4. Conclusion

Four methanol extracts sourced from *Decaspermum gracilentum*, *Uvaria simaensis*, *Syzygium attopeuense*, and *Cratoxylum cochinchinensis*, originating from Vietnam, exhibit noteworthy antioxidant potential, as evidenced by their IC_{50} values falling within the approximate range of 60 to 100 µg/mL. In terms of standard equivalent, their antioxidant capacity spans from 0.15 to 0.25 mg AA equivalents/mg sample and from 0.03 to 0.05 mg GA equivalents/mg sample.

Two specific compounds, namely quercetin 3-*O*-(6-*O*-*α*-L-rhamnopyranosyl)-*β*-D-glucopyranoside and epi-catechin, manifest robust antioxidant potential, as reflected by their IC_{50} values of 84.15 µM and 111.24 µM, respectively. In the context of standard equivalent, the former demonstrates values of approximately 0.31 mg AA equivalents/mg sample and 0.06 mg GA equivalents/mg sample, while the latter exhibits values of approximately 1.11 mg AA equivalents/mg sample and 0.21 mg GA equivalents/mg sample, respectively.

Our results suggest a promising antioxidant potential in the tested extracts and compounds. However, it is imperative to conduct further *in vitro* assays to validate their antioxidant properties before proceeding with subsequent *in vivo* investigations.

References

1. WHO/noncommunicable-diseases. 2024: Access date 27 March, 2024. **2.** Banothu V., Neelagiri C., Adepally U., Lingam J., Bommareddy K. (2017), Phytochemical screening and evaluation of *in vitro* antioxidant and antimicrobial activities of the indigenous medicinal plant *Albizia odoratissima*, *Pharmaceutical Biology*, 55(1), 1155-61. **3.** Siddeeg A., AlKehayez N. M., Abu-Hiamed H. A., Al-Sanea E. A., Al-Farga A. M. (2021), Mode of action and determination of antioxidant activity in the dietary sources: An overview, *Saudi Journal of Biological Sciences*, 28(3), 1633-44. **4.** Becker M. M., Nunes G. S., Ribeiro D. B., Silva F. E., Catanante G., Marty J. (2019), Determination of the antioxidant capacity of red fruits by miniaturized spectrophotometry assays, *Journal of the Brazilian Chemical Society*, 30, 1108-1114. **5.** Olugbami J., Gbadegesin M., Odunola O. J. (2014), *In vitro* evaluation of the antioxidant potential, phenolic and flavonoid contents of the stem bark ethanol extract of *Anogeissus leiocarpus*, *African journal of medicine and medical science*, 43, 101-109. **6.** Dueñas M., González-Manzano S., González-Paramás A., Santos-Buelga C. (2010), Analysis B. Antioxidant evaluation of O-methylated metabolites of catechin, epicatechin and quercetin, *Journal of Pharmaceutical and Biomedical Analysis,* 51(2), 443-449. **7.** Olszowy-Tomczyk (2021), How to express the antioxidant properties of substances properly?, *Chemical Papers*, 75(5), 6157-6167. **8.** Yen G. C., Duh P. D., Tsai H. L. (2002), Antioxidant and pro-oxidant properties of ascorbic acid and gallic acid, *Food Chemistry*, 79(3), 307-313.

Journal of Medicinal Materials, 2024, Vol. 29, No. 3 (pp. 170 - 176)

ANTIOXIDANT AND ANTI-INFLAMMATORY EFFECTS OF *OCHNA INTEGERRIMA* LEAF EXTRACTS

Tu Quoc Thang[1], Nguyen Phuc Khanh[2], Nguyen Thi Huong Nguyen[2], Le Pham Thuy An[2], Vu Huynh Kim Long[2], Ton Nu Lien Huong[1], Nguyen Minh Hien[2,*]

[1]College of Natural Sciences, Can Tho University, Vietnam;

[2]Faculty of Pharmacy, Ton Duc Thang University, Ho Chi Minh City, Vietnam

*Corresponding author: nguyenminhhien@tdtu.edu.vn

(Received March 18th, 2024)

Summary

Antioxidant and Anti-Inflammatory Effects of *Ochna integerrima* Leaf Extracts

The objective of this study was to investigate the antioxidant and anti-inflammatory properties of *n*-hexane, ethyl acetate (EtOAc), *n*-butanol, aqueous, and methanol (MeOH) extracts of *Ochna integerrima* leaves. The total phenolic (TPC) and flavonoid contents (TFC) were measured using the Folin-Ciocalteu (FC) and aluminum chloride assays. The antioxidant potential was determined through 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), ferric reducing antioxidant power (FRAP), and reducing power (RP) assays. The anti-inflammatory activity was assessed by measuring the inhibition of nitric oxide (NO) production using RAW 264.7 macrophage cells. The results showed that the EtOAc extract exhibited significant levels of total phenolic content (TPC) and total flavonoid content (TFC) values at (232.85 ± 3.06 mg GAE/g) and (60.73 ± 0.38 mg QE/g), respectively. Moreover, all extracts demonstrated significant antioxidant activity, except for the *n*-hexane extract. Out of five extracts, the EtOAc extract exhibited remarkable antioxidant potential using DPPH and ABTS assays with IC_{50} values of 9.21 ± 0.12 µg/mL and 7.87 ± 0.44 µg/mL, respectively. Similarly, it also displayed the highest FRAP and RP values of 532.94 ± 2.80 mg AAE/g and 78.84 ± 2.85 µg/mL, respectively. Regarding anti-inflammatory activity, the EtOAc extract showed the most potent activity with the IC_{50} value of 53.10 ± 2.34 µg/mL, followed by aqueous extract (IC_{50} 67.33 ± 2.05 µg/mL) in inhibiting NO production without causing cell damage. The remaining extracts did not exhibit significant inhibition of NO production. In summary, *O. integerrima* leaves exhibit potential benefits for treating oxidative and inflammatory disorders.

Keywords: *Antioxidant effect, Anti-Inflammatory effect, O. integerrima leaf extracts.*

1. Background

High levels of free radicals-reactive oxygen species (ROS) and reactive nitrogen species (RNS) are important to inflammatory processes, often originating from oxidative stress [1],[2],[3]. Nitric oxide (NO), is synthesized by NO synthase (NOS) enzymes during inflammation. NO production is often increased as part of the immune response. Moreover, when there are excessive amounts of free radicals and insufficient antioxidants leading to oxidative stress [4]. Under conditions of oxidative stress, NO can interact with free radicals to form complex reactions, including peroxynitrite ($ONOO^-$), causing inflammatory processes [5],[6]. When inflammation gets out of control, it can cause serious health problems like infections, heart disease, cancer, and diabetes. According to estimates from the World Health Organization (WHO), 80% of people have received their main medical care from traditional medicine, and most of these treatments involve the use of plant extracts [7]. Nevertheless, due to the lack of systematic analysis, most of the use of Vietnamese medicinal herbs is not supported by research.

Ochna integerrima (Loureiro) Merrill is generally known in Vietnam as the lucky flower. This species is frequently utilized for therapeutic and ornamental uses [8]. It is mostly found in Southeast and South Asia. Flavonoids, cyanoglycosides, and sterol glycosides have been identified as the major components in the *Ochna* genus. These groups possess considerable pharmacological actions, including anti-inflammatory and antioxidant capabilities [9],[10]. In conventional therapies, the bark and root are used as digestive tonics and antibiotics, respectively while the leaf is used as an antipyretic and an antidysentery [11]. Previously, we reported that the MeOH extracts from flower, leaf, and stem of *O. integerrima* had good antioxidant activity using the DPPH analysis, with IC_{50} values of 48.51 ± 1.43, 17.2 ± 0.15, and 10.05 ± 0.07 µg/ml, respectively [8],[11],[12]. For anti-inflammatory activity, we also found that the *n*-hexane extract from *O. integerrima* stems inhibited BSA denaturation by 57.11 ± 0.53 % at a concentration of 125 µg/mL [12]. To explore its protective effects in further detail, this study conducted investigations into antioxidant capabilities using ABTS, DPPH, RP, and FRAP, in conjunction with TPC and TFC assays from *n*-hexane, EtOAc, *n*-butanol, aqueous, and MeOH extracts. Additionally, we also carried out the anti-inflammatory effect of *O. integerrima* leaf extracts on NO production by LPS-induced macrophages RAW 264.7. To the best of our knowledge, this is the first report of the antioxidant and anti-inflammatory activities of *O. integerrima* leaf extracts.

2. Object and research method

2.1. Plant material and extraction

The leaves of *O. integerrima* were collected in An Giang province, Vietnam, in October 2023 and, identified by botanist Dr. Van Son Dang at the Institute of Tropical Biology, Vietnam Academy of Science and Technology, Ho Chi Minh City, Vietnam. A voucher specimen (AG-OIL) was deposited at the Faculty of Pharmacy, Ton Duc Thang, Vietnam.

Extraction: 2.4 kg dried leaves of *O. integerrima* were macerated four times with 4.0 L of MeOH for one night to give the MeOH extract (96.54 g). After partitioning with organic solvents, the following extracts were obtained: *n*-hexane (8.59 g), ethyl acetate (EtOAc) (33.75 g), *n*-butanol (38.31 g), and aqueous (2.02 g) extracts.

2.2. General procedures

The absorbance of antioxidant tests was measured using a Thermo Fisher Scientific UV-Vis spectrophotometer. The following reagents were acquired from Sigma Aldrich (USA): vitamin C (BCCH6669; 99.0%), quercetin (10230364; 97.0%), DPPH, ABTS, Folin-Ciocalteau (FC) reagent, and 2,4,6-Tri(2-pyridyl)-s-triazine (TPTZ). Gallic acid (5995-86-8; 98.0%) was obtained from Himedia.

For anti-inflammatory activity, the optical density (OD) values were measured using the BioTek Elx800 spectrophotometer. Dulbecco's Modified Eagle's Medium (DMEM) and fetal bovine serum (FBS) were sourced from Life Technologies, Inc. (Gaithersburg, MD, USA). Lipopolysaccharides (LPS), sodium nitrite, sulfanilamide, *N*-1-napthylethylenediamine dihydrochloride, dimethyl sulfoxide (DMSO), and dexamethasone were obtained from Sigma Chemical Co. (St. Louis, MO, USA). The RAW 264.7 cells were provided by Prof. Domenico Delfino, University of Perugia, Italy.

2.3. Antioxidant assays of the extracts

2.3.1. Total phenolic content (TPC):

The procedure was carried out with a few minor modifications by the FC assay [13]. Gallic acid (1.5625 to 25.0 µg/mL) was employed to construct a calibration curve. The extracts were dissolved in DMSO and subsequently diluted with H_2O to yield 50 µg/mL. After adding 0.25

mL of 25% FC, 2 mL of the extract at a final concentration of 50 µg/mL was applied. 0.25 mL of 10% Na_2CO_3 was added and thoroughly mixed after 3 minutes. The mixtures were then measured at 760 nm after 30 minutes of dark incubation. The experiment was carried out three times. The TPC was determined using the gallic acid standard curve and given as mg gallic acid equivalent (GAE)/g.

2.3.2. Total flavonoid content (TFC):

With a few minor modifications, the experimental protocol was carried out using Chang *et al.* (2002) [14]. Quercetin (6.25 to 100 µg/mL) was used to build a calibration curve. The extracts were dissolved in DMSO and diluted with H_2O to yield a solution of 2.0 mg/mL. 1.5 mL of methanol was mixed with 0.5 mL of this extract. The mixture above was well mixed with 0.1 mL of 10% $AlCl_3$ after 5 minutes. Following a 6-minute incubation, 0.1 mL of 1 M CH_3COOK and 2.8 mL of distilled water were added to the mixture, which was then allowed to sit at room temperature for 45 minutes in the dark. The experiment was carried out three times. The TFC was determined using the quercetin standard curve and given as mg quercetin equivalent (QE)/g.

2.3.3. DPPH radical scavenging assay:

With a few adjustments, the protocol was executed using the Masuda J. *et al.* (2014) [15]. After dissolving the extracts in DMSO solution to a concentration of 10 mg/mL, the extract was diluted with 0.1 mM DPPH solution to have a range of concentrations (6.25 to 100.0 µg/mL). The mixture was allowed to sit in the dark for half an hour. The absorption was read at 517 nm. Vitamin C (0.625 to 10.0 µg/mL) was used as a positive control. The findings were displayed as IC_{50} values, which represent 50% of DPPH radical scavenging inhibition. The following formula was used to calculate its radical scavenging capacity:

DPPH inhibition (%) = [(Ac - As)/Ac] × 100

where the absorbances of the extract and control, respectively, are As and Ac.

2.3.4. ABTS radical scavenging assay:

With a few minor adjustments, the protocol was carried out following Re et al. (1999)'s procedure [16]. The color change from dark blue to light blue served as an indicator for the ABTS assay. First, a 1:1 mixture of 2.45 mM $K_2S_2O_8$ and 7 mM ABTS was used to form the $ABTS^{\bullet+}$ cation. At room temperature, this mixture was incubated overnight. After diluting with acetate buffer (pH = 4.5), the absorbance was measured at 0.74 ± 0.03. The extracts were diluted in this ABTS solution to give the concentration (6.25 to 100 µg/mL). The extract solution was assessed using UV-Vis at 734 nm after being incubated for 7 minutes at room temperature in the dark. Vitamin C (0.625 to 10.0 µg/mL) was used as a positive control. Without extracts, the control sample was made in the same procedure. The results were shown as the IC_{50} values. Its radical scavenging capacity was calculated according to the following formula:

ABTS inhibition (%) = [(Ac - As)/Ac] × 100

2.3.5. Ferric reducing antioxidant power (FRAP) assay:

The experimental protocol was carried out with minor adjustments by Benzie and Strain's (1996) method [17]. First, the following chemicals were combined in a 1:1:1 ratio to obtain the TPTZ solution: 300 mM acetate buffer, 10 mM TPTZ in 40 mM HCl, and 20 mM $FeCl_3.6H_2O$. Vitamin C (3.215 to 50 µg/mL) was used as a positive control. The extracts were dissolved in DMSO and diluted with water to produce a 50 µg/mL solution. When the extract reached a final concentration of 50 µg/mL, 1.5 mL of TPTZ solution was added and well mixed with 0.5 mL of the extract. The experiment was carried out three times. The FRAP was determined using the vitamin C standard curve and was given as mg vitamin C equivalent (AAE)/g.

2.3.6. Reducing power (RP) assay:

The method of Oyaizau (1986) with some modifications was used to determine the reducing power [18]. The extracts were dissolved in DMSO and diluted with H_2O to achieve concentrations from 18.75 to 300 µg/mL. 0.2 M phosphate buffer (pH 6.6), and 1% $K_3Fe(CN)_6$ were added and incubated in a water bath set at 50°C for 20 minutes. 0.5 mL of 10% CCl_3COOH was added, and then centrifuged for 10 minutes at 3000 rpm. The upper layer was filled with 1 mL of H_2O and 0.2 mL of 0.1% $FeCl_3$. The absorption was observed at 700 nm. Quercetin (5.0 to 80.0 µg/mL) was used as a positive control.

2.4. Anti-inflammatory activity

Griess reagent was used to measure the amount of NO emission with minor adjustments [19]. The RAW264.7 cells were cultured for 24 hours at 37°C and 5% CO_2 at a density of 2×10^5 cells/well using Dulbecco's Modified Eagle Medium (DMEM). Subsequently, different doses of extracts (100-2.65 µg/mL) were applied to the

cells when LPS (1 μg/mL) was generated. The untreated cell was performed as the blank. The cells that were exposed to LPS + DMSO without extracts were used as the negative control group. NO was examined after a 24-hour treatment. Next, 100 μL of its supernatant was moved to a different 96-well culture plate, combined with 100 μL of Griess reagent (0.1% (w/v) naphthyl ethylenediamine dihydrochloride and 1% (w/v) sulfanilamide in 5% (v/v) phosphoric acid). This mixture was incubated for 15 minutes at room temperature. The absorbance was then determined using a microplate reader at 540 nm. The IC_{50} value (50% of NO inhibition) was determined using the Table-Curve 2Dv4 software. The MTT assay was used to assess the cytotoxic activity. The formazan crystals were dissolved in DMSO for 60 minutes at 37°C. The quantity of formazan was measured at 590 nm. Dexamethasone (0.8 to 100 μM) was used as a positive control.

2.5. Statistical analysis

The data are expressed as mean ± standard deviation (n=3). The statistical analyses were performed using GraphPad Prism 9.3.1 (San Diego, CA, USA).

3. Results and discussion

3.1. Antioxidant activity of the extracts

3.1.1. Total polyphenolic, flavonoid content assays:

Through the construction of a calibration curve ($y = 0.0752x + 0.0533$, $R^2 = 0.9994$) based on various gallic acid concentrations, the TPC was found using the FC procedure. TPC values were the highest in the EtOAc extract (232.85 ± 3.06 mg GAE/g). The following order was displayed by the extracts: MeOH (181.0 ± 1.69), *n*-BuOH (145.97 ± 1.07), aqueous (130.32 ± 1.15), and *n*-hexane (13.94 ± 0.85) mg GAE/g (Fig. 1)

TFC was expressed as mg QE per gram of sample in dry weight (mg/g) using the calibration curve regression equation ($y = 0.0157x-0.0407$, $R^2 = 0.9987$) with statistical *significance* of *p*-value (0.00002). The TFC of the EtOAc extract was 60.73 ± 0.38 mg QE/g, the highest value among the extracts, followed by the MeOH (49.86 ± 2.78), aqueous (25.08 ± 0.21), and *n*-BuOH (20.68 ± 0.13) ones (mg QE/g). The *n*-hexane extract had the lowest TFC value, measuring 12.5 ± 0.16 mg QE/g (Fig. 1).

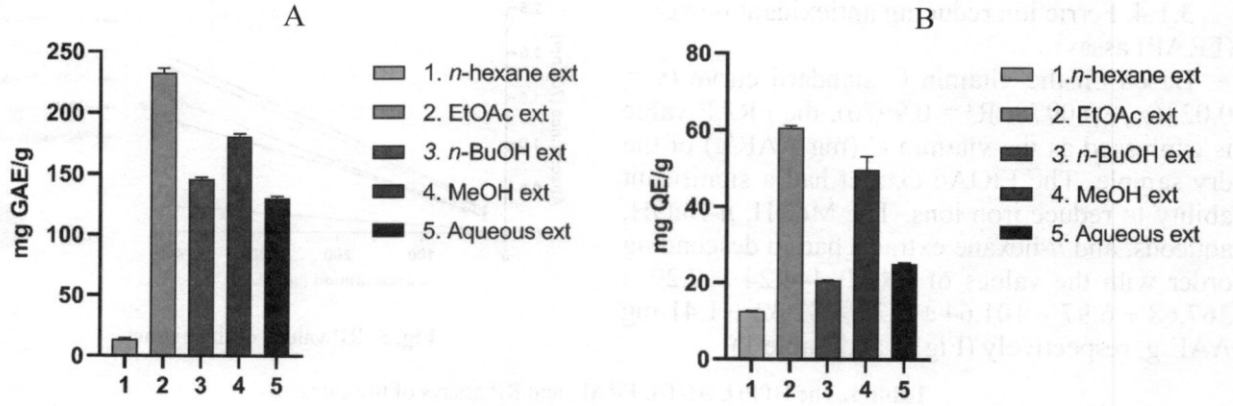

Fig. 1. TPC (A) and TFC (B) of the extracts

3.1.2. DPPH assay:

With an IC_{50} value of 9.21 ± 0.12 μg/mL, the EtOAc extract had the highest antioxidant activity, followed by the MeOH (IC_{50} 12.74 ± 0.38 μg/mL), *n*-BuOH (IC_{50} 15.33 ± 0.43 μg/mL) and aqueous (IC_{50} 34.23 ± 0.89 μg/mL) extracts compared to positive control vitamin C (IC_{50} 1.19 ± 0.01 μg/mL). The *n*-hexane extract, on the other hand, did not show any antioxidant activity with IC_{50} more than 100 μg/mL (Fig. 2 and Table 1).

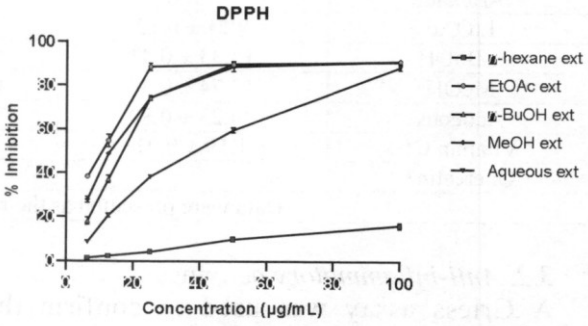

Fig. 2. DPPH radical scavenging activity (% inhibition) of the extracts

3.1.3. ABTS assay:

The pattern of antioxidant activity observed in the ABTS assay closely resembled that observed in the DPPH assay. Specifically, the EtOAc extract displayed the most potent antioxidant activity (IC_{50} 7.87 ± 0.44 µg/mL), followed by the MeOH (IC_{50} 10.61 ± 0.57 µg/mL), *n*-BuOH (IC_{50} 11.77 ± 0.09 µg/mL), and aqueous extracts (IC_{50} 24.67 ± 0.77 µg/mL), in descending order. However, the *n*-hexane extract did not exhibit any antioxidant activity, with an IC_{50} value exceeding 100 µg/mL (Fig. 3 and Table 1).

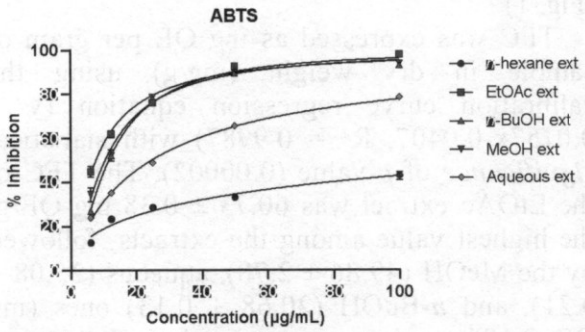

Fig. 3. ABTS radical scavenging activity (% inhibition) of the extracts

3.1.4. Ferric ion reducing antioxidant power (FRAP) assay:

Based on the vitamin C standard curve ($y = 0.0236x + 0.0978$; $R^2 = 0.9976$), the FRAP value is expressed as the vitamin C (mg AAE/g) of the dry sample. The EtOAc extract had a significant ability to reduce iron ions. The MeOH, *n*-BuOH, aqueous, and *n*-hexane extracts had in descending order with the values of FRAP 444.24 ± 7.29 > 367.68 ± 6.97 > 101.64 ± 5.29 > 32.32 ± 1.41 mg AAE/g, respectively (Fig. 4 and Table 1).

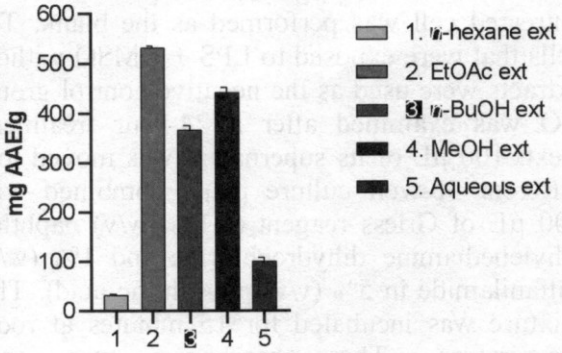

Fig. 4. FRAP values of the extracts

3.1.5. Reducing power (RP) assay:

The results indicated that the RP values of the tested extracts vary significantly. The aqueous extract exhibited the highest RP value of 69.09 ± 2.45 µg/mL, indicating strong reducing potential. Following this, the EtOAc extract demonstrated the next highest activity with an RP value of 78.84 ± 2.85 µg/mL. With an RP value over 400 µg/mL, the *n*-hexane extract showed less antioxidant activity (Fig. 5 and Table 1).

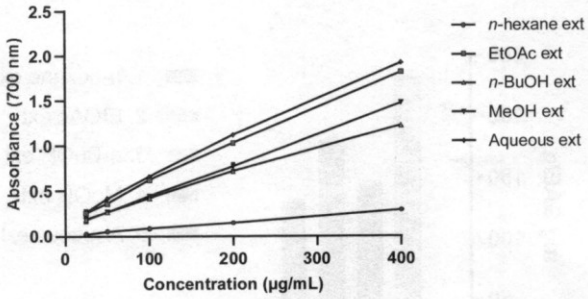

Fig. 5. RP values of the extracts

Table 1. The DPPH, ABTS, FRAP, and RP assays of the extracts

Extracts	DPPH ± SD (IC_{50} µg/mL)	ABTS ± SD (IC_{50} µg/mL)	FRAP ± SD (mg AAE/g)	RP± SD (µg/mL)
n-Hexane	>100	>100	32.32 ± 1.41	>400
EtOAc	9.21 ± 0.12	7.87 ± 0.44	532.94 ± 2.8	78.84 ± 2.85
n-BuOH	15.33 ± 0.43	11.77 ± 0.09	367.68 ± 6.97	131.68 ± 4.47
MeOH	12.74 ± 0.38	10.61 ± 0.57	444.24 ± 7.29	123.45 ± 12.48
Aqueous	34.23 ± 0.89	24.67 ± 0.77	101.64 ± 5.29	69.09 ± 2.45
Vitamin C*	1.19 ± 0.01	1.06 ± 0.01		
Quercetin*				13.66 ± 0.89
Data were presented as the mean ± SD (n=3). *Positive controls				

3.2. Anti-inflammatory activity

A Griess assay was used to confirm the effect of the extracts on NO generation in LPS-induced RAW 267.4 cells. With IC_{50} values of 53.10 ± 2.34 and 67.33 ± 2.05 µg/mL against NO production, the EtOAc and aqueous extracts showed good inhibitory efficacy among the extracts. These extracts

did not exhibit any cytotoxic effects on RAW 264.7 cells with a cell survival rate of above 80%. In contrast, the *n*-hexane extract caused cell harm, implying cytotoxic effects on RAW 264.7 cells with less than 80% cell viability (Table 2).

Table 2. IC_{50} values for the inhibition of NO production of the extracts

Extracts	IC_{50}	% Cell viability at 100 µg/mL
n-hexane	NA	15.38 ± 2.31
EtOAc	53.10 ± 2.34	80.96 ± 1.94
n-BuOH	>100	78.47 ± 2.33
MeOH	>100	87.25 ± 2.14
Aqueous	67.33 ± 2.05	100.52 ± 1.28
Dexamethasone*	12.64 ± 1.28	93.92 ± 0.19

Data were presented as the mean ± SD (n=3); *Positive control (µM); Extracts (µg/mL)
NA: Not available due to cell death.

Polyphenolic compounds, including flavonoids, are commonly present in plants and renowned for their antioxidant and anti-inflammatory properties. They play a crucial role in reducing harmful reactive oxygen species and preventing lipid peroxidation. Several flavonoids were isolated from the leaves, flowers, and stems of this species [10]. However, limited research has been conducted on the antioxidant and anti-inflammatory effects of extracts from each part. Our previous publication highlighted the antioxidant potential of extracts derived from the flowers and stems of *O. integerrima*, effectively neutralizing free radicals, and protecting against oxidative damage [8],[11],[12]. Continuing from our earlier work, where we emphasized the promising antioxidant activity of the MeOH extract from leaves via the DPPH assay [11], our ongoing investigation aims to broaden the *in vitro* antioxidant properties using different assays. Our findings indicated that high levels of TPC and TFC were detected in the EtOAc and *n*-BuOH extracts. These extracts exhibited scavenging potential in DPPH and ABTS assays indicating the strong antioxidant abilities of the plant. Additionally, these extracts demonstrated high reducing power as evidenced by the results of RP and FRAP assays. In this investigation, the potential antioxidant and anti-inflammatory properties displayed a strong positive correlation, particularly highlighted by the good inhibition of NO production from the EtOAc extract. In summary, our findings regarding the antioxidant and anti-inflammatory activities of leaf extracts from *O. integerrima* are consistent with those reported for flowers and stems.

4. Conclusion

This study represents the initial exploration into the antioxidant and anti-inflammatory potential of the leaf extracts from *O. integerrima*. Using ABTS, DPPH radical scavenging, RP, and FRAP assays, all extracts showed considerable antioxidant activity, except for the *n*-hexane extract. The IC_{50} values ranged from 9.21 ± 0.12 to 34.23 ± 0.89 µg/mL for DPPH and from 7.87 ± 0.44 to 24.67 ± 077 µg/mL for ABTS. Furthermore, the EtOAc extract had the highest TPC value (232.85 ± 3.06 mg GAE/g) and TFC value of 60.73 ± 0.38 mg QE/g. It also displayed strong reducing potential. These results were consistent with anti-inflammatory effects, indicating that the EtOAc extract had the strongest anti-inflammatory effect by suppressing the LPS-induced production of NO. Thereby, the EtOAc extract exhibited potential for controlling diseases associated with inflammation and oxidative stress. Thus, additional chemical investigations are necessary to provide solid evidence supporting these activities in future research.

Acknowledgments: *This research is funded by Ton Duc Thang University under grant number FOSTECT.2023.39*

References

1. Phaniendra A., Jestadi D. B., Periyasamy L. (2015), Free radicals: properties, sources, targets, and their implication in various diseases, *Indian Journal of Clinical Biochemistry*, 30(1), 11-26. **2.** Jomova, K., Raptova R., Alomar S. Y., Alwasel S. H., Nepovimova E., Kuca K., Valko M. (2023), Reactive oxygen species, toxicity, oxidative stress, and antioxidants: chronic diseases and aging, *Archives of Toxicology*, 97(10), 2499-2574. **3.** Vona R., Pallotta L., Cappelletti M., Severi C., Matarrese P. (2021), The impact of oxidative stress in human pathology: focus on gastrointestinal disorders, *Antioxidants*, 10(2), 201. **4.** Sharifi-Rad M., Anil Kumar N. V., Zucca P., Varoni E. M., Dini L., Panzarini E., Rajkovic J., Tsouh Fokou P. V., Azzini E., Peluso I., Prakash Mishra A., Nigam M., El Rayess Y., Beyrouthy M. E., Polito L., Iriti M., Martins N., Martorell M., Docea

A. O., Setzer W. N., Sharifi-Rad J. (2020), Lifestyle, oxidative stress, and antioxidants: back and forth in the pathophysiology of chronic diseases, *Journal Frontiers in Physiology*, 11, 694-715. **5.** Lobo V., Patil A., Phatak A., Chandra N. (2010), Free radicals, antioxidants and functional foods: impact on human health, *Pharmacognosy Reviews*, 4(8), 118-126. **6.** Engwa G. A., EnNwekegwa F. N., Nkeh-Chungag B. N. (2022), Free radicals, oxidative stress-related diseases and antioxidant supplementation, *Alternative Therapies in Health Medicine*, 28(1), 114-128. **7.** Wilson E. O., Frances M. P. (1988), Screening plants for new medicines, *Biodiversity*, 9, 83-97. **8.** Buranasudja V., Kobtrakul K., Vimolmangkang S., Binalee A., Sanookpan K., Vu T. Y., Vu H. K. L., Le B., Nguyen H. T., Do K. M., Dang, V. S., Nguyen H. M. (2022), Some antioxidant properties of components from the flower of *Ochna integerrima* and their beneficial effects on HaCaT keratinocytes and *in silico* analysis on tyrosinase, *Chemistry & Biodiversity*, 19(4), e202100882. **9.** Likhitwitayawuid K., Rungserichai R., Ruangrungsi N., Phadungcharoen T. (2001), Flavonoids from *Ochna integerrima, Phytochemistry*, 56(4), 353-357. **10.** Bandi A. K., Lee D. U., Tih R. G., Gunasekar D., & Bodo B. (2012), Phytochemical and biological studies of *Ochna* species, *Chemistry & Biodiversity*, 9(2), 251-271. **11.** Ton N. L. H, Vu H. K. L., Thach U. D., Tran T. T. T., Le T. N. T., Do D. T., Le T. K. C., Nguyen M. H. (2022), Microscopic characteristics, antioxidant activities, and chemical compositions of *Ochna integerrima* leaves and stems, *Journal of Medicinal Materials*, 27(4), 227-234. **12.** Nguyen T. H. N., Phan T. L., Le. P. T. A., Nguyen P. K., Nguyen H. A., Ton N. L. H., Nguyen B. A., Le T. K. C., Nguyen M. H. (2023), Antioxidant and anti-inflammatory potential from *Ochna integerrima* stems, *Journal of Medicinal Materials*, 28(3), 185-190. **13.** Zielinski H., Kozlowska, H. (1998), Antioxidant activity and total phenolics in selected fruits, vegetables, and grain products, *Journal of Agricultural and Food Chemistry*, 46(10), 4113-4117. **14.** Chang C. C., Yang M. H., Wen H. M., Chern J. C. (2002), Estimation of total flavonoid content in propolis by two complementary colometric methods, *Journal of Food Drug Analysis*, 10(3), 178-182. **15.** Masuda T., Yonemori S., Oyama Y., Takeda Y., Tanaka T., Andoh T., Shinohara A., Nakata M. (1999), Evaluation of the antioxidant activity of environmental plants: activity of the leaf extracts from seashore plants, *Journal of Agricultural Food Chemistry*, 47(4), 1749-1754. **16.** Re R., Pellegrini N., Proteggente A., Pannala A., Yang M., Rice-Evans C. (1999), Antioxidant activity applying an improved ABTS radical cation decolorization assay, *Journal Free Radical Biology Medicine*, 26(9-10), 1231-1237. **17.** Benzie I. F., Strain J. J. (1996), The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay, *Analytical Biochemistry*, 239(1), 70-76. **18.** Oyaizu M. (1986), Studies on products of browning reaction antioxidative activities of products of browning reaction prepared from glucosamine, *Japanese Journal of Nutrition and Dietetics,* 44, 307-315. **19.** Natalia R. B., Marlon H., Isabela F. J. C. B., Fabricio M. A., Eduardo P. A., Elena B. L., Michelle F. M., Daniela B. O. (2014), Nitric oxide production, inhibitory, antioxidant and antimycobacterial activities of the fruits extract and flavonoid content of *Schinus terebinthifolius, Revista Brasileira de Farmacognosia*, 24(6), 644-650.

Journal of Medicinal Materials, 2024, Vol. 29, No. 3 (pp. 176 - 184)

STUDY ON ANTI-FATIGUE AND IMMUNO-ENHANCEMENT EFFECTS OF THE EXTRACTS FROM *PARIETARIA DEBILIS* G. FORST. RHIZOME IN MICE

Nguyen Quynh Nhu, Nguyen Dieu Thuan, Pham Hoang Thuy Van, Dinh Thi Vy Vy, Dinh Truong Son, Cao Ngoc Giang, Nguyen Hoang Minh*

Research Center of Ginseng and Medicinal Materials Ho Chi Minh City - National Institute of Medicinal Materials, Vietnam

*Corresponding author: hoangminhtkd90@gmail.com

(Received February 03st, 2024)

Summary

Study on Anti-Fatigue and Immuno-Enhancement Effects of the Extracts from *Parietaria debilis* G. Forst. Rhizome in Mice

Parietaria debilis G. Forst. is applied to traditional medicine as a medicinal liquor or a medicinal decoction which has effects of liver detoxification, antipyretics, anti - nephritis, anti-arthritis, and so on. The aim of this research is to study the effects of anti-fatigue via Brekhman's swimming test, and immunostimulation via the immunosuppressive model by cyclophosphamide (at a single dose of 150 mg/kg, i.p) - induced in mice by the aqueous extract (at the oral dose of 0.74g/kg - 1.48g/kg) and 70% ethanol extract (at the oral dose of 0.28g/kg - 0.56g/kg) from *P. debilis* rhizome. The result showed that aqueous extract and 70% ethanol extracts from *P. debilis* rhizome at the oral doses markedly increased the mouse swimming times. Besides, the extracts from *P. debilis* rhizome enhanced the abilities of phagocytes, immune cell-mediated responses (delayed type hypersensitivity), and white blood cells.

Keywords: *Parietaria debilis* Forst. F, *Immunosuppression, Immuno - Enhancement, Anti - Fatigue, Cyclophosphamide, Mice.*

1. Introduction

Fatigue and immunity are interrelated. As we know, when people feel tired, their immunity will decrease significantly, and they will easily become infected; when the human body is infected, people also easily feel fatigued. Fatigue is a complex process involving physiological and biochemical changes, long-time or intense exercise leads to energy consumption such as glucose, muscle glycogen, HG and as well as the accumulation of metabolites including lactic acid and others, which is accompanied by immune