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Original Research Article

Study on Phytochemical Constituents and Evaluation of Radical Scavenging Activity of Myanmar Traditional Medicinal Plant *Stemona curtisii* Hook f.

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ABSTRACT

In this study, rhizomes of *Stemona curtisii* Hook f., family Stemonaceae were selected for chemical investigations. The rhizomes of *S. curtisii* Hook f., Myanmar named Tha-myar were collected from Kalay Township, Chin State, Northern-west of Myanmar. The constituents of phytochemical compounds from selected plant were isolated and their structures were elucidated. Two alkaloid compounds, stemofoline (1), isostemofoline (2) and other three compounds (3-5) were isolated from ethyl acetate portion of *S. curtisii* Hook f. by Thin Layer and Column Chromatography methods at Meijo University, Nagoya, in Japan. The structures of these compounds were elucidated by 1D and 2D NMR spectroscopy and mass spectrometry. The radical scavenging activity of ethyl acetate extract of rhizome of *S. curtisii* Hook f. was evaluated by DPPH assay method.

Keywords: *Stemona curtisii* Hook f., Stemonaceae, 1D and 2D NMR spectroscopy, DPPH Assay method, radical scavenging activity.

INTRODUCTION

Stemona curtisii Hook f. family stemonaceae is widely distributed in Southeast Asia. The roots of stemona plants have been used as insecticides and antitussive remedies in China, Japan, and Southeast Asian for some 2000 years. Herbal extracts from plants belonging to the stemonaceae family have been used in folk medicine in East Asia for thousands of years, with three species of the stemona genus. [1]

Traditional medicine has been used for generations and is more affordable and easily obtainable - even in rural areas - therefore it continues to be widely used and plays a significant role in health care in Myanmar. Myanmar traditional knowledge and medicine is believed to be able to cure all of these diseases by using ingredients such as fresh or dried roots, stems, leaves, buds, and flowers. Myanmar is conducting research on treatment of six major diseases - diabetes, hypertension, malaria, tuberculosis, diarrhea, and dysentery through traditional medicine. At the recent time, many researchers discover the more and more effective medicinal plant and potency of containing chemical constituents. Hence, the isolation of new and more potent

compounds on serious diseases (such as HIV, cancer) is the most popular in the field of natural product organic chemistry. [2] A wide variety of plants and herbs with specific therapeutic qualities are used for making medicines, ideally using every parts of the plant. [3] Traditional medicines are an integral part of people's culture and are used extensively by the peoples in developing countries for their primary health care. [4]

One Myanmar indigenous medicinal plant *S. curtisii* Hook. f. has been reported that various parts and different kinds of extracts of this plant showed the several biological activities, such as, antibacterial, antifungal activities, insecticidal activities, [5] P-glycoprotein (P-gp) modulator activities, antiplasmodial activities, antimalarial activity, cytotoxic activity, [6] and acetylcholine -esterase (AChE) inhibitory activities. The study of p-gp function test indicates that stemofoline could be developed as a specific potent modulator to overcome P-gp-mediated MDR in cancer cells. [7]

In the present research work, one Myanmar indigenous medicinal plant, *S. curtisii* Hook f. was chemically investigated at Meijo University, Nagoya in Japan. Two alkaloids compounds (stemofoline and isostemofoline), one lignin compound (sesamine), and two dihydrostebene compounds (stilbostemin-A and D) were isolated and their structures were elucidated by advanced spectroscopic methods. Stemofoline and isostemofoline are major components of this plant species. This research focuses on pharmaceutical knowledge of tribal communities of ASEAN peoples and aimed to document their traditional herbal use of plants.

Botanical Description

Family Name: Stemonaceae
Botanical Name: *Stemona curtisii* Hook f.
Myanmar Name: Tha-myar
Part used : Rhizome
Medicinal uses: To treat respiratory disorders, including pulmonary tuberculosis, bronchitis, insecticides and antitussive remedies, treatment of cancer

Figure 1 Plant and Rhizome of *Stemona curtisii* Hook f.

MATERIALS & METHODS

^{1}H- and ^{13}C-NMR spectra were recorded on a BRUKER ECP-600 (^{1}H: 600 MHz and ^{13}C: 150 MHz). Chemical shifts for ^{1}H- and ^{13}C-NMR are given in parts per million (δ). EI-Mass spectrum was obtained with a JEOL JMS MS-700 and HX-110, respectively. Optical rotation was recorded on a JASCO P-1020 polarimeter (cell length 100 mm). Analytical TLC was performed on Silica gel 60 F254 (Merck). Column chromatography was carried out on silica gel BW-820MH (Fuji Silysia Chemicals, Co. Ltd, Seto, Japan).

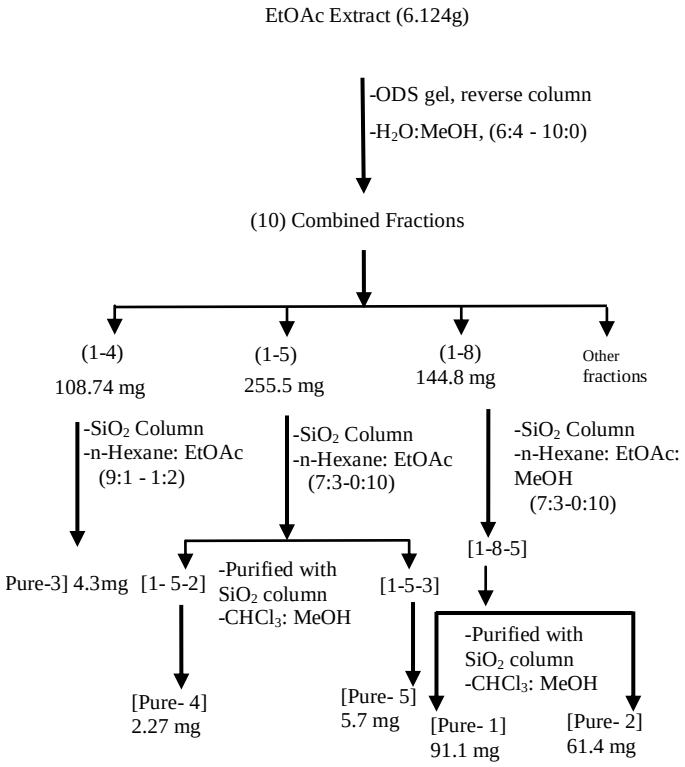
Sample Collection

The rhizomes of Stemona crtisii Hook f. were collected from Kalay Township, Chin State in Myanmar.

Extraction and Isolation

The air-dried rhizome of *S. curtisii* Hook. f. was percolated with 95% methanol at room temperature for one month. The methanol extract was concentrated and the residue (30.0 g) was suspended in water. This suspension was successively extracted with n-hexane, ethyl acetate, and *n*-butanol. The ethyl acetate soluble extract was concentrated by rotary evaporator to produce a residue (6.124 g). The extract was fractionated on a reverse column using methanol and water gradient to afford ten fractions (frs. 1-1 to 1-10)

Isolation of Pure Compounds from Ethyl Acetate Extract of *S. curtisii* Hook. f.



Determination of Radical Scavenging Activity by Spectrophotometric Method

Chemicals

DPPH, 95% Ethanol, Ascorbic acid

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 colored reagent bottle. It must be kept in the fridge for no longer than 24 hours before use.

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 2 μg/mL, 4 μg/mL, 6 μg/mL, 8 μg/mL and 10 μg/mL respectively and the same volume 5.0 mL of standard ascorbic acid solution was prepared for each concentration.

Preparation of test sample solution

The ethyl acetate extract of selected sample was diluted with 50% ethanol in various ratios to obtain four ranges of concentration, such as 12.5 μg/mL, 25 μg/mL, 50 μg/mL, 100 μg/mL, and 200 μg/mL respectively. Then, 5.0 mL of ethanol solution was prepared for each concentration.

Measurement of DPPH radical scavenging activity by spectrophotometric method

The control solution was prepared by mixing 2.0 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 test sample solution and 2.0 mL of 50% 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 Spectrophotometer. The absorbance values

obtained were applied to calculate percent inhibition by the following formula.

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

% inhibition= percent inhibition of test sample

Sample = absorbance of test sample solution

$\text{DPPH}_{\text{alone}}$ = absorbance of control solution

Blank = absorbance of blank solution

RESULTS AND DISCUSSION

In this research work, pure compounds (1-5) were isolated from the ethyl acetate extract of rhizome of *S. curtisii* by using Thin Layer and Column Chromatographic separation method. The structures of these isolated compounds were elucidated by ^{1}H NMR, ^{13}C NMR, DEPT, HMQC, DQF COSY, HMBC, NOESY and EI Mass spectral data. The assignments of spectral data and the elucidated structures are described as follows.

Compound-(1): Stemofoline, Pale yellow amorphous solid. $[\alpha]_D^{25}$= 246.678 (*c* 0.112, $CHCl_3$). ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 2.09 (3H, s, H-16), 0.93 (3H, t ; *J* = 6.9 Hz,H-4′), 1.38 (3H, d ; *J* = 6.5 Hz, H-17), 1.39, 1.37 (2H, m, H-3′), 1.93, 1.85 (2H, m; H-1′), 1.43, 1.28 (2H, m, H-6), 1.58 (2H, m, H-2′), 1.97, 1.72 (2H, m ; H-1), 3.1 (1H, dd ; *J* = 6.5, 4.8 H-9), 1.82 (1H,dd; *J* = 7.1,5.3Hz, H-5), 3.01,3.15 (2H, ddd; *J* = 12.4, 6.3, 4.2 Hz, H-9), 2.7 (1H, d ; *J* =6.0 Hz, H-7), 4.14, (3H, s), 3.48 (1H, br s), 4.26 (1H, br s). ^{13}C-NMR (125 MHz, $CDCl_3$) δ:9.08 (C- 16), 13.91 (C-4′), 18.26 (C-17), 23.06 (C- 3′), 26.60 (C- 1′), 27.24 (C- 6), 31.56 (C- 2′), 33.21 (C - 1), 34.51 (C-4), 47.49 (C-5), 47.56 (C-9), 49.88 (C-7), 58.79 (OCH_3), 60.88 (C-9a), 78.49 (C-2), 82.78 (C-3), 98.49 (C-14), 112.65 (C-8), 127.80 (C-12), 148.40 (C-11), 162.79 (C-13), 169.65 (C-15). EIMS ***m*/*z*** (rel. int.): 387 $[M^+]$ ($C_{22}H_{29}NO_5$).

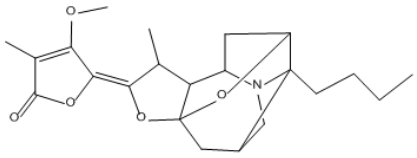
Compound-(2): Isostemofoline, is pale yellow solid compound and it is Z isomer of compound (1). The structure of isostemofoline was confirmed by NOESY spectrum.

Compound (3) : Sesamin, White amorphous solid. $[\alpha]_D^{25}$= + 0.0595 (*c* 0.058, $CHCl_3$) ^{1}H-NMR (600 MHz, $CHCl_3$) δ: 6.80 (1H, dd, J= 7.98,1.0 Hz; H-2,2′), 6.78 (1H, dd, J= 7.98; H-3,3′), 6.84 (1H, d, J=1.0 Hz; H-6,6′), 5.95 (2H, s; H-10,10′), 4.71 (1H, d, *J* = 4.2 Hz; H-7,7′), 3.82,4.25 (2H,dd, *J* = 1.78,9.12 Hz; H-9,9′), 3.05 (1H, dd, J =1.0 Hz, 7.98 Hz; H-8,8′) ^{13}C-NMR (125 MHz) δ: 147.98 (C- 5,5′), 147.12 (C- 4,4′), 135.08 (C-1,1′), 119.35 (C-2,2′), 108.19 (C- 3,3′),106.49 (C-6,6′), 101.06 (C-10,10′),85.80 (C- 7,7′), 71.72 (C- 9,9′), 54.35 (C-8,8′). EIMS ***m*/*z*** (rel. int.): 354 $[M^+]$ ($C_{20}H_{18}O_6$); Melting point is 127-129°C.

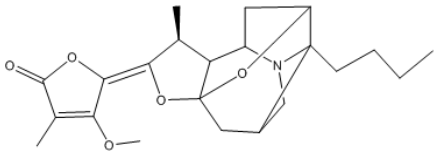
Compound (4): Brown amorphous solid. ^{1}H-NMR (600 MHz, $CDCl_3$) δ: 7.08 (1H, dd, *J* = 1.68,7.38 Hz; H-2′), 6.58 (1H,s ; H-6′), 6.58 (1H, s ; H-2″), 6.58 (1H, s ; H-6″), 4.73 (1H, d, *J* = 4.5 Hz; H-6), 7.16 (1H, dt, *J* = 8.1,1.7 Hz; H-4), 6.83 (1H, dt, *J* = 8.1, 1.68 Hz; H-5), 6.92 (1H, d, *J*= 8.1Hz; H-3), 6.17 (1H, d, *J* = 2.23; H-6′), 6.17 (1H, d, *J* = 2.23; H-2′), 6.12 (1H, d, *J* = 2.23; H-4′) 3.85 (3H, s ; C-5″-O$\underline{CH_3}$), 2.69 (2H, m ; C-1″), 2.85 (2H, m ; C-2’’).^{13}C-NMR (125 MHz,$CDCl_3$) δ: 159.27 (C- 3′), 159.27 (C-5′), 158.91 (C- 2), 146.04 (C- 1′), 131.40 (C- 1) 130.88 (C- 6), 128.24 (C- 4), 121.40 (C - 5), 111.44 (C- 3), 108.02 (C- 6′), 108.02 (C- 2′), 101.09 (C- 4′), 55.77 (C-2-O$\underline{CH_3}$), 37.58 (C-1″), 33.50 (C-2″). EIMS ***m*/*z*** (rel. int.): 244 $[M^+]$ ($C_{15}H_{16}O_3$).

Compound (5): Reddish brown amorphous solid. ^{1}H-NMR (600 MHz, $CDCl_3$) δ: 6.99 (1H, t, *J* = 7.87 Hz; H-5), 6.78 (1H, dd, *J* = 7.68,1.5 Hz; H-6), 6.86 (1H, dd, *J* = 8.04,1.56 Hz; H-4), 6.4 (1H, d, *J* = 2.52 Hz; H-2′), 6.31 (1H, d, *J* = 2.52 Hz; H-6′), 3.82(3H, s ; C-2,O$\underline{CH_3}$),3.77 (3H, s ; C-5′-O$\underline{CH_3}$), 2.93 (2H, m ; C-2″), 2.91 (2H, m ; 1″),5.57(s,C-3,OH),4.67(s, C- 3′ OH). ^{13}C-NMR (125 MHz,$CDCl_3$) δ: 158.19 (C- 5′), 154.59 (C- 3′), 149.02 (C- 3), 145.30 (C- 2), 142.48 (C- 1′) 134.85 (C- 1), 124.91 (C-5), 121.44 (C - 6), 113.97 (C- 4′), 113.69 (C- 4), 107.32 (C- 2′), 99.27 (C- 6′), 61.28

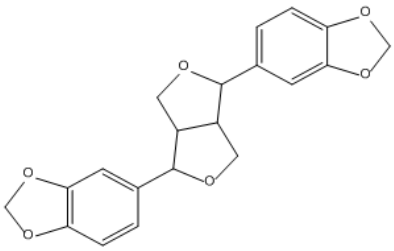
(C-2-O<u>C</u>H$_3$), 55.26(C-5′-O<u>C</u>H$_3$),35.00 (C-2″), 30.82 (C-1″),10.42(CH$_3$). EIMS ***m/z*** (rel. int.): 288 [M$^+$] ($C_{17}H_{20}O_4$).



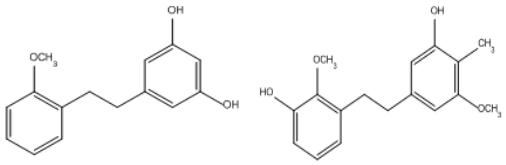
Structure of Stemofoline Alkaloid Compound (1)



Structure of Isostemofoline Alkaloid Compound (2)



Structure of Sesamin Lignan Compound (3)



Structure of Stilbostemin-A and Stilbostemin-D Compounds (4 & 5)

DPPH Assay of standard ascorbic acid

The present study was done for the investigation of the radical scavenging activity of ethyl acetate extract of rhizome of *S. curtisii* Hook. f.. Ascorbic acid was used as a standard antioxidant. Ascorbic acid is a water soluble antioxidant that maintains many cofactors in the reduced state. The potential antioxidant activities of selected plant were assessed on the basis of the scavenging activity of the stable DPPH free radicals. Antioxidant activity of ethyl acetate extract of selected sample was expressed as percentage of DPPH radical inhibition and IC_{50} values (μg/mL). The IC_{50} value is a parameter used to measure antioxidative activity and it is defined as the extract concentration required for 50% scavenging of DPPH radicals under experimental condition employed. A smaller IC_{50} value corresponds to a higher antioxidant activity. The results of antioxidant activity of ethyl acetate extract of sample by DPPH method using ascorbic acid as a positive control are showed in Table 1, 2 and Figure 2, 3.

Table 1 Absorbance Values and % Inhibition of Standard Ascorbic Acid

Concentration (μg/mL)	2	4	6	8	10
% Inhibition	12.42	33.33	60	75.15	79.09

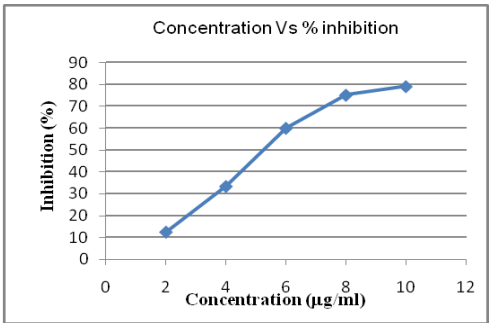


Figure 2 Concentration Vs % Inhibition Curve of Standard Ascorbic Acid

Table 2 Absorbance Values and % Inhibition of Ethyl Acetate Extract of *S. curtisii* Hook. f.

Concentration (μg/mL)	12.5	25	50	100	200
% Inhibition	10.27	22.92	42.45	64.84	89.32

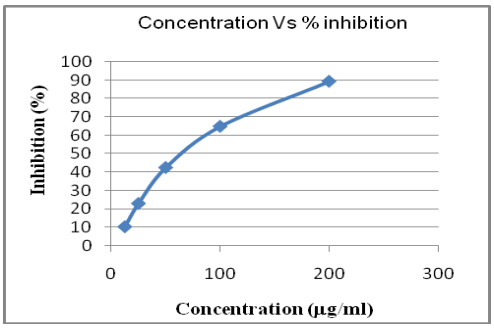


Figure 3 Concentration Vs % Inhibition of Ethylacetate Extract of *S. curtisii*

In accordance with experimental data, the ethyl acetate extract of selected plant showed scavenging against DPPH radical. These results revealed that antioxidant activity of ethyl acetate extract of selected

plant (IC_{50} = 64.42 µg /mL) shows significant antioxidant property which is comparable to IC_{50} value of standard ascorbic acid as positive control (IC_{50} = 5.51 µg /mL). Therefore, the study suggests that the selected plant might be a potential source of natural antioxidants.

CONCLUSION

In the present study, rhizome of *S. curtisii* Hook. f. was chemically investigated.

The five pure compounds were isolated from ethyl acetate extract of selected plant and their structures were elucidated by advance spectroscopic methods. The significant potencies of these isolated compounds were studied by previous reported data. Among them Stemofoline is major component in selected plant which has potent anticancer property. The radical scavenging activity of ethyl acetate extract of selected plant was determined by DPPH assay method. The IC_{50} value of plant extract was found to be 64.42 µg/mL which responds significant antioxidant activity compare with standard ascorbic acid. Other effective phytochemical compounds such as Sesamine, Stilbostemin - A and D were observed in this selected plant.

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