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Original Research Article

Anti-inflammatory and Antioxidant Activities of Root Extract of *Morinda officinalis* from Quang Binh Province, Vietnam

Thanh Pham[1*], Thi Y. Van[2], Minh D. Tran[2], Dien Dinh[3], Thi H.Q. Hoang[2], Nam T. Tran[2]*

[1]*Department of Biology, University of Education, Hue University, 34 Le Loi, Hue 530000, Vietnam*
[2]*Faculty of Forestry, University of Agriculture and Forestry, Hue University, 102 Phung Hung, Hue 530000, Vietnam.*
[3]*Phong Dien Nature Reserve, ThuaThien Hue province, Vietnam*

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ABSTRACT

In Asia, *Morinda officinalis* F.C. How, a member of the Rubiaceae family, is frequently used as a medicinal plant and is thought to have several biological properties. This study aimed to evaluate the anti-inflammatory and antioxidant of *M. officinalis*. Nitric oxide (NO) generation in RAW 264.7 macrophage cells was used to measure the anti-inflammatory effects, while the antioxidant activity of the extract was determined using the 2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonate (ABTS) assay. The results show that the root extract of the plant dramatically reduced the amount of NO produced, with IC_{50} values of 37.11 ± 2.6 µgmL^{-1} (compared to IC_{50} 14.20 ±1.1 µgmL^{-1} for dexamethasone). The antioxidant activity against ABTS has an SC_{50} value of 318.37 ± 12.21 µgmL^{-1} (Trolox, IC_{50} 7.02 ± 0.24 µgmL^{-1}). These results gave important background data for future research on the anti-inflammatory and antioxidant benefits of *M. officinalis*.

Keywords: *Morinda officinalis*, Nitric oxide, ABTS, Anti-inflammatory, Antioxidant.

Introduction

The lianoid shrub *Morinda officinalis* How. var. *officinalis* (Rubiaceae), an important medicinal plant, is most commonly found in China's tropical and subtropical regions, particularly the Fujian, Hainan, and Guangdong provinces.[1] Wild populations of *M. officinalis* can also be found in the Vietnamese provinces of Cao Bang, Lao Cai, Ha Giang, Quang Binh, Thua Thien Hue, and Quang Tri.[2-4]

The primary chemical components of *M. officinalis* are anthraquinones, which have bioactivities against osteoporosis[5] and Alzheimer's disease[6]. There have also been reports of iridoid glycosides, saccharides, organic acids, volatile oils, and triterpenoids in *M. officinalis*. Additional bioactivities associated with the saccharide components include antiosteoporotic, immunomodulatory, and depressive effects.[7]

Several previous research studies on the anti-inflammatory activity of *M. officinalis*.[8] According to oriental medicine, rheumatoid arthritis, dermatitis, and endocrine conditions like impotence have all been cured with *M. officinalis* roots.[8] *M. officinalis* inhibits the expression of iNOS, COX-2, and TNF by downregulating NF-KB binding activity.[9] It also has anti-inflammatory and analgesic properties.[9] According to Huan et al. (2021), findings on anti-inflammatory phytochemicals of the plant revealed that 2 pairs of new methyl-2-naphthoate enantiomers, 1 new anthraquinone, 3 new natural unknown anthraquinones, and 18 known anthraquinones were isolated and characterized from the root extract of *M. officinalis*. These compounds substantially decreased the amount of NO generated. This result was confirmed by immunoblotting, quantitative real-time PCR, and immunofluorescence labelling studies. The compounds inhibited the nuclear translocation of NF-B and dose-dependently suppressed the production of COX-2 and iNOS, two pro-inflammatory proteins activated by lipopolysaccharide.[10]

Furthermore, the oligosaccharides in *M. officinalis* may also possess beneficial effects on the immune system, angiogenesis, and reproductive, antiosteoporosis, antioxidant, antidepressant, and antidementia qualities.[11-14] The antioxidant, anti-chromosomal mutagenesis, anticancer, and analgesic properties of *M. officinalis* iridoids have also been reported.[15-17]

In Vietnam, the focus on *M. officinalis* involves its development in breeding procedure[3], cultivation[2], and evaluation of species genetic diversity[4,18], while assessing the species' active compounds has not been investigated or given attention. This study aimed to evaluate the biological potential of the root extract of *M. officinalis* in line with our goal of finding bioactive natural compounds from traditional therapeutic plants.

*Corresponding author. E mail: phamthanh@hueuni.edu.vn
Tel: +84911346679

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Materials and methods

Plant material

M. officinalis root was purchased in February 2023 from the province of Quang Binh's Bo Trach region. Dr. Tran Minh Duc of the Forestry Faculty, Hue University of Agriculture and Forestry, Hue University, Vietnam, 530000, identified the sample, and a voucher specimen was deposited at the Biology lab at Hue University of Education, Hue University, Vietnam, 530000.

Reagent

Escherichia coli lipopolysaccharides (LPS) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Fetal bovine serum (FBS) and Dulbecco's Modified Eagle's Medium (DMEM) were bought from Life Technologies, Inc. (Gaithersburg, MD, USA). Sodium nitrite, sulfanilamide, n-1-napthylethylenediamine dihydrochloride, and dimethyl sulfoxide (DMSO) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Sigma, GIBCO, Invitrogen, and Promega provided further essential chemicals. Sigma Chemical Co. (St. Louis, MO, USA) is the source of 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonate) (ABTS) and 2,4,6-tripyridyl-s-

triazine (TPTZ). A 96-well plastic plate (Corning, USA), pipettes (Eppendorf), and an ELISA 96-well plate reader (Bio-rad) were used.

Methanol extraction

The root bark of *M. officinalis* (3 g) was extracted with methanol (90 mL x 3) by heating in a water bath for 3 h until exhaustive extraction was attained and then filtered. The combined filtrates were evaporated to dryness under reduced pressure in a rotatory evaporator at 50°C to obtain the crude extract.

Determining the ability to inhibit NO production in RAW 264.7 macrophage cells

The RAW 264.7 cell line was provided by Prof. Dr. Domenico Delfino from the University of Perugia in Italy. This cell line was cultured in DMEM media supplemented with 2 μL^{-1} glutamine, 10 µM HEPES, and 1.0 µM sodium pyruvate. In addition, 10% FBS from GIBCO was used as a supplement. The cells were housed in a CO_2 incubator at 37°C with 5% CO_2 and passaged every 3–5 days at a ratio of 1:3.

2×10^5 RAW 264.7 cells were planted in each well of the 96-well plate, and the cells were then cultured for 24 h at 37°C and 5% CO_2 in a CO_2 incubator. The culture medium was removed, and the DMEM medium without FBS was added and then incubated for 3 h. Subsequently, the cells were preincubated at different concentrations for 2 h before being stimulated with LPS (10 μgmL^{-1}) to induce NO production for 24 h. Some wells were not preincubated with samples and were utilized as negative controls. The positive control was dexamethasone (Sigma) at concentrations of 100, 20, 4, and 0.8 μgmL^{-1}. Nitrite (NO_2^-) was considered an indicator of NO production, determined using the Griess Reagent System (Promega Cooperation, WI, USA). Specifically, 100 µL of cell culture medium (sample) was transferred to a fresh 96-well plate and combined with 100 µL of Griess reagent: 50 µL of 1% (w/v) sulfanilamide in 5% (v/v) phosphoric acid and 50 µL of 0.1% (w/v) N-1-naphthylethylenediamine dihydrochloride in water. This mixture was incubated at room temperature for 10 min, and the nitrite content was measured using a microplate reader at a wavelength of 540 nm. FBS-free DMEM medium was used as a blank. The nitrite levels in each experimental sample are determined by comparing them to a standard curve of $NaNO_2$ and expressed as a percentage of the negative control (LPS).

The corresponding percentage of inhibition of NO generation for each sample was calculated using the following formula:

$$\% \text{ inhibition} = 100\% - (NO_{sample} \text{ concentration}/NO_{control} \text{ concentration}) \times 100.$$

The experiment was done in triplicates. The IC_{50} value (the concentration that inhibits 50% of NO generation) was calculated using the TableCurve 2Dv4 computer program.

The MTT method was used to determine cell viability under the influence of the test substance. This approach assesses cell viability by measuring the formation of a coloured formazan product when MTT is applied to the cell wells under the influence of enzymes in live cells. Specifically, the following steps are involved: After collecting the supernatant for NO measurement, 90 µL of cell culture medium and 10 µL of MTT (final concentration of 5 mg/mL) was introduced into each cell culture plate used for the NO expression experiment. After 4 h, the medium was removed, and the formazan crystals were dissolved by adding 50 µL of 100% DMSO. The optical density (OD) value was determined at a wavelength of 540 nm using a BioTek Elx800 spectrophotometer.

The cell viability in the presence of the test substance was determined using the following formula:

$$\% \text{ cell viability} = (OD_{(sample)} - OD_{(blank)})/(OD_{(DMSO)} - OD_{(blank)}) \times 100.$$ [19-23]

Antioxidant Determination using ABTS assay

The determination of antioxidant activity utilizing the TEAC method of a test sample was performed following the method of Saeed et al. (2012) with slight adjustments.[24] Briefly, different concentrations (80, 400, 2000, 10000 μgmL^{-1}) of the sample solution were prepared in ion-free water. Trolox (reference control) was prepared in concentrations of 16, 80, 400, & 2000 μgmL^{-1} using ion-free distilled water. 7 mM ABTS and 2.45 mM potassium persulfate were mixed and allowed to stand in the dark for 16 h at room temperature. The ABTS+ solution was diluted in acetate buffer to achieve an OD value of 0.70 ± 0.02 at a wavelength of 734 nm. 1900 µL of the ABTS+ solution was added to 100 µL of the prepared sample (final concentrations in the wells were 4, 20, 100, & 500 μgmL^{-1}). DMSO (1%) was used as the negative control. Wells containing only ion-free purified water are considered blank wells. The OD was read at a wavelength of 734 nm using a BioTek 96-well plate reader.

The antioxidant activity of the test samples was determined from the formula below:

$$ABTS+ (\%) = (A0 - A)/A0 \times 100,$$

Where A0: OD value of the control well − OD value of the blank well; A: OD value of the well containing the test sample − OD value of the blank well.

The antioxidant activity of the test samples was expressed as the EC50 value, which is the concentration required to decrease the ABTS+ radicals by 50%.

The anti-inflammatory and antioxidant activity studies were conducted at the Institute of Biotechnology, Vietnam Academy of Science and Technology.

Result and Discussion

Extract yield

The crude methanol extract yield was 6.75% (w/w).

Effects of M. officinalis root Extract on NO Production

The inhibitory activity of different concentrations of the extracts and dexamethasone (positive control drug) against NO generation is shown in Table 1. The measurements include the proportion of inhibition of NO generation and the percentage of living cells. At a concentration of 100 μgmL^{-1}, *M. officinalis* root extract showed 85.50% inhibition of NO production compared to 81.13% for dexamethasone. In addition, at a concentration of 20 μgmL^{-1}, the root extract also showed 35.11% suppression of NO generation compared to dexamethasone (52.66%). Furthermore, *M. officinalis* root extract exhibits 19.08% and 8.00% inhibition of NO production at 4 μgmL^{-1} and 0.8 μgmL^{-1}, respectively.

The results from this study show that at 100 μgmL^{-1}, both *M. officinalis* root extract and dexamethasone show comparable inhibitory effects on NO production, with slightly higher inhibition observed for the *M. officinalis* root extract. At a concentration of 20 μgmL^{-1}, dexamethasone exhibits a higher inhibitory effect than the *M. officinalis* root extract. The IC_{50} values for *M. officinalis* root extract and dexamethasone were 37.11 ± 2.59 μgmL^{-1} and 14.20 ± 1.11 μgmL^{-1}, respectively.

One of the crucial signalling molecules in the pathophysiology of inflammation is NO. Nitric oxide generation inhibitors are thought to have anti-inflammatory properties.[9,10,16,17,25] *Morinda* and its components were reported to have anti-inflammatory qualities. *M. officinalis* inhibited this NO production in a concentration-dependent manner over the 40–80 μgmL^{-1} concentration range with an IC_{50} of 61.83 μgmL^{-1}.[9,10] However, IC_{50} values for methyl-2-naphthoate enantiomers were moderate at 34.32 ± 4.87 μgmL^{-1}, and other compounds had IC_{50} values greater than 40 μgmL^{-1}.[10] In addition, the anti-inflammatory effects of *Morinda citrifolia* have an IC_{50} of 70.21 μgmL^{-1}.[25]

3.3. Effects of *M. officinalis* root Extract on ABTS antioxidant activity

Table 2 compares the percentage of free radical scavenging activities with the activity of Trolox at different concentrations. At a concentration of 100 μgmL^{-1}, the average percentage of free radical scavenging activities was 65.72%, while the mean percentage activity of Trolox was much greater at 92.84%. Similar trends were observed at lower concentrations as well. At 20 μgmL^{-1}, the average free radical scavenging activity was 24.77%, whereas the mean percentage activity of Trolox was noticeably greater at 70.98%. At 4 μgmL^{-1} and 0.8 μgmL^{-1} concentrations, the free radical scavenging activities were 7.90% and 2.71%, respectively. In contrast, Trolox demonstrated more

scavenging activity at these concentrations, with percentage activity of 40.19% and 13.37%, respectively. These results indicate that the tested samples had moderate free radical scavenging activities compared to Trolox.

Table 1: Inhibitory effects of *M. officinalis* root extract on the production of NO in RAW 264.7 macrophage cells

Concentration (μgmL^{-1})	*Morinda officinalis* root extract				Dexamethasone (Positive control)			
	% inhibition NO		% living cells		% inhibition NO		% living cells	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
100	85.50	2.70	98.81	1.13	81.13	1.45	98.47	1.57
20	35.11	1.62	100.88	0.43	52.66	1.08	101.23	2.25
4	19.08	1.05			41.95	0.97		
0.8	8.00	0.70			32.77	1.12		
IC_{50}	37.11±2.59		-		14.20±1.11			

Table 2: Effects of *M. officinalis* root extract on ABTS antioxidant activity

Concentration (μgmL^{-1})	% free radical scavenging activities		% Activity of Trolox	
	Mean	SD	Mean	SD
100	65.72	1.23	92.84	1.06
20	24.77	0.93	70.98	1.19
4	7.90	0.74	40.19	1.02
0.8	2.71	0.27	13.37	0.84
SC_{50}	318.37 ± 12.21		7.02 ± 0.24	

Earlier studies on the ABTS antioxidant capacity of the *Morinda* genus showed antioxidant activities with the IC_{50} 70.21 μg mL[25,26]. An investigation into the effects of extracting phenolic antioxidants from *M. citrifolia* was performed using a single-factor experiment. Antioxidant capacity was measured by analyzing the scavenging impact on ABTS.[26] Experimental results showed that extraction conditions significantly affected phenolic compounds' extraction and antioxidant capacities. The ideal conditions were 40% ethanol for 80 min at 65°C, with values of 791.71 μmol TEAC/100 g DW for ABTS.[26] It has been shown that reactive oxygen species (ROS), reactive nitrogen species (RNS), and chlorine species are involved in the pathophysiology of many human diseases, which may assist in explaining why traditional Chinese remedies are said to have favourable health effects.[27] Because they are major sources of phenolic compounds, herbal extracts, particularly traditional Chinese remedies, would be anticipated to have antioxidant properties.[28] Multiple antioxidant entities that interact with ABTS+ at different rates are probably present in the extracts.[27]

Conclusion

The *M. officinalis* root extract demonstrated inhibitory action on NO generation with IC_{50} values of 37.11 μgmL^{-1} while showing antioxidant activity against ABTS with an SC_{50} value of 318.37 ± 12.21 μgmL^{-1}. In conclusion, extracts of *M. officinalis* could provide a rich source of new anti-inflammatory and antioxidant agents.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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