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Determination of Total Phenolic Contents and Antioxidant Activity of the Roots of *Pimpinella candolleana* Wight & Arnott

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Pimpinella candolleana Wight & Arnott belonging to family Apiaceae is a valuable medicinal plant. The plant specimens were collected from Pindaya Township, Southern Shan State in July 2014. The total phenolic contents in the aqueous, ethanolic and methanolic extracts of the roots of *Pimpinella candolleana* Wight & Arnott were determined at Food and Drug Administration Department, Mandalay by Folin-Ciocalteu colorimetric method using gallic acid as the standard. The antioxidant activity of the different concentrations (100 µg/ml, 200 µg/ml, 300 µg/ml, 400 µg/ml and 500 µg/ml) of three extracts was evaluated at Department of Medical Research (Pyin Oo Lwin Branch) by DPPH free radical scavenging method. The total phenolic contents of aqueous, ethanolic and methanolic extracts of roots were 83.63 mg GAE/g extract, 93.87 mg GAE/g extract and 118.85 mg GAE/g extract, respectively. The IC_{50} values of aqueous, ethanolic and methanolic extracts were 69.183 µg/ml, 63.096 µg/ml and 31.622 µg/ml, respectively. The results showed that there is a positive correlation between free radical scavenging effect and total phenolic contents. Thus, this study scientifically proved that this resource sample is rich in total phenolic contents and significantly has antioxidant activity.

Keywords: Pimpinella candolleana roots, Folin-Ciocalteu, Phenolic, Antioxidant, DPPH

INTRODUCTION

Pimpinella candolleana Wight & Arnott belonging to family Apiaceae is a valuable medicinal plant. This family consists of 400-450 genera and 3500-3700 species and is found in most parts of the world mainly in the Northern Hemisphere.[1] There are 150 species in the genus *Pimpinella* distributed in Africa, Asia, and Europe. This species is grown in the pinus forest margins, among shrubs, grassy slopes, streamsides, 1300-3500 m above sea level in China, South India and Southeast Asia and native is peninsular India.[2] Certain members of Apiaceae, 28 genera and 48 species and, 26 genera and 40 species were described in Myanmar plant checklists[3, 4] but *P. candolleana* Wight & Arnott has not been reported. In Myanmar, this species is grown in temperate and hilly region 1100 m above sea level especially Southern Shan State.

In China, the subterranean part's decoction or infusion of *P. candolleana* Wight & Arnott is used for treatment of cold and flu, musculoskeletal system disorders, digestive system disorders, urticarial, rheumatism and in skin care products for its anti-inflammatory and whitening properties.[5, 6] It was also proposed that the ethyl acetate and methanolic extracts of aerial parts of *P. candolleana* Wight & Arnott had shown the *α*-glucosidase inhibitory and antioxidant activities *in vitro*. The luteolin and isovitexin isolated from the ethyl acetate extracts showed *α*-glucosidase inhibitory activity and luteolin had antioxidant activity.[7] It had been indicated that different extracts of the *Pimpinella* species such as *P. anisum* L.,[8]

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P. tragioides (Boiss) Benth.,[9] *P. brachycarpa* (Kom.) Nakai.,[10] *P. tirupatiensis* Bal. & Subr., had antioxidant activity.[11]

It had been reported that medicinal plants rich in flavonoids and phenolic acids could be a good source of natural antioxidants.[12] It was described that the various bioactivities of phenolic compounds are responsible for their chemopreventive properties such as anti-oxidant, anticarcinogenic and anti-inflammatory activities.[13] It was considered that the cut-off point for antioxidant activity as IC_{50} = 50 µg/ml. IC_{50} >50 µg/ml was as moderately active and IC_{50} <50 µg/ml was judged as high antioxidant capacity.[14]

In Myanmar, local people in Southern Shan State use the leaves of *P. candolleana* Wight & Arnott as vegetables and the root as tonic. Annually, about 11000 kg of the roots of this plant have been traded as a commercial raw material for traditional crude drug by local people. It is needed to identify for standardization on the quality and authenticity of raw materials used as commercial raw material in Myanmar traditional crude drug markets. However, there is a lack of scientific studies about these roots distributed in Southern Shan State of Myanmar. Therefore, this study was conducted to determine total phenolic contents and to evaluate the free radical scavenging activity of the aqueous, ethanolic and methanolic extracts of roots of *P. candolleana* Wight & Arnott.

MATERIALS AND METHODS

This study was carried out as a laboratory-based experimental design. The sample was extracted at Research Division, University of Traditional Medicine, Mandalay. The total phenolic contents and the antioxidant activity of various extracts of roots were determined at Food and Drug Administration Department, Mandalay and at the Department of Medical Research (Pyin Oo Lwin Branch), respectively.

Preparation of sample

The plant specimens were collected from Pindaya Township, Southern Shan State in July 2014. Pindaya is located between North latitude 20º 42' and 21º 13' and East longitude 96º 25' and 96º 57'and the altitude is above 1100 meter. The plant specimens were identified by a taxonomist from Department of Botany, University of Mandalay according to the method of Menglan, *et al*.[2] The root pieces of *P. candolleana* Wight & Arnott (100 g) were mixed with 1000 ml of distilled water and heated by reflux extractor at 80°C for 3 hours. They were then allowed to cool at room temperature, filtered and evaporated to dry under reduced pressure at 50°C by rotary evaporator and made into powder by freeze drier (FD 1). For ethanolic and methanolic extracts, the crude pieces (100 g) were mixed with 1000 ml of 95% ethanol and methanol, respectively, and agitated and sonicated for 3 hours. The extracted liquid was filtrated and evaporated to dry under reduced pressure at 50°C by rotary evaporator and dried in water bath until constant weight was achieved.

Determination of total phenolic contents in various solvent extracts of roots

The total phenolic contents of the aqueous, ethanolic and methanolic extracts of roots were determined by Folin-Ciocalteu colorimetric method using gallic acid as the standard.[15] The absorbance was measured at 765 nm by UV spectrophotometer 1601.

Table 1. Absorbance of standard compound (gallic acid)

Concentration (µg/ml)	Absorbance (Mean)λ_{max}=765 nm
5	0.359
10	0.745
20	1.4135
30	2.2853
40	3.2148

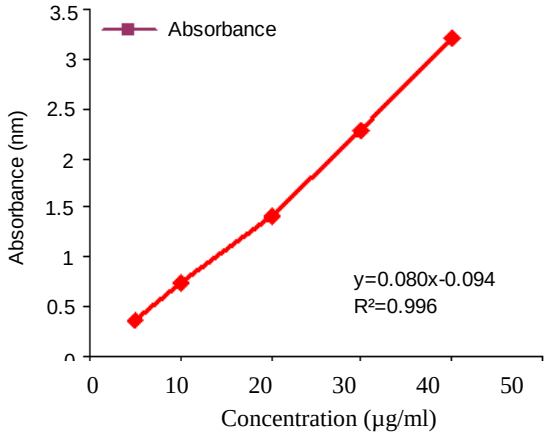

Fig. 1. Standard calibration curve of different concentrations of gallic acid

A standard calibration curve was prepared using different concentrations of gallic acid in ethanol (5, 10, 20, 30 and 40 µg/ml). In a 20-ml test tube, 1 ml gallic acid of each concentration

was added to 5 ml of 10% Folin-Ciocalteu reagent and 4 ml of 7% Na_2CO_3 to get a total volume of 10 ml. The blue colored mixture was shaken well and incubated for 30 minutes at 40°C in a water bath. The average absorbance values obtained at different concentrations of gallic acid were used to plot the calibration curve (Table 1 & Fig. 1).

Total phenolic contents of the three extracts were calculated from the regression equation of calibration curve (y=0.080x-0.094, R^2=0.996) and expressed as mg gallic acid equivalent (GAE) per gram of sampleas gallic acid equivalent by the following equation:

$$C = \frac{cV}{m}$$

C = Total phenolic content mg GAE/g dry extract
c = Concentration of gallic acid obtained from calibration curve in mg/ml
V = Volume of extract in ml
m = Mass of extract in gram

Determination of antioxidant activity of the root extracts

The antioxidant activity of the aqueous, ethanolic and methanolic extracts of the roots was determined by using the DPPH (1,1-diphenyl-2-picrylhydrazyl). The stock solution was prepared by dissolving root extract with 95% ethanol at concentration of 1 mg/ml. From this stock solution, different concentrations (100, 200, 300, 400 and 500 µg/ml) were prepared. The sample solutions were prepared by mixing thoroughly with 1 ml of 60 µM DPPH solution (2.36 mg of DPPH in 100 ml of 95% ethanol) and 1 ml of different concentrations of each plant extract prepared from stock solution was mixed vigorously by a vortex mixer. Ascorbic acid (1 µg/ml, 2.5 µg/ml, 5 µg/ml, 7.5 µg/ml and 10 µg/ml) was used as standard. Blank solution was prepared without the addition of DPPH solution.

All solutions were allowed to stand at room temperature (27ºC) for 30 minutes and then the measurement of absorbance was done at 517 nm using UV-Visible 1601 PC, Shimadzu, Spectrophotometer in triplicate and the absorbance was calculated to obtain the percent inhibition by using the following formula:

$$\text{Scavenging activity} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \text{ X } 100\%$$

Absorbance of control = Absorbance of 60 µM DPPH solution

Absorbance of sample = Absorbance of test sample solution

Statistical analysis

All the experiments were carried out in triplicates and data were reported as mean. For the total phenolic content, the linear correlation coefficient was calculated by using Microsoft Office Excel (2007). The concentrations of extracts were calculated from this regression equation. The total phenolic content of each extract at that concentration was calculated. For antioxidant activity, the mean, standard deviation (SD) and the linear regression of percent inhibition of different extracts were analyzed by using the computer package SPSS version 20 software. The 50% inhibitory concentration (IC_{50}) of free radical scavenging activity was calculated from linear regression line according to log concentration.

RESULTS

In the extractability, the yield percentages of aqueous, ethanolic, and methanolic extracts of the root of *P. candolleana* Wight & Arnott were 33.4%, 18.8% and 8.98%, respectively.

Total phenolic contents in the various extracts of roots

The total phenolic content in aqueous, ethanolic and methanolic extracts of roots were 83.63 mg GAE/g extract, 93.87 mg GAE/g extract and 118.85 mg GAE/g extract, respectively. The total phenolic profile was significantly found in three extracts of root.

Table 2. Percent inhibition for free radical scavenging activity of various extracts of roots at different concentrations

Extract	Percent inhibition (Mean±SD) at different concentrations (n=3) and IC_{50} values					
	100 µg/ml	200 µg/ml	300 µg/ml	400 µg/ml	500 µg/ml	R^2 linear
Aqueous	52.75 ±0.32	56.85 ±0.09	60.11 ±1.78	62.85 ±0.28	63.21 ±0.23	0.992
Ethanol	52.73 ±0.30	56.91 ±0.09	58.85 ±1.78	62.66 ±0.28	63.05 ±0.23	0.992
Methanol	65.31 ±0.37	75.62 ±3.61	87.24 ±1.33	87.63 ±1.87	88.47 ±1.05	0.973

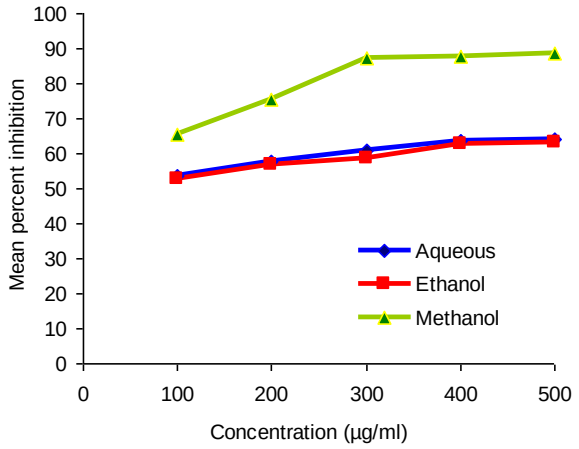

Fig. 2. Percent inhibition in different concentrations of various extracts of roots

Antioxidant activity of the various extracts of roots

In the determination of antioxidant activity of aqueous, ethanolic and methanolic extracts of the roots by DPPH assay method, the percent inhibitions (Mean±SD) of each extract solutions at different concentrations (100 µg/ml, 200 µg/ml, 300 µg/ml, 400 µg/ml and 500 µg/ml) are mentioned in Table 2 and Figure 2. The linear regression for free radical scavenging activity of various extracts and ascorbic acid were more than 0.9. The IC_{50} values of methanolic, ethanolic and aqueous extracts were 31.622 µg/ml, 63.096 µg/ml and 69.183 µg/ml, respectively. However, the percent inhibitions for ascorbic acid at different concentrations (1 µg/ml, 2.5 µg/ml, 5 µg/ml, 7.5 µg/ml and 10 µg/ml) were 48.91±0.44, 57.12±0.44, 76.39±1.64, 83.59±0.63 and 84.91 ±0.12, respectively, and its IC_{50} value was 1.23 µg/ml.

DISCUSSION

This study was carried out to determine the total phenolic contents and free radical scavenging activity of aqueous, ethanolic, methanolic extracts of the root of *P. candolleana* Wight & Arnott by laboratory-based experimental design. The yield percentages of aqueous, ethanolic and methanolic extracts were different.

In the determination of phenolic content, it was observed that methanolic extract (118.85 mg GAE/g extract) had the highest total phenolic content followed by ethanolic (93.87 mg GAE/g extract) and aqueous extract (83.63 mg GAE/g extract). Therefore, this species may contribute to antioxidant activity due to the rich total phenolic contents.

In antioxidant activity, all concentrations (100 µg/ml, 200 µg/ml, 300 µg/ml, 400 µg/ml and 500 µg/ml) of aqueous, ethanolic and methanolic extracts had free radical scavenging effect. The free radical scavenging effects of all extracts increased in order: methanolic extract > ethanolic extract > aqueous extract. However, the IC_{50} values of three extracts were lower than that of standard ascorbic acid. The cut-off point for antioxidant activity was mentioned as IC_{50}=50 µg/ml and samples with IC_{50} >50 µg/ml were classified as moderately active but samples with IC_{50} <50 µg/ml were judged as possessing high antioxidant activity.[14] Therefore, it can be considered that the methanolic extract with IC_{50} <50 µg/ml was highly antioxidant and the other two extracts with IC_{50} >50 µg/ml showed moderate antioxidant activity.

Regarding the various extracts of different *Pimpinella* species, it was reported that the methanolic extract of *P. tragioides* (Boiss) Benth. showed significantly the highest antioxidant activity.[9] It was also described that the water and ethanolic extracts of *P. anisum* L. and the aqueous extract of *P. tirupatiensis* Bal. & Subr. also showed antioxidant activity.[8, 11] It had been stated that the methanolic and ethyl acetate extracts of aerial parts of *P. candolleana* Wight & Arnott had shown antioxidant activity and then luteolin, one of flavones, isolated from that species showed antioxidant activity.[7] In this study, the methanolic extracts of the root possess significantly higher antioxidant activity than the aqueous and ethanolic extracts. Therefore, the methanolic extract of *Pimpinella* species is more likely to be contributed to free radical scavenging activity. This result suggests that the pure compounds isolated from the methanolic extract of that species should be investigated for the antioxidant activity.

It had been reported that the flavonoids and phenolic acids are the beneficial natural antioxidant components that can scavenge harmful active oxygen species.[12] It was also proposed various bioactivities of phenolic compounds are responsible for their chemo-preventive properties such as antioxidant, anticarcinogenic, and anti-inflammatory.[13] It was observed that three extracts of the root of *P. candolleana* Wight & Arnott had antioxidant activity as well as the phenolic compounds

which were believed to have the ability of free radical scavenging and antioxidation.

The methanolic extract had the highest phenolic content and highest scavenging activity among three tested extracts. It was observed that sample containing large amount of total phenolic compounds has better free radical scavenging effect. The results showed there is a positive correlation between free radical scavenging effect and total phenolic contents. Thus, this study scientifically proved that this root rich total phenolic and antioxidant activity.

Competing interests

The authors declare that they have no competing interests.

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