Determination of Antioxidant Activity of Bamboo Leaves (*Bambusa vulgaris* Schrad. ex J.C. Wendl.)

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Abstract

Bamboo is one of the most valuable, naturally occurring plants worldwide because its different edible parts contain important nutrients.

Bamboo-Leaf extract has multiple biological effects, such as anti-free radical, anti-oxidation, anti-aging and the prevention of cardiovascular diseases (Zhang and Ding, 1996, 1997; Tang and Ding, 2000). In this research work, bamboo leaves (*Bambusa vulgaris* Schrad. ex J.C.Wendl.) (shwe-wa) was selected for chemical analysis. The preliminary phytochemical test of bamboo leaves was carried out. Ash and moisture contents in bamboo leaves were determined by using oven drying method and Muffel furnace method. Vitamin C content in bamboo leaves was quantitatively determined by iodometric titration method. Antimicrobial activity in bamboo leaves was determined by agar well diffusion method. In antioxidant activity, ethanolic extract of bamboo leaves was used to investigate the radical scavenging activity by (1,1-diphenyl-2-picryl-hydrazyl) DPPH assay. Antioxidant contains in the leaves of Shwe-wa but antioxidant activity of ethanol extract of leaves of Shwe-wa is lower than that of ascorbic acid.

Keywords: bamboo leaves, vitamin C content, antimicrobial activity, antioxidant activity, iodometric titration method, DPPH assay

Introduction

Bamboo was once a symbol of the Orient that grows throughout the world and has originated from Southeast Asia, where it is considered as a natural component of the forest ecosystem. They are classified under the Poaceae family and subfamily Bambusoideae which is composed of woody and some herbaceous bamboo of about 1575 species and includes two tries.

Bamboo is an important grass that has significant uses in many parts of the world especially as a raw material for construction. Popular among the common people as "Poor man's timber" since ancient times, because its innumerable applications have attained the status of "Green Gold of Forests". From ancient time bamboo has been an important ingredient of traditional Asian Medicines in general and Chinese medicine in particular. Modern scientific approaches are now used to validate the traditional uses and researchers from round the globe have been successful in isolating active chemical constituents from different parts of the green gold.

Bamboo is one of the most valuable, naturally occurring plants worldwide because its different edible parts contain important nutrients. Bamboo leaves have been used in traditional Chinese medicine for treating fever and for detoxification for over 1000 years (Zhang and Ding, 1996). In addition, the antioxidant contained in bamboo leaves have been included in China's National Standard (GB-2760) as a kind of food antioxidant. Bamboo-Leaf extract has multiple biological effects, such as anti-free radical, anti-oxidation, anti-aging and the prevention of cardiovascular diseases (Zhang and Ding, 1996, 1997; Tang and Ding, 2000).

According to the International Network for Bamboo and Rattan, INBAR, 21 genus and 102 species have been identified and cultivated in Myanmar.

Bambusa vulgaris Schrad. ex J.C.Wendl. (Poaceae) a rhizomatous plant commonly known as Golden Bamboo, is widely distributed and grows in tropical and subtropical areas. A few members of the genus *Bambusa* species have presented numerous benefits to science and communities.

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Bamboo leaves have many active substance that is flavonoids and their glucosides, active polysaccharides, special amino acids and their peptides, chlorophyll, vitamins, aromatic elements as well as microelement such as manganese, zinc and selenium which can reduce blood fat and cholesterol and may reduce the oxidation antioxidant, can be as anti-aging ingredient for skin, and can maintain the stamina body and prevent cardiovascular disease. The composition is very similar flavonoid bamboo leaf structure of hemoglobin. Therefore, bamboo leaves can be directly injected into a vein. Bamboo leaf flavonoids are also very safe and non toxic.

Bioactive compounds are normally accumulated in all parts of plants, but their concentration varies according to the part of plants. Not all natural antimicrobial agents could inhibit *E. coli*. A recent investigation reported that *E. coli* was the most resistent bacteria to the natural antimicrobial agent from edible mushroom (*Dictyophora indusiata*). Leaf extract of different species of bamboo showed on antimicrobial against some bacteria which causes diseases in human.

Aim and Objectives

Aim

The aim of this research work is to determine the antioxidant activity of bamboo leaves (*Bambusa vulgaris* Schrad. ex J.C.Wendl.)

Objectives

- To collect the leaves of golden bamboo from University of Traditional Medicine, Mandalay
- To perform the phytochemical screening of bamboo leaves
- To determine the moisture and ash content of bamboo leaves
- To determine the vitamin C content of bamboo leaves by iodometric titration method
- To determine the antimicrobial activity of bamboo leaves by agar well diffusion method
- To determine the antioxidant activity of ethanol extract of bamboo leaves by DPPH assay

Botanical Description

Botanical name - *Bambusa vulgaris* Schrad. ex J.C.Wendl.
Family - Poaceae
English name - Golden Bamboo
Myanmar name - Shwe-wa
Part use - Leaves

Materials and Methods

Sample Collection

Leaves of *Bambusa vulgaris* were collected from the University of Traditional Medicine, Mandalay. Firstly, the sample was dried in the shade and cut into small pieces.

Preliminary Phytochemical Test on Sample

Preliminary phytochemical analysis was performed in order to know different types of chemical constituents present in the plant sample. The results are expressed in Table (1).

Determination of Moisture Content

The moisture content of sample was determined by oven drying method. The moisture content of sample is the weight lost due to the evaporation of water at the drying temperature.

Procedure

Sample (ca. 2.00 g) was placed in the crucible. The crucible with the sample was placed in oven and dried at 100°C for 30 minutes. Then, they were removed from the oven and cooled in the air-tight desiccator to room temperature and weighed. The procedure was repeated until the loss in weight had not been changed. The moisture content can be calculated by the following formula.

$$\text{Moisture (\%)} = \frac{\text{Loss in weight (g)} \times 100}{\text{weight of sample (g)}}$$

Determination of Ash Content

The ash content of a sample is the inorganic residue remaining after the organic matter has been burnt away.

Procedure

Sample (ca. 2.00 g) was introduced into a predried and cooled porcelain crucible in air-tight desiccator and accurately weighed. Then, it was heated gently over a burner until the sample was thoroughly charred. The crucible was transferred to the electric furnace at 600°C for two hours until the residue was free carbon. Then the crucible containing residue was cooled in a desiccator and weighed. Heating, cooling and weighing were repeated until constant weight was obtained. The ash content of the sample was calculated by using the following equations.

$$\text{Ash (\%)} = \frac{\text{weight of residue (g)} \times 100}{\text{weight of sample (g)}}$$

Determination of Antimicrobial Activities of Leaves of *Bambusa vulgaris* Schard. ex J.C.Wendl. (Shwe-wa)

Antimicrobial activities of the crude extract of the leaves of *Bambusa vulgaris* Schard. ex J.C.Wendl. were tested in various solvent systems by using Agar-well diffusion method on six selected organisms at PRD (Pharmaceutical Research Department), Insein, Yangon.

Determination of Ascorbic Acid Content in Leaves of *Bambusa vulgaris* Schrad. ex J.C.Wendl. (Shwe-wa) By Iodometric Titration Method

Preparation of Sample

Sample fresh leaves of *Bambusa vulgaris* Schrad. ex J.C.Wendl. (Shwe-wa) was prepared. The samples were washed and wiped. Then, they were cut into pieces. The pieces (100 g) were crushed to paste state for approximately 2 min using a blender. The homogenized sample was transferred into a 100 mL volumetric flask and distilled water was added to the mark. The mixture was shaken manually for 10 minutes.

Preparation of Iodine Solution (0.001 N)

KIO_3 (10.00 g) was placed in an oven for one hour at 100°C. Then, 0.268 g KIO_3 was weighed. KIO_3 (0.268 g) and 5 g of KI were dissolved into 500 mL beaker with 200 mL distilled water, 30 mL of 3 M concentrated sulphuric acid was added into the beaker and then diluted with distilled water until 500 mL solution.

Preparation of Ascorbic Acid Solution

Ascorbic acid (0.500 g) was dissolved with 100 mL in the beaker. The solution was transferred into 500 mL beaker and diluted with distilled water until 500 mL solution.

Preparation of Starch Indicator Solution

Starch (1.00 g) was dissolved into boiling distilled water.

Standardization of Ascorbic Acid Content

The ascorbic acid content was determined by iodometric titration. 25 mL of ascorbic acid solution was placed into a conical flask and then starch indicator (10 drops) was added into a conical flask. The solution was titrated with iodine solution. When the solution turned into pale blue color, the ascorbic acid content was computed.

Determination of Vitamin C Content of Fresh Sample

Fresh sample (25 mL) solution was transferred into a 250 mL conical flask by using pipette and 10 drops of starch indicator was added. The solution was directly titrated with 0.001 N standardized iodine solution. The solution turned reddish-brown color, the vitamin C content was computed. The analysis was done for three times. Finally, the vitamin C content of fresh sample could be calculated.

Determination of Antioxidant Activity of *Bambusa vulgaris* Schrad. ex J.C.Wendl. (Shwe-wa) by DPPH Radical Scavenging Assay

DPPH (1,1-diphenyl-2-picryl-hydrazyl) radical scavenging assay was chosen to assay the antioxidant activity of plant materials.

Preparation of Crude Extract

Ethanol extract of bamboo leaves was prepared by the following procedure. About 50 g of air dried *Bambusa vulgaris* Schrad. ex J.C.Wendl. was extracted with 200 mL of ethanol by using continuous hot extraction at 60°C. After 3 hours of extraction, it was cooled at room temperature and then the mixture was filtered using filter paper. The filtrate was concentrated at 70°C to get the constant weight in a water bath.

Preparation of Reagents

Preparation of 60 μM DPPH Solution

DPPH powder (2.364 mg) was dissolved in 95 % ethanol and made the volume up to 100 mL. The solution was freshly prepared in the brown colored flask and kept in a refrigerator for no longer than 24 hours.

Preparation of Test Sample Solution

The crude extract (4 mg) to be tested was dissolved in 50 % ethanol (20 mL). The desired concentration of test sample solutions (12.5, 25, 50, 100, 200 μg/mL.) was prepared by dilution the stock solution with 50 % ethanol.

Procedure

(i) Control solution was prepared by mixing 60 μM DPPH solution (1.5 mL) and 95 % ethanol (1.5 mL)

(ii) Similarly, the blank solution was prepared by mixing test solution (1.5 mL) and 50 % (1.5 mL).

(iii) The sample solution was also prepared by mixing the test solution (1.5 mL) with 60 μM DPPH solution (1.5 mL).

All these solutions were allowed to stand at room temperature for 30 minutes. Then, the absorbance was measured at λ 517 nm using UV-7504 spectrophotometer. Absrobance of individual solutions was measured λ_{max} triplicate and calculated the % inhibition by using the following formula.

$$\% \text{ inhibition} = \frac{\text{DPPH alone} - (\text{Sample} - \text{Blank})}{\text{DPPH alone}} \times 100\ \%$$

$$\text{Average } \bar{X} = \frac{x_1 + x_2 + \ldots + x_n}{n}$$

Ascorbic acid was used as a standard antioxidant for comparison purpose. Then, IC_{50} values were also calculated by liner regressive excel program.

DPPH alone = absorbance of control solution
Sample = absorbance of sample solution
Blank = absorbance of blank solution
$\bar{X}$ = average % inhibition
$x_1, x_2, \ldots, x_n$ = % inhibition of test sample solution
n = number of times

Results and Discussion

Phytochemical Constituents of Bamboo Leaves (Shwe-wa)

Table (1) Results of Preliminary Phytochemical Test on Bamboo Leaves (Shwe-wa)

No	Constituent	Extract	Reagents	Observations	Result
1.	Alkaloid	1 % HCl	Dragendroff's	Orange-red ppt	+
2.	Flavonoid	1 % HCl	Mg, conc;HCl	green color	+
3.	Glycoside	Distilled water	10 % lead acetate	No ppt	–
4.	Saponin	Distilled water	-	No foam	–
5.	Polyphenol	ethanol	1% $FeCl_3$ + 1%$K_3Fe(CN)_6$	green color	+
6.	Sugar	Distilled water	Benedict's solution	orange ppt	+
7.	Terpene	pet-ether	Acetic anhydride chloroform, conc; H_2SO_4	No ppt	–
8.	Tannin	Distilled water	10% $FeCl_3$, dilute H_2SO_4	yellowish brown ppt	+
9.	Phenolic	ethanol	10% $FeCl_3$	bluish black color	+
10.	Steroid	pet-ether	Acetic anhydride, conc; H_2SO_4	No color	–

(+) = present of constituent (-) = absence of constituent

According to the preliminary phytochemical test, the leaves of *Bambusa vulgaris* Schrad. ex J.C.Wendl. (Shwe-wa) contained except glycoside, saponin, terpene and steroid.

Ash Content and Moisture Content in Leaves of *Bambusa vulgaris* Schrad. ex J.C.Wendl. (Shwe-wa)

Ash content and moisture content in this sample was determined and the results are shown in Table (2).

Table (2) Ash Content and Moisture Content in Leaves of *Bambusa vulgaris* Schrad. ex J.C.Wendl. (Shwe-wa)

No.	Content	Values (%)
1.	Ash	10.13
2.	Moisture	9.30

Ash content is a measure of the total amount of mineral present within a food, whereas the mineral content is a measure of specific inorganic components present with a food such as calcium, sodium, potassium and chlorine. According to this table, the leaves of Shwe-wa present mineral. Water content or moisture content is the quality of water contained in a material. It is type of nutrient. According to this table, the leaves of Shwe-wa contained nutrient.

Vitamin C Content in Bamboo Leaves by Iodometric Titration Method

Table (3) Vitamin C Content in Bamboo Leaves

Sample	Ascorbic acid (%)
Bamboo Leaves	0.074

Ascorbic acid (vitamin C) acts as antioxidant and also provides health benefits. According to this table, the leaves of Shwe-wa show antioxidant activity.

Antimicrobial Activity in Leaves of *Bambusa vulgaris* Schrad. ex J.C. Wendl. (Shwe-wa)

Antimicrobial activity of n-hexane, ethanol and ethyl acetate extract of leaves of Shwe-wa were determined by Agar-well diffusion method on six organisms such as *Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus pumilus, Candida albican* and *E-coli*. These results were shown in Table (4).

Table (4) Results of Antimicrobial Activity in Leaves of *Bambusa vulgaris* Schrad. ex J.C.Wendl. (Shwe-wa)

Sample	Solvent	Organisms					
		I	II	III	IV	V	VI
Leaves of Bamboo (Shwe-wa)	n-hexane	–	-	–	–	-	-
	EtOAc	-	-	-	-	12 mm (+)	-
	EtOH	12 mm (+)	13 mm (+)	12 mm (+)	-	13 mm (+)	11 mm (+)

Organisms	Agar well – 10 mm
(1) *Bacillus subtilis*	10 mm ~ 14 mm (+)
(2) *Staphylococcus aureus*	15 mm ~ 19 mm (++)
(3) *Pseudomonas aeruginosa*	20 mm above (+++)
(4) *Bacillus pumilus*	
(5) *Candida albican*	
(6) *E. coli*	

According to this table, the leaves of Shwe-wa have antimicrobial activity.

Antioxidant Activity of Crude Extract from Leaves of *Bambusa vulgaris* Schrad. ex J.C.Wendl. (Shwe-wa)

To thoroughly examine the antioxidant activity of bamboo leaves, radical scavenging activity screening was carried out by using DPPH assay. This assay was chosen to apply because of its simplicity and applicability either an antioxidant in its pure state or in a mixture (e.g; natural extract). According to pre-test, it was found that ethanol extracts could change the

color of DPPH from purple into yellow. Therefore, it can be inferred that ethanol extracts may possess the DPPH free radicals scavenging activity. 4 mg of ethanol crude extract was dissolved in 20 mL of ethanol get (200 µg/mL) concentration and then it was diluted with ethanol to obtain (12.5, 25, 50, 100, 200 µg/mL) concentration. After mixing with DPPH solution, the absorbance of each solution was measured at 517 nm. Ascorbic acid was used as a standard antioxidant. On the basic of absorbance values, % inhibition of ethanol extract in different concentrations was calculated. The results obtained are tabulated in Table (6). The inhibition of ethanol extract of these samples were also compared with that of ascorbic acid. In addition, from these results, IC_{50} values (50 % inhibition concentration) for ethanol extract of these samples and ascorbic acid were determined by linear regressive excel program and the results are described in Table (5). The IC_{50} value was found to be 164.11µg/mL for ethanol extract of bamboo leaves sample. According to the data, IC_{50} value of bamboo leaves extract is greater than that of standard ascorbic acid (IC_{50} = 17.99 µg/mL).

Table (5) % Inhibition of Various Concentration and IC_{50} Value for Standard Ascorbic Acid

Sample Concentration (µg/mL)	Mean Absorbance	Mean % inhibition	IC_{50} (µg/mL)
50	0.297	68.50	17.99
25	0.350	61.61	
12.5	0.483	48.78	
6.5	0.562	40.41	
3.125	0.608	35.52	

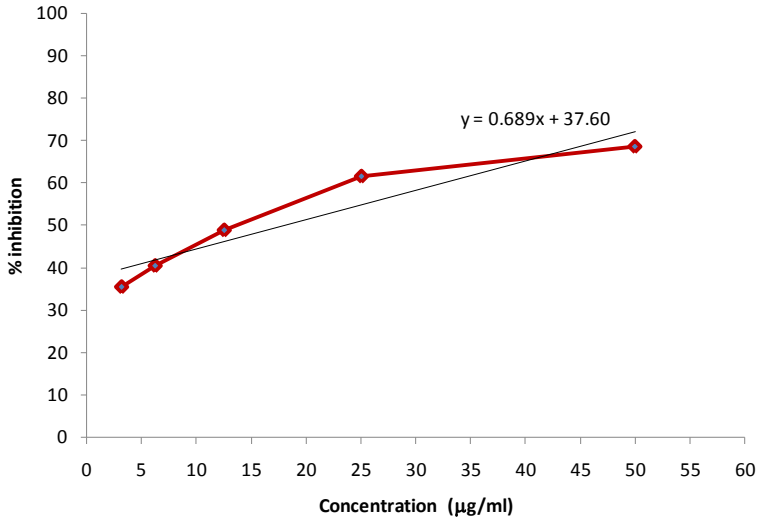

Figure 1. % Inhibition Vs Concentration (µg/mL) of Standard Ascorbic Acid

Table (6) % Inhibition of Various Concentration of Crude Extract and IC_{50} Value of Sample (Bamboo Leaves)

Concentration (µg/mL)	Mean Absorbance	Mean % inhibition	IC_{50} (µg/mL)
200	0.321	54.59	164.11
100	0.413	41.58	
50	0.473	33.09	
25	0.482	31.82	
12.5	0.546	22.77	

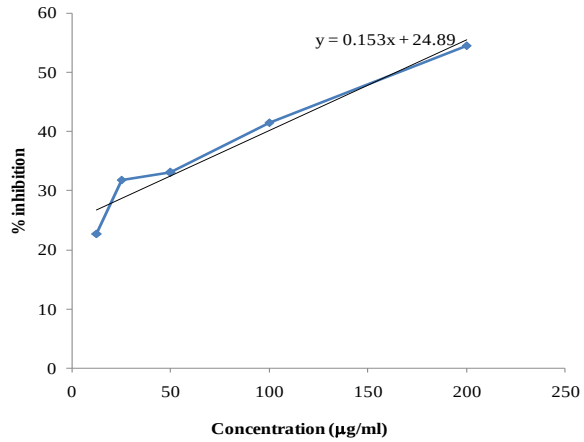

Figure (2) % Inhibition Vs Concentration (µg/mL) of Crude Extract Bamboo Leaves

Conclusion

In this research work, the leaves of Shwe-wa (Golden bamboo), *Bambusa vulgaris* Schrad. ex J.C.Wendl. was selected for chemical investigation. According to the phytochemical screening, leaves of Shwe-wa contains alkaloid, flavonoid, polyphenol, sugar, tannin and phenolic compound. In phyto-test, leaves of Shwe-wa contain flavonoid, polyphenol and phenolic compound which are antioxidant activity. Phenolic compounds including flavonoids act as antioxidants because of their structure that consists of at least one hydroxyl groups attached to a benzene ring. It can donate hydrogen atom to free radical so that the chain reaction is interrupted. Ash and moisture content of leaves of Shwe-wa were determined and found that ash content was (10.13 %) and moisture content was (9.30 %). Vitamin C content of leaves of Shwe-wa was quantitatively determined by iodometric titration method. Vitamin C content of leaves of Shwe-wa was 0.074%. Antimicrobial activity of crude extract of n-hexane, ethanol and ethyl acetate from leaves of Shwe-wa were investigated on six strains of bacteria which include *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus pumilus*, *Candida albican* and *E. coli* by agar well diffusion method. It was found that ethanol extract shows low antimicrobial activity, ethyl acetate and n-hexane extract did not show the antimicrobial activity. Antioxidant activity of ethanol crude extract was determined by DPPH assay. The ascorbic acid was used as a standard antioxidant in the assay. IC_{50} values were also calculated and the comparison of antioxidant activity of ethanol crude extract with ascorbic acid was described. Antioxidant contains in the leaves of Shwe-wa but antioxidant activity of ethanol extract of leaves of Shwe-wa is lower than that of ascorbic acid.

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Online Materials

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http://www.ijpab.com

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