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STUDY ON PHYTOCHEMICAL OF THE STEMS OF *MORINDA PERSICAEFOLIA* COLLECTED IN AN GIANG PROVINCE

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ABSTRACT

Objectives: *To determine the phytochemical of the stems of Morinda persicaefolia collected in Tan Phu, An Giang Province, Vietnam; to fractionate and isolate pure compounds from the anthranoid-enriched fractions.* ***Methods:*** *stems powder was extracted with ethanol 70% by percolation and liquid-liquid partition. The chloroform fraction was further isolated by vacuum liquid chromatography (VLC), fractional partition with different pH solution and column chromatography (CC) to obtain pure compound(s). The isolated compound(s) were structurally identified on the basis of UV-Vis spectra.* ***Results:*** *the pure compound isolated from anthranoid-enriched fractions was identified as an alizarin derivative.* ***Conclusion:*** *the results provide informative references to conduct research on biological effects afterward.*

Keywords: *Morinda persicaefolia, Anthranoid, An Giang.*

I. INTRODUCTION

Morinda persicaefolia Ham., locally known as Nhau nuoc or Nhau nho, was traditionally used as a laxative or antihypertensive therapies. Contemporary, studies on phytochemical of *Morinda persicaefolia* were still limited. In order to contribute information in phytochemical, biological effects and to take the advantages of Vietnamese botanical drugs in treatment, the study on *Morinda persicaefolia,* which was collected in Tan Phu, An Giang Province, was carried out with a main focus on:

- Investigating the qualitative phytochemical of the stems of *Morinda persicaefolia*
- Fractionating and isolating pure compound(s) from the anthranoid-enriched fractions.

II. MATERIALS AND METHODS

2.1 Materials

The whole plant of *Morinda persicaefolia* Ham (MP) was collected on July 2018 in Phu Tan district, An Giang Province, Vietnam. The stems were washed, dried at 50 – 60ºC and grounded into powder. The samples were stored at a cool and dry place for further investigation. A voucher specimen has been deposited at the Department of Pharmacognosy - Traditional Pharmacy - Botany, Can Tho University of Medicine and Pharmacy.

Chemical reagents and laboratory materials

Morphological and microscopic procedures: camera, ruler, microscope. Thin-layer chromatography (TLC) was performed with aluminum pre-coated silica gel 60 F_{254} nm (Merck). CC and VLC were carried out on silica gel 60 (Merck, 40 – 63μm). The UV spectra were recorded on a UV-Vis Jasco – V730 (Japan). Ethanol, benzene, n-hexane, toluene, petroleum ether 60-90, dichloromethane, chloroform, ethyl acetate, diethyl ether, methanol was purchased from Chemsol (Vietnam). Other chemicals, solvents, and reagents are at an analytical reagent standard.

2.2 Methods

2.2.1 Study of botanical characteristics

Identifying scientific name based on morphological characteristics with comparison to references [1], [2], [3], [4].

Microscopic method: The internal structure of stems, stems powder characteristics were conducted according to the methods outlined in Appendix 12.18 of the Vietnamese Pharmacopeia fifth edition [5].

Preliminary phytochemical screening: the process of analyzing the phytochemical was based on Ciuley's method.

2.2.2 Extractives assays

Quantify the extractive content in herbal by extracting with ethanol 96%, 70%, 50% and 25% and water according to two methods (hot and room temperature) outlined in the Appendix 12.10 of the Vietnamese Pharmacopoeia fifth edition [5].Then, the extracts of each solvent were investigated using a TLC plate.

2.2.3 Extraction, isolation and purification

Application of percolation and liquid-liquid partition, VLC and CC and purifying (recrystallized method) to obtain pure compound(s). The TLC method was used for qualitative analysis to each fraction and to initially determine the purity of the target compound(s).

The target compound(s) were analyzed by UV-Vis.

III. RESULTS

3.1. Botanical characteristics

3.1.1 Morphological characteristics

Morinda persicaefolia is a small shrub, 0.5 – 1 m height. The bark is dark brown, smooth, and glabrous. The root is light brown and the inner root is yellowish. The leaf arrangement is the opposite. Leaves are light green, elliptic, lanceolate, oblong or fiddle-like shaped, 0.5 - 4.5 cm width and 4 - 13 cm length; base cuneate; margin entire; apex acute or acuminate. Leaf-opposed inflorescence: capitula solitary; peduncle at anthesis is sessile or inconspicuous. Flowers are white or pinkish, develop in clusters which are adherent by

calyx tube to form a cylinder 1 – 2 cm length and 5 – 8 mm width; corolla is hypocrateriform. Fruits are green berries, ovoid syncarp of many drupes, 2 – 2.5 cm height, which have a rough surface.

Figure 1. Morphological characteristics of *Morinda persicaefolia.*

1. Whole body; 2. The leaes; 3. The roots; 4. The fruits; 5. The flowers; 6. The flower buds

3.1.2. Microscopic characteristics of stems of Morinda persicaefolia

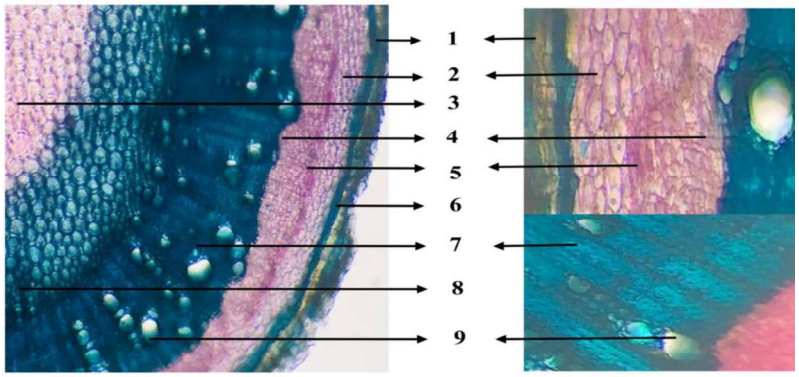

Figure 2. Internal structure of stems.

1. Cork; 2. Cortex parenchyma; 3. Pith parenchyma; 4. Secondary phloem; 5. Primary phloem; 6. Sclerenchyma; 7. Secondary xylem; 8. Xylem parenchyma (parenchyme vasicentrique); 9. Vessels

Figure 3. Stems powder characteristics.

1. Pieces of xylem vessels; 2. Fibres; 3. Parenchyma; 4. Calci oxalate crystal; 5. Starch grains

3.1.3 Preliminary phytochemical screening

The main phytochemical groups were tested with their specific reagents. The results were presented in Table 1. The results gave the positive reactions of triterpenoid, anthraglycoside, saponin, organic acid, and reductant.

Table 1. Phytochemical screening of MP stem.

Ingredients	Qualitative results of the extract			Positive / Negative
	Petroleum ether extract	Alcoholic extract	Water extract	
Fat	-			Negative
Carotenoid	-			Negative
Oil	-			Negative
Free triterpenoid	+			Positive
Alkaloid	-	-	-	Negative
Coumarin	-	-		Negative
Antraglycosid	+	+	+	Positive
Flavonoid	-	-	-	Negative
Cardiac glycosid		+/-	+/-	Unclear
Anthocyanosid		-	-	Negative
Proanthocyanidin		-	-	Negative
Tanin		-	-	Negative
Hydrolysis triterpenoid		+	+	Positive
Saponin		+	+	Positive
Organic acid		+	+	Positive
Reductant		+	+	Positive
Polyuronic acids			-	Negative

3.2 Extractive assays

MP was extracted with water, ethanol 25 %, ethanol 50 %, ethanol 70 %, ethanol 96 % with 2 methods (hot extraction and room temperature extraction). Each extraction was repeated triplicate to give the average concentration. The extract was tested on TLC for the composition evaluation. In combination with extractive concentration, the results were illustrated in table 2, figure 4, and figure 5.

Table 2. Extractive contents with two extraction methods.

Extraction method	Extractive concentration (%)				
	Distilled water	Ethanol 25%	Ethanol 50%	Ethanol 70%	Ethanol 96%
Hot extraction	9.85	9.57	9.04	8.5	4.92
Room temperature extraction	4.85	9.53	9.3	7.98	4.61

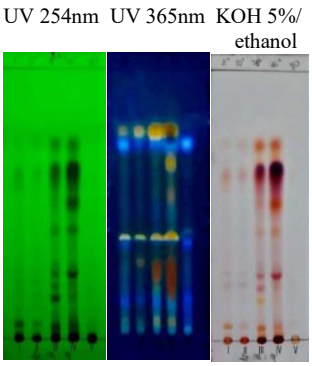

Figure 4. Chromatogram of room extraction temperature extraction.

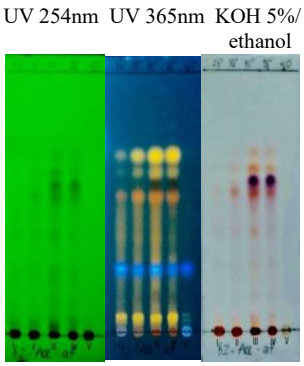

Figure 5. Chromatogram of hot.

Note: Mobile phase was benzene – acetone – acid formic (75-24-1).
I. Ethanol 25%; II. Ethanol 50%; III. Ethanol 70%; IV. Ethanol 96%; V. Distilled water

Although the extraction yield was highest in distilled water, it has some disadvantages: conditions that favour bacterial and mold growth, unselective soluble ability that leads to the impurities in the extract, the high boiling point which is difficult to evaporate. Notably, the anthraquinone spots of distilled water extract were light or almost invisible to UV 254nm or KOH 5%/ethanol reagent. Meanwhile, in addition to overcoming the weakness of distilled water, anthraquinone spots of ethanol 70% extract were darker, fewer impurities compared to other solvents, and had a relatively high extraction yield. Therefore, ethanol 70% was chosen as an ideal extracting solvent.

3.3 Extraction, isolation and purification

15 kg of dried stems powder of *MP* (humidity: 10.96%) were extracted with ethanol 70% by percolation. The extract was evaporated under low pressure to give 1047.7 g residue, which was suspended in distilled water and then partition with chloroform and ethyl acetate, respectively to yield chloroform extract (101.9 g) and ethyl acetate extract (40.9 g).

The chloroform extract was subjected to VLC over silica gel (350 g) with elution of chloroform – acid formic (100: 3 drops, v: v) to give 15 fractions (F1 – F15). The fraction from F11 to F15 was continually partitioned with different pH solutions (NaHCO3 5 %, NaOH 5 %, and then concentrated HCl with pH of 5-6) to obtain 5 fractions (I – V). Fraction I (175.4 mg) was further separated on column chromatography with n-hexane – chloroform (100:0→0:100) to yield 8 subfractions (I1 – I8). The subfraction I7 was purified to obtain X compound (1.2 mg) (TLC plates had been illustrated in supplemented materials).

Purification: compound X was crystallized and re-crystallized in benzene-chloroform (1-2, v: v), then n-hexane.

The obtained compound X (1.2 mg) is the reddish-orange needle crystals and react with KOH 5%/ ethanol reagent to produce a violet solution as anthraquinone dyes. TLC was used to determine the purity of compound X with 3 solvents: (1) n-hexane – chloroform – formic acid (60 – 40 - 3 drops); (2) benzene– methanol – formic acid (94 – 6 - 3 drops); (3) chloroform – methanol – formic acid (90 - 10 - 3 drops). The compound was considered as TLC pure compound. (Fig. 6)

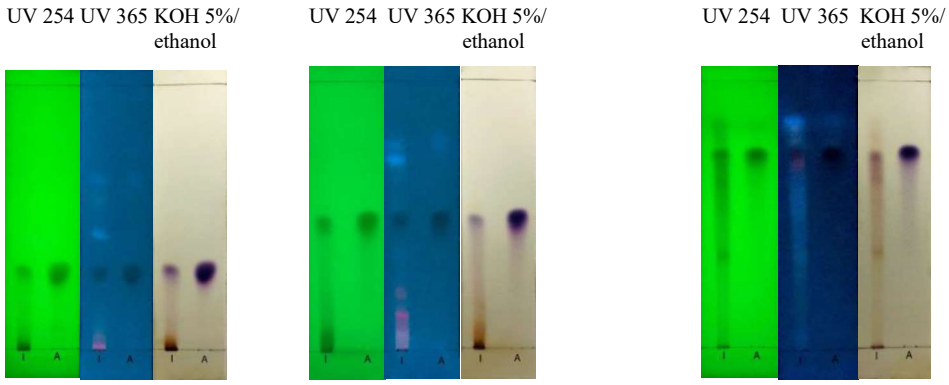

Figure 6. Chromatogram of checking the purity of compound X.

Fig. 7 showed the UV-Vis spectrum of compound X which had 3 peaks. 2 peaks were at 230 nm and 259 nm in the ultraviolet region and 1 peak at 443 nm in the visible region. In alkaline solution (KOH 5% in methanol), the bathochromic effect occurred at peak 259→262 nm and peak 443→551 nm.

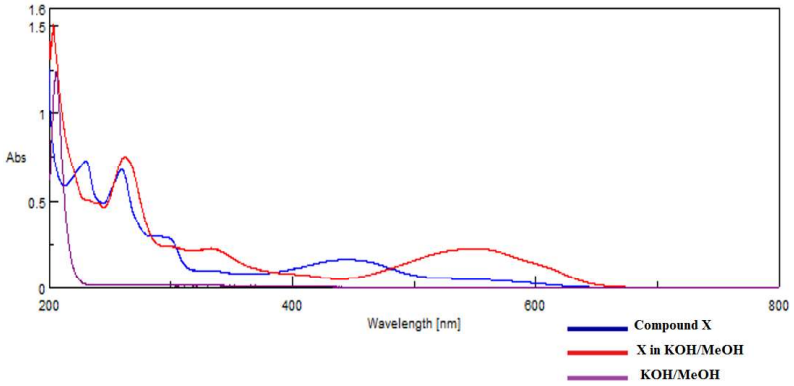

Figure 7. Overlay UV-Vis spectrum of compound X in methanol and KOH 5%.

IV. DISCUSSION

4.1 Botanical characteristics

MP differs to *Morinda citrifolia (*a famous species in *Morinda* genus*)* in some morphological characteristics: the height is shorter (about 0.5 – 1 m) in comparison to the other (6 – 10 m); the size of fruits is smaller (2 – 2.5 cm) while the other is 5 – 6 cm. Thus, it's easy to identify and differentiate between MP and *Morinda citrifolia.*

The microscopic characteristics of our samples were similar to the description in previous studies [3], [4].

There was no considerable difference between our present research and other published references. Notably, the positive results of anthraquinone and triterpenoids were clearly visualized. According to the Nguyen My Hanh's research, the content of anthraquinone and triterpenoids was highly in stems and roots [4]. Therefore, our research was aimed to conducted in appropriate extraction and isolation methods.

4.2 Extractives assays

Compared to the room temperature extraction method, the hot extraction method resulted in an increase in extraction yield. The extraction yield was highest in distilled water, followed by ethanol 25%, ethanol 50%, ethanol 70%, and ethanol 96%. Although the distilled water extraction is higher than the other, the ethanol 70 % extraction at room temperature was chosen for further investigation because of its high density of anthranoid (target composition).

4.3 Extraction and isolation

Chloroform fraction contains less polar compounds in comparison to ethyl acetate extract. Fig. S1 shown that anthranoid spots of chloroform extract were extremely intense. Because of the large amount of anthranoid content and extraction yield, VLC and CC method were used to fractionate complex mixtures into simple fractions. These fractions were treated by a liquid-liquid partition with different pH solutions ($NaHCO_3$ 5 %, NaOH 5 %, and then concentrated HCl with pH of 5-6) based on the solubility of free aglycons and anthraquinone glycosides.

In comparison with the UV-Vis spectra of certain alizarin derivatives of genus *Morinda* (e.g morindin, damnacanthal, nordamnacanthal, rubiadin, etc.) reported in previous studies [3], [6], the UV-Vis spectra of compound X showed the similarities in the absorption signals in a range of 200 - 300 nm and longer wavelengths (350 - 450 nm) with the decrease in the molar absorptivity. According to above results, compound X was supposed as an alizarin derivative. Due to the limitation of the obtained sample amount, more advanced spectrometry was not implemented.

V. CONCLUSION

Morpho-microscopical characteristics of the stems were described. The results of extractive content in MP were used to investigate the suitable solvent and extraction methods. Phytochemical investigation resulted in the isolation of pure compound X based on VLC and CC methods. The pure compound X was hopefully identified as alizarin derivative on the basis of UV-Vis spectra recorded and in comparison with published literature [3].

Conflict of Interest: The authors declare that they have no conflict of interest.

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SUPPLEMENTED MATERIALS

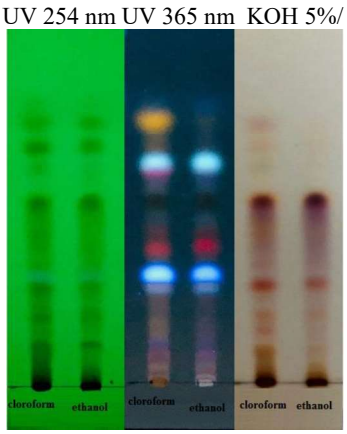

Figure S1. Chromatogram of Chloroform and Ethanol extracts.

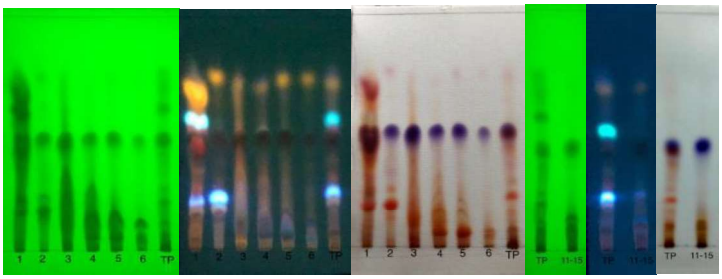

Figure S2. Chromatogram of fraction F1 – F15.

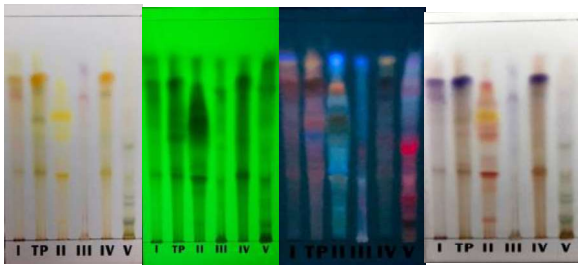

Figure S3. Chromatogram of fraction I – V.

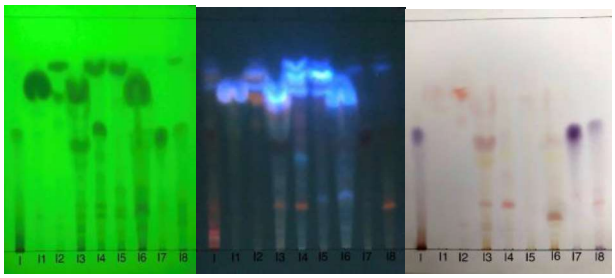

Figure S4. Chromatogram of fraction I1 – I8.

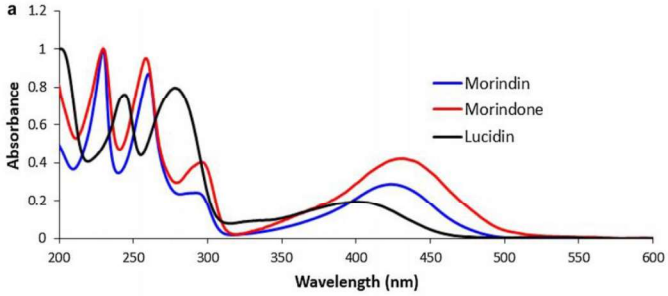

Figure S5. Overlay UV-Vis spectrum alizarin derivatives.

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ISOLATION AND IDENTIFICATION OF THE ENDOPHYTIC BACTERIA IN *HOUTTUYNIA CORDATA THUNB* AND THEIR ANTIBACTERIAL ACTIVITY AGAINST *STAPHYLOCOCCUS AUREUS* OF HUMAN FURUNCLES IN CAN THO CITY

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ABSTRACT

Background: *Endophytic bacteria from medicinal plants are sources of biologically active material for pharmaceutical industries to treat symptoms of numerous diseases. No history indicates that the Houttuynia cordata Thunb in these areas had ever been studied for endophytic bacteria previously.* ***Objectives:*** *To isolate and identify the endophytic bacteria from Houttuynia cordata Thunb and evaluate the antimicrobial activity to Staphylococcus aureus of human furuncle in Can Tho city.* ***Methods:*** *The entire Houttuynia cordata Thunb plant was used to isolate endophytic bacteria and their antibacterial ability against Staphylococcus aureus was done. Based on the sequence of 16SrRNA, some bacterial strains with antimicrobial activities were identified. Besides, comparing the sequence of endophytic bacteria strains with the sequence of bacterial strains found in the NCBI data bank.* ***Results:*** *The results showed that 60 endophytic bacteria strains in*