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Research Article

Total Phenolic and Flavonoid Contents, Anti-tyrosinase and Antioxidant Activities of *Pachyrhizus erosus* Extracts

Pornpun Laovachirasuwan, Methin Phadungkit*

ABSTRACT

Background: The storage roots of *Pachyrhizus erosus* (PE) have been traditionally used as a skin whitening agent in Indonesia and are also consumed fresh in many Southeast Asian countries, including Thailand. However, research on the biological activities of PE is limited. **Objective:** This study aimed to measure the total phenolic and flavonoid contents and to test the anti-tyrosinase and antioxidant activities of PE extracts. The Folin-Ciocalteu colorimetric, the Aluminium chloride colorimetric, the DPPH scavenging, and the Dopachrome assays were used for the experiments. **Results:** The dichloromethane extract had the highest anti-tyrosinase activity (IC_{50}= 2.08 ± 0.40 mg/mL), total flavonoid content (9.93 ± 0.02 mg QE/g extract), and antioxidant activity (IC_{50}= 40 ± 0.02 µg/mL). The largest total phenolic concentration was found in the 80% ethanol extract, albeit (11.97 ± 0.55 mg GAE/g extract). **Conclusion:** Based on its remarkable activities, the dichloromethane extract is recommended for future development in skin-lightening products. The study's findings conclude that PE extracts may be used as an alternate source of antioxidants and anti-tyrosinase agents.

Key words: *Pachyrhizus erosus*, Total phenolic content, Total flavonoid content, Anti-tyrosinase, Free radical scavenging.

Pornpun Laovachirasuwan, Methin Phadungkit*

Assistant Professor, Faculty of Pharmacy, Mahasarakham University, THAILAND.

Correspondence

Methin Phadungkit

Assistant Professor, Faculty of Pharmacy, Mahasarakham University, THAILAND.

E-mail: phadang_p@hotmail.com

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INTRODUCTION

Skin whitening products have become increasingly popular worldwide, particularly in Asian markets where pale skin is viewed as a sign of attractiveness. Tyrosinase inhibitors and antioxidants have become important components of many cosmetic formulations as a result of the demand for such goods.[1] Tyrosinase inhibitors prevent the synthesis of melanin, the pigment that gives color to the skin, while antioxidants protect the skin from oxidative damage caused by free radicals. Skin pigmentation has been reported to be prevented or delayed by both of these mechanisms.[2]

Due to their lesser toxicity as compared to their synthetic counterparts, natural sources of tyrosinase inhibitors and antioxidants have become more and more popular in recent years. Particularly in plants, phenolic substances are abundant and have been shown to have considerable antioxidant and tyrosinase inhibitory effects.[3]

Numerous plants, including herbs, fruits, and roots, have been investigated for their ability to serve as free-standing sources of antioxidants and tyrosinase inhibitors.

Pachyrizus erosus (PE), also known as jicama or yam bean, is a legume plant that is widely grown in tropical and subtropical regions, including Thailand. This plant's root is frequently eaten as food and is prized for its high energy content. Due to their ability to whiten skin, PE root extracts have also been utilized traditionally in Indonesian cosmetics.[4] Previous investigations into the phytochemistry of PE have shown that its extracts include a variety of bioactive substances. For instance, Lukitaningsih *et al.*[4] reported isolating phenolic and flavonoid chemicals from PE's root extracts, including daidzein, 8,9-furanyl-pterocarpan-3-ol, daidzein-7-O-glucopyranose, and 5-hydroxy-daidzein-7-O-glucopyranose. These substances are recognized for their anti-inflammatory and antioxidant properties, which are linked to a variety of skin advantages, including the prevention of damage and aging, the decrease of redness and irritation, the stimulation of wound healing, and the general enhancement of skin health.[5]

The purpose of this research is to investigate the total phenolic and flavonoid content of PE extracts as well as their anti-tyrosinase and antioxidant activity. The findings of this study could help to advance PE as a possible source of natural antioxidants and tyrosinase inhibitors for use in the formulation of skincare and cosmetic products. Furthermore, the findings may shed light on the possible health benefits of consuming jicama, thereby promoting its use in dietary supplements or functional foods.

MATERIALS AND METHODS

Plant material and extraction

The storage roots of Pachyrhizus erosus, commonly known as Yam Bean, were collected from the Maha Sarakham province, Thailand, in January 2020. The plant material was botanically identified and authenticated by Dr. Phadungkit and stored at room temperature until further use. For extraction, the dried and pulverized plant material was subjected to a soxhlet extraction using dichloromethane, 95% ethanol, and 80% ethanol as solvents. The extracts were obtained by separating the constituents through the extraction process. Subsequently, the extracts were filtered and concentrated using a rotary evaporator. The resulting crude extracts were utilized for the analysis of chemical components and the investigation of bioactivities.

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Total phenolic content assay

Total phenolic content was determined using the Folin-Ciocalteu assay.[6] In a 25 mL volumetric flask, 9 mL of distilled water was added to an aliquot of plant extract (1 mL) or standard solution of Gallic acid (100, 200, 300, 400, and 500 g/mL). With distillated water, a blank reagent was produced. Next, 1 mL of Folin-Ciocalteu phenol reagent was added to the mixture. Five minutes later, 10 mL of a 7% Na_2CO_3 solution was added to complete the volume. Before measuring the absorbance against the reagent blank at 550 nm with a UV-Visible spectrophotometer, the mixture was incubated at room temperature for 90 minutes. Total phenolics content was expressed as mg Gallic acid Equivalents (GAE).

Total flavonoid content assay

The total flavonoid content was measured using a colorimetric aluminum chloride assay.[7] In a 10 mL volumetric flask, an aliquot of plant extract (1 mL) or standard solutions of quercetin (20, 40, 60, 80, and 100 g/mL) were mixed with 4 mL of distilled water. After five minutes, 0.30 mL of 5% $NaNO_2$ and 0.30 mL of 10% $AlCl_3$ were added to the vessel. Five minutes later, 2 mL of 1M NaOH was added, and the volume was brought to 10 mL using distilled water. After mixing the solution, the absorbance was measured against the blank at 510 nm. The total flavonoid content was expressed in terms of mg equivalents of quercetin (QE).

Anti-tyrosinase activity assay

The assay for tyrosinase inhibition was conducted using the dopachrome method with L-DOPA as the substrate, with minor modifications.[8] Briefly, the plant extracts and kojic acid (Sigma) were dissolved in 0.1 mg/mL DMSO. Using an ELISA microplate reader (VersaMax Molecular Devices, USA), absorbance was measured at 475 nm using a 96-well microplate for the reaction. Each well was prepared by adding 40 L of plant extract dissolved in DMSO, 80 L of phosphate buffer (pH 6.8), 40 L of tyrosinase enzyme, and 40 L of L-Dopa (Sigma) to 80 L of phosphate buffer (pH 6.8). Each sample was accompanied by a blank that lacked L-Dopa but contained all other components. Kojic acid was utilized as the comparison standard inhibitor. components except for L-Dopa. Kojic acid was used as the reference standard inhibitor for comparison. The percentage of tyrosinase inhibition (I%) was calculated as follows: I% = [(A control – A sample)/A control] × 100, where A control is the absorbance of the control reaction, and A sample is the absorbance of the plant extract. The sample concentration that provides 50% inhibition (IC_{50}) was calculated by plotting inhibition percentages against the sample concentrations. Analyses were run in triplicate, and the results were expressed as average values with standard deviations.

Antioxidant activity assay

The antioxidant activity of the extracts was evaluated using a modified version of the DPPH scavenging technique.[9] Briefly, stock solutions of each sample (1000 µg/mL) were serially diluted to concentrations of 500, 250, 125, 62.5, 31.3, 15.63, 7.81, 3.90,1.95 µg/mL. Then, 0.2 mL of each sample solution was then added to 3.8 mL of 50 M DPPH methanolic solution, and the mixture was permitted to react at room temperature for 30 minutes. At 517 nm, the absorbance of the resulting mixtures was measured, and a control without sample or standard was prepared and measured at 0 minutes. In a reaction mixture, lower absorbance values indicate higher free radical scavenging activity, and vice versa. I% = [(Ablank Asample) / Ablank] x100, where Ablank is the absorbance value of the control reaction (consisting of all reagents except the test compound) and Asample is the absorbance value of the test compound. In order to ascertain the sample concentration at which 50% inhibition occurs (IC_{50}), inhibition percentages were plotted against sample concentrations. The analyses were conducted three times, and the results were reported as means with standard deviations.

Statistical analysis

Statistical analysis was performed on triplicate experiments, and the results were expressed as mean ± SD. Significance was considered at $p<0.05$. The statistical significance between groups was determined using one-way analysis of variance (ANOVA), followed by the Duncan post hoc test.

RESULTS

Total phenolic and flavonoid contents assay

Table 1 displays the total phenolic content and total flavonoid content of the herbal extracts. The ethanol extract at 80% had the highest total phenolic content with 11.97± 0.55 mg GAE/g extracts, followed by dichloromethane with 9.45 ± 1.47 mg GAE/g extracts. In contrast, the ethanol extract at 95% had the lowest total phenolic content, measuring 8.27 0.49 mg GAE/g extract.

The dichloromethane extract had the highest total flavonoid content at 9.93 ± 0.66 mg GAE/g extracts, which was significantly higher than the content in the 80% ethanol extract (5.06 ± 0.14 mg GAE/g extracts) and 95% ethanol extract (4.20 ± 0.39 mg GAE/g extracts). According to these results, the dichloromethane extract has the highest concentration of total flavonoids among the tested extracts.

Anti-tyrosinase activity assay

The current study revealed that plant extracts inhibited the oxidation of L-DOPA catalyzed by mushroom tyrosinase in a dose-dependent manner, with the enzyme activity declining rather than being inhibited. Table 1 demonstrates that plant extracts inhibited mushroom tyrosinase by 50% at concentrations spanning from 0.14 to 0.47 mg/mL. The dichloromethane extract of YB's root was the most potent, with the highest total flavonoid content and an IC_{50} value of 2.08 ± 0.40 mg/mL. In contrast, the IC_{50} value for the kojic acid standard was 0.10 ± 0.02 mg/mL.

Antioxidant activity assay

The antioxidant activity of PE extracts was assessed through the DPPH free radical scavenging assay in this study. The IC_{50} values for

Table 1: Total phenolic and flavonoid contents, anti-tyrosinase, and antioxidant activities of the PE extracts.

The herbal extracts	Total phenolic content (mg GAE/g extracts)	Total flavonoid content (mg GAE/g extracts)	Anti-tyrosinase activity (IC_{50}, mg/mL)	Antioxidant (IC_{50}, µg/mL)
80% ethanol	11.97±0.55^b	5.06 ± 0.14^a	24.31 ± 1.058^c	79 ± 0.10^c
95% ethanol	8.27±0.49^a	4.20 ± 0.39^a	10.90 ± 0.83^b	174 ± 0.02^d
dichloromethane	9.45 ±1.47^a	9.93 ± 0.66^b	2.08 ± 0.40^a	40 ± 0.02^b
Kojic acid	-	-	0.10± 0.02^a	-
ascorbic acid	-	-	-	3.6 ± 0.02^a

Values are expressed as mean ± standard deviation. Different letters indicate significant differences at $p < 0.05$)

both ascorbic acid and the herbal extracts were determined and are presented in Table 1. The results revealed that the dichloromethane extract exhibited the highest antioxidant activity among the tested extracts, with an IC_{50} value of 40 ± 0.02 µg/mL. This finding suggests that the dichloromethane extract possesses stronger antioxidant activity in comparison to the other extracts. Moreover, the positive control, ascorbic acid, demonstrated the most potent antioxidant activity, as indicated by its remarkably low IC_{50} value.

DISCUSSION

Total phenolic and flavonoid content assays

In total phenolic content assay, the 80% ethanol extract possessed the highest total amount of phenolic content when compared to other extracts. This may be due to the polarity of the solvent. The solvent polarity is very important in increasing the solubility of phenolic compounds [j]. The 80 % ethanol has the highest polarity solvent in the current study. For the present study, the highest flavonoid yields were found in dichloromethane extract (9.93 ± 0.66 mg GAE/g extracts). The results of this study demonstrated that the use of medium polarity solvents resulted in higher yields of total flavonoids extracted from PZ. These findings are consistent with those reported in a previous study by Lukitaningsih E.,[4] where flavonoid compounds were isolated from the ethyl acetate extract of PZ, which is also a medium polarity solvent. The results of the current study indicated that PE extracts contain significant amounts of phenolic and flavonoid compounds. Phenolics and flavonoids are well-known for their antioxidant and anti-inflammatory properties, which have been linked to numerous health benefits, including the prevention of chronic diseases such as cancer, cardiovascular disease, and neurodegenerative disorders.[10-12] In addition to their health-promoting effects, phenolics and flavonoids are also widely used in the cosmetics industry due to their potential skin-brightening and anti-aging properties.[13]

Anti-tyrosinase activity assay

The present study investigated the potential of PZ root extracts as a source of natural tyrosinase inhibitors. The results of the tyrosinase inhibition assay demonstrated that the plant extracts had a dose-dependent inhibitory effect on the oxidation of L-DOPA by mushroom tyrosinase. The enzyme activity was observed to rapidly decrease rather than being suppressed. This is consistent with previous studies that have reported similar inhibitory effects of plant extracts on tyrosinase activity.[14,15]

The inhibitory effect of PZ extracts on mushroom tyrosinase activity may be attributed to the presence of phenolic and flavonoid compounds. Flavonoids are well-known inhibitors of tyrosinase activity and have been shown to effectively reduce melanin synthesis in melanocytes.[16] The most potent extract in this study was the dichloromethane extract obtained from the root of YB, which had the highest total flavonoid content and exhibited an IC_{50} value of 2.08 ± 0.40 mg/mL. This is consistent with previous studies that have demonstrated the potent tyrosinase inhibitory effects of flavonoids.[17,18]

This study is in line with a previous study conducted by Lukitaningsih *et al.*,[4] which found that the major inhibitory compounds in PZ were predominantly flavonoids, such as daidzein and 5-hydroxyldaidzein-7-O-ß-glucopyranose. These important compounds were shown to effectively inhibit tyrosinase activity. Similarly, Lukitaningsih *et al.* reported that ethyl acetate extracts of PZ exhibited the highest inhibitory activity against tyrosinase, further supporting the findings of this study.[19]

Antioxidant activity assay

Antioxidant activity is a crucial parameter for assessing the potential health benefits of natural extracts. In this study, the antioxidant activity of PE extracts was evaluated using the DPPH free radical scavenging assay. The IC_{50} values, representing the concentration required to scavenge 50% of the DPPH free radicals, were determined for both ascorbic acid and the herbal extracts.

The results obtained from the antioxidant activity assay revealed that the dichloromethane extract exhibited the highest antioxidant activity among the tested extracts, as indicated by its lowest IC_{50} value of 40 ± 0.02 µg/mL. The observed high antioxidant activity of the dichloromethane extract may be attributed to its rich flavonoid content, as it exhibited the highest total flavonoid content compared to other extracts. Flavonoids are well-known for their potent antioxidant activity, attributed to their hydrogen-donating ability and free radical scavenging properties.[20]

The present findings are consistent with previous studies that have demonstrated the antioxidant activity of PE extracts.[21] However, the current study expands upon these previous findings by identifying the specific extract (dichloromethane) with the highest antioxidant activity and linking it to the flavonoid content.

CONCLUSION

In conclusion, this study demonstrated the diverse bioactive properties of the herbal extracts from *Pachyrhizus erosus* (PZ). The 80% ethanol extract exhibited the highest total phenolic content, while the dichloromethane extract showed the highest total flavonoid content. Additionally, the dichloromethane extract had the highest IC_{50} value among the tested extracts, demonstrating its strongest anti-tyrosinase activity. Additionally, based on its lowest IC_{50} value, the dichloromethane extract demonstrated the highest antioxidant activity. These findings underscore the potential of PZ extracts as a valuable source of bioactive compounds with notable antioxidant and anti-tyrosinase activities. Further investigations are necessary to identify and characterize the specific compounds responsible for these activities and to explore their potential applications in various fields, including the cosmetic industry.

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ABBREVIATIONS USED

PE	*Pachyrhizus erosus*
IC_{50}	Inhibitory concentration 50%
DPPH	2,2-diphenyl-1-picrylhydrazyl,
QE	quercetin equivalent
GAE	GAE stands for gallic acid equivalent
L-DOPA	L-3,4-dihydroxyphenylalanine

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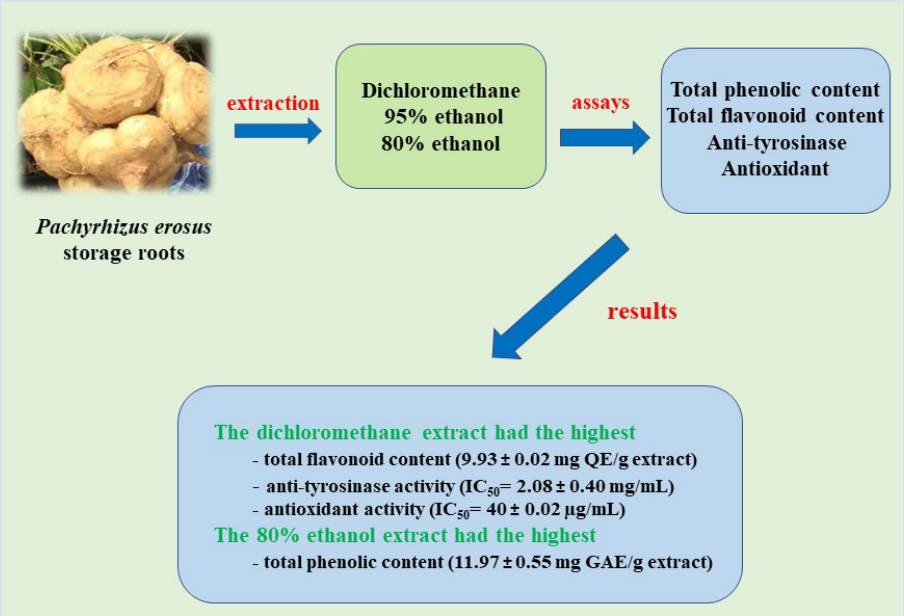
GRAPHICAL ABSTRACT



ABOUT AUTHORS

Methin Phadungkit, Ph.D., is an Assistant Professor within the Faculty of Pharmacy at Mahasarakham University, Thailand. His research expertise encompasses natural products chemistry, biological activity testing, and the formulation of dosage forms for natural products.

Pornpun Laovachirasuwan, Ph.D., is an Assistant Professor affiliated with the Faculty of Pharmacy at Mahasarakham University, Thailand. Her research focuses on pharmaceutical technology and the development of dosage forms for natural products.

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