



Research Article

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Antioxidant and acute anti-inflammatory activities of aerial parts of *Morinda umbellata* L

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ABSTRACT

This study investigated *in vitro* antioxidant and acute anti-inflammatory effect of aerial parts of *Morinda umbellata* L. (ML). Extracts of ethanol 70% were used to evaluate pharmacological activities. Total flavonoid content (TFC), and the scavenging effect on DPPH radical were exhibited. The effect on acute anti-inflammation was evaluated by *in vivo* models. It was found that the TFC, antioxidant ability and acute anti-inflammatory effect of the ML leaf extract was higher and more effective than this of the stem extract.

Keywords: Antioxidant, *Morinda umbellata* L, Acute anti-inflammatory.

INTRODUCTION

Free radicals can be created in biological systems in the character of reactive oxygen species (ROS) [1]. High free radical levels or oxygen species induce oxidative stress [2]. The ROS had been implicated that it is the cause of various chronic diseases such as cancer, diabetes and obesity [3], alzheimer's and parkinson's diseases [4]. The research to find new safe and economical antioxidant sources being of natural origin is required to reduce side effects of synthetic antioxidants such as the toxicity and carcinogenic. For this reason, in the past decades many medicinal plants have been commodiously studied for their antioxidant activity and free radical inhibiting activity [5, 6, 7].

Morinda umbellata L. (ML), an annual herbaceous herb, belongs to the Rubiaceae family. The earlier studies have indicated that this herb has high therapeutic and medicinal value and chemical constituents potential. The aerial parts contain quinones, sesquiterpene and enantiomers [8], the racemic trimeric quinone and polycyclic quinones [9], 11-noriridoids, umbellatolides A and B [10]. This herb has versatile pharmacological effects including rheumatic, stomachache, and diabetes, etc. [8]. In traditional Vietnamese medicine, the aerial parts of the ML were used to treat rheumatoid arthritis (RA) being a chronic inflammatory autoimmune disease. In human, RA is defined by a series of pathological processes of the joints. The most frequently prescribed medication to treat RA is non-steroidal anti-inflammatory drugs [11]. While these drugs effectively reduce the symptoms, they may generate serious side effects including gastrointestinal ulcer and renal morbidity [12, 13]. Accordingly, herbal medicine therapeutics for RA to control side effects was encouraged. Thus, the work was aimed to study and compare the TFC, the antioxidant ability effect on acute anti-inflammation from the leaf and stem ethanol extracts of *Morinda umbellata* L.

MATERIALS AND METHODS

Chemicals and reagents

Quercetin, 1,1-diphenyl-2-picrylhydrazyl (DPPH), butylated hydroxytoluene (BHT), aluminum chloride (AlCl₃) and potassium acetate (CH₃COOK) were bought from Showa Chemical Co., LTD. (Japan). Dimethyl sulfoxide (DMSO) and ethanol (95%) were procured from J.T. Baker (Avantor Performance Inc, USA) and Echo Chemical Co., LTD. (Taiwan), respectively. Carrageenan was procured from Sigma-Aldrich (St Louis, USA) and other reagents.

Preparation of samples

Two grams of 50-mesh sieved dry powder of leaf and stem were separately extracted doubly with ethanol 70% at 30°C by ultrasonic. The filtrates were obtained and concentrated in a vacuum evaporator at 45°C. The dehydrated fractionation was weighted to calculate yield, then diluted in DMSO to a regular concentration.

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Determination of total flavonoid content

The TFC was measured by following the spectrophotometric method of Arvouet-Grand et al. (1994)^[14]. The 0.5 mL of 70% ethanol extract solution (1 mg/mL) was added with 1.5 mL of 95% ethanol, 2.3 mL of distilled water, 0.1 mL of 1M CH₃COOK, 0.1 mL of 10% AlCl₃, and 1mL of 1M NaOH. After incubating at room temperature for 90 min, absorbance was measured at 415 nm. The TFC was calculated from a calibration curve of quercetin solution with $y = 0.0089 - 0.0366x$, $R^2 = 0.9723$ and standardized by the calculation of quercetin equivalent per gram of dry extract (mg of QUE/g of dry extract). The estimation of the TFC in the extracts was performed in triplicate and the results were averaged.

DPPH free radical scavenging ability

The DPPH inhibitory effect was performed as earlier work with some modifications^[15]. The DPPH solution was diluted with ethanol 95% to obtain a concentration of 0.002% (w/v). 0.1 mL sample in methanol at different concentrations of 5 to 160 µg/mL was added 0.9 mL of DPPH solution. The mixture was incubated at 37°C for various intervals until the reaction reached the steady state, and DPPH radical scavenging ability was determined by measuring the absorbance at 517 nm. Butylated hydroxytoluene (BHT) was used as positive control. DPPH radical scavenging activity (%) = $(1 - A_s/A_o) \times 100$. Where A_s and A_o were the absorbance of the sample and the blank (without sample), respectively. IC₅₀ value was determined. After evaluating the antioxidant effect of stem and leave extract. The results show that the leave extract has better antioxidant effect. Therefore, the leave extract was chosen to evaluate anti-inflammatory activity.

Carrageenan-induced paw edema in rats

Swiss rats weighing (220 ± 20g) of either sex were procured from the National Institute of hygiene and epidemiology. All the experiments were carried out in the morning according to current guidelines for the care of the laboratory animals and the ethical guidelines. Experiment was performed as earlier work with some modifications^[16]. Edema in the right hind paw of rats was induced by injecting 0.1 mL of carrageenan 1% (w/v) in distilled water subcutaneously in the plantar side. The paw diameter was determined before injecting and the each hour up to three times then after 3 and 5 h. The rats were randomly divided into four groups (n=8). The first group (control group) was treated distilled water (3 ml/kg body weight p.o.), while the second group was treated the standard anti-inflammatory drug; the indomethacin (10 mg/kg body weight p.o.). The third group was treated with the leave extract of the ML (100 mg/kg body weight p.o.). The fourth group was treated with twice dose of the third group. The rats were pretreated 1 h before the administration of carrageenan. Percentage of paw volume increase is: $X\% = (V_1 - V_o/V_o) \times 100$; in which X% is percentage of paw volume increase; V_o is paw volume after carrageenan injection; V_1 is paw volume at 3 and 5 after carrageenan injection. Edema inhibiting percentage was measured by $Y\% = (M_c - M_t/M_t) \times 100$, in which, Y% is percentage of paw edema decrease, M_c is percentage of paw edema increase of control group and M_t is percentage of paw edema increase of leave extract treated group.

RESULTS AND DISCUSSION

Total flavonoid content

The extract dehydrated fractionation was weighted to calculate the yield. Ten grams of leaves and stem dry powder after extracting were combined, respectively, 1.32 grams (13.2 percent of weight) and 0.77 grams (7.7 percent of weight) of dry extract powder. The leave extract exhibited higher the yield compared to the stem extract (5.5 percent). The total flavonoid contents were found in ethanol 70% of the leave and stem extracts (113.22±1.43 mg QUE/g dry extract and 86.16 ±1.15 mg QUE/g dry extract, respectively).

Antioxidant properties

DPPH radical scavenging ability in a dose-dependent manner were shown in Figure 1. The IC₅₀ values of stem, leave and BHT were, respectively, 41.68, 23.80 and 10.72 µg/mL and ranked stem>leave>BHT. The higher IC₅₀ value shows the lower antiradical capacity. Among the extracts tested, the leave extract was shown to have the higher antioxidant activity compared to the stem extract. This result could be explained that the total flavonoid contents of the leave extract are higher than the stem extract and more compounds with strong antioxidant capacity.

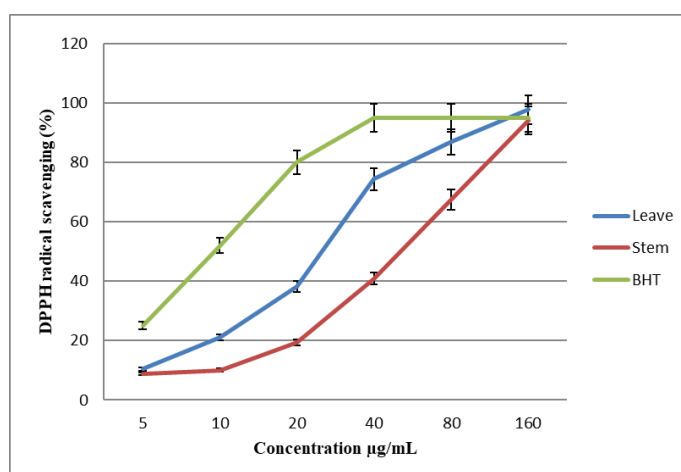


Figure 1: DPPH radical scavenging capacity of leave and stem extract of *Morinda umbellata* L.

Acute anti-inflammation effect

The results of acute anti-inflammation effect were shown in Table 1. It was found that the leave extract reduced 35.46% and 38.21% of inflammation in a carrageenan-induced paw edema assays at oral dose of 100 mg/kg/day after 3 and 5 hours injecting carrageenan, respectively. At 200 mg/kg/day oral administered, this extract also exhibited acute inflammation with an inhibitory effect of 45.82% and 54.26% after 3 and 5 hours injecting carrageenan. This study suggests that the leave extract possesses the anti-inflammatory activity and could be the active principle of the plant *Morinda umbellata* L in ethnomedicinal uses.

Table 1: The acute anti-inflammation effect of the leave extract

Treatments and doses	Number of rats (n)	After 3 hours injecting carrageenan		After 5 hours injecting carrageenan	
		Percentage of paw edema increase (%)	Percentage of paw edema decrease compared to control group (%)	Percentage of paw edema increase (%)	Percentage of paw edema decrease compared to control group (%)
Control group	8	36.97±1.86	-	31.09±1.25	-
Indomethacin (20 mg/kg/day)	8	10.75±0.64	-	8.56 ±1.31	-
Leave extract (100 mg/kg/day)	8	23.86±1.06	35.46	19.21±1.11	38.21
Leave extract (200 mg/kg/day)	8	20.03±0.72	45.82	14.22±1.02	54.26

CONCLUSION

The leave extract of the ML has the higher total flavonoid content, antioxidant and anti-inflammation capacity compared to the stem extract. Therefore, the findings obtained in the present work could enhance knowledge the antioxidant and anti-inflammation capacity of aerial parts of *Morinda umbellata* L.

Conflict of interest

None declared.

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None declared.

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