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Optimization of Pagoda (*Clerodendrum paniculatum* L.) Extraction Based By Analytical Factorial Design Approach, Its Phytochemical Compound, and Cytotoxicity Activity

Budiman Yasir[a,b,c], Ayun D Astuti[a,c], Muhammad Raihan[c], Rosdiana Natzir[d], Subehan[c], Abdul Rohman[e], Gemini Alam[c*]

[a]*Department of Medical Science, Faculty of Medicine, Hasanuddin University, Makassar 90245, Indonesia*

[b]*Sekolah Tinggi Ilmu Farmasi, Makassar 90242, Sulawesi Selatan, Indonesia*

[c]*Department of Pharmacognosy-Phytochemistry, Faculty of Pharmacy, Hasanuddin University, Makassar 90245, Indonesia*

[d]*Department of Biochemistry, Faculty of Medicine, Hasanuddin University, Makassar 90245, Indonesia*

[e]*Departement of Pharmaceutical Analysis and Medicinal Chemistry, Faculty of Pharmacy, Gadjah Mada University, Indonesia*

Abstract

One of the successful determinants of active compounds extraction from plant material depends mainly on the extraction procedures, including the type of solvents, plant materials, and extraction techniques used in the extraction procedure. This research aimed to optimize Pagoda's extraction yields (*Clerodendrum paniculatum* L.) using several types of solvents and extraction techniques and to evaluate the cytotoxic activity of extracts obtained. The various solvents include water, methanol, hexane, and ethanol, while the extraction techniques used were maceration, microwave-assisted extraction (MAE), and reflux. The flowers, leaves, and stems were also extracted to represent parts of the plants. Screening of secondary metabolites was carried out using thin-layer chromatography (TLC), while cytotoxic activity was tested against *Artemia salina*. The highest yield of extract was obtained using water, followed by methanol > ethanol > hexane. The most effective extraction methods were the reflux method, followed by MAE and maceration. Analysis of variance (ANOVA) showed that the linear model of plant parts, solvents, and interactions between factors showed significant effects on yield ($P < 0.05$). The studied extracts contain secondary metabolites of terpenoids, flavonoids, alkaloids, and tannins. These extracts were also very toxic to *Artemia salina*; therefore, they can be developed as anticancer.

Keywords: *Pagoda, Factorial Design, Phytochemical, Artemia salina, Cytotoxicity*

1. Introduction

The pagoda plant is a plant under Verbenaceae's family and other 560 species within the genus *Clerodendrum*. The plants can grow in tropical and subtropical areas as small trees, shrubs, or herbs. It is divided into four large groups based on the geographical distribution: the Asian clade, the African clade, which comprises coastal species from Africa, Asia, Central America, and the Pantropical Coastal clade [1,2]. In terms of the Pagoda plant's ethnomedicinal uses, there were several reports that we have identified. In Thailand, Pagoda root's practical benefits were antipyretic and anti-inflammatory [3]. The flowers and leaves of this plant have been utilized to treat hemorrhoids in the northern part of Thailand [4]. In India, Pagoda's roots were used for the traditional treatment of typhoid fever. The roots and leaves of this plant were also used for the treatment of rheumatism, antipyretic, anthelmintic, venereal diseases, and malaria [5], eye pain treatment [6], treatment of jaundice, body aches, snake bites, and dizziness [7].

Several types of compounds have been successfully isolated. These include terpenes, flavonoids, tannins, alkaloids, phenolic acid, sterols, and glycosides [8,9], quercetin [10], 24S-methyl cholesta-5,22,25-triene-3β-ol, α-amyrin, β-sitosterol [11], oleanolic aldehyde acetate, β-sitosterol, lupeol,

*Corresponding author e-mail: daengta007@yahoo.com.; (Gemini Alam).

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stigmasta-4,25-dien-3-one-22E-stigmasta-4,22,25-trien-3-one, 3β-stigmasta-4,22,25-trien-3-ol [12]. Leaves contain tannins, phenols, sterols, routine, and quercetin [10]. Terpenes such as triacatane, clerodin, clerodendrin A, 3β-acetylloleanolicacid, 3β-acetylloleanolic aldehyde, glutinol, poriferasta-5,22E, 25-trien-3β-ol were also reported [11]. This study optimizes the extraction of Pagoda plant parts using a range of extraction methods and solvents. There are many research experiments involving the study of the effects of two or more factors. In general, the factorial design was most efficient for this type of analysis, and the possible combination of factors was investigated deeply [13].

The use of the factorial design techniques on the natural product is essential to understanding variable factors. The interaction between variables and there are many research experiments involving the study of the impact of two or more elements. In general, the factorial design was most efficient for this type of analysis, and the possible combination of factors was investigated deeply [13]. The use of the factorial design techniques on the natural product is essential to understand the effect of variable factors, the interaction between variables, and the importance of variables to the influencing the targeted response for designed and selected to the optimal experiment procedures and get factors for developed of subsequent research procedures in the field of natural products which have many variable factors and difficult to understand it. The importance of variables influencing the targeted response for designed and selected to the optimal experiment procedures and get elements for developing subsequent research procedures in natural products has many variable factors and difficult to understand. This study also aimed to identify classes of each extract's secondary metabolites and their cytotoxic bioactivities against the shrimp larvae *Artemia salina* Leach.

2. Methods

2.1 Materials

The new C. *paniculatum* were collected from Masamba, North Luwu District, and South Sulawesi Province, Indonesia. Parts of the plant such as flowers, leaves, and stems were separated in an oven at around 40-50 ºC for three days. The dried plant materials were then ground with a blender and passed through a 40/60 mesh, then stored in a container. The plant identification was carried out at the Department of Biology, Mathematics, and Natural Sciences at the Botanical Laboratory, Universitas Negeri Makassar, Indonesia.

2.2 Extraction

The summary of the extraction is described in **Table 1.** For each of the dried leaves, flowers, and stems of the C. *paniculatum*, approximately 25 g of the plant materials were extracted with each type of solvents and techniques. There were four types of solvent used in this experiment: methanol, 96% ethanol, hexane, and water. The plant materials were immersed with solvents in a 1:10 (w/v) ratio between plant parts and solvents [14]. Four different extraction techniques were used include maceration with occasional stirring (1-3 times within 24 hours), microwave-assisted extraction (MAE) with 30 watts of power [15] for 19 minutes, and reflux method for 19 minutes. All soluble extracts were then separated and collected by vacuum filtration so that 36 types of pagoda extract were obtained.

2.3 Physical examination

The extracts' color was observed after the solvent was evaporated with a rotary evaporator and water bath. The dried and viscous extract were weighed using an analytical balance, and the %yield of the extract [16] was calculated based on the following formula.

$$\%\ Yield\ extract = \frac{Weight\ of\ dried\ extracts}{Weight\ of\ dried\ materials}\ x\ 100\%$$

2.4 Identification of Phytochemical compounds

For identify phytochemicals, all extracts were spotted on a TLC plate PF60 254 nm and developed using a mixture of n-hexane and ethyl acetate as the mobile phase [17]. For n-hexane, ethanol, and methanol extract, the mobile phase was developed by mixing n-hexane to ethyl acetate at a ratio of 5:1, 3:1, and 3:2 ml.

*Alkaloid identification***:** Screening of alkaloid content was performed by spraying the Dragendorff reagent to the spotted TLC plate. The plate was further observed under the UV 365 nm, and the alkaloid compounds that appear as a bright orange color was classified as a positive result [18,19] and observed on the UV 254 nm, visible, and UV 365 nm before spraying the reagent.

*Flavonoids***:** The TLC plates were sprayed with a 5-10 ml solution of citric acid-boric acid reagent. The TLC plates were then heated for 3-5 minutes at 110 ºC. Yellow, green, or orange colors that appeared under UV 365 nm were determined as a positive result of flavonoids compounds [18,20) and observed on the UV 254 nm, visible, and UV 365 nm as before spraying the reagent.

*Tannin***:** Tannin was detected by spraying the TLC plates with a solution of 5-10 ml of iron III chloride solution (FeCl3) reagent for 3-5 minutes, and were heated at 110 oC. The positive result was blue-blackish color under visible light [18, 21] and observed on the UV 254 nm, visual, and UV 365 nm before spraying the reagent.

Table 1. The optimization of extracts using different solvents and extraction methods

Pagoda plant parts	Solvent	Extraction method	Extraction specific	Dry simplicia (g)	Viscous extract (g)	Liquid extract colors	Viscous extract colors	Yield extract (%)
Leaf	Methanol	Maceration	1-3 stirring time, 24 hours	25	1.62	Light green	Blackish/green	6.48
	Water			25	3.16	Red maron	Red maron/brown	12.64
	Hexane			25	0.36	Yellow	Yellow/brown	1.44
	Ethanol			25	0.93	Dark green	Green/black/brown	3.72
Leaf	Methanol	Microwave	Power 30, 19 minutes	25	2.66	Light green	Blackish/green	10.64
Leaf		Reflux	19 minutes					
	Hexane			25	0.62	Yellow	Yellow/brown	2.48
	Ethanol			25	1.01	Dark green	Green/black/brown	4.04
	Methanol			25	2.39	Light green	Blackish/green	9.56
Flower	Water	Maceration	1-3 stirring time, 24 hours	25	5.16	Red maron	Red maron/brown	20.64
	Hexane			25	0.56	Yellow	Yellow/brown	2.24
	Ethanol			25	1	Dark green	Green/black/brown	4
	Methanol			25	1.99	Light green	Blackish/green	7.96
Flower	Water	Microwave	Power 30, 19 minutes	25	4.04	Red maron	Red maron/brown	16.16
	Hexane			25	0.18	Yellow	Yellow/brown	0.72
	Ethanol			25	2.9	Dark green	Brown/blackish	11.6
	Methanol			25	1.99	Light green	Brown/blackish	7.96
Flower	Water	Reflux	19 minutes	25	5.31	Red maron	Red maron/brown	21.24
	Hexane			25	0.16	Yellow	Yellow/brown	0.64
	Ethanol			25	2.41	Dark green	Brown/blackish	9.64
	Methanol			25	1.95	Light green	Brown/blackish	7.8
Stem	Water	Maceration	1-3 stirring time, 24 hours	25	8.01	Red maron	Red maron/brown	32.04
	Hexane			25	0.31	Yellow	Yellow/brown	1.24
	Ethanol			25	2.62	Dark green	Brown/blackish	10.48
	Methanol			25	0.99	Light green	Green/orange/black	3.96
Stem	Water	Microwave	Power 30, 19 minutes	25	1.87	Red maron	Red maron/brown	7.48
	Hexane			25	0.19	Yellow	Green/yellow/black	0.76
	Ethanol			25	1.62	Dark green	Green/orange/black	6.48
	Methanol			25	1.23	Light green	Green/orange/black	4.92
Stem	Water	Reflux	19 minutes	25	1.55	Red maron	Red maron/brown	6.2
	Hexane			25	0.12	Yellow	Green/yellow/black	0.48
	Ethanol			25	1.69	Dark green	Green/orange/black	6.76
	Methanol			25	0.99	Light green	Green/orange/black	3.96
	Water			25	2.5	Red maron	Red maron/brown	10
	Hexane			25	0.15	Yellow	Green/yellow/black	0.6

	Ethanol			25	1.3	Dark green	Green/orange/black	5.2

*Terpenoids***:** The TLC spotted plates were sprayed approximately 5-10 ml of vanillin in glacial acetic acid followed by heating (110 ºC) for 3-5 minutes. A positive result was indicated by the occurrence of blue-purplish or red-purplish color, or brown-orange or blue-orange under visible light [18, 22] and observed on the UV 254 nm, visual, and UV 365 nm before spraying the reagent.

2.5 Evaluation of Cytotoxicity activity

Preparation of seawater as the hatching medium was carried out by dissolving 30 g of sea salt in 1 L (3%) of distilled. Any solid particles were removed, bypassing the liquid to vacuum filtration. Yeast suspension was prepared by weighing approximately 30 mg of dry yeast powder was suspended in 10 ml of artificial seawater as stock food for shrimp larvae.

2.5.1 Preparation of shrimp larvae A. *salina*

A. *salina* eggs were obtained from shrimp breeding center in Pangkep District, South Sulawesi Province, Indonesia. A total of 500 mg was suspended in 500 ml of seawater in a container with an aerator that circulates air around the compartment. Above the case, a 10-watt yellow light bulb for lighting and mild heating. After 24 hours, the eggs will hatch and ready to be used for the Cytotoxicity test these procedure modifications from the method [23].

2.5.2 Cytotoxicity

For each extract, a series of concentrations of 10, 100, and 1000 ppm [24] was prepared in calibrated vials and this mild procedure modification from the method by [25]. Initially, 0.1 ml of dimethylsulfoxide (DMSO) was to dissolve the extracts. Subsequently, 5 ml artificial seawater and ten shrimp larvae were added to the vial. Each treatment was replicated three times. A vial containing seawater without any added extract was used as the negative control. All testing vials were then stored beside a yellow light lamp. Observations were carried out after 24 hours. LC50 was then calculated, and the mortality percentage was obtained using the following equation [26].

$$\% \, Mortality = \frac{The \ average \ number \ of \ larvae \ deaths}{Total \ number \ of \ larvae} \ x \ 100\%$$

2.5.3 Statistical analysis

The value of the % yield of the extracts obtained from processes that involved a variety of solvents and extraction methods was analyzed statistically using Minitab® version 18. Full factorial design of experiment was used as the analytical approach.

3. Result and Discussion

3.1 Extraction optimization

Optimization of the %yield extract of leaves, flowers, and stems of the pagoda plant with a variation of solvent and the extraction method is given in **Table 1**. The organoleptic properties of each extract are observed based on different parts of the plants and the techniques. The leaves extract from maceration, microwave, and reflux methods, for instance, consists of liquid and viscous extract with light green and blackish-green color. The ethanol extracts showed dark green and green or brownish-black color, while hexane extracts are commonly yellow and yellow-brown. The water extracts, on the other hand, showed a distinct red maroon and blackish-red color. The flower extracted with methanol also gave the viscous light green and blackish-brown section. Extraction with ethanol resulted in greenish colored and brown to thick black extract, while hexane and water extract showed relatively similar organoleptic properties with the leaves extract. The stems extracted with methanol have a light green to orange and brown to black color extract. The ethanol extract showed dark green-colored extract and sometimes orange or dark green thick liquid. Extraction with hexane gave a yellow and black green-yellow section, while aqueous extraction resulted in red pale and brownish-red extracts. The color of areas may indicate the characteristics of the chemical components in the unit.

Figure 1 showed a summary of our findings. In the analysis of the main effect of distinctive plant parts, it was found that the flowers' extraction contributed to the highest yield compared to the leaves and stemmed respectively. The variation of solvent also showed differences in the result of extraction. More polar solvents such as water are highly useful to the extraction yield of Pagoda. This was also shown by the extraction using methanol as the solvent, which gave a higher result than n-hexane. The extraction techniques were, however, showed insignificant differences between one and other methods. The extraction was obtained at relatively similar yields for the three extraction techniques used in this experiment.

The one-way ANOVA results **Figure 1** showed that between each variable (plant parts, solvent types, and extraction techniques), the use of different methods was not statistically significant ($p>0.05$). However, various solvents with particular methods showed statistically significant extraction yields ($p<0.05$). The model describes 97.11% of the data's variance indicated that the model fits perfectly with the data. The plot of the main effect **Figure 2** on Pagoda plant parts, i.e., flower, water solvent, and

reflux extraction method, seem to be associated with the highest average strength, indicating that the main effect is statistically significant. Interactions between the pagoda plant parts, the solvent, and its extraction methods are statistically significant. The portion of the plant gives the high and low yield of the flower then leaf and stem, methanol and ethanol solvents that influence the %yield of the extract insignificant, and the lowest in the hexane solvent. Simultaneously, the extraction method maceration and the microwave also show an insignificant influence on the %yield of the extract. There is an interaction of factors that significantly affect each other or an insignificant increase in the %yield of the extracted result.

The use of various types of solvent and the plant parts caught our interest as the water and reflux method showed the highest percent yield, while the hexane was the lowest in all extraction methods and components of the plant. The interaction between plant parts and solvent in the three extraction methods resembled relatively similar results. From these findings, a large percentage of the %yield seems to be influenced by the type of solvent used. Water and reflux resulted in the most extensive extraction product than other solvents and methods, as shown in many phytochemical extractions in which water has been used as a universal solvent [14] with a dielectric constant of 78.5 [28].

The higher polarity of water and at high extraction temperatures in the reflux extraction method has been correlated with the increasing yield of plants' extraction [27]. Ethanol and methanol as the extraction solvent having dielectric constants 24.3 and 32.6, respectively [28], produced a %yield of the extraction that is not significant. While extraction with hexane yielded the lowest amount of extract, it has a dielectric constant of 1.89, indicating a more non-polar property. The quantity and the compositions of secondary metabolites of an extract depend largely on the type of extraction techniques, duration, temperature, solvent properties, solvent concentrations, and polarity [14].

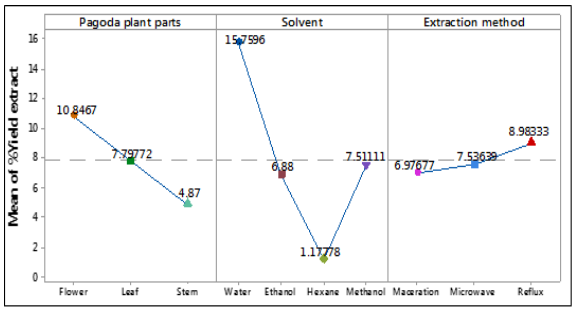

Figure 1. The Pareto effect of the variables is essential to the impact of %yield extract results (Response is % yield extract, α = 0.05)

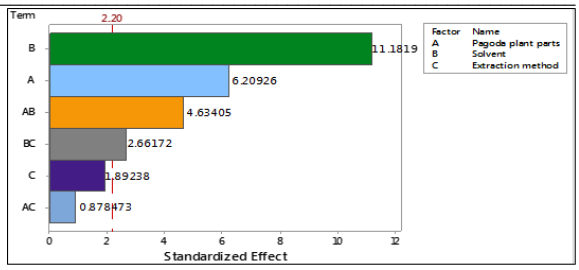

Figure 2. The correlation plot of main effects to the increase in the average %yield of the extracts.

The optimization plot for the %yield of the extracts has a parameter design to produce maximum parameters with target 32.04 is considered excellent, and the value below 0.48 is unacceptable. The fitted values are point estimates of the mean response for the given values (y=29.17) and thus gave the desired value (d=0.91). Composites that are approaching 1 indicates that the setting achieved favorable results for all responses and have multiple prediction response variables such as flowers, solvent water, and extraction method reflux. The standard error of fit to measure the accuracy of average response estimation was 1.68, indicating a 95% confidence interval used to assess the data estimate for the variable values observed depending on the number of degrees of freedom, the 95% confidence interval extends about two errors standards above and below the predicted average obtained (25.48, 32.87), and predictive interval 95% to assess the accuracy of the prediction obtained (23.38, 34.96) indicates that 95% is confident that the actual value of the predicted optimization response will be in between about 23.38 and 34.96 value.

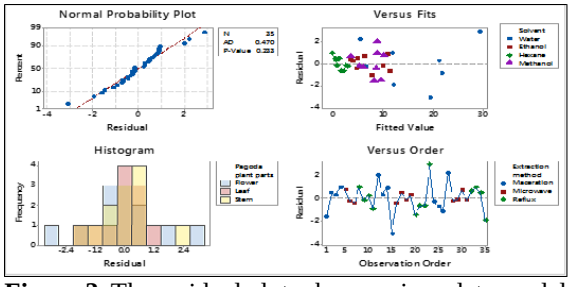

Figure 3. The residual plots show various data models with the normal distribution of the factor variables against to %yield of the extracts.

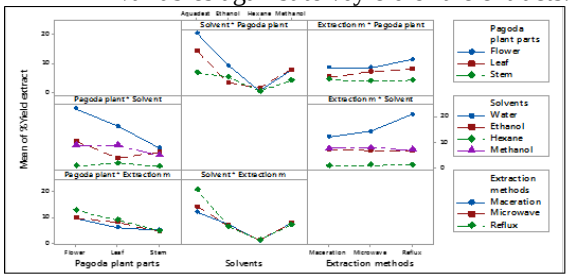

Figure 4. The interaction plots show various data models with each factor variable against the %yield of the extracts.

3.2 Phytochemical compound

Based on a qualitative test of the Pagoda plant's secondary metabolite, as seen in **Table 2**, there were at least several metabolites identified in the stem, leaves, and flower. Screening of the secondary metabolites in the hexane extract obtained from all types of extraction methods gave a positive result of terpenes, flavonoids, and alkaloids. However, tannin did not seem to be detected in this extract. In most cases, hexane is mainly used to remove non-polar lipids and unwanted glycosides, as well as chlorophyll [29].

Table 2. The qualitative test results of the secondary metabolite variation of the solvent and extraction methods

Pagoda plant parts	Solvents	Extraction methods	Reagent			Total of spots compounds							
			VLN	STB	DGF	VLN Vsl	STB UV 366 nm	DGF UV 366 nm	FeCl3 UV 366 nm	Vsl	UV 254 nm	UV 366 nm	Mobile and Stationary phase; DC.
Leaf	Hexane	Maceration	+	+	+	16	6	8	3	4	7	3	5:1 (hexane: etyl acetate) and silica gel PF60; 2.67
		Microwave	+	+	+								
		Reflux	+	+	+								
Stem		Maceration	+	+	+	9	7	10	2	5	8	6	
		Microwave	+	+	+								
		Reflux	+	+	+								
Bunga		Maceration	+	+	+	11	8	7	2	4	7	4	
		Microwave	+	+	+								
		Reflux	+	+	+								
Leaf	Ethanol	Maceration	+	+	+	7	12	9	7	10	11	8	2:1 (hexane: etyl acetate) and silica gel PF60; 3.3
		Microwave	+	+	+								
		Reflux	+	+	+								
Stem		Maceration	+	+	+	8	11	6	3	7	8	9	
		Microwave	+	+	+								
		Reflux	+	+	+								
Bunga		Maceration	+	+	+	10	9	7	1	6	7	9	
		Microwave	+	+	+								
		Reflux	+	+	+								
Leaf	Methanol	Maceration	+	+	+	7	6	5	2	9	10	7	3:2 (hexane: etyl Acetate) and silica gel PF60; 3.6
		Microwave	+	+	+								
		Reflux	+	+	+								
Stem		Maceration	+	+	+	8	4	4	2	4	5	3	
		Microwave	+	+	+								
		Reflux	+	+	+								
		Maceration	+	+	+	6	4	3	1	0	4	1	
		Microwave	+	+	+								
		Reflux	+	+	+								

Description: (VLN) Vanillin sulphate acid; (SBT) sitroborat; (DGF) Dragendorff; ($FeCl_3$) Iron III Chloride; (Vsl) Visible, (UV) Ultraviolet; (TLC) Thin Layer Chromatography; (DC) Dialectric Constant.

However, hexane also has been used to extract flavonoid compounds, saponins, tannins [28], alkaloids, and terpenes [30]. Classes of compounds such as terpenes, flavonoids, alkaloids, and tannins were identified from extraction using more polar solvents like ethanol and methanol. Praveen et al. (2012) have also demonstrated that tannin, phenols, sterols, carbohydrates, and cardiac glycosides were present in the Pagoda leaves. In the other study, ethanol is capable of extracting tannins, polyphenols, polyacetylenes, flavonol, terpenes, sterols, alkaloids, and methanol was able to extract the alkaloid, flavonoids, tannins. Methanol was also used to extract terpenes and saponins [32], xanthoxillines, totarol, quassinoid, and lactones flavones, phenols, polyphenols [14] from this plant. It has been acknowledged that different types of solvent may affect the extraction result. However, the specific type of compounds and the resulting concentration may vary. Less polar solvents are useful for extracting flavonoids aglycones, and more polar solvents could be used to extract flavonoids glycosides. Hexane can extract flavonoids, such as 1,7-hydroxy-5-methoxy flavone and 5,7-dihydroxyflavone [33].

Further examination of the total flavonoids, equivalent to caffeic acid, was the highest when hexane was used (68.02 %) while methanol was only 39.27 % in *Cordyline terminalis* leaves [34]. In ethanol and methanol as solvents, there is also a difference in the types of extracted secondary metabolites. For instance, ethanol extracted five flavonoid compounds, including catechins, epicatechin, routine, luteolin, and

apigenin. The use of methanol extracted seven flavonoid compounds, including catechins, epicatechin, routine, myricetin, luteolin, apigenin, and naringenin from Spearmint leaves. Higher concentrations of bioactive compounds of flavonoids were obtained when 70% of ethanol was used, as its polarity is higher than 96% ethanol [35]. The observation in the variation of the extraction methods with the same solvents showed that similar types of compounds might be identified in each extract's chromatographic profile. Some of the only differences are that the appearance of various stain spots compound and the density related to the concentration of the compounds.

3.3 Cytotoxicity activity

The cytotoxic activity was conducted using shrimp larvae *Artemia salina* L. to test the toxicity of various concentrations of extracts: 10 ppm, 100 ppm, and 1000 ppm. Results showed in **Table 3** indicated that all plant parts indicated the toxicity effect of LC_{50} < 1000 µg/ml, except the flower extracted with water by reflux technique, as well as the leaves extracted with ethanol and water by maceration exhibited LC_{50} >1000 µg/ml. According to the bioassay standard of shrimp larvae test, the value of LC_{50} < 1000 µg/ml is considered bioactive in evaluating plant extract toxicity [36].

A study conducted by Mayilsami and Geetharamanan (2016) showed that methanol and hexane extract of three plant leaves of angiosperm: *Andrographis paniculata*, *Argemone mexicana*, and *Vitex negundo* exhibited larger cytotoxicity than the other solvent extracts. This might be due to the presence of an active antitumor agent that correlates quite well with the cytotoxic properties of some extract against human cancer cells [38].

The presence of terpenes on the solvent extract of hexane has been shown to correlates to its potency as an antitumor and anticancer agent. Cytotoxicity of natural products also varied according to the types of compounds that present in plant extracts. These include alkaloids [39, 40] phenolic compounds [41].

In this study, the cytotoxicity testing using the shrimp larvae of *A. salina* L, at various concentrations, was observed by the difference in larvae morphology **Figure 5**. In the testing of negative control, shrimp larvae indicated normal development. The larvae of *A. salina* that died after exposure to 10 ppm of the extract were characterized by irregularities of body structures where the larvae became elongated.

Table 3. The cytotoxic activity of the extract against *Artemia salina* L.Methods

Pagoda plant parts	Extraction methods	Solvents	%Mortality			LC_{50} (µg/ml)	Coefficient of Determination	Regression equations (y=ax+b)
			10 ppm	100 ppm	1000 ppm			
Flower	Maceration	Hexane	76.66	86.66	90.00	0.02	$R^2 = 0.953$	y = 0.275x + 5.49
Flower	Reflux	Hexane	20.00	23.33	70.00	323.59	$R^2 = 0.810$	y = 0.68x + 3.29
Flower	Microwave	Hexane	56.67	70.00	90.00	6.3	$R^2 = 0.956$	y = 0.555x + 4.546
Flower	Maceration	Methanol	60.00	86.67	90.00	1.94	$R^2 = 0.869$	y = 0.515x + 4.85
Flower	Reflux	Methanol	76.67	86.67	90.00	0.02	$R^2 = 0.953$	y = 0.275x + 5.49
Flower	Microwave	Methanol	76.67	86.67	90.00	0.02	$R^2 = 0.953$	y = 0.275x + 5.49
Flower	Maceration	Ethanol	16.67	23.33	90.00	141.25	$R^2 = 0.790$	y = 1.12x + 2.586
Flower	Reflux	Ethanol	13.33	20.00	90.00	151.36	$R^2 = 0.833$	y = 1.195x + 2.386
Flower	Microwave	Ethanol	16.67	23.33	90.00	134.9	$R^2 = 0.826$	y = 1.12x + 2.623
Flower	Maceration	Water	16.67	20.33	90.00	933.25	$R^2 = 0.868$	y = 1.045x + 1.88
Flower	Reflux	Water	3.33	6.67	80.00	>1000	$R^2 = 0.999$	y = 0.705x + 2.463
Flower	Microwave	Water	3.33	13.33	33.33	416.87	$R^2 = 0.843$	y = 1.34x + 1.486
Leaf	Maceration	Hexane	63.33	83.33	90.00	1.48	$R^2 = 0.967$	y = 0.47x + 4.92
Leaf	Reflux	Hexane	20.00	23.33	70.00	323.59	$R^2 = 0.810$	y = 0.68x + 3.29
Leaf	Microwave	Hexane	43.33	63.33	83.33	20.89	$R^2 = 0.996$	y = 0.565x + 4.246
Leaf	Maceration	Methanol	63.33	70.00	90.00	3.09	$R^2 = 0.887$	y = 0.47x + 4.773
Leaf	Reflux	Methanol	56.67	73.33	90.00	5.62	$R^2 = 0.988$	y = 0.555x + 4.58
Leaf	Microwave	Methanol	30.00	73.33	86.66	3.98	$R^2 = 0.949$	y = 0.815x + 3.773
Leaf	Maceration	Ethanol	10.00	20.00	40.00	>1000	$R^2 = 0.993$	y = 0.515x + 3.18
Leaf	Reflux	Ethanol	16.67	26.67	90.00	123.03	$R^2 = 0.860$	y = 1.12x + 2.66
Leaf	Microwave	Ethanol	20.00	30.00	86.67	117.49	$R^2 = 0.869$	y = 0.975x + 2.966
Leaf	Maceration	Water	6.67	10.00	63.33	758.58	$R^2 = 0.838$	y = 0.915x + 2.353
Stem	Reflux	Hexane	60.00	73.33	90.00	3.98	$R^2 = 0.974$	y = 0.515x + 4.686
Stem	Microwave	Hexane	10.00	20.00	90.00	165.96	$R^2 = 0.874$	y = 1.28x + 2.16
Stem	Maceration	Hexane	73.33	70.00	90.00	0.74	$R^2 = 0.846$	y = 0.38x + 5.046
Stem	Maceration	Methanol	73.33	73.33	90.00	3.47	$R^2 = 0.750$	y = 0.33x + 5.18
Stem	Reflux	Methanol	70.00	83.33	86.67	0.13	$R^2 = 0.925$	y = 0.295x + 5.273

Stem	Microwave	Methanol	13.33	90.00	90.00	38.9	$R^2 = 0.750$	y = 1.195x + 3.093
Stem	Maceration	Ethanol	13.33	13.33	90.00	177.83	$R^2 = 0.750$	y = 1.195x + 2.296
Stem	Reflux	Ethanol	10.00	13.33	90.00	194.98	$R^2 = 0.799$	y = 1.28x + 2.07
Stem	Microwave	Ethanol	10.00	10.00	90.00	213.8	$R^2 = 0.750$	y = 1.28x + 2.013
Stem	Maceration	Water	3.33	6.66	10.00	>1000	$R^2 = 0.999$	y = 0.28x + 2.883
Stem	Reflux	Water	3.33	10.00	83.33	323.59	$R^2 = 0.892$	y = 1.4x + 1.48
Stem	Microwave	Water	3.33	63.33	73.33	173.78	$R^2 = 0.837$	y = 1.23x + 2.243

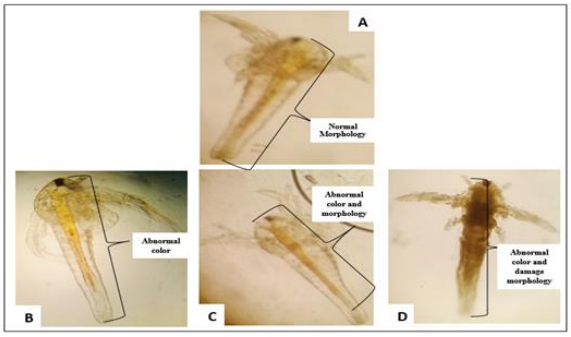

Figure 5. The condition of shrimp morphology larvae's *Artemia salina* after administration of the extracts for 24 hours. A, without treatment, standard; B, extract ten ppm; C, extract 100 ppm; and D, extract 1000 ppm.

The color turned pale yellow. The observation of extract toxicity at a concentration of 100 ppm showed abnormalities of larvae morphology such as elongation of the body and color changes such as yellow or dark brown. The concentration of 1000 ppm of extract, body elongation, persistent dark color, and failure in developing the antenna and the third complement (mandibular feeding component) were observed. The criteria of growth disorders proposed by Bustos-Obregon and Vargas (2010) correlate to the larvae's mortality rate and the plant's potential as anticancer agents.

4. Conclusions

Optimization percent of %yield of the extracts of the Pagoda plant parts with a variation of solvent and the extraction method showed that the highest result in terms of plant parts of Pagoda was obtained from the extraction of the flowers. Among the solvent system, higher yields were obtained subsequently from water > Methanol > Ethanol > Hexane. In terms of the extraction method, it was shown that reflux> extraction > maceration. The analysis of variance and interactions between variables resulted in a significant P-value < 0.05 and an insignificant factor in the variable of extraction methods. The factorial model gave 97.11% of the data variance, indicating that the model fits the data relatively well. Optimation plots percent of the highest extracts of the predicted value y=29.1725, and d=0.90914 with targets 32.04 and should not be under 0.48. Phytochemical screening showed that the extract at least contains terpenes, flavonoids, alkaloids, and tannins. The cytotoxic test using A. *salina*, hexane, and methanol extract obtained by maceration, microwave, and reflux were relatively toxic to *Artemia salina*. It could be potentially tested further for their anticancer activities.

5. Conflicts of interest

The authors have no conflict of interest.

6. Author Contributions

Budiman Yasir, Ayun Dwi Astuti, and Muhammad Raihan conducted the experiment, Subehan and Rosdiana Natzir conducted the calculations, Budiman Yasir, Gemini Alam, and Abdul Rohman wrote and revised the manuscript. All authors agreed to the final version of this manuscript.

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