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DPPH RADICAL SCAVENGING ACTIVITY OF SOME MEDICINAL PLANT EXTRACTS AND COMPOUNDS

Nguyen Xuan Bac[1], Nguyen Trinh Quynh Anh[1], Nguyen Thi Hoai[2], Phung Thanh Huong[1], Nguyen Khac Tiep[1,]*

[1]*Faculty of Biotechnology, Hanoi University of Pharmacy, Vietnam;*

[2]*Faculty of Pharmacy, Hue University of Medicine and Pharmacy, Vietnam*

*Corresponding author: tiepnk@hup.edu.vn

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Summary

DPPH Radical Scavenging Activity of some Medicinal Plant Extracts and Compounds

The DPPH$^{\bullet}$ radical scavenging activity assay was used to screen and evaluate the antioxidant activities of eight methanol extracts and 22 pure compounds. The results revealed the high antioxidant potential of four extracts from *Decaspermum gracilentum*, *Uvaria simaensis*, *Syzygium attopeuense,* and *Cratoxylum cochinchinensis* with the greatest ascorbic acid equivalent and gallic acid equivalent of 0.25 mg ascorbic acid (AA) equivalents/mg sample, and 0.05 mg gallic acid (GA) equivalents/mg sample, respectively. Two pure compounds quercetin 3-*O*-(6-*O*-*α*-L-rhamnopyranosyl)-*β*-D-glucopyranoside isolated from *Anodendron paniculatum* and epi-catechin isolated from *Uvaria fauveliana* had ascorbic acid equivalent greater than 0.30 mg AA equivalents/mg sample. Epi-catechin expressed higher antioxidant activity than ascorbic acid. This is the first time the antioxidant activity of these extracts and compounds has been reported. Furthermore, our adapted DPPH assay employing a microplate reader offers a convenient and relatively high-throughput approach for antioxidant screening.

Keywords: *Antioxidant, DPPH assay, Radical scavenging, Herbal extract, Natural compounds.*

1. Introduction

In recent years, there has been a notable surge in the prevalence of non-communicable diseases (NCDs) such as cardiovascular diseases, diabetes mellitus, bronchial asthma, Alzheimer's disease, Parkinson's disease, depression, rheumatoid arthritis, and, notably, various forms of cancer. According to the World Health Organization (WHO), NCDs account for the demise of approximately 41 million individuals annually, constituting a staggering 74% of all reported deaths [1]. The pathogenesis of these maladies, as well as the aging process itself, is intricately linked to the phenomenon of "oxidative stress" [2]. Consequently, antioxidants play a pivotal role in disease prevention and treatment, thereby contributing to the enhancement of human life quality. Medicinal herbs stand out as a promising source of natural compounds due to their rich composition, encompassing various active ingredients characterized by complex structures and auspicious attributes, including antioxidant activity. Vietnam, a nation endowed with abundant reserves of medicinal herbs and a rich history of utilizing these botanical resources in traditional medicine, occupies a unique position in this regard. Nonetheless, there remains a trove of invaluable plant species awaiting discovery, necessitating the confirmation of their biological activities. To fully harness the potential of medicinal plants, it is imperative to continually expand our ethnopharmacological knowledge through the systematic screening and evaluation of their pharmaceutical properties.

Currently, various *in vitro* methods based on the mechanism of hydrogen atom transfer (HAT) or single electron transfer (SET) are used to assess the antioxidant activities of substances. Among these, DPPH assay, a SET method, is one of the most common techniques for antioxidant screening [3] because it is simple, rapid, low-cost, and possibly high-throughput [4].

In searching for plants that possess potential pharmaceutical activities, we collected samples that had not been investigated in terms of antioxidant effects from some different areas of Vietnam. From these samples, eight extracts and 22 pure compounds were tested for their antioxidant ability. This work aimed to find plants and substances with antioxidant potential so that they can be used for food technology, cosmetic, and pharmaceutical purposes.

2. Materials and methods

2.1. Chemicals and reagents

1,1-Diphenyl-2-picrylhydrazyl (DPPH, CAS 1898-66-4), ascorbic acid (CAS 50-81-7), gallic acid (CAS 149-91-7), and dimethyl sulfoxide (DMSO, CAS 67-68-5) were purchased from Sigma-Aldrich (USA), absolute ethanol (CAS 64-17-5) was purchased from Merck (Germany). All chemicals and reagents are analytical grade.

2.2. Samples

Eight extracts (Table 1) and 22 compounds (Table 2) isolated from herbal plants were

provided by the Department of Pharmacognosy, Hue University of Medicine and Pharmacy. Briefly, the collected plants were cleaned and shade-dried at room temperature. The dried samples were ground into fine powders before being extracted with methanol. The extraction process with methanol was conducted using the hot extraction method in a water bath with reflux at the solvent's boiling temperature, which was set at 75°C in this case. The extracts were dried under reduced pressure using a rotary evaporator (RV 10 digital V - IKA, Germany). The final extracts were stored at -80°C until use.

Table 1. Ethnobotanical data and yield of the tested extracts

Code	Name	Harvesting location	Harvest time	Parts used
DG001	*Decaspermum gracilentum* (Hance) Merr. et Perry, Myrtaceae	Đakrong, Quangtri province	08/2020	Leaves and branches
UV002	*Uvaria siamensis* (Scheff.) L.L.Zhou, Y.C.F.Su & R.M.K.Saunders, Annonaceae	Hue city, Thuathienhue province	07/2021	Leaves and branches
SA003	*Syzygium attopeuense* (Gagnep.) Merr. & L.M.Perry, Myrtaceae	Đakrong, Quangtri province	08/2020	Leaves and branches
CC004	*Cratoxylum cochinchinensis* Blume, Clusiaceae	Hue city, Thuathienhue province	07/2021	Leaves and branches
ATK1	*Alphonsea tonkinensis* A.DC., Annonaceae	Đakrong, Quangtri province	07/2017	Leaves and branches
ALD2	*Aspidistra letreae* Aver., Tillich & T.A.Le, Asparagaceae	Vinhlinh, Quangtri province	01/2019	Aerial parts
GPP3	*Glycosmis parviflora* (Sims) Little, Rutaceae	Đakrong, Quangtri province	01/2019	Aerial parts
PAL4	*Pogostemon auricularius* (L.) Hassk., Lamiaceae	Huonghoa, Quangtri province	02/2016	Aerial parts

The compounds (Table 2) were isolated from various medicinal herbs using a combination of chromatography methods, including gel filtration chromatography, reverse-phase column chromatography, normal-phase column chromatography, ion exchange chromatography, and crystallization techniques. The chemical structures of these compounds were determined using spectroscopic methods, including nuclear magnetic resonance spectroscopy (NMR) and mass spectrometry (MS). The purity of these compounds was confirmed by using the thin layer chromatography (TLC) which resulted in only one spot on a developed TLC plate for each compound.

Table 2. Tested compounds and their origins

Code	Name	Name of original plants
AL1	2*H*-chromene-2-one	*Aspidistra letreae* Aver., Tillich & T.A.Le, Asparagaceae
AP10	Vaniline	*Anodendron paniculatum* A.DC., Apocynaceae
AP5	Kaempferol 3-*O*-(6-*O*-*α*-L-rhamnopyranosyl)-*β*-D-glucopyranoside	
AP6	Quercetin 3-*O*-(6-*O*-*α*-L-rhamnopyranosyl)-*β*-D-glucopyranoside	
AP9	3-(3,5-Dimethoxy-4-*O*-*β*-D-glucopyranosyl phenyl)-2*ζ*,3*ζ*-epoxipropanol	
DK2	Ursolic acid	*Diospyros kaki* Thunb., Ebenaceae
H10-9-4a	Betulinic acid	*Hedyotis pilulifera* (Pit.) T. N. Ninh, Rubiaceae
H13-3-1	Oleanic acid	
H22-1	Rotungenic acid	
HP3	Asperuloside	
HP6	Paederosidic acid methyl ester	
PPE1	Pongapin	*Pongamia pinnata* (L.) Pierre,

Code	Name	Name of original plants
PPE3	Ovalichromene B	Fabaceae
PPE7	Gamatin	
PPE8	Pongaglabrone	
SAC18	Euscaphic acid	*Sarcosperma affinis* Gagnep., Sapotaceae
SAC22	Myrianthic acid	
UFE3A	Oxoanolobine	*Uvaria fauveliana* (Fin. et Gagnep.) Ast, Annonaceae
UFE7B	Epi-catechin	
UGC5	(-)-Zeylenone	*Uvaria grandiflora* (Lesch. ex DC.) Roxb., Annonaceae
UGC6	(-)-Zeylenol	
W4-10	3-Prenyl-4-*O*-β-D-glucopyranosyloxy-4-hydroxylbenzoic acid	*Anodendron paniculatum* (Roxb.) A. DC., Apocynaceae

2.3. Methods

DPPH radical scavenging activity assay

The DPPH$^{\bullet}$ radical scavenging activity of samples was conducted as described by Magda M. Becker et al with some modifications [4]. Briefly, DPPH was dissolved in ethanol to make a 0.6 mg/mL stock solution. DMSO was used to make 1.8 mg/mL stock solutions of extracts and 0.9 mg/mL stock solutions of compounds (tested compounds and two standards-ascorbic acid and gallic acid). For the screening purpose, the reaction mixtures contained 700 µL ethanol, 200 µL DPPH stock solution and 10 µL stock solutions of extract/compound solutions (reaction mixture concentration was 200 µg/mL and 100 µg/mL for extracts and compounds, respectively),. The mixture was incubated for 30 minutes in the dark. Then, 250 µL of the mixture was transferred to each well of a 96-well plate to measure absorbance at 520 nm using a plate reader (iMark, BioRad, Japan). The scavenging activity (antioxidant effect) expressed as % inhibition of DPPH$^{\bullet}$ radical was calculated using the following formula:

Inhibition of DPPH$^{\bullet}$ (%) = $(A_{control} - A_{sample})/(A_{control} - A_o) \times 100$

Where $A_{control}$ is the absorbance of the negative control, A_{sample} is the absorbance of the test sample and A_o is the absorbance of the solution where DPPH$^{\bullet}$ was completely converted to DPPH.

Based on the results of the screening experiments, only extracts/compounds that had greater than 50% inhibition of DPPH$^{\bullet}$ radical were selected to determine the concentrations required to scavenge 50% of DPPH$^{\bullet}$ radicals (IC_{50}) by the same method for the screening test using various concentrations of extracts/compounds diluted by the factor of two starting at 1.8 mg/mL and 0.9 mg/mL for the extracts and compounds, respectively. These values were calculated on the inhibition data using the Fit spline/LOWESS method in the Graph Pad Prism Software, Version 8.4.3 (GPPS Inc., La Jolla, CA).

The results were also expressed as the equivalent antioxidant capacity of standards (hereafter called standard equivalent) i.e. mg standard equivalents/mg extract or compound, which was generated using the following formula [5]:

$IC_{50\ (standard)}$(µg/mL)/$IC_{50\ (sample)}$(µg/mL) = X mg standard equivalents/mg extract or compound

Statistical analysis

All assays were performed in triplicates. Values for each sample are expressed as the mean ± standard deviation. Statistical analysis to compare mean values was conducted using Graph Pad Prism Software, Version 8.4.3 (GPPS Inc., La Jolla, CA). $p < 0.05$ is considered a statistically significant difference.

3. Results and discussions

3.1. Antioxidant screening of extracts/compounds and IC_{50} value determination

For screening the effect of the extracts and the compounds on DPPH$^{\bullet}$ radical, we conducted DPPH$^{\bullet}$ assay using a 1.8 mg/mL solution of each extract (reaction mixture concentration was 200 µg/mL), and 0.9 mg/mL solution of each compound or positive standards including ascorbic acid and gallic acid (reaction mixture concentration was 100 µg/mL). The results (Table 3 and Table 4) show that the four extracts from *Decaspermum gracilentum* (DG001), *Uvaria simaensis* (UV002), *Syzygium attopeuense* (SA003), *Cratoxylum cochinchinensis* (CC004) and the two compounds quercetin 3-*O*-(6-*O*-α-L-rhamnopyranosyl)-β-D-glucopyranoside (AP6), epi-catechin (UFE7B) significantly inhibited DPPH$^{\bullet}$ radical, i.e., greater than 50%. The extracts from two plants *Glycosmis parviflora* and *Pogostemon auricularius* showed a moderate antioxidant effect, i.e., greater than 20% and less than 50% inhibition of DPPH$^{\bullet}$ radical at a concentration of 200 µg/mL. The two other plants and 20 rest compounds did not show noticeable effect (less

than 20% inhibition of DPPH$^{\bullet}$ radical) at a concentration of 200 μg/mL for plant extracts and 100 μg/mL for compounds.

Accordingly, we chose four extracts (DG001, UV002, SA003, and CC004) and two compounds (AP6 and UFE7B) that had greater than 50% inhibition of DPPH$^{\bullet}$ radical and compounds for the next experiments to determine their 50% inhibition concentration (IC_{50}) on DPPH$^{\bullet}$ radical.

Table 3. DPPH$^{\bullet}$ radical scavenging effects of the extracts

Code of plant	Name of plant	Extract concentration	% inhibition of DPPH$^{\bullet}$ radical	Experiment note
DG001	*Decaspermum gracilentum* (Hance) Merr. et Perry, Myrtaceae	200 μg/mL	101.1 ± 1.22	Eligible for further study to determine IC_{50} value
UV002	*Uvaria siamensis* (Scheff.) L.L.Zhou, Y.C.F.Su & R.M.K.Saunders, Annonaceae	200 μg/mL	98.34 ± 3.09	Eligible for further study to determine IC_{50} value
SA003	*Syzygium attopeuense* (Gagnep.) Merr. & L.M.Perry, Myrtaceae	200 μg/mL	92.43 ± 2.40	Eligible for further study to determine IC_{50} value
CC004	*Cratoxylum cochinchinensis* Blume, Clusiaceae	200 μg/mL	99.71 ± 2.03	Eligible for further study to determine IC_{50} value
ATK1	*Alphonsea tonkinensis* A.DC., Annonaceae	200 μg/mL	1.71 ± 1.03	
ALD2	*Aspidistra letreae* Aver., Tillich & T.A.Le, Asparagaceae	200 μg/mL	-0.71 ± 1.13	
GPP3	*Glycosmis parviflora* (Sims) Little, Rutaceae	200 μg/mL	22.78 ± 5.13	
PAL4	*Pogostemon auricularius* (L.) Hassk., Lamiaceae	200 μg/mL	30.76 ± 7.89	
Std1	Ascorbic acid	100 μg/mL	101.05 ± 2.12	
Std2	Gallic acid	100 μg/mL	102.05 ± 4.35	

Note: Ascorbic acid and gallic acid are used as the standards. The former exhibits high hydrophilicity, while the latter demonstrates pronounced hydrophobic characteristics.

Table 4. DPPH$^{\bullet}$ radical scavenging effects of the compounds

Code of compound	Group	Name of compound	% inhibition of DPPH$^{\bullet}$ radical*	Experiment note
AL1	Coumarin	2H-chromen-2-one	0.25 ± 0.39	
AP10	Aromatic	Vaniline	0.42 ± 0.24	
AP5	Flavonoid	Kaempferol 3-*O*-(6-*O*-α-L-rhamnopyranosyl)-β-D-glucopyranoside	10.2 ± 2.19	
AP6	Flavonoid	Quercetin 3-*O*-(6-*O*-α-L-rhamnopyranosyl)-β-D-glucopyranoside	99.5 ± 3.35	Eligible for further study to determine IC_{50} value
AP9	Phenyl propanoid	3-(3,5-Dimethoxy-4-*O*-β-D-glucopyranosyl phenyl)-2ζ,3ζ-epoxipropanol (sargentol)	7.43 ± 1.15	
DK2	Triterpenoid	Ursolic acid	4.22 ± 1.31	
H10-9-4a	Triterpenoid	Betulinic acid	6.69 ± 1.51	
H13-3-1	Triterpenoid	Oleanic acid	7.49 ± 2.33	
H22-1	Triterpenoid	Rotungenic acid	7.56 ± 1.36	
HP3	Iridoid	Asperuloside	5.09 ± 1.31	
HP6	Iridoid	Paederosidic acid methyl ester	4.65 ± 0.81	
PPE1	Furanoflavonoid	Pongapin	3.14 ± 1.65	
PPE3	Pyranoflavonoid	Ovalichromene B	3.71 ± 0.91	
PPE7	Furanoflavonoid	Gamatin	2.44 ± 1.01	
PPE8	Furanoflavonoid	Pongaglabrone	1.63 ± 0.73	
SAC18	Triterpenoid	Euscaphic acid	2.23 ± 1.32	
SAC22	Triterpenoid	Myrianthic acid	3.14 ± 2.11	
UFE3A	Alkaloid	Oxoanolobine	16.7 ± 5.35	

Code of compound	Group	Name of compound	% inhibition of DPPH• radical*	Experiment note
UFE7B	Flavonoid	Epi-catechin	102.8 ± 5.31	Eligible for further study to determine IC_{50} value
UGC5	Polycarbonate	(-)-Zeylenone	5.79 ± 2.55	
UGC6	Polycarbonate	(-)-Zeylenol	6.84 ± 1.47	
W4-10	Prenylbenzoic acid	3-Prenyl-4-*O*-*β*-D-glucopyranosyloxy-4-hydroxylbenzoic acid	7.06 ± 2.66	
Std1	Ascorbic acid		101.05 ± 2.12	
Std2	Gallic acid		102.05 ± 4.35	

*Note: * The final concentration of DPPH• in the reaction mixture was 1.33 mg/mL*

To determine the IC_{50} value of the four selected extracts and the two compounds that had greater than 50% inhibition of DPPH• radical in the screening experiments, we evaluated five different concentrations of the extracts ranging from 12.5 µg/mL to 200 µg/mL and five different concentrations of the compounds ranging from 6.25 µg/mL to 100 µg/mL. Based on the % inhibition on DPPH• radical, IC_{50} of each extract or compound was calculated using the GraphPad Prism 8. The results (Table 5) indicated that all the extracts but SA003 have IC_{50} values lower than 100 µg/mL. The IC_{50} value of two tested compounds AP6 and UFE7B is close to that of the standard compound ascorbic acid.

Table 5. IC_{50} value of the extracts and compounds

Code of plant/compound	Name of plant/compound	IC_{50}
DG001	*Decaspermum gracilentum* (Hance) Merr. et Perry, Myrtaceae	65.90 ± 1.12 (µg/mL)
UV002	*Uvaria siamensis* (Scheff.) L.L.Zhou, Y.C.F.Su & R.M.K.Saunders, Annonaceae	64.63 ± 1.38 (µg/mL)
SA003	*Syzygium attopeuense* (Gagnep.) Merr. & L.M.Perry, Myrtaceae	110.05 ± 3.32 (µg/mL)
CC004	*Cratoxylum cochinchinensis* Blume, Clusiaceae	74.06 ± 1.99 (µg/mL)
AP6	Quercetin 3-*O*-(6-*O*-*α*-L-rhamnopyranosyl)-*β*-D-glucopyranoside	51.33 ± 3.88 (µg/mL) 84.15 ± 6.36 (µM)
UFE7B	Epi-catechin	15.24 ± 2.57 (µg/mL) 111.24 ± 18.76 (µM)
Std1	Ascorbic acid	16.21 ± 1.02 (µg/mL) 92.1 ± 5.8 (µM)
Std2	Gallic acid	3.18 ± 0.27 (µg/mL) 18.71 ± 1.59 (µM)

The outcomes of our antioxidant potential screening, encompassing methanol extracts derived from eight herbal plants and 22 isolated compounds from an additional eight plant sources, revealed four extracts and two compounds displaying considerable antioxidant activity. Specifically, these substances exhibited IC_{50} values below 200 µg/ml for extracts and less than 200 µM for pure compounds. It is noteworthy that the plants subjected to testing are not commonly used in Vietnamese traditional medicines, and there exists limited available data on their biological activities. Consequently, it becomes imperative to scrutinize their biological attributes, particularly about their antioxidant potential. The present study represents the inaugural investigation into the DPPH scavenging ability of four medicinal plants: *Decaspermum gracilentum* (DG001), *Uvaria simaensis* (UV002), *Syzygium attopeuense* (SA003), and *Cratoxylum cochinchinensis* (CC004). The documented antioxidant properties of these plants furnish compelling impetus for further exploration into their antioxidant activity.

Regarding the 22 pure compounds investigated, it is pertinent to note that the

majority of prior publications have suggested their possession of antioxidant capabilities. However, within the current study, only two of these compounds exhibited discernible DPPH• radical scavenging activity. These compounds are specifically identified as quercetin 3-O-(6-O-α-L-rhamnopyranosyl)-β-D-glucopyranoside (AP6), isolated from *Anodendron pniculatum*, and epi-catechin (UFE7B), isolated from *Uvaria fauveliana*. The antioxidant prowess displayed by AP6 and UFE7B in our study aligns with findings reported in other investigations [6]. Consequently, these results underscore the importance of conducting further research employing alternative antioxidant evaluation methodologies to corroborate the antioxidant potential of the remaining compounds.

3.2. Ascorbic acid and gallic acid equivalent determination

Ascorbic acid and gallic acid equivalents of the extracts and compounds were calculated based on their IC_{50} values as mentioned in the method section. The results (Fig. 1 and 2) showed that among the evaluated extracts/compounds, compound UFE7B had the highest antioxidant activity expressed by the standard equivalent, which was significantly higher than all the other samples. By contrast, the extract SA003 expressed the least antioxidant effect.

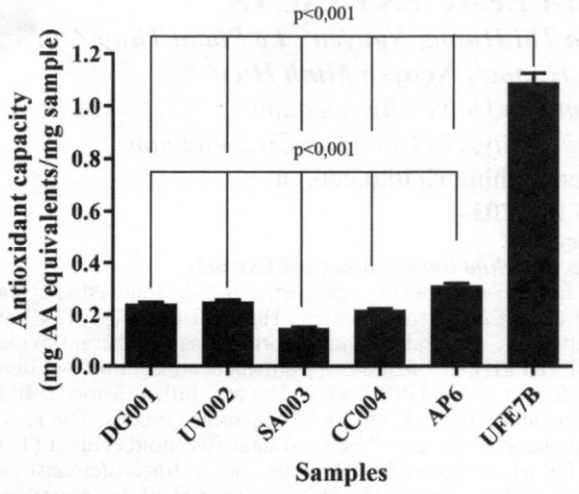

Fig. 1. Antioxidant capacity of samples (extract or compound), expressed as mg ascorbic acid (AA) equivalents/mg sample.
The data are expressed as mean ± standard deviation of the mean; n = 3.

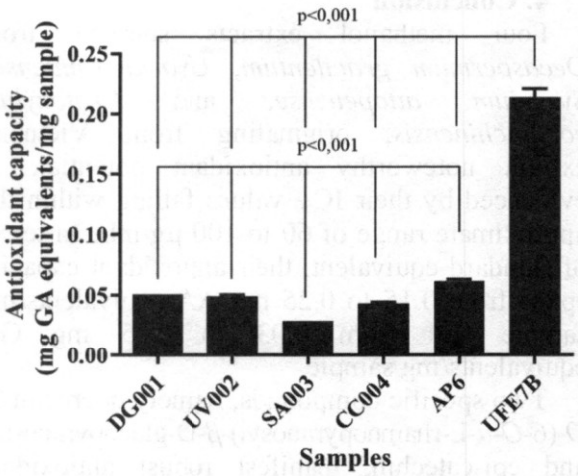

Fig. 2. Antioxidant capacity of samples (extract or compound), expressed as mg gallic acid (GA) equivalents/mg sample.
The data are expressed as mean ± standard deviation of the mean; n = 3.

Although the IC_{50} value remains a widely used parameter for expressing antioxidant efficacy, its utilization is associated with several limitations. The IC_{50} value is contingent upon various experimental conditions, such as the initial DPPH concentration, incubation time, and the choice of solvent, making it inherently challenging to standardize. Consequently, comparing IC_{50} values across different studies is a problematic endeavor, often leading to confusion among readers [7]. To provide a more robust representation of antioxidant activities, we ascertained the standard equivalent of the tested samples, choosing ascorbic acid and gallic acid as reference standards, both renowned for their high antioxidant potential [8].

Our results reveal that all the tested extracts and compounds exhibited relatively high standard equivalents. Specifically, concerning ascorbic acid equivalent, all values for the extracts and compounds exceeded 0.10 mg AA equivalents/mg sample, with UFE7B notably surpassing 1.00 mg AA equivalents/mg sample. In terms of gallic acid equivalent, the values for all extracts and compounds exceeded 0.025 mg GA equivalents/mg sample. This determination of standard equivalents offers valuable insights, enabling users and manufacturers to calculate the requisite sample quantities to meet specific demands in the realms of food, cosmetic, or pharmaceutical industries, particularly concerning their antioxidant properties.

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.

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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

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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.*