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Phytochemical Screening and Alkaloid Identification of Cabomba Furcata

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Abstract. Ekor Kucing (*Cabomba furcata*) is a dominating water plant, which is rapidly proliferating in Lake Chini, Pahang, Malaysia. If left uncontrolled, the prevalence of this plant would threaten other plants, such as by inhibiting the growth of lotus (*Nelumbo nicifera* and *Nympae lotus*). It is also suspected as being a cause of pollution in Lake Chini. Steps to eradicate or control *C. furcata* plants first require a comprehensive study of its phytochemical content through methods of chemical screening, extraction/fractionation and analysis of individual compounds. Phytochemical screening carried out on the extracts detected flavonoid, saponin and alkaloid groups, but not triterpene and tannin. Different extraction yields were obtained from various plant parts, with the highest yield (%g/g sample) obtained before and after alkaloid fractionation were from the leaf (10.1%, 6.9%), followed by stem (9.6%, 4.5%) and flower (0.8%, 0.5%). Identification of alkaloids in *C. furcata* extracts by TLC showed the presence of nicotine, tomatine, thebaine and quinine.

Introduction

Lake Chini is the second largest natural lake in Malaysia, with an area of 202 hectares. It is rich in flora and fauna, with 288 plant species, 25 species of aquatic plants, 92 bird species and 144 species of fish[1]. Recently, the existence of aquatic plants has been disrupted by the dominance of Ekor Kucing plant (*Cabomba furcata*), which also was reported to have an adverse effect on some aspects of water quality. This plant is an aquatic weed that has the ability to grow rapidly. It has spread throughout the lake, becoming the dominant plant and one of contributors to pollution.

The plant domination has endangered the lotus population (*Nelumbo nicifera* and *Nympae lotus*), which is an attraction for visitors [2]. Plants that have the ability to grow and develop into a dominant position usually produce compounds that inhibit the growth and development of other plants. These are known as allelopathic effects [3]. Plants may also produce compounds that are beneficial to health and well being [4]. For example, alkaloids from plants such as atropine, codeine and nicotine are widely used in the production of drugs [5].

The purpose of this study is to identify the phytochemicals in *C. furcata* through a chemical screening. In addition, this study aims to determine the extraction yield from leave, stem and flower parts of *C. furcata*, and to identify alkaloid types found before and after fractionation.

Materials and Method

Preparation. The raw material used was *C. furcata* from Lake Chini. First, the sample was washed, and then dried in an oven at a temperature of 45°C until the sample weight was constant.

Solvent Selection. A sample that had been milled and dried, weighing 10 grams, was then fed into the Soxhlet extractor (Hamilton, United States) using 200 ml of solvent (methanol, water, methanol + water). Extraction was carried out for 3 hours, with sampling of 10 ml every 30 minutes. The resulting extracts were dried using a vacuum evaporator (Heidolph, Germany) at 80 rpm and 65°C (with a methanol solvent), 100°C (with water) and 90°C (with water + methanol) to determine the percentage of extract yield produced.

Phytochemical Screening Test

Flavonoids Test (Mg Test). A few drops of concentrated hydrochloric acid (HCl) and 0.5g of metal magnesium (Mg) were added to 5 ml of ethanolic extract (containing 1g of the material sample). A pink or red/purple coloration would indicate the presence of flavonoid substances within the material [6].

Saponin Test. A total of 5g of small slices of plant material was extracted with hot methanol, then dried and extracted again with ether. Materials that did not dissolve in the ether were shaken vigorously with water. The formation of static froth would indicate saponin content [7].

Triterpene/Steroid Test. A total of 5g of small slices of plant material was extracted with hot ethanol, then dried and extracted again with ether. The ethanolic extract was tested with sulphuric acid or anhydrous acetic (Burchad Lieberman). Purple, red or pink coloration would indicate the presence of triterpene/steroids [7].

Alkaloid Test. A total of 5-10 g of small slices of fresh or dried sample was milled with sand. Once fine, 10 ml of chloroform was added and then this was shaken. The filtered sample solution and the solution of sulphuric acid 2M (10 drops) were added to a test tube and shaken vigorously. The aqueous phase was carefully separated and put into a test tube with the addition of a few drops of Meyer test materials (pottassiomercuri solution). The formation of a turbid solution would indicate the presence of an alkaloid [7].

Tannin Test. 1 g of ethanolic extract was evaporated and the rest extracted with 10 ml NaCl (9%). This was filtered and divided into three equal parts. A solution of sodium chloride (NaCl) was added to the first part, a solution of gelatin (1%) to the second and gelatin salt added to the third. Formation of precipitation would indicate the presence of tannins. A $FeCl_3$ solution was then added to the extracts. A blue, dark blue, green or blue-green coloration would indicate the presence of tannins.

Extraction and Fractionation. *C. furcata* that had been milled and dried, weighing up to 4g, was placed in an Erlenmeyer flask with 40 ml of methanol solvent (10% acetic acid). Extraction was carried out in darkness by closing the Erlenmeyer with aluminum foil. This was then placed on a shaker for 4 hours at a speed of 150 rpm at room temperature (27°C) [8]. The resulting extract was concentrated using a vacuum evaporator with a speed of 55 rpm at 55° C for 10 minutes.

After the extract was concentrated, NH_4OH was added drop by drop until clotting occurred and sediment formed. The sediment was separated using a centrifuge at a speed of 3000 rpm for 10 minutes, and was then mixed with 1% NH_4OH and diluted with a little chloroform. If the sediment did not form after drizzling with NH_4OH, the alkaloid was extracted three times using chloroform (40 ml).

The result was concentrated using a vacuum evaporator at a speed of 55 rpm and a temperature of 55°C for 10 minutes. The result was then placed in an oven at a temperature of 45°C to obtain a solid extract.

Identification of Alkaloid Type Using TLC. TLC analysis was performed on G60 silica gel plates. Distances from spot/points were measured to calculate an R_f value. R_f (Retardation factor/ Retention factor) describes the ratio of time in the stationary phase relative to time spent in the mobile phase. R_f can be mathematically described by the ratio of migration distance of substance to migration distance of solvent front. The R_f values obtained were compared with the reference value shown in Table 1. From this comparison, initial estimates were made for the type of alkaloids present in the extract of *C. furcata*.

Table 1. Reference for retention factor of alkaloid type on TLC plate [9]

Alkaloid	R_f	Reagen for detection
Cytisine	32	*Dragendorff*
Nicotine	57	*Iodoplatinate*
Tomatine	62	*Iodoplatinate*
Morphine	34	*Iodoplatinate*
Solanine	52	*Marquis*
Codeine	35	*Iodoplatinate*
Berberine	7	*Iodoplatinate*
Strychnine	22	*Iodoplatinate*
Thebaine	41	*Iodoplatinate*
Atropine	18	*Iodoplatinate*
Quinine	52	*Iodoplatinate*
Conniine	26	*Iodoplatinate*

Results and Discussion

Determination of Phytochemical Presence in *C. furcata*

Flavonoid. A pink coloration occurred briefly, which changed to a brown color when concentrated hydrochloric acid and Mg powder were added to the methanolic extracts. This indicates that the phytochemical substances in *C. furcata* contain flavonoids. The results obtained are in line with the results by Donald and Donna [10]. This result therefore confirmed *C. furcata* as a potential source of antioxidants.

Saponin. Saponin test results showed the formation of permanent froth inside the tube. This shows that *C. furcata* contains saponin, although different results obtained by Watson and Dallwitz (1992), who stated that the cabomba genus did not contain saponin [11]. This difference may be due to the fact that the Watson and Dallwitz (1992) study was not carried out on the *C. furcata* species. The saponin content in *C. furcata* is very useful because it contains compounds that may be beneficial to the human body such as by helping to reduce both cholesterol and the risk of cancer, as well as acting as antioxidants.

Triterpene/Steroid. The bioactive phytochemical compound triterpene/steroid was not detected in *C. furcata* extracts since no color change in the ethanolic extract was observed after the addition of sulphuric acid.

Alkaloid. Evidence of the alkaloid group of bioactive phytochemical compounds was found in *C. furcata*, marked by cloudiness in the solution when Meyer reagents were added. This result is similar to what found in the genus Cabomba [11] and Nymphaeales [12].

Tannin. The tannin group was not detected in *C. furcata* since no color change occurred after the addition of $MgCl_3$ to the extract. In principle, this is not possible because flavonoids, which are tannin that has undergone condensation, were detected in the samples. The failure to detect tannin may be due to the test method not being suitable to detect condensed tannins.

Extract Yields. Table 2 show that the percentage of extract before fractionation was higher than after fractionation for all three types of samples (leaf, stem, flower). This is because prior to fractionation, various other components in the *C. furcata* sample were also extracted, whereas after fractionation, only specific group of compounds were fractionated. In this study, the specific component was an alkaloid component. This is emphasized by Silva *et al*. (1998) who stated that if

chloroform is used during fractionation, it may react with the extract mixtures to produce some alkaloids, salt and other artifacts [13]. It was also observed that the leaf produced the highest yield before and after fractionation, followed by stem and flower. Thus, based on the results after fractionation, alkaloids are mostly present in the leaves of *C. furcata*.

Table 2. Yield before and after fractionation

Sample type	Yield of extracts (% g extract/g sample)	
	Before Fractionation	After Fractionation
Leaf	10.1	6.92
Stem	9.62	4.5
Flower	0.8	0.5

TLC Analysis Before Fractionation. From the TLC analysis conducted on leaves, stems and flowers, no alkaloid was obtained before fractionation. This may be due to the alkaloid components not yet being separated from the other components contained in the sample. Before fractionation was performed, the results obtained showed the presence of all components in the sample, without indicating any particular alkaloid. This complicates the process of detecting alkaloid components within the samples. According to Sarker *et al* (2005), it was incredibly difficult to separate individual components from a crude mixture using only one separation step [14].

Another factor which may have caused the non-detection of alkaloids could be due to the unsuitability of the chromatography method used. This is because there are too many components in the sample, complicating the process of isolating the components to be studied. Other factors such as the mobile phase used, specifically methanol/ammonium hydroxide for the chromatography, may also not be appropriate for examining the presence of alkaloids before fractionation.

TLC Analysis After Fractionation. The spots obtained on the TLC plate are very sensitive to various factors, including the sample concentration, the way the sample is dropped onto the plate and the solvent type used. Different extract concentrations affect the resulting distance of the spot and so affect the R_f value. One should note the appropriate concentration needed to obtain the proper R_f value where the R_f value indicates the alkaloid type [15]. Thus, observations are repeated to confirm the actual type of alkaloid existing in the extract. Iodoplatinate reagents were used for reference of nicotine, thebaine, quinine and tomatine alkaloids. The R_f and color of spots was compared with the reference color of Clarke [9] and Waldi et al. [16], respectively.

R_f value was determined for the leaf, stem and flower extracts after fractionation. Table 3 shows that at a concentration of 0.005 g/ml, alkaloids of nicotine type are traceable. At a concentration of 0.01 g/ml, alkaloids of thebaine, nicotine and quinine types were identified, whereas at a concentration of 0.02g/ml, alkaloids of nicotine, quinine and tomatine types were detected. This also shows the presence of nicotine and tomatine in all three concentrations of *C. furcata* leaf and stem, whereas only tomatine could be identified in all three concentrations of *C. furcata* flower.

Table 3. TLC analysis of extracts after fractionation at different extract concentrations

Plant Part	Replicate	R_f Extract Concentration (g/ml)			Color of Iodoplatinate	Alkaloid Identified
		0.005	0.01	0.02		
	1		46.3		Blue	Thebaine
			55.0	55.9	Purple/Dark blue	Nicotine
		67.6	66.3	64.8	UD	UD
	2		50.0		Green Blue	Quinine
		57.5	58.8	57.0	Purple/Dark blue	Nicotine
		66.3	68.8	65.8	UD	UD
Leaf			92.5	91.1	UD	UD

	3		44.6	46.1	UD	UD
			51.8	52.6	Green Blue	Quinine
		57.7		57.9	Purple/ Dark blue	Nicotine
				63.2	Dark Blue	Tomatine
		64.4	68.7		UD	UD
			85.5		UD	UD
	4	58.0			Purple/Dark blue	Nicotine
				60.1	Dark Blue	Tomatine
			68.7	67.6	UD	UD
			90.7		UD	UD
	1	56.8	58.2	57.1	Purple/Dark blue	Nicotine
			63.3		Dark Blue	Tomatine
		67.6	72.2		UD	UD
	2	54.4			UD	UD
			55.0	58.6	Purple/Dark Blue	Nicotine
		63.3			Dark Blue	Tomatine
			65.0		UD	UD
			75.0		UD	UD
Stem						
	3	55.1		55.3	UD	UD
			57.3		Purple/Dark blue	Nicotine
				61.8	Dark Blue	Tomatine
		64.1	69.3		UD	UD
	4	56.0			Purple/Dark blue	Nicotine
		63.7		62.8	Dark Blue	Tomatine
			76.4		UD	UD
	1	63.3			Green Blue	Tomatine
			65.4	70.9	UD	UD
			87.3		UD	UD
	2		67.1		UD	UD
		78.5	87.3	88.6	UD	UD
Flower						
	3	63.8	63.9	62.1	Green Blue	Tomatine
		76.0		76.0	UD	UD
	4	64.0		61.8	Green Blue	Tomatine
		86.1	87.3	81.0	UD	UD

UD = unidentified

Table 3 shows that nicotine and tomatine alkaloids had the same color as the reference color. However, the existence of spots in the flower extract showed no similarity with the reference color. These spots were not the same color as the reference, probably because they were the result of an incomplete separation process. Careful observation of the leaves analysis reveals that spot/points of thebaine and quinine were obtained only at a concentration of 0.01 g/ml.

Conclusions

This study identified alkaloid, flavonoid and saponin components in *C. furcata* extracts through chemical screening. Analysis by TLC showed there was no alkaloid detected in the extracts before fractionation but after fractionation several alkaloids such as nicotine, tomatine, thebaine and quinine were observed. The presence of these alkaloids in *C. furcata* extracts showed that this plant has a potential for pharmaceutical drugs, such as nicotine in helping to stop smoking and tomatine as an agitator agent of steroids reduction. Further study is being carried out for the utilization of *C. furcata* through the bioactivity and the allelopathic effects of various extracts obtained by different solvent and extraction methods.

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