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Original Article

Total Phenolic Contents and Free Radical Scavenging Activity of *Guaiacum officinale* L. Extracts

Suthira Maneechai[1] and Vachiraporn Pikulthong[2]*

ABSTRACT

Backgound: *Guaiacum officinale* L. is an alien species to Thailand. It is used as anti-arthritis and anti-rheumatoid agents in Indian folklore medicine. **Objective**: The present study was aimed to investigate total phenolic contents and free radical scavenging activity of the extracts from *Guaiacum officinale* L. (Zygophyllaceae). **Methods:** The plant parts including bark, twig and leaf were extracted using different solvents (ethyl acetate, water and ethanol). Total phenolic contents were determined by Folin-Ciocalteu Colorimetry method while free radical scavenging activity of the extracts was investigated by 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity assay. **Results:** The results show that the highest total phenolic content is found in the ethyl acetate twig extracts (20.3±0.0031 µg GAE/1 µg extracts). The ethanolic twig extracts had the highest free radical scavenging activity with IC_{50} of 0.45±0.0188 mg/ml. **Conclusion**: The extracts from *Guaiacum officinale* L. exhibit good anti-oxidant activity and may be suitable for development as drugs and supplementary food.

Key words: Total Phenolic Contents, Free Radical Scavenging Activity, *Guaiacum officinale* L, Ethanolic twig extracts, Ethyl acetate twig extracts.

Suthira Maneechai[1] and Vachiraporn Pikulthong[2]*

[1]Department of Biology, Faculty of Science, Mahasarakham University, THAILAND
[2]Division of Biology, Department of Science, Faculty of Science and Technology, Suan Sunandha Rajabhat University, THAILAND

Correspondence

Dr. Vachiraporn Pikulthong

Division of Biology, Department of Science, Faculty of Science and Technology, Suan Sunandha Rajabhat University, Bangkok 10300, THAILAND

Phone numbers +6621601143
Facsimile numbers +6621601146

E-mail: vachiraporn.pi@ssru.ac.th

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INTRODUCTION

Guaiacum officinale L. (Thai name: Keaw Jao Jom) is a plant belonging to the family Zygophyllaceae. It is a tree with grayish bark. The leaves are opposite with round apex and terete petiole. The flowers are light-blue. For phytochemical constituents, it is reported that bark,[1] flowers[2] and leaves[3] of *Guaiacum officinale* L. mainly contain saponin such as guaianin and guaiacin. Saponin is found to have pharmacological activities including immunostimulant, anti-carcinogenic properties, anti-rheumatoid and hypocholesterolaemic effect.[4,5]

Recently, treatments of many diseases by alternative medicine or natural products are interesting. There are reports on biological activities of *Guaiacum officinale* L. such as anti-HIV activity of methanolic leaf, twig and seed extracts.[5] The anti-oxidant activity of wood resin has been carried out.[6] However, there is no report on the anti-oxidant activity of ethyl acetate, ethanol and water extracts of this plant. Therefore, the objectives of this study are to investigate total phenolic contents and free radical scavenging activity of the extracts from *Guaiacum officinale* L. The results from this study may be helpful for value addition and development of this plant as cosmetic, drugs and supplementary food.

MATERIALS AND METHODS

Chemicals

Gallic acid (Sigma-Aldrich), ascorbic acid (Sigma-Aldrich), sodium carbonate (Na_2CO_3), 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Sigma-Aldrich), Folin-Ciocalteu (Sigma-Aldrich), ethanol AR grade (Merck) and ethy acetate AR grade (Merck)

Plant preparation and extraction

Bark, twig and leaf of *Guaiacum officinale* L. were collected from campus of Suan Sunandha Rajabhat University, Bangkok, Thailand. Voucher specimens were deposited and identified at herbarium of National Park, Wildlife and Plant Conservation Department, Ministry of Natural Resources and Environment, Bangkok, Thailand. Plant parts were dried in hot air oven at 60 °C until absolutely dry. They were then ground as fine powder. 600 ml of different solvents (ethyl acetate, water and ethanol) were added to 30 g plant powder (bark, twig and leaf). The plant powders were then extracted by Soxhlet extraction apparatus at 60 °C for 4 hours. The extracts were evaporated in water bath at 60 °C and frozen dry in order to obtain crude extracts. The crude extracts were kept in dark container at room temperature until uses.

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Determination of total phenolic contents

Total phenolic contents were determined by Folin-Ciocalteu Colorimetry method (adapted from Amin *et al*., 2006).[7] Gallic acid is used as standard (concentration of 12.5-100 mg/ml). The crude extracts were dissolved in methanol. Folin-Ciocalteu reagent was added to 0.5 ml of different extracts (1 mg/ml) and left for 5 minutes. 2 ml Na_2CO_3 was then added to the solution. The solutions were mixed and adjusted volume as 5 ml by distilled water and left at room temperature for 2 hours. The solutions were then measured the absorbance at 760 nm wavelength. Total phenolic contents in the extracts were determined by comparison of standard curves between mg of Gallic acid equivalent/g extract.

DPPH free radical scavenging activity study

Free radical scavenging activity of the extracts was investigated by 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity assay which is followed the study by Likhitwitayawuid *et al*. (2006).[8] 100 µm DPPH was prepared in methanol. The extracts were prepared in methanol at the concentration of 1 mg/ml. 180 µl DPPH solution and 20 µl extract solution were pipetted into microplate. The solutions were then incubated in dark room at room temperature for 20 minutes. These solutions were measured for absorbance by microplate reader (SPECTRO star Nano, BMG LabTech) at 517 nm wavelength. The absorbance of samples (As) was obtained. Methanol was used as blank and vitamin C was used as standard. Percentage inhibition of free radical was calculated by the following equation; % radical scavenging = [(Ac – As)/Ac] × 100. The graph illustrating the relationship between concentrations of extracts and % radical scavenging is plotted and then IC_{50} was calculated.

Statistical analysis

Total phenolic contents and free radical scavenging activity were measured in 3 replicates (n=3). All values were expressed as mean±S.D. The difference among means was analyzed by one-way ANOVA. The significant difference is at $p<0.05$.

RESULTS

The dry weight and percentage of yield

The dry weight and percentage of yield of plant parts (bark, twig and leaf) extracted by different solvents (ethyl acetate, ethanol and water) is shown in Table 1. The percentages of yield of ethyl acetate, ethanol and water bark extracts are 0.83, 1.23 and 4.67 %, respectively. The percentages of yield of ethyl acetate, ethanol and water twig extracts are 1.97, 15.03 and 8.99 %, respectively. The percentages of yield of ethyl acetate, ethanol and water leaf extracts are 1.44, 14.50 and 7.07 %, respectively.

Total phenolic contents

Total phenolic contents were calculated from standard curve of gallic acid ($y=4.1867x+0.0215$, $R^2=0.996$). It is found that ethyl acetate twig extracts have the highest total phenolic content (20.3±0.0031 µg GAE/1 µg extracts), followed by ethanol and water twig extracts (12.9±0.0006 and 10.3±0.0010 µg GAE/1 µg extracts, respectively).

DPPH free radical scavenging activity

The results found that ethanolic twig extracts have the highest anti-oxidant activity with IC_{50} of 0.45±0.0188 mg/ml, followed by ethyl acetate twig extracts and ethanolic bark extracts (IC_{50} of 1.16±0.0054 and 1.96±0.0243 mg/ml, respectively). However, the standard vitamin C has anti-oxidant activity with IC_{50} of 0.0034±0.0002 mg/ml (Table 2.)

DISCUSSION

According to the results, it is found that twig extracts from *Guaiacum officinale* L. have the highest total phenolic content and anti-oxidant activity in comparison to those of bark and leaf extracts. Interestingly, ethyl acetate twig extracts have the highest total phenolic content followed by those of ethanol and water twig extracts. DPPH free radical scavenging activity study revealed that ethanolic twig extracts have the highest anti-oxidant activity with the lowest IC_{50} value in comparison to those of other extracts. When compared to those of vitamin C, the plant

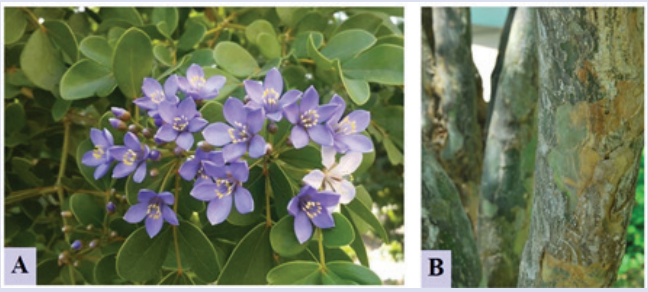
Table 1: The dry weight and percentage of yield of *Guaiacum officinale* L. extracts

Solvent/ Part of plants	Ethyl acetate		Ethanol		water	
	Dry weight (g)	% yield	Dry weight (g)	% yield	Dry weight (g)	% yield
Bark	0.25	0.83	0.37	1.23	1.40	4.67
Twigs	0.59	1.97	4.51	15.03	2.70	8.99
Leave	0.53	1.44	4.35	14.50	2.12	7.07

Table 2: Total phenolic contents and DPPH free radical scavenging activity of *Guaiacum officinale* L. extracts

Solvent	Plants part	DPPH (IC_{50} mg/ml) ± SD	Total phenolics (µg GAE/1 µg extracts)
Ethyl acetate	Bark	2.11 ± 0.0901^c	2.9 ± 0.0032
	Twigs	1.16 ± 0.0054^b	20.3 ± 0.0031
	Leave	3.03 ± 0.0698^d	6.4 ± 0.0002
Ethanol	Bark	1.96 ± 0.0243^c	7.6 ± 0.0004
	Twigs	0.45 ± 0.0188^a	12.9 ± 0.0006
	Leave	5.71 ± 0.1126^e	5.8 ± 0.0009
water	Bark	>9.86	0.1 ± 0.0001
	Twigs	2.65 ± 0.2389^d	10.3 ± 0.0011
	Leave	3.32 ± 0.0539^d	8.4 ± 0.0004
Ascorbic acid		0.0034 ± 0.0002	

Values are expressed as mean ± SD (n = 3) of triplicate measurements. Different letters in the column indicate significant differences at $P < 0.05$



Guaiacum officinale L. A: flower, leave and twig; B: bark

extracts have higher IC_{50} value. This indicates that *Guaiacum officinale* L. extracts have lower anti-oxidant capacity than those of vitamin C.

Lowe *et al.* (2014) reported anti-HIV activity of methanol and ethyl acetate extracts of leaf, twig and seed of *Guaiacum officinale* L. It was found that ethyl acetate extracts has higher HIV inhibitory activity than those of methanol extracts and leaf extracts have the highest anti-HIV activity.[5] This report correspond with the present study in which ethyl acetate leaf extracts exhibit the higher anti-oxidant capacity than those of ethanol and water leaf extracts of this plant.

There is a study on anti-rheumatoid and anti-oxidant activity of resin from wood of *Guaiacum officinale* L. which was found that the extracts have high anti-rheumatoid and anti-oxidant activity in an animal model.[6] This findings is similar results to the anti-oxidant activity of this plant in the *in vitro* study.

The results from this study revealed that twig extracts have higher total phenolic content and anti-oxidant activity in comparison to those of bark and leaf extracts. However, there is a few reports on twig extracts of this plant. The studies mainly focused on the biological activity and phytochemical constituents of bark, wood (resin) and flower extracts. Therefore, there should be further study on phytochemical constituents of twig extracts of *Guaiacum officinale*.

CONCLUSION

The results from this study revealed that *Guaiacum officinale* L.extracts exhibit anti-oxidant capacity. Therefore, there should be the investigations on other biological and pharmacological activities such as anti-microbial activity and anti-inflammatory activity. In additions, phenolic compound and flavonoids are beneficial to human health and disease prevention including cardio-vascular disease and cancers .[9,10] The findings from this study provide information for selection of this plant for health promotion and further development as supplementary food.

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CONFLICT OF INTEREST

Authors declare no conflict of interest.

ABBREVIATION USED

DPPH: 2,2-diphenyl-1-picrylhydrazyl; **GAE:** Gallic acid equivalent; **SD:** Standard deviation; **As:** Absorbance of samples; **Ac:** Absorbance of controls.

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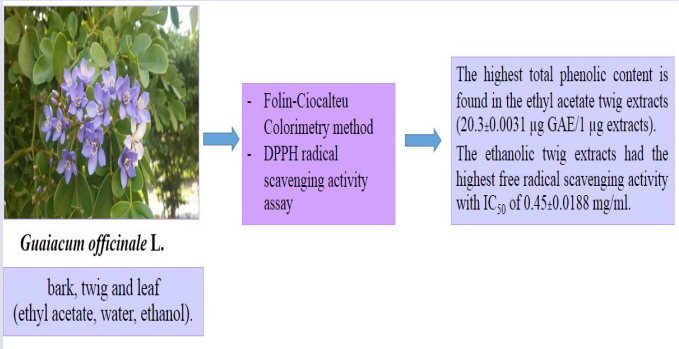
GRAPHICAL ABSTRACT



SUMMARY

- The twig extracts from *Guaiacum officinale* L. have the highest total phenolic content and anti-oxidant activity in comparison to those of bark and leaf extracts.
- The ethyl acetate twig extracts have the highest total phenolic content followed by those of ethanol and water twig extracts.
- DPPH free radical scavenging activity study revealed that ethanolic twig extracts have the highest anti-oxidant activity with the lowest IC_{50} value in comparison to those of other extracts.

ABOUT AUTHORS

Dr.Vachiraporn Pikulthong: Obtained her Ph.D. in Bioscience, Faculty of Science, Kasetsart University, Thailand. Currently, she is the lecturer at Division of Biology, Department of Science, Faculty of Science and Technology, Suan Sunandha Rajabhat University, Thailand. She has experiences in the area of Biology working mainly in plant anti-oxidant, plant molecular biology and somatic embryogenesis.

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