Investigation of Antioxidant Activity and Isolation of an Organic Compound from Tubers of *Stemona burkillii* PRAIN. (THAMYA)

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Abstract

Thamya, a local name for *Stemona burkillii* has been chosen to study the antioxidant activity and isolate an organic compound. The Folin-Ciocalteu reagent (FCR) method was used to measure the total phenolic contents of the ethanol and watery extracts of *S. burkillii* tuber, and the results showed 135.46 g GAE/mg and 35.75 g GAE/mg, respectively. The DPPH free radical scavenging experiment was used to test the antioxidant activity, and the results revealed that both the ethanol extract (IC_{50} 42.54 g/mL) and the watery extract (IC_{50} 50.32 g/mL) exhibited moderate antioxidant activity when compared to the standard ascorbic acid (IC_{50} 1.74 g/mL). By utilizing column chromatography, an organic compound called stigmasterol (41 mg, 0.014%) was extracted from *S. burkillii* tubers' alkaloid-free ethyl acetate extract. It was then identified by FT IR spectroscopy, and its spectra were compared to those previously reported for stigmasterol.

Keywords: *S. burkillii* tubers, total phenol content, antioxidant activity, column chromatography

Introduction

The medicinal plant (Thamya), *Stemona burkillii*, belonging to the family Stemonaceae, has herbaceous stems up to one meter long from an underground tuber. It is a flowering plant family that is monocotyledonous (monocotyledonous). This family consists of four genera and 24 species distributed in areas with seasonal climates across Southeast Asia and tropical Australia (Aye Thida Win, 2014). This species is widely distributed in upper Myanmar and is grown in Gangaw Township, Chin State, Magway, Bago, Mandalay, and Sagaing Regions (Inthachub *et al*., 2010). It is the only source of the stemona alkaloids, including the four main compounds, such as stemofoline, 2'-hydroxystemofoline, dihydrostemofoline, and stemoburkilline, and it possesses many other bioactive compounds (Kongkiatpaiboon, S, 2010).

Traditional medicine utilizes several sections of the tuberous plant *S. burkillii* (Thamya), which has significant therapeutic effects. Additionally, it is said to have a number of biological effects, including antibacterial, antifungal, insecticidal, P-glycoprotein modulator, and anti-cancer activity. Insecticides, anti-helminthic worm medications, and pulmonary tuberculosis are all still treated with extracts from the fleshy tuberous roots (Chanmahasathien, 2011).

Botanical Description

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Family : Stemonaceae
Genus : *Stemona*
Species : *S.burkillii*
Botanical name : *Stemona burkillii*
Myanmar name : Thamya
Part of plants : Tubers
Habit : Herb

Figure 1. Plant and roots of *Stemona burkillii* (Thamya).

Materials and Methods

I. Collection and Preparation of Plant Samples

The tubers of *Stemona burkillii* were collected from Gangaw Township, Magwe Region, Myanmar. The samples were washed, cut into small pieces and then air-dried at room temperature. The dried samples were ground into powder by an electric blender and stored in an airtight container for further investigation.

II. Preparation of Crude Extracts by the Solvent Extraction Method

The different crude extracts of the tubers of *S. burkillii* were prepared by the solvent extraction method using different polarities of solvents such as PE, EtOAc, EtOH, and H_2O. Extractions of dried powder samples were made in ethanol at room temperature and in an on-water bath for 48 h. These extracts were filtered and the filtrates were evaporated to dryness with a rotatory evaporator at 40˚C. In this way, the remaining three crude extracts of the tubers of *S. burkillii* were prepared according to the above procedure for further investigation.

III. Determination of Total Phenol Content by the FCR Method

In the present study, the total phenol content of the ethanol and watery crude extracts was determined by the Folin-Ciocalteu Reagent method. 10 % FCR reagent (5 mL) was added to 1 mL of each standard Gallic acid solution (20, 10, 5, 2.5, 1.25, and 0.625 µg/mL) and allowed to stand for 5 minutes. And then, these solutions were mixed with 1 M of Na_2CO_3 (4 mL) at room temperature for 2 hrs. After that, measurements of absorbance at 765 nm were made of these solutions using a UV spectrophotometer. Similarly, the sample solution was prepared by dissolving 1 mg of the respective extract in 10 mL of distilled water. 1 mL of the extract solution was mixed with 10 % FCR reagent (5 mL) and 1 M of Na_2CO_3 (4 mL) in the same procedure as described earlier. Absorbance measurements were done in triplicate for each solution.

The total phenol content of the extract was calculated by using a linear regressive excel program.

IV. Screening of Antioxidant Activity

The free radical scavenging activity of ethanol and watery extracts of tubers of *S. burkillii* was investigated by the DPPH free radical scavenging assay as described in Brand-Williams (1995).

Preparation of DPPH Solution

2 mg of DPPH powder and 100 mL of 95 % ethanol were thoroughly mixed by a vortex mixer to get 20 µg/mL of DPPH solution. This solution was freshly prepared in the brown-colored flask. Then it must be stored in the fridge for no longer than 24 hours.

Preparation of Sample and Standard Solutions

The stock solution of the test sample was prepared by dissolving 2 mg of the sample in 10 mL of 95 % ethanol. This stock solution was two-fold serially diluted with ethanol to get the sample solutions with concentrations of 200, 100, 50, 25, 12.5, and 6.25 µg/mL by using the vortex mixer.

The stock solutions of standard ascorbic acid with concentrations of 200, 100, 50, 25, 12.5, 6.25, 3.12, and 1.56 µg/mL were also prepared by the procedure described for the sample solution.

Preparation of a Blank Solution

The blank solution was prepared by mixing 1.5 mL of each sample solution with 1.5 mL of ethanol using the vortex mixer.

Procedure

The control solution was prepared by mixing 1.5 mL of 20 µg/mL DPPH and 1.5 mL of ethanol in the brown bottle. The sample solution (1.5 mL of test sample solution mixed with 1.5 mL of 20 µg/mL DPPH) and the blank sample solution (1.5 mL of test sample solution mixed with 1.5 mL of ethanol) were prepared in the brown bottle. The solutions were allowed to stand at room temperature and shaken on the shaker for 30 min. After incubation, the absorbance of these solutions was measured at 517 nm using a UV spectrophotometer.

$$\% \text{ Inhibition} = [Abs_{Control} - (Abs_{Sample} - Abs_{Blank}) / Abs_{Control}] \times 100$$

Where % Inhibition = percent inhibition of test sample

$Abs_{Control}$ = absorbance of DPPH in ethanol solution

Abs_{Sample} = absorbance of sample + DPPH solution

Abs_{Blank} = absorbance of sample + ethanol solution

V. Extraction and Isolation of an Organic Compound from the Tubers of *S. burkillii*

Extraction of Ethyl Acetate Crude Extract

The dried powder samples of the tubers of *S. burkillii* were defatted with pet-ether solvent. The defatted sample was further extracted with 70 % EtOH at room temperature for 72 h. And then, the extract was filtered and evaporated to get residue. This residue was separated using an ethyl acetate solvent to produce an ethyl acetate extract, which was then digested with 2 N of HCl and separated by saturated NaCl to remove the alkaloid.

Isolation of an Organic Compound from the Ethyl Acetate Extract

The ethyl acetate extract (2.8 g) was fractionated using a column chromatographic method with a gradient elution of PE: EA solvent system, obtaining seven ($F_1 – F_7$) fractions.

The fraction F_2 was washed with methanol to get the pure isolated compound F_{2b} and it was then identified by FT IR spectroscopy.

Results and Discussion

I. Determination of Total Phenol Contents

The total phenol contents of the ethanol and watery extracts of the tubers of *S. burkillii* were determined by the FCR method, using gallic acid as the standard. The absorbance of the solutions was measured at wavelengths of 765 nm. The total phenol contents of the crude extracts were calculated from the regression equation of the calibration curve and expressed as µg Gallic acid equivalents (GAE) per milligram of sample in dry weight. The results are presented in Table 1. It was found that the total phenol content of ethanol extract (135.46 µg GAE/mg) was higher than that of watery extract (35.75 µg GAE/mg). Thus, the ethanol extract of the sample seems to contain more phenolic compounds than the watery extract, and it may have more potency in treating aging-related problems and cancers. Therefore, the tuber of *S. burkillii* may be used for the formulation of antiseptic and sore throat remedies.

Table 2. Total Phenol Contents of EtOH and H_2O Extracts of Tubers of *S. burkllii*

No	Crude Extracts	µg of GAE/mg ± SD of Extract
1	Ethanol	135.46 ± 0.036
2	Water	35.75 ± 0.004

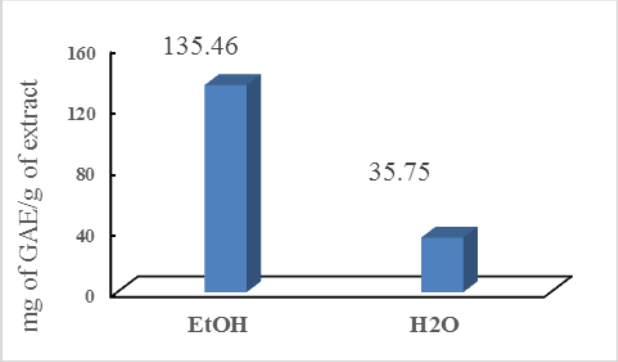


Figure 2. Comparison of total phenol content of ethanol and watery extracts

II. Screening of Antioxidant Activity of *S. burkillii* Tubers

The antioxidant activity of ethanol and watery extracts of *S. burkillii* tubers was determined by a DPPH free radical scavenging assay using ascorbic acid as the standard. The various concentrations of test samples were prepared using the half-dilution method. The absorbance values of the test samples were measured at wavelengths of 517 nm, and the percentage of DPPH radical inhibition activity was expressed as IC_{50} values (µg/mL). The lower the IC_{50} values, the higher the antioxidant activity.

According to the results, the IC_{50} value of the ethanol extract (42.54 µg/mL) was lower than that of the watery extract (50.32 µg/mL) but they both have been found to have milder antioxidant potency than the standard ascorbic acid (IC_{50} 1.74 µg/mL). However, the selected plant possessed the antioxidant potency necessary for the treatment of aging-related problems.

Table 3. Radical Scavenging Activity (% RSA) and IC_{50} Value of Standard Ascorbic acid

Test Sample	% Inhibition at Various Concentrations (µg/mL)						IC_{50} (µg/mL)
	1.56	3.12	6.25	12.5	25	50	
Ascrobic acid	45.95 ± 0.69	80.34 ± 0.69	97.54 ± 0.69	99.51 ± 0.34	99.14 ± 0.17	99.88 ± 0.17	1.74

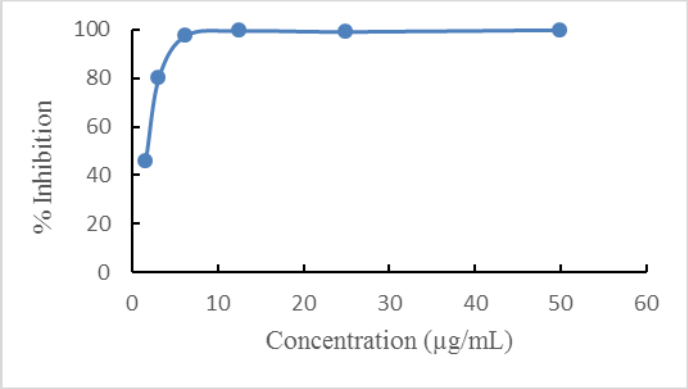


Figure 3 A plot of the % RSA of standard ascorbic acid on antioxidant activity

Table 4. Radical Scavenging Activity (% RSA) and IC_{50} Values of Ethanol and Watery Extracts of the tubers of *S. burkillii*

Test Sample	% Inhibition at Various Concentrations (µg/mL)						IC_{50} (µg/mL)
	6.25	12.5	25	50	100	200	
EtOH	26.44 ± 0.43	32.41 ± 0.43	45.16 ± 0.01	52.06 ± 0.29	71.60 ± 0.29	87.35 ± 1.9	42.54
H_2O	27.47 ± 0.14	30.86 ± 0.0	41.56 ± 0.87	49.90 ± 0.72	66.36 ± 0.14	85.80 ± 0.9	50.32

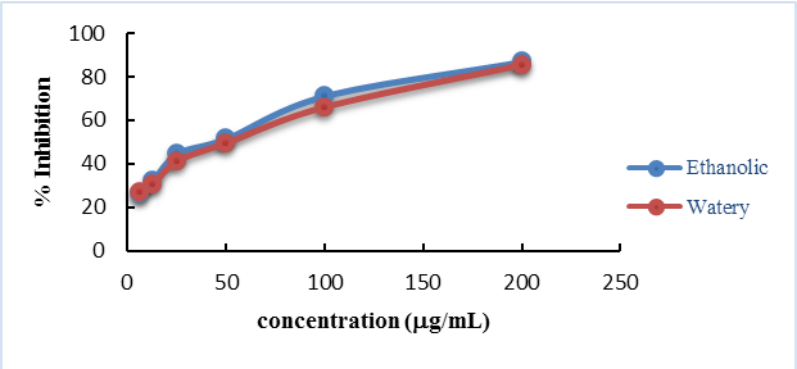


Figure 4. A plot of the % RSA of the ethanol and watery extracts of the tubers of *S. burkillii* on antioxidant activity

Isolation of an Organic Compound

An isolated compound, F_{2b}, was obtained from the alkaloid-free ethyl acetate extract of the tuber of *S. burkillii* as a white crystal compound (41 mg, 0.014 %). It is UV inactive at both 254 nm and 365 nm. Its R_f value is 0.38 with the solvent system PE: EA (8:2). It gave a blue color spot on TLC after spraying with H_2SO_4-anisaldehyde reagent followed by heating, which showed the characteristics of a steroid or terpenoid. Its melting point is 161-164 ºC which is nearly consistent with that of reported stigmasterol.

In the FT IR spectrum of an isolated compound F_{2b} (Figure 6), the absorption at 3425 and 3300 cm^{-1} indicated the presence of -OH stretching vibration for the OH group, and it was confirmed by the C-O stretching, which appeared at 1052 and 1023 cm^{-1} for the 2º alcohol. The absorption at 3045 cm^{-1} showed the =C-H stretching for the C=C-H group, and the =C-H bending that appeared at 970 cm^{-1} and 959 cm^{-1} indicated the presence of trans-di-substituted alkene and tri-substituted alkene. The band appeared at 1633 $cm^{-1,}$ indicating the -C=C- stretching vibration. The strong absorption at 2934 and 2869 cm^{-1} indicated asymmetric and symmetric -CH stretching for the $-CH_2$ and CH_3 groups, respectively, and the asymmetric and symmetric -CH bending for the $-CH_2$ group at 1458 cm^{-1}, and the symmetric -CH bending for the iso-propyl group at 1381 and 1364 cm^{-1}. The FT-IR spectrum of the isolated compound F_{2b} is very consistent with that of stigmasterol (Figure 8). According to the chemical characteristics, melting point, and FT IR spectral data, the isolated compound F_{2b} may be deduced as stigmasterol.

Compound F_{2b} PE: EA, (8:2)

White crystal $Rf = 0.38$

(41 mg, 0.014 %) H_2SO_4-Anisaldehyde, Δ

Figure 5. Photograph and thin layer chromatogram of an isolated compound

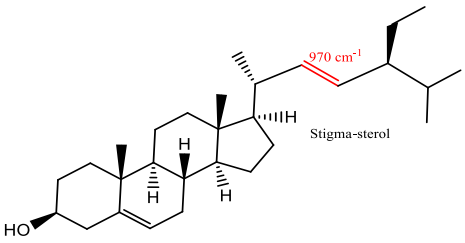


Figure 6. Structure of stigmasterol ($C_{29}H_{48}O$)

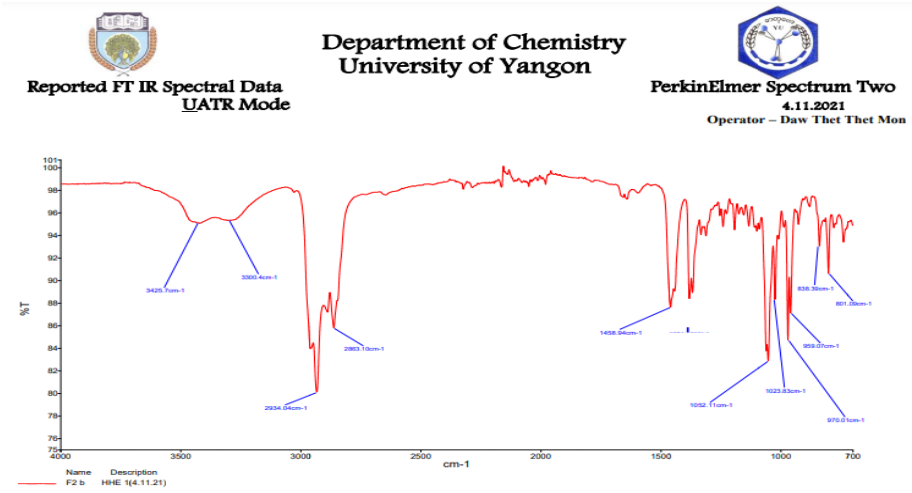
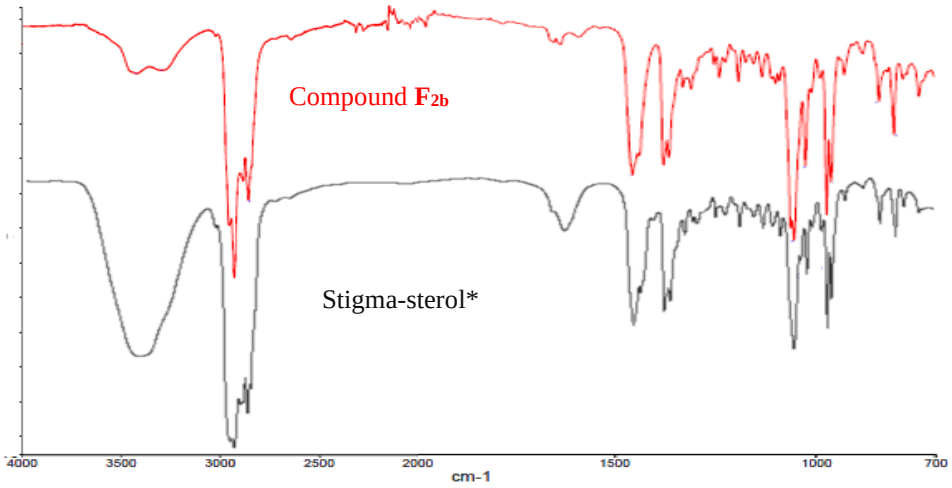


Figure 7. FT IR spectrum of the isolated compound F_{2b}



*(AIST: Spectral Database for Organic Compounds, SDBS)

Figure 8. Comparison of the FT IR spectra of the isolated compound F_{2b} and stigmasterol*

Conclusion

The overall assessment of the present study indicates the useful and valuable results of the tuber of *S. burkillii* (Thamya) regarding the total phenolic contents and antioxidant activity. According to the experimental results, the selected medicinal plant, *S. burkillii* (Thamya), showed significant antioxidant activity when compared with the standard ascorbic acid. Therefore, the selected plant may be used in the treatment of aging-related diseases, tumors, and cancers. In addition, an organic compound, stigmasterol, was isolated from the alkaloid-free ethyl acetate extract and identified by the facts, which are based on the chemical characteristics, melting point, and FT IR spectral data.

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SDBS (Spectral Database for Organic Compounds) (https://sdbs.db.aist.go.jp) the National Institute of Advanced Industrial Science and Technology