Quantification of Total Phenolic Content in the Root of *Millettia extensa* (Benth.) Benth. ex Baker and Its Potential Application as Natural Antioxidant

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Abstract

In the present study, the root of *Millettia extensa* (Benth.) Benth. ex Baker, Myanmar name Wunu, was selected to evaluate the total phenolic content and its radical scavenging potential. Firstly, the phytochemical constituents of this sample were carried out. Moreover, total phenolic content of the root of analyzed sample was evaluated by the Folin-Ciocalteau reagent using UV Visible spectrophotometer (UV- 1800, SHIMADZU, UV spectrophotometer) at 765 nm. The total phenolic content of this selected sample was determined as 40.53 ± 0.058 mg gallic acid equivalent (GAE) per g dry weight. Furthermore, the free radical scavenging potential of MeOH extract of root of *M. extensa* (Benth.) was analyzed by 2,2-diphenyl-1-picrylhydrazyl Assay method. IC_{50} value of MeOH extract of the root of analyzed sample was 25.75 µg/mL. The root of *M. extensa* (Benth.) was found to be considerable radical scavenging potential which is comparable to standard ascorbic acid (4.92 µg/mL).

Keywords: *Millettia extensa* (Benth.) Benth. ex Baker, phenolic content, Folin- Ciocalteau reagent, UV Visible spectrophotometer, radical scavenging potential.

1. Introduction

Medicinal plants are rich source of novel drugs that forms the ingredients in traditional system of medicine, modern medicines, pharmaceutical intermediates and lead compounds in synthetic drugs [1]. The reason for using them as medicine lies in the fact that they contain chemical components of therapeutic value [2]. These compounds are naturally occurring in the medicinal plants, leaves, vegetables and roots that have defense mechanism and protect from various diseases. The medicinal value of plants lies in some chemical substances (usually secondary metabolites) that produce a definite physiological action as the human body [3].

Phenolic compounds are commonly found in plants, and they have been reported to have multiple biological effects, including antioxidant activity [4]. Many studies had revealed that phenolic content in plants could be correlated to their antioxidant activities. Plants contained phenolic and polyphenol compounds can act as antioxidant [5].

Antioxidants help organisms deal with oxidative stress caused by free radical damage. Free radicals are chemical species, which contain one or more unpaired electrons due to which they are highly unstable and cause damage to other molecules by extracting electrons from them in order to attain stability [6].

There is an increasing interest in natural antioxidants, namely phenols, present in medicinal and dietary plants, that might help prevent oxidative damage [7, 8]. Presently, the use of synthetic antioxidants has been criticized. It is usually implied that regular consumption of natural antioxidants from vegetables, fruit, tea, and herbs may contribute to a shift in balance toward an ample antioxidant status. The interest in natural antioxidants, especially phytochemicals has greatly increased in recent years [9].

Millettia extensa (Benth.) Benth. ex Baker family Fabaceae is usually a large climbing shrub that can grow 20 meters tall, but when growing in a more open situation it is sometimes found as a small, almost erect shrub. The roots were extensively used to cure fracture and treat inflammation of joints. It was used as a fish poison. Some research works were done an antispermatogenic effect and spermicidal effect using 80% ethanol extract showing negative results [10, 11].

In the present work, *M. extensa* (Benth.) was selected for the assessment of phytochemical compounds, total phenolic content and radical scavenging activity because it is one of the rich sources of phenols which possess radical scavenging potential.

1.1 Botanical Description

Family	:	Fabaceae
Genus	:	*Millettia*
Species	:	*M. extensa*
Botanical name	:	*Millettia extensa* (Benth.) Benth. ex Baker

Figure 1. Plant and root of *Millettia extensa* (Benth.) Benth. ex Baker

2. Material and Methods

2.1. Sample Collection

The roots of *M. extensa* (Benth.) were collected from Patheingyi Township, Mandalay in Myanmar (Figure 1). Then they were chopped into tiny pieces, and dried in shade at room temperature for about five weeks.

2.2. Phytochemical Screening of Root of *M. extensa* (Benth.)

The phytochemical constituents of the root of *M. extensa* (Benth.) were measured by the test tube method.

2.3. Extraction of Phenol from Root of *M. extensa* (Benth.)

1 g of dried plant sample was ground in a mortar and pestle. It was extracted with 10 mL of 0.3 % HCl in methanol. The mixture was centrifuged at 5000 rpm for 30 min. The supernatant was decanted to a small beaker. The extraction procedure was repeated for two times. The supernatant was poured to the same container. The supernatant was evaporated to dryness and it was dissolved in distilled water. This solution was made up to 20 mL with distilled water. This extract contains the phenols [12, 14].

2.4. Qualitative Test for Phenols

2.4.1. Acid Properties Test:

The extract solution of selected sample was tested by blue litmus paper. This blue litmus paper turns red [12, 14].

2.4.2. Colour with $FeCl_3$:

1 mL of extract solution was taken and a few drops of very dilute solution of ferric chloride were added. The colour changes to brown which indicates the presence of phenol [12, 14].

2.5. Quantitative Determination of Total Phenolic Content

2.5.1. Principle

Phenols in alkaline medium react with phosphomolybdic acid of Folin - Ciocalteau reagent producing a blue coloured complex [13].

2.5.2. Estimation of λ_{max} for Gallic Acid

To determine the absorption maximum, standard solution of gallic acid in concentration 7.5 µg/mL was prepared. And then, 100 µL of Folin–Ciocalteau reagent and 300 µL of saturated Na_2CO_3 (20 %) solution were added. This standard solution was heated in the water bath at 40 ºC for 30 min and then it was cooled at room temperature. The spectrum of this solution was measured in the wavelength interval 700 to 800 nm [13, 14].

2.5.3. Preparation and Determination of Standard Gallic Acid

10 mg of the standard gallic acid was taken in a test tube. 10 mL of distilled water was added to the standard compound. 1 mL of this standard solution was taken in another test tube. The volume of this solution was made up to 10 mL with distilled water. The standard solution was taken by micro-pipette into a series of test tubes 20 µL, 40 µL, 60 µL, 80 µL and 100 µL respectively. The solutions were prepared and measured as previous procedure. [13, 14].

2.5.4. Determination of Total Phenolic Content of Root of *M. extensa* (Benth.)

The total phenolic content of extract solution of root of *M. extensa* (Benth.) was measured with the Folin-Ciocalteau reagent. Firstly, 2 µL of extract solution was taken in a test tube. The solution was prepared and measured as previous procedure. The assay was carried out in triplicate [13, 14].

2.6. Determination of Antioxidant Activity by Spectrophotometric Method

2.6.1. Chemicals. 2,2-diphenyl-1-picrylhydrazyl (DPPH), 95 % EtOH, Ascorbic acid [14, 15].

2.6.2. Preparation of 60 µM DPPH Solution

0.0024 g (2.4 mg) of DPPH powder was weighed and it was thoroughly and gently dissolved in 100 mL of 95 % ethanol and stored in brown coloured reagent bottle. It must be kept in the fridge for no longer than 24 hours before use [14, 15].

2.6.3. Preparation of Standard Ascorbic Acid Solution

0.01 g (10 mg) of ascorbic acid was weighed and was dissolved in 100 mL of 95 % ethanol. It was diluted with 50 % ethanol in various ratios to obtain five ranges of concentration, such as 1 µg/mL, 2 µg/mL, 4 µg/mL, 8 µg/mL and 16 µg/mL respectively and the same volume 5.0 mL of standard ascorbic acid solution was prepared for each concentration [14, 15].

2.6.4. Preparation of Methanolic Extract Solution of Root of *M. extensa* (Benth.)

The methanolic extract of root of *M. extensa* (Benth.) was diluted with methanol in various ratios to obtain five ranges of concentration, such as 6.25 µg/mL, 12.5 µg/mL, 25 µg/mL, 50 µg/mL and 100 µg/mL respectively. Then, 5.0 mL of methanol solution was prepared for each concentration [14, 15].

2.6.5. Measurement of DPPH Radical Scavenging Activity

The control solution was prepared by mixing 2 mL of 60 µM DPPH solution and 2.0 mL of 95 % ethanol using vortex mixer. Moreover, the blank solution could be prepared by mixing 2.0 mL of methanolic extract solution of root of *M. extensa* (Benth.) and 2.0 mL of 95 % ethanol thoroughly in the vortex mixer. Furthermore, the prepared standard ascorbic acid solutions and the test sample solutions were also prepared by mixing gently each of 2.0 mL of 60 µM DPPH solution and 2.0 mL of test sample solution with various concentrations by applying vortex mixer. After that, the solutions were allowed to stand for 30 minutes at room temperature. Then, the absorbance value of each solution at 517 nm was measured by UV Visible spectrophotometer [14, 15].

3. Results and Discussion

According to the phytochemical analysis on the root of *M. extensa* (Benth.), the presence of chemical constituents such as alkaloids, flavonoids, glycosides, phenolic compounds, sugars, saponins, tannins, steroids and terpenes were detected in the root of *M. extensa* (Benth.).

From the experimental results of qualitative tests, the phenol compounds were present in the root of *M. extensa* (Benth.).

The maximum absorbance (λ_{max}) of standard gallic acid in a wavelength range from 700 nm to 800 nm was observed to be 765 nm as described in Figure 2.

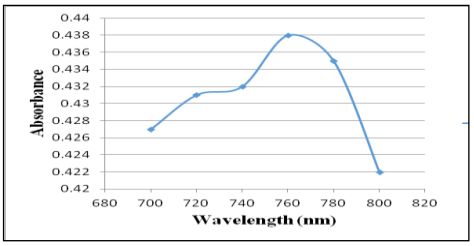

Figure 2. Maximum wavelength of standard gallic acid

By applying the measured data of standard gallic acid solutions, the standard graph of gallic acid could be constructed. This calibration curve is shown in Figure 3.

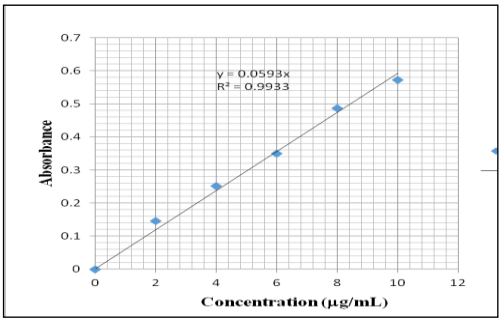

Figure 3. Absorbance concentration calibration curve for standard gallic acid

The total amount of phenol present in root of *M. extensa* (Benth.) was evaluated by spectrophotometric method applying UV- 1800, SHIMADZU, UV spectrophotometer. The amount of total phenol compounds of analyzed sample was calculated by using the standard calibration curve. The calculated values are tabulated in Table 1.

Table 1. The results of total phenolic content of root of *M. extensa* (Benth.)

Name of Sample	Phenol (mg/g)	Phenol (mg/g) Mean ± Standard Deviation
Root of *M. extensa* (Benth.)	40.5	40.53 ± 0.058
	40.6	
	40.5	

According to above table, the amount of total phenol content presented in the root of *M. extensa* (Benth.) was 40.53 ± 0.058 mg gallic acid equivalent (GAE) per g dry weight.

The experimental resulted data showed that the root of *M. extensa* (Benth.) had the considerable amount of total phenols.

DPPH, (2,2-diphenyl-1-picrylhydrazyl) assay is one of the most used methods because it is practical, fast and stable. The free radical scavenging potential of MeOH extract of root of *M. extensa* (Benth.) was exhibited as % inhibition and IC_{50} values (µg/mL). The observed data are described in Figure 4, 5 and Table 2.

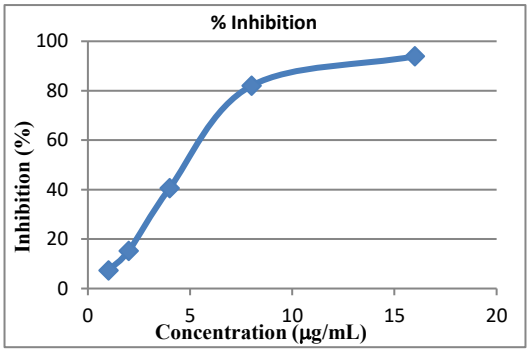

Figure 4. Plot of % inhibition vs concentration of standard ascorbic acid

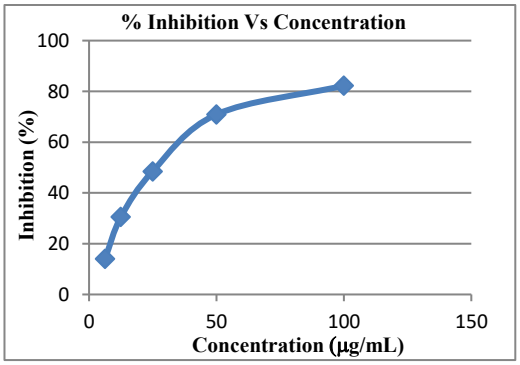

Figure 5. Plot of % inhibition vs concentration of methanolic extract of root of *M. extensa* (Benth.)

Table 2. IC_{50} Values of standard ascorbic acid and methanolic extract of root of *M. extensa* (Benth.)

No.	Test Samples	IC_{50} Values (µg /mL)
1.	Ascorbic acid	4.92
2.	Methanolic extract of root of *M. extensa* (Benth.)	25.75

The resulted values of the current study showed that the root of *M. extensa* (Benth.) responded the good antioxidant potential in accordance with the IC_{50} value of ascorbic acid which is a positive control.

In this study, the free radical scavenging potential of methanolic extract of root of *M. extensa* (Benth.) was described to be affected by the amount of phenol compounds present in it. The root of *M. extensa* (Benth.) containing significant amount of phenols expressed high radical scavenging potential. Thus, radical scavenging activity of methanolic extract of root of *M. extensa* (Benth.) is directly correlation with total phenol content. Therefore, the study suggests that the root of *M. extensa* (Benth.) might be a potential source of natural free radical scavengers.

4. Conclusion

The present study is focused on the phytochemical investigation and assessment of total phenolic content in the root of *M. extensa* (Benth.). It was observed that the total phenolic content was 40.53 ± 0.058 mg gallic acid equivalent per g of dry weight. Furthermore, the free radical scavenging activity of methanolic extract of root of *M. extensa* (Benth.) was studied. The experimental result showed that the methanolic extract of root of *M. extensa* (Benth.) gave the considerable free radical scavenging potential. Based on the obtained results, it can be concluded that phenol enriched root of *M. extensa* (Benth.) was effective on DPPH radicals significantly. Thus, the potential application of extract of root of *M. extensa* (Benth.) Benth. ex Baker (Wunu) as natural antioxidant is more beneficial because of its considerable total phenolic content and significant radical scavenging potential by comparing with standard ascorbic acid.

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