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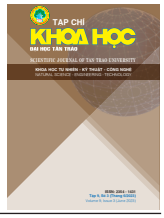

CHEMICAL COMPOSITION AND ANTI-INFLAMMATORY ACTIVITITES OF MORINDA OFFICINALIS COLLECTED IN THAI NGUYEN

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Abstract

From the stems of *Morinda umbellata* trees, using HPLC and according to the IV Pharmacopoeia, the content of major chemical compounds was determined to be tannin (2.6%), saponin (1.7%), phytosterol (26.3%), flavonoid (2.1%), and alkaloid (2.9%). The in vitro anti-inflammatory activity was tested through the inhibition of NO production on the macrophages of *Morinda umbellata* (RAW264.7). The results showed that the high-concentration extract (MU) exhibited good anti-inflammatory activity with an IC_{50} value of 36.2 µg/ml and high cell survival rate. The ethyl acetate fraction (MUE) demonstrated the best anti-inflammatory activity, with an IC_{50} value of 18.1 µg/ml and higher cell survival rate compared to the positive control [Cardamonin], which had an IC_{50} value of 10.5 µM. These promising findings, along with further studies, may contribute to unlocking the potential of *Morinda umbellata* for functional food and pharmaceutical applications, thus utilizing this plant species effectively.

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THÀNH PHẦN HÓA HỌC VÀ HOẠT TÍNH KHÁNG KHÁNG VIÊM IN VITRO CỦA CAO CHIẾT CÂY NHÀU TÁN (MORINDA UMBELLATA L.) Ở THÁI NGUYÊN

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Từ khóa

Morinda umbellata;
Kháng viêm; Thái Nguyên

Tóm tắt

Từ thân cành cây Nhàu tán (*Morinda umbellata*), bằng phương pháp HPLC và theo dược điển IV đã xác định được hàm lượng các nhóm các nhóm hợp chất hóa học chính là tanin (2.6%), saponin (1.7%), phytosterol (26.3%), flavonoid (2.1%) và alkaloid (2.9%). Hoạt tính kháng viêm *in vitro* được thử nghiệm thông qua ức chế sản sinh NO trên đại thực bào của cây Nhàu tán (*Morinda umbellata*) RAW264.7, kết quả cho thấy cao chiết tổng (**MU**) có hoạt tính kháng viêm tốt với giá trị IC_{50} là 36.2 μg/ml và tỷ lệ sống sót tế bào cao. Phân đoạn ethyl acetate (**MUE**) thể hiện hoạt tính kháng viêm tốt nhất, với giá trị IC_{50} là 18.1 μg/ml và tỷ lệ sống sót tế bào cao so với chất đối chứng (+) [Cardamonin] là 10.5 μM . Kết quả khả quan này cùng với các nghiên cứu sâu hơn sẽ góp phần mở ra tiềm năng khai thác cho cây Nhàu tán (*Morinda umbellata*) vào thực phẩm chức năng và dược phẩm. Từ đó góp phần sử dụng hiệu quả loài cây này.

1. Introduction

The genus *Morinda* L. belongs to the Rubiaceae family, and has the shape of a slithering or climbing shrub, with roughly 13 species and 4 varieties. The genus *Morinda* is recognized in Vietnam to include 9 species and 3 varieties, which are spread in dry terrain near woodland and sandy coastal regions [1, 2, 3]. Certain species in folk medicine are extremely beneficial to healing, such as *Morinda citrifolia*, which has laxative, nerve-regulating, and blood pressure-lowering properties; *Morida officinalis* is used to feed the brain and strengthen the tendons and bones, save in cases of rheumatism. *Morinda umbellata* is becoming increasingly popular in the plains, midlands, and mountains, where it is used to cure rheumatism, diarrhea, swelling, rashes, worms, stomachache, fever, cough, severe hepatitis, and other ailments. Van Kieu and Muong people harvest *Morinda umbellata* roots in spring and fall, wash them, remove the rootlets, soak them in warm water, cut them short, and dry them as medicine. The Pako people cure boils, rashes, scabies, and deworming using the whole tree, yellow star, and decoction. *Morinda umbellata* root is commonly used to cure rheumatism when combined with mother-of-pearl bark, scraped grass root, and sharp broom root [4].

Chemical studies have been conducted on various parts of *Morinda umbellata*, revealing the presence of several chemical constituents. The preliminary phytochemical analysis of the leaf extract identified the presence of flavonoids, phenolic compounds, alkaloids, steroids, tannins, and carbohydrates [5]. From the ethanolic extract of the vines of *Morinda umbellata*, 22 compounds were isolated and identified. These compounds include anthraquinones (1,6-dihydroxy-2-methoxymethylanthraquinone, 6-hydroxy-7-methoxy-2-methoxymethylanthraquinone, 3,6-dihydroxy-7-methoxy-2-methylanthraquinone, 6-hydroxy-2-methoxymethylanthraquinone, 3-hydroxy-2-methoxy-6-methylanthraquinone, 2-hydroxy-6-methoxyanthraquinone, 3,6-dihydroxy-2-methylanthraquinone, 6-hydroxy-2-methylanthraquinone, 3-hydroxy-2-methylanthraquinone, 3-hydroxy-2-hydroxymethylanthraquinone, soranjidiol, robustaquinone D, pustaline, 6-hydroxyrubiabin, anthragallol-1,2-dimethylether, rubiadin, alizarin-1-methylether), flavonoid (chrysoeriol), coumarin (scopoletin), benzaldehyde (gallaldehyde, *p*-oxybenzaldehyde), and triglyceride (2-monolinolein) [6]. Additionally, the first report of 11-noriridoids (umbellatolides A-B) was discovered in the methanol extract of the aerial parts of Vietnamese *Morinda umbellata* [7]. Chinese scientists investigated the chemical constituents of the aerial parts of *Morinda umbellata* and found quinones to be the main compounds present. These quinones include 2-Hydroxy-6-hydroxymethylanthraquinone, 2-Hydroxymethyl-6-methoxyanthraquinone, 1,6-Dihydroxy-2-hydroxymethylanthraquinone, 1,3-Dihydroxy-7-methoxymethylanthraquinone, 3-Hydroxy-1-methoxy-7-methoxymethylanthraquinone, 3-Hydroxy-1,7-dimethoxyanthraquinone, 2,3-Dihydroxy-6-methoxymethylanthraquinone, 2,6-Dihydroxy-1-methoxyanthraquinone, 6-Hydroxy-1,2-methylenedioxyanthraquinone, 3-Hydroxy-1,2-methylenedioxyanthraquinone, Methyl 4,6-dihydroxy-1-methoxynaphthalene-2-carboxylate, Dimethyl 1,1′-dihydroxy-4,4′-dimethoxy-2,2′-binaphthalene-3,3′-dicarboxylate, Methyl 7,12-dihydro-2,9-dihydroxy-5-methoxy-7,12-dioxodinaphtho[1,2-*b*:2′,3′-*d*]furan-6- carboxylate, Methyl 7,12-dihydro-2,5,9-trihydroxy-7,12-dioxodinaphtho[1,2-*b*:2′,3′-*d*]furan-6-carboxylate, Methyl 7,12-dihydro-9-hydroxy-5-methoxy-7,12-dioxodinaphtho[1,2-b:2′,3′-d]furan-6-carboxylate, Methyl-7,12-dihydro-10-hydroxy-5-methoxy-7,12-dioxodinaphtho[1,2-*b*:2′,3′-*d*]furan-6-carboxylate, 1,4a,13,14a-tetramethoxy-4,4a,14,14a-tetrahydro-3*H*-naphtho[2,3-*b*]xanthene-3,7,12-trione, 2-ethoxy-5-methoxy-6,11-dioxo-6,11-dihydro-2H-naphtho[2,3-*g*]chromene-3-carboxamide, dimethyl 4-hydroxy-1'-((1-hydroxy-4-methoxy-3-(methoxycarbonyl) naphthalen-2-yl)oxy)-4'-methoxy-1-oxo-1,2-dihydro-[2,2'-binaphthalene]-3,3'-dicarboxylate, 2,2-dimethyl-1,2,3,4-tetrahydroanthracene-9,10-dione, 5-methoxy-2-methyl-6,11-dioxo-6,11-dihydro-2*H*-naphtho[2,3-g]chromene-3-carboxamide. Moreover, there are several other compounds present in *Morinda umbellata*, such as sesquiterpene ((*R*,*E*)-6-hydroxy-3-(3-hydroxybut-1-en-1-yl)-2,4,4-trimethylcyclohexa-2,5-dien-1-one), phenol (4-hydroxy-2-(2-hydroxybenzoyl)-3-methoxybenzoic acid, 2,14-dihydroxy-12-methoxy-6-oxo-6*H*-benzo[h]naphtho[2,3-c]chromen-7-yl methyl carbonate, (2*S*)-3-(4-hydroxy-3-methoxyphenyl)-2-(hydroxymethyl)-5-methoxy-2,3-dihydro-9*H*-[1,4]dioxino[2,3-h]chromen-9-one, (2*S*)-3-(4-hydroxy-3,5-dimethoxyphenyl)-2-(hydroxymethyl)-5-methoxy-2,3-dihydro-9*H*-[1,4]dioxino[2,3-h]chromen-9-one). These compounds, along with the previously mentioned quinones and other compounds, contribute to the chemical composition of *Morinda umbellata* [8, 9, 10].

Inflammation is the body's natural physiological reaction to external (physical, chemical, viral, immunological, etc.) or internal factors that induce inflammation (closed necrosis causing inflammation, etc.) inflammation around cancer tissue, enhanced inflammatory metabolism such as blood urea generating inflammation of the pleura and pericardium, etc. Chronic illnesses may be exacerbated by uncontrolled inflammation [11].

Activated inflammatory cells (neutrophils, eosinophils, monocytes, and macrophages) release enormous quantities of nitric oxide (NO), prostaglandin E2 (PGE2), and other chemicals during inflammation. Proinflammatory cytokines such as IL-1, IL-6, and

TNF-α aid with the destruction or inhibition of invading microbes or malignant tissue. Overproduction of these chemicals, on the other hand, not only causes tissue and cell damage but also activates macrophages in rheumatoid arthritis and chronic hepatitis [12, 13, 14]. The purpose of this study was to undertake a preliminary evaluation of the anti-inflammatory efficacy of Morinda umbellata extracts by decreasing NO generation on the murine macrophage cell line RAW264. contribute to new evidence on *Morinda umbellata* anti-inflammatory efficacy that is comprehensive and thorough. The findings will add to the scientific foundation for successful utilization.

2. Research object and method

2.1. Research subjects

In April 2021, *Morinda umbellata* was gathered in the Phu Binh District of Thai Nguyen Province, Vietnam. Dr. Nguyen Quoc Binh, Vietnam National Museum of Nature - VAST identifier, recognized the specimen.

Morinda umbellata stems were used.

2.2. Method of making the extract

Following collection, *Morinda umbellata* samples were cut, dried in the shade, dried at 60 ^{o}C to constant weight, and ground. At room temperature, the sample was extracted three times with methanol in an ultrasonic device. To get a methanol residue, the whole solution was distilled to the solvent at decreased pressure and at 50 ^{o}C. The methanol extract was mixed with water before being extracted with the increasing polarity solvents *n*-hexane and ethyl acetate. After solvent removal, *n*-hexane, ethyl acetate, and methanol residues were recovered.

2.3. Several components of the Morinda umbellata sample were quantified.

The Institute of Life Sciences - Thai Nguyen University measured reducing sugar, cellulose, total ash, moisture content, and phytosterol, flavonoids, and triterpenoids groups in total ethanol extracts of *Morinda umbellata* stems using the Pharmacopoeia IV and HPLC methods.

2.4. In vitro anti-inflammatory activity assay

The Institute of Natural Product Chemistry - VAST investigated anti-inflammatory action of *Morinda umbellata* RAW264.7 on macrophages by inhibiting NO generation. The testing procedure is as follows:

Materials and chemicals

ATCC gave the cell RAW264.7 and the chemicals (American Type Culture Collection, Manassas, VA, USA). Sigma-Aldrich supplied the cell culture media DMEM (Dulbecco's Modified Eagle Medium), MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide), Griess reagent, and LPS (lipopolysacharide). Thermo Fisher Scientific (now Merck KGaA, Darmstadt, Germany) (Waltham, MA, USA).

RAW264.7 cells were grown for 48 hours in DMEM media at 37^{o}C, 5% CO_2, and 10% FBS. The cell fluid was then transferred at a density of 2.5 x 105 cells/well to plate well 96. Cells were stimulated for 24 hours with 2 L LPS (0.1 mg/mL) and various drug/reagent doses were applied. Cardamonin was used as a positive control. To create the calibration curve, the cell suspension was treated with Griess reagent and $NaNO_2$ at various doses. At λ = 570 nm, take a reading of the reaction mixture.

The rate of NO production inhibition (%) was calculated using the formula: $\%UC=([X_{TB}]_{sample}-[X_{TB}]_{LPS})/([X_{TB}]_{DC}-[X_{TB}]_{LPS})$ x100

Where [XTB] is the average NO concentration determined by the $NaNO_2$ standard curve.

After being used to test *in vitro* activities, the leftover cells were treated with MTT solution (0.5 mg/ml in PBS) and incubated for 4 hours at 37°C in a 5% CO_2 incubator. The formazan crystalline conversion product was diluted in dimethyl sulfoxide (DMSO, Sigma-Aldrich), and the optical density was measured on an Infinite F50 (Tecan, Männedorf, Switzerland) at λ = 540/720 nm.

Comparison to the control, the cell survival rate was CS% (Cell survival) in%. (%) = $[(OD_{[sample]}/OD_{[control\ (-)]})$ x 100] ± The formula is used to compute the standard deviation:

$$\sigma = \sqrt{(\sum(xi - \bar{x})\text{^}2)/(n-1)}$$

3. Discussion and results

3.1. Sample preparation

The chemical composition and biological activities of *Morinda umbellata* extracts were investigated. Figure 1 depicts the extract manufacturing process.

Tabel 1. Some fundamental indicators for assessing the Morinda umbellata

Targets	Content (%)	Targets	Content (%)
Moisture content	50.5	Tanin	2.6
Total Ashes	6.2	Saponin	1.7
Reducing sugar consumption	9.8	Flavonoid	2.1
Starch	15.2	Alkaloid	2.9
Cellulose	16.8	Phytosterol	26.3

Table 2. Anti-inflammatory activity of Morinda umbellata extracts tested

No.	Sample	Highest sample concentration	Inhibition rate NO production	Cell rate survive (%)	IC_{50} Value
	Control (-)	-	100.0 ± 0.70	102.45 ± 0.28	
	Control (+) [Cardamonin]	3.0 μM	85.12 ± 1.46	70.85 ± 0.51	10.5 μM
		0.3 μM	64.88 ± 0.95	88.44 ± 0.66	
	LPS	-	0.0 ± 0.50	100.0 ± 0.13	
1	**MU**	50 μg/ml	61.23 ± 1.34	63.11 ± 1.89	36.2 μg/ml
		25 μg/ml	40.76 ± 0.55	72.24 ± 1.59	
2	**MUH**	50 μg/ml	54.88 ± 1.29	43.45 ± 1.32	66.4 μg/ml
		25 μg/ml	29.13 ± 1.77	55.43 ± 1.67	
3	**MUE**	50 μg/ml	71.34 ± 0.85	66.54 ± 1.32	18.1 μg/ml
		25 μg/ml	55.66 ± 1.43	79.07 ± 1.68	
4	**MUW**	50 μg/ml	32.35 ± 1.21	65.23 ± 1.46	-
		25 μg/ml	16.20 ± 1.42	82.34 ± 1.10	

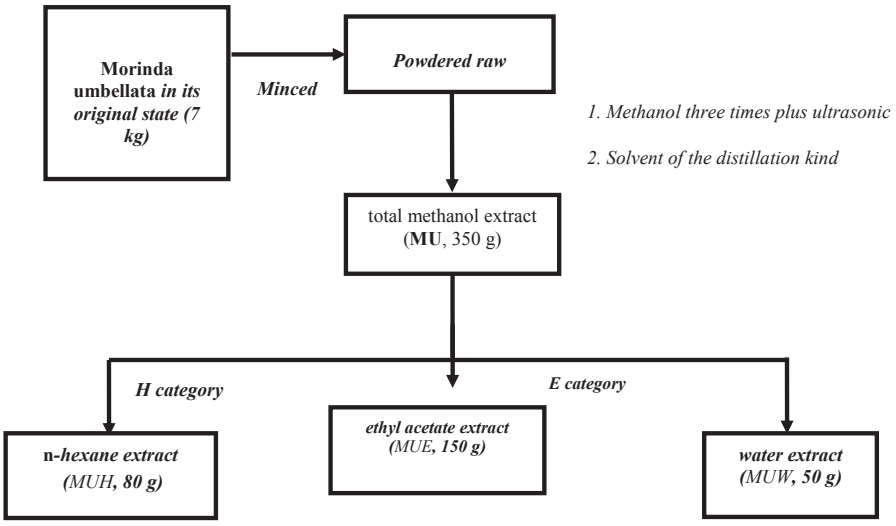

Image 1. Morinda umbellata sample processing diagram

3.2. Quantification results of various substance groups in the Morinda umbellata

Table 2 shows the findings of a quantitative study of chemical classes performed on the whole extract (MU) of the *Morinda umbellata*. *Morinda umbellata* medicinal plants include the following ingredients: Natural reducing sugars (9.8%), starch (15.2%), cellulose (16.8%), total ash (6.2%), moisture (50.5%), tannin (2.6%), saponin (1.7%), phytosterol (26.3%), flavonoid (2.1%), alkaloid

3.3. In vitro anti-inflammatory activity of Morinda umbellata extract

Table 2 shows the findings of an *in vitro* anti-inflammatory activity test on *Morinda umbellata*. The total methanol extract (**MU**) exhibits anti-inflammatory effects as evidenced by its capacity to suppress NO generation. The IC_{50} value was 36.2 g/ml, and the cell

survival rate was high. The ethyl acetate extract (**MUE**) had the strongest anti-inflammatory effect, with an IC_{50} value of 18.1 g/ml and a high cell survival rate, followed by *n*-hexane, which had an IC_{50} value of 66.4 g/ml. The water extract, on the other hand, exhibited essentially little action. This implies that the *Morinda umbellata* has strong anti-inflammatory action, and good anti-inflammatory chemicals are mostly found in ethyl acetate extract. Direction for future research on the chemical composition and anti-inflammatory effectiveness of *Morinda umbellata*.

4. Conclusion

By chemical composition study of the trunk of the *Morinda umbellata*, we found the primary chemicals as tannin content (2.6%), saponin content (1.7%), phytosterol content (26.3%), flavonoid content (2.1%), and alkaloid content (2.9%).

The in vitro anti-inflammatory activity test findings of *Morinda umbellate* revealed that the whole extract (**MU**) exhibited good anti-inflammatory activity with an IC_{50} value of 36.2 g/ml and cell survival rate. High. With an IC_{50} value of 18.1 g/ml and a high cell survival rate, the ethyl acetate extract (**MUE**) displayed the highest anti-inflammatory efficacy. This encouraging finding, together with more research, will help to unleash the potential of the *Morinda umbellata* for functional foods and medications. As a result, this plant is being used more effectively.

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