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Original Article

Total Phenolic Content and Tyrosinase Inhibitory Potential of Extracts from *Cajanus cajan* (L.) Millsp.

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ABSTRACT

Background: *Cajanus cajan* (L.) Millsp. (Fabaceae) is a legume plant which used for human food and animal feed. **Objective**: The present study was aimed to investigate total phenolic content and tyrosinase inhibitory potential of the root, stem and seed parts of *C. cajan*. **Method**: Crude extracts were prepared by reflux extraction using methanol, dichloromethane and water as solvents. Total phenolic contents of the obtained extracts were analyzed by Folin-Ciocalteu colorimetric method and the inhibitory effect on tyrosinase activity was determined spectrophotometrically. **Results**: The results revealed that dichloromethane seed extract had more total phenolic content than those of methanol and water seed extracts, respectively. Dichloromethane seed extract had the highest total phenolic content of 92.00 ± 1.24 mg GAE/ g extracts. However, the highest tyrosinase inhibitory activity was found in the root methanol extract with IC_{50} of 3.55 ± 0.35 mg/ml. **Conclusion**: Type of extraction solvent was an important factor on phenolic contents of *C. cajan* extracts. The extracts of *C. cajan* inhibit slightly tyrosinase inhibitory.

Key words: *Cajanus cajan*, Total phenolic content, Tyrosinase inhibitor, Dichloromethane seed extract, Root methanol extract.

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INTRODUCTION

Medicinal plants are used in an alternative medicine for centuries. One of the most interesting medicinal plants is *Cajanus cajan* (L.) Millsp. (Fabaceae) or pigeon pea. It is an erect, branched, hairy shrub, 1-2 meters high. Leaves are oblong-lanceolate to oblanceolate with three leaflets. Flowers are yellow, in sparse peduncled racemes. Pod is hairy containing two to seven seeds. It is a multipurpose plant as extensively eaten as a dal for Indian people. In Thailand, young shoots of this plant are eaten as vegetables and the mature fruits are steamed for edible seeds. In traditional Chinese medicine, *C. cajan* is indicated for pain relief and used as a sedative agent. It is also used to treat ischemic necrosis, wound healing, sores, skin irritations, hepatitis, measles, jaundice and stabilizing menstrual period.[1]

Phenolic compounds are the most abundant secondary metabolites in plants. Phytochemical studies revealed that *C. cajan* contained various types of phenolic compounds including genistein, cajanol, biochanin A, longistylin A and C.[2-6] Biochanin A has been reported to exhibit tyrosinase inhibition.[7] The melanogenesis inhibitory effect occurs due to the 20 phenolic compounds determined in the extract from *Morus alba*.[8] Phenolics have similar structures to tyrosine which are oxidized by tyrosinase and they can act as substrate analog inhibitors against melanogenesis.[9] Moreover, tyrosinase is commonly found in living organisms. It activates the color change of mushroom, vegetables and fruits into dark brown. This enzyme is responsible for molting process in insects and regulates melanin synthesis process resulting in color determination of skin, hair and eyes in animals.[10] Anti-tyrosinase agents usually lower melanin synthesis process. It helps to prevent of melanin accumulation. This advantage has been applied in the whitening cosmetic industries. Currently, medicinal plant derived cosmetics are widely interested. Many research groups are interested in the investigation and screening of effective medicinal plants for developing as whitening agent. *C. cajan* has been reported various biological activities including anti-microbial, anti-oxidant and anti-cancer.[2-6] However, there is no report on its tyrosinase inhibitory activity. Therefore, the present study was aimed to determine total phenolic contents and to investigate the tyrosinase inhibitory activity of the root, stem and seed extracts of *C. cajan*. The findings from this study would be scientific information for value addition of Thai local plants and used as basic data for further application and development of cosmetic and food supplementary product industries.

MATERIALS AND METHODS

Chemicals

Folin-Ciocalteu (Sigma-Aldrich), sodium carbonate (Na_2CO_3), DMSO (Sigma-Aldrich), Kojic acid

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(Sigma-Aldrich), L-DOPA (Sigma-Aldrich), Mushroom tyrosinase enzyme (Sigma-Aldrich), Gallic acid (Sigma-Aldrich), phosphate buffer, dichloromethane AR grade (Merck) and methanol AR grade (Merck).

Plant materials and extract preparation

The root, stem and seed parts of *C. cajan* were collected from the cultivation area in Sila sub-district, Muang district, Khon Kean province, Northeastern part of Thailand. The plant specimens were identified using keys to genus and species and compared to type specimens. The voucher specimens were deposited in Bangkok herbarium, Bangkok, Thailand. The plant parts were dried in hot air oven at 60°C until dry. Then, they were homogenized as the fine powders. 10 g of plant powder (root, stem and seed) was dissolved in 300 ml of different solvents including methanol, dichloromethane and water. The plant extracts were prepared by reflux extraction at 60°C for 30 min. The solutions were evaporated by water bath at 60°C and freeze dried as crude extracts. The extracts were kept in freezer at -20°C until use.

Determination of total phenolic contents

Total phenolic contents in the plant extracts were analyzed by Folin-Ciocalteu colorimetric method modified from Amin *et al.*[8] Gallic acid at the concentrations of 12.5, 25, 50, 80 and 100 mg/ml was used as a standard. The plant extracts were dissolved in methanol. 0.5 ml of extracts (at the concentration of 1 mg/ml) was mixed with 2.5 ml Folin-Ciocalteu solution and left at room temperature for 5 min. Then, 2 ml Na_2CO_3 solution was added to the solution. The solution was mixed well and adjusted volume into 5 ml by distilled water and left at room temperature for 2 h. The solutions were measured for the absorbance at wavelength of 760 nm. Finally, total phenolic contents of extracts were calculated by comparison to gallic acid's standard curve (mg of gallic acid equivalent (GAE)/ g extract)

Tyrosinase inhibitory activity study

Tyrosinase inhibitory activity study was carried out by the methods modified from Alam *et al.*[9] Tyrosinase enzyme was extracted from mushroom. The plant extracts were dissolved in 50% DMSO at the concentration of 5 mg/ml. 40 µl extracts were mixed with 80 µl sodium phosphate buffer (0.1 M, pH 6.8) and 40 µl of 31 units/ml tyrosinase enzyme (dissolved in sodium phosphate buffer). The solutions were added to the microplate (mixed well) and incubated at room temperature for 10 min. Then, the solutions were mixed with 40 µl of 2.5 mM L-DOPA and left at room temperature for 10 min. The solutions were measured for the absorbance by microplate reader (SPECTRO star Nano, BMG LabTech) at wavelength of 475 nm. Kojic acid was used as a standard. Percentage inhibitions of tyrosinase activity were calculated by using the following equation:

$$\% \text{ Inhibition} = 100\ [(A-B)-(C-D)]\ /\ (A-B)$$

When A = absorbance of blank; B = absorbance of control; C = absorbance of extracts in the presence of tyrosinase; D = absorbance of extracts in the absence of tyrosinase

Statistical analysis

Total phenolic contents and tyrosinase inhibitory activity were carried out in 3 replicates (n = 3). The results were expressed as mean ± S.D.

RESULTS

Crude extracts of root, stem and seed parts of *C. cajan* using methanol, dichloromethane and water were prepared by reflux extraction. The methanol and dichloromethane extracts were dried by using rotary evaporator while water extracts were dried by using freeze dryer. The dry weights of crude extracts were used for calculation of percentage yield (% yield). The results showed that the water extracts from root, stem and seed had the highest percentage yield. However, the methanol seed extracts had the highest percentage yield as shown in Table 1.

Total phenolic contents

Total phenolic contents of *C. cajan* extracts were ranged from 4.27 – 92.00 mg GAE/g extracts. Total phenolic contents of dichloromethane, methanol and water seed extracts are 92.00 ± 1.24, 50.03 ± 2.78 and 4.27 ± 0.91 mg GAE/g extracts, respectively. The dichloromethane, methanol and water root extracts contained the total phenolic contents of 80.28 ± 4.49, 41.02 ± 0.77 and 5.95 ± 0.99 mg GAE/g extracts, respectively. The stem extracted with dichloromethane, methanol and water has the total phenolic contents of 72.46 ± 4.09, 30.57 ± 1.33 and 9.16 ± 0.69 mg GAE/g extracts, respectively (Table 2).

Tyrosinase inhibitory activity

Tyrosinase inhibitory activity of *C. cajan* extracts was expressed as IC_{50} values. The results revealed that the extracts exhibited tyrosinase inhibitory activity with IC_{50} values ranging from 3.55 – 12.43 mg/ml. However, the IC_{50} value of the standard kojic acid was 0.73 ± 0.0019 mg/ml as shown in Table 2.

DISCUSSION

Plants extracts usually contain different classes of phenolic compounds which are soluble in the selective solvents. Selection of the appropriate solvents is the key to a successful biological guided extraction. In this study, solvents of different polarity were used to extract the root, stem and seed parts of *C. cajan*. The dichloromethane seed extract of *C. cajan* had the highest total phenolic content and dichloromethane was a solvent that provides the highest total phenolic content in comparison to those of methanol and water. Our result was correlated with the prior research work. Al-Saeedi and Hossain[10] reported the following order of total phenolic obtained among the crude extracts of *C. cajan* seed was hexane >chloroform>methanol>ethyl acetate>butanol>water. It was implied the non-polar organic solvent was more appropriate to extract phenolic compounds of *C. cajan* than polar solvent. Plant parts did not demonstrate the effect on the total phenolic content. The obtained results indicated that type of extraction solvent was an important factor on phenolic contents of *C. cajan* extracts.

The tyrosinase inhibitory activity study showed the methanol root extract have the greatest tyrosinase inhibitory capacity (IC_{50} = 3.55 mg/ml). However, it was found that the standard kojic acid (IC_{50} = 0.73 mg/ml) had potent activity than the most tyrosinase inhibitory extract from *C. cajan* approximately 5 folds. It had been reported that methanol seed extracts from *C. cajan* had more tyrosinase inhibitory activity than those of the ethanol extracts.[11]

Lin *et al.*[12] investigated melanogenesis inhibition of Biochanin A from *C. cajan*. The results revealed that Biochanin A had melanogenesis inhibitory activity *in vitro* and *in vivo* with the similar inhibitory capacity to those of the standard arbutin without statistical difference. In the present study, it was found that root and stem extracts have a good tyrosinase inhibitory activity. This may due to the presence of Biochanin A in the root and stem of *C. cajan*.

It was reported that flavonoids including quercetin (IC_{50} = 0.07 mM), luteolin (IC_{50} = 0.19 mM) and kaempferol (IC_{50} = 0.13 mM) had a potent tyrosinase inhibitory activity which were similar to those of kojic acid (IC_{50} = 0.014 mM). Interestingly, flavonoid with the structure of 3-OH and 4-carbonyl (3-hydroxy-4-keto moiety) in quercetin which had the similar structure to kojic acid, support a good tyrosinase inhibitory capacity.[13]

Table 1: Percentage yield of *C. cajan* extracts.

Plant parts	%yield		
	water	Dichloromethane	Methanol
Stem	5.186	1.324	1.79
Root	5.962	1.401	1.745
Seed	10.075	1.572	4.389

Table 2: Total phenolic contents and tyrosinase inhibitory activities of *C. cajan* extracts.

Extracts	Total phenolic contents (mg GAE/ g extracts)	IC_{50} of Tyrosinase inhibition (mg/ml)
Root water	5.95 ± 0.997	7.36 ± 0.926
Root dichloromethane	80.28 ± 4.498	4.62 ± 0.455
Root methanol	41.02 ± 0.779	3.55 ± 0.346
Stem water	9.16 ± 0.698	3.94 ± 0.033
Stem dichloromethane	72.46 ± 4.099	3.82 ± 0.362
Stem methanol	30.57 ± 1.334	12.43 ± 0.679
Seed water	4.27 ± 0.918	4.39 ± 0.830
Seed dichloromethane	92.00 ± 1.243	4.81 ± 0.757
Seed methanol	50.03 ± 2.786	7.48 ± 0.655
Kojic acid (standard)		0.73 ± 0.019

Values are expressed as mean ± SD of triplicate measurements.

Phytochemical studies in *C. cajan* confirmed the presence of phenolic compound, flavonoids and stilbene i.e. cajanin, quercetin, genistein, biochanin A, betuinic acid and cajanol[14-15] which may be related to the tyrosinase inhibitory capacity. Therefore, there should be further study on phytochemistry of this plant in more details.

CONCLUSION

The present study can be concluded that the extracts from different parts of *C. cajan* have different total phenolic contents. There should be further studies on the other biological activities of this plant such as anti-cancer and anti-microbial activities. Phenolic compounds are beneficial for health and disease prevention. The findings from the present study can be used as the alternatives for consumption of local vegetable for health promotion and further development as food supplementary products.

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CONFLICT OF INTEREST

Authors declare no conflict of interest.

ABBREVIATIONS

GAE: Gallic acid equivalent; **SD:** Standard deviation; **DMSO:** Dimethyl sulfoxide

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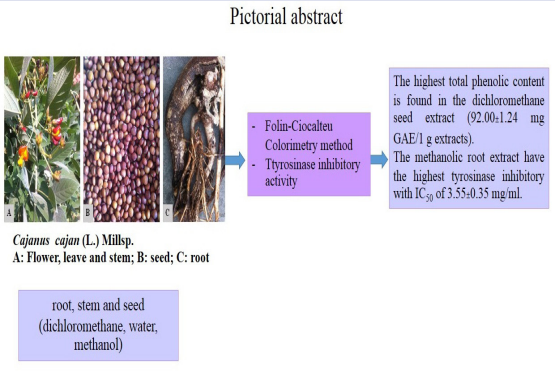
GRAPHICAL ABSTRACT



SUMMARY

- The seed extracts from *Cajanus cajan* (L.) Millsp. have the highest total phenolic content and tyrosinase inhibitory activity in comparison to those of stem and root extracts.
- The dichloromethane seed extract have the highest total phenolic content followed by those of methanol and water extracts.
- Type of extraction solvent was an important factor on phenolic contents of *C. cajan* extracts.
- Tyrosinase inhibitory activity study revealed that methanolic root extract have the highest tyrosinase inhibitory activity with the lowest IC_{50} value in comparison to those of other extracts.

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