glycoside-3-flavonol)^{7,8}. The extract in the second tube was evaporated to dryness, dissolved in 1 mL of 96% ethanol, added 100mg of magnesium powder and 10mL of concentrated HCl, if red orange was formed until the red purple color indicated the presence of flavonoids. If orange yellow is formed, indicates the presence of flavones and chalcones^{7,8}.

Identification of Glycosides

The ethanol extract (3g) was putted into the erlenmeyer, then added 30mL of the mixture of 90% ethanol (7:3), concentrated sulfuric acid was added until the pH of solution 2 was obtained, then refluxed by using a ball cooler for 10 minutes, then cooled, then filtered. After that, 20mL of filtrate was taken, and then 25mL of water and 25mL of lead (II) acetate 0.4 M were added. Next, shaken and left for 5 minutes, then filtered. The filtrate was extracted 3 times with 20mL of a solvent mixture of chloroform: isopropanol (3:2), then two layers were obtained. In extracting, each organic solvent sodium sulfate anhydrous was added and filtered. Afterward, the filtrate was evaporated at no more than 50°C, then the residue was dissolved in 2mL of methanol, then divided into 3 test tubes^{7,8}.

The extract in the first tube was boiled in water and added a Liebermann-Burchard reagent, then a blue or green color was occurred that indicating the presence of glycosides^{7,8}.

The third tube of sample was boiled in water, then cooled and filtered. After that, added a lot of Fehling A and Fehling B solution and heated, then a red brick precipitate was formed that exhibit the presence of reducing sugar^{7,8}.

The extract in the second tube was evaporated over a water bath then added 2mL of water and 5 drops of Molish reagent. Next, added 2mL of concentrated sulfuric acid, then a purple ring was formed at the fluid boundary that indicating a sugar bond^{7,8}.

Identification of Saponins

The ethanol extract (500mg) was putted into a test tube. After that, 10mL of hot water was added, then cooled and shaken with vigorously for 10 seconds. If the compound was examined in the form of a liquid preparation, diluted 1mL of that liquid preparation with 10mL of distilled water and shaken vigorously for 10 minutes. A positive result was exhibited with a constant froth for no less than 10 minutes, as high as 1cm to 10cm then by addition 1 drops of HCl 2 N, the froth was not disappear^{7,8}.

Identification of Tannins

The ethanol extract (500mg) was extracted with 10mL of distilled water for 15 minutes. Then filtered, the filtrates were diluted with distilled water until they were almost colorless. Taken 2mL of filtrate, and then added 2 drops of 10% FeCl₃ solution. Note the color that occurs, blue or green indicates tannin, blue indicates 2 hydroxy groups in the aromatic ring of tannin^{7,8}.

Identification of Steroids and Terpenoids

The ethanol extract (1g) was added with ether or n-hexane, let stand for 2 hours, then filtered. Next, the filtrate was evaporated. The result of extraction was added anhydride acetic acid, then pressed concentrated sulfuric acid (Liebermann-Bouchardart reagent). Occurrence of red or red or turn blue green indicates a triterpenoids and steroids^{7,8}.

Brine Shrimp Lethality Bioassay

Preparation of Artemia salina by 1 g of egg is placed in a container containing salt water, left for 1-2 hours. After that, the soaked eggs are transferred into 2 L of salt water to drop. When hatching eggs for 2 days, during the hatching process they are illuminated with 40-60 watt incandescent light so that the hatching temperature is maintained. The test solution was made with concentrations of 20, 40, 60, 80 and 100 ppm in DMSO (dimethyl sulfoxide) and aquadest solvents. For the negative control, DMSO and aquadest solvents were used without extract (0%). All test solutions are prepared in a test tube. Each tube is inserted into 10 Artemia salina larvae, leave for 24 hours, then observe and count the number of larval deaths in each test tube^{9,10}. Based on the number of deaths of Artemia salina in each group, the value of lethality concentration of 50% (LC₅₀) was calculated using the probit analysis method.

RESULTS AND DISCUSSION

Results

The results of the research presented below are the results of a qualitative analysis of the bioactive content of the pagoda flower ethanol extract. The test results of the bioactive potential of pagoda flower ethanol extract are

Table 1: The results of phytochemical screening of ethanol extract of pagoda flowers

No	Bioactives	Result
1	Alkaloids	+
2	Flavonoids	+
3	Steroids/Triterpenoids	+
4	Tannins	+
5	Saponins	+
6	Glycosides	+

Explanations: + : exist, - : unexist

presented in a table that shows the average brine shrimp mortality value per concentration, then the number of deaths from each of these concentrations was calculated LC₅₀ value by means of probit analysis using SPSS program. The results of the identification of bioactive ethanol extract of pagoda flowers can be seen in table 1.

Table 2: The results of the test of brine shrimp lethality

No	Groups	Average of Lethality Values \pm Standard Error
1	Control (0 ppm)	0.00 \pm 0.00
2	20 ppm	1.33 \pm 0.33
3	40 ppm	4.67 \pm 0.33
4	60 ppm	6.33 \pm 0.33
5	80 ppm	7.00 \pm 0.00
6	100 ppm	9.67 \pm 0.33

The LC₅₀ value based on probit analysis using the SPSS program can be seen in table 3.

Table 3: The LC₅₀ value based on probit analysis

Probability	95% Confidence Limits for Concentrations	95% Confidence Limits for log (Concentrations)
	Estimate	Estimate
0.100	18.036	1.256
0.200	24.777	1.394
0.300	31.153	1.493
0.400	37.885	1.578
0.500	45.487	1.658
0.600	54.615	1.737
0.700	66.418	1.822
0.800	83.508	1.922
0.900	114.721	2.060

Discussions

Research that has been done on the largest chemical composition of the genus *Clerodendrum* was steroids, such as: β -sitosterol, γ -sitosterol octacosanol, clerosterol, bungein A, acteoside, betulinic acid, clerosterol 3-O- β -Dglucopyranoside, colebrin AE, campesterol, 4 α -methylsterol, cholesta-5-22-25-trien-3- β -ol, 24- β -cholesta-5-22-25-trien, cholestanol, 24-methyl-22-dihydrocholestanol, 24- β -22-25-bis-dehydrocholesterol, 24- α -methyl-22-dehydrocholesterol, 24- β -methyl-22-dehydrocholesterol, 24-ethyl-22-dehydrocholesterol, 24-ethylcholesterol, 22-dehydroclerosterol, 24-methylathosterol, 24- β -ethyl-25-dehydrolathosterol, (24S)-ethylcholesta-5-22-25-triene-3 β -ol which has been isolated from various species of *Clerodendrum* such as *C. inerme*, *C. phlomidis*, *C. infortunatum*, *C. paniculatum*, *C. cyrtophyllum*, *C. fragrans*, *C. splendens*, *C. campbellii* and *C. splendens*¹.

Other content is terpen compounds, including monoterpenes, diterpenes, triterpenes, iridoids and sesquiterpenes. The flavonoids found in the genus *Clerodendrum* include cynaroside, 5-hydroxy-4'-7-dimethoxymethyl flavone, kaempferol, salvigenin, 4-methyl scutellarein, 5,7,4 O-trihydroxyflavone, apigenin, luteolin, acacetin-7-O-glucuronide, Hispidulin, 2'-4-4'-trihydroxy-6'-methylchalcone, 7-hydroxy flavone, and

others. The glycosides contained in *Clerodendrum* are cyanogenic glycosides such as lucumin and prunasin¹.

Based on the probit analysis of the number of brine shrimp deaths carried out, the LC₅₀ value of the ethanol extract of the pagoda flower (*Clerodendrum paniculatum* L.) was 45,487 ppm. The same study was conducted by Waliullah et al in 2015 on ethanol and chloroform extract from roots, leaves and stems of *Clerodendrum infortunatum* Linn. The results showed that LC₅₀ values of ethanol extract from roots, leaves and stems were 20,845, 24,017 and 31,379 ppm, LC₅₀ values of chloroform extract were 30,702, 32,907 and 42,559 ppm¹¹.

The evaluation of the toxic action of plant extracts is indispensable in order to consider a treatment safe; it enables the definition of the intrinsic toxicity of the plant and the effects of acute overdose^{12,13}. The procedure was adopted to determine the lethality of crude plant extracts to brine shrimp. *Artemia* bioassay has been demonstrated to provide a viable alternative to the mouse bioassay, which is expensive and associated with ethical constraints. In bioactivity evaluation of plant extracts by brine shrimp bioassay, an LC₅₀ value lower than 1000 ppm is considered cytotoxic^{14,15}.

CONCLUSION

The pagoda flower ethanol extract contains alkaloid compounds, flavonoids, steroids/triterpenoids, tannins, saponins, and glycosides. Pagoda flower ethanol extract has strong potential activity.

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