ISOLATION, IDENTIFICATION OF PHYTOCONSTITUENTS FROM THE RHIZOMES OF *GLOBBA SHERWOODIANA* (PADEIN-GNO) AND THEIR BIOLOGICAL ACTIVITIES*

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Abstract

The aim of the present study was to isolate and to identify the phytoconstituents from the rhizomes of *Globba sherwoodiana* (Padein-gno) and to screen their biological activities. The active $CHCl_3$ extract of Padein-gno was separated by silica gel column chromatography which afforded four compounds namely campest-4-en-3-one (**A**, 4 mg, colourless needle crystal), stigmasta-4,22-dien-3-one (**B**, 15 mg, colourless needle crystal), stigmast-4-en-3-one (**C**, 8 mg, colourless needle crystal), and docosyl ferulate (**D**, 1.0 g, white amorphous powder). All of the isolated compounds, **A**–**D** were identified by modern spectroscopic techniques including FT IR, ^{1}H NMR, ^{13}C NMR, and HR ESI MS as well as by comparing with the reported data. The antiproliferative activity of the crude extracts of Padein-gno rhizomes and its constituents were screened against four human cancer cell lines [human cervical cancer cell line (HeLa), human lung cancer cell line (A549), human breast cancer cell line (MCF-7), and human gastric cancer cell line (GSU)] and normal human fibroblast cells (WI) by 3-(4,5-dimethylthiazol-2-yl)-2,5-dimethyltetrazolium bromide (MTT) assay. The screening results revealed that the $CHCl_3$ extract showed a higher than the MeOH extract against all the tested cancer cell lines. In addition, the isolated compounds **A** to **D** also showed the potent activity with IC_{50} values ranging from 13.9 to 98.3 μg/mL. In the antibacterial activity, both of the crude extracts exhibited a moderate activity against Gram-positive bacteria *B. subtilis* and *S. aureus* with MIC values ranging from 25.0 to 100 μg/mL. The isolated compounds, **A-C** were found to show significant antibacterial activity against *B. subtilis* and *S. aureus* with MIC values ranging from 3.1 to 25.0 μg/mL, respectively.

Keywords: *Globba sherwoodiana*, antiproliferative activity, antibacterial activity, isolated compounds

Introduction

Cancer has become the second leading cause of death worldwide. For many years, chemotherapeutic agents have been developed and used to treat cancer. However, there is no drug that shows good selectivity for cancer cells. To date, about 70% of anti-cancer drugs were developed from natural resources, and world populations still rely mainly on anti-cancer drugs derived from medicinal plants. Medicinal plants are known to produce certain bioactive molecules which react with other organisms in the environment, inhibiting bacterial and fungal growth (Chopra *et al*., 1992). Bacteria and fungi are various types of organisms. They can also cause tissue damage and hamper our body functions to the point of causing disease (Vogt and Dippold, 2005). Medicinal plants include a various types of plants used in herbalism and some of these plants have medicinal activities. These medicinal plants consider as a rich resources of ingredients which can be used in the development and synthesis of drugs. Beside that these plants play a critical role in the development of human cultures around the whole world (Hassan, 2012). Plants, especially used in Ayurveda can provide biologically active molecules and lead structures for the development of modified derivatives with enhanced activity and reduce toxicity.

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Globba sherwoodiana (Zingiberaceae) is a small perennial herb from 38 to 45 cm in height with rhizomes and compact and tall leafy shoots. It was identified as a new species in 2012, based on its obvious morphology. In Myanmar, it is cultivated and locally known as 'Padein-gno'. It is also cultivated in Thailand and India. The flowers of the Padein-gno are sold in markets and used for Buddhist offerings in Myanmar and Thailand. The previous phytochemical studies revealed that the genus *Globba* contains labdane diterpenoids, sesquiterpenoids, steroids, lipids, and phenolic compounds. Labdane diterpenoids were reported to possess antibacterial and antifungal activities. To the best of our knowledge, no phytochemical and biological studies have been reported from any part of *G. sherwoodiana*. Therefore, in this paper the isolation and identification of four compounds from the rhizomes of Padein-gno and the screening of their biological activities were firstly reported.

Materials and Methods

The experimental works were conducted at the Division of Natural Products Chemistry, University of Toyama, Japan. The fresh Padein-gno rhizomes (10.0 kg) were collected from Pyin Oo Lwin Township, Mandalay Region, Myanmar, in October 2018. The plant was identified as *Globba sherwoodiana* by authorized botanist at the Botany Department, University of Yangon. The rhizomes were cleaned by washed with water and air-dried at room temperature. The dried rhizomes were ground into powder and stored in air-tight container. The following instruments were used for structure elucidation of isolated compounds: Nuclear magnetic resonance (NMR) spectra were measured at 500 MHz (^{1}H NMR) and 125 MHz (^{13}C NMR) on a JEOL ECA500II spectrometer (Wako Pure Chemical Industries, Ltd., Osaka, Japan) in $CDCl_3$. High-resolution mass spectrometry (HRESIMS) data were taken on a SHIMADZU LCMS-IT-TOF (Shimadzu, Kyoto, Japan) instrument. Normal phase silica gel (silica gel 60 N, spherical, neutral, 40–50 μm; Kanto Chemical, Tokyo, Japan) and reversed phase silica gel (Cosmosil 75C_{18}-OPN; Nacalai Tesque, Kyoto, Japan) were used for open column chromatography (C.C.). Thin layer chromatography (TLC) was performed using silica gel GF_{254} precoated (Merck) plates. The fractions were detected by visualization using a UV lamp at 254 and 365 nm, followed by spraying with 1% Ce $(SO_4)_2$–10% aqueous H_2SO_4, *p*-anisaldehyde stain solution and heating to 150 °C for 5–10 min in a drying cabinet. Normal-phase HPLC column chromatography with COSMOSIL 5SL-II (10 × 250 mm) columns together with an Agilent Technologies 1260 quat pump with a JEOL detector, was used for separation of the compounds. The biological assay was measured at 570 nm using a SH-1200 Microplate Reader (Corona, Hitachinaka, Japan).

Extraction and Isolation of the rhizomes of *G. sherwoodiana*

The dried rhizome powder of *G. sherwoodiana* (3.2 kg) was sonicated in chloroform (7.0 L, 90 min, × 5) at room temperature and the extract was concentrated under reduced pressure to give a residue (80.4 g). The chloroform extract (80.0 g) was subjected to silica gel C.C. eluted with *n*-hexane: EtOAc (from 9.5:0.5 to only EtOAc) and EtOAc: MeOH (9:1 and 7:3) to give 28 fractions of 500 mL each, and these fractions were combined into ten main fractions (F1-F10) after TLC profiling. Fraction F8 (7.4 g) was subjected to Cosmosil 75C18 C.C., eluted with MeOH: H_2O (3:1 to 7:1) to yield three subfractions (Fr. 8-1–8-3). Subfraction 8–3 was purified by normal-phase HPLC, eluted with a linear gradient solvent system (*n*-hexane–EtOAc, 3:7 to

1:4), and monitored at the wavelength of 254 nm, to afford compound **A** (2.0 mL/min, t_R = 50.65 min, 4.0 mg), compound **B** (2.0 mL/min, t_R = 51.23 min, 15.0 mg), and compound **C** (2.0 mL/min, t_R = 57.23 min, 8.0 mg). Fraction F9 (6.9 g) was chromatographed on Cosmosil 75C_{18} C.C. eluted with MeOH:H_2O (3:1 to 7:1) to give five main subfractions (Fr. 9-1–9-5). The subfraction 9–2 (2.5 g) was rechromatographed on silica gel C.C., using the *n*-hexane:CH_2Cl_2:EtOAc (20:20:1) isocratic solvent system to obtain compound **D** (1.0 g). All of **A**–**D** were isolated in this investigation for the first time from this plant species.

Structural Elucidation

The structures of isolated compounds **A**, **B**, **C**, and **D** were elucidated by modern spectroscopic techniques, namely FT IR, ^{1}H NMR, ^{13}C NMR and HRESIMS.

Investigation of some Biological Activities of the Crude extracts of Padein-gno Rhizomes and Its Isolated Compounds

Antiproliferative activity by MTT assay

The crude extracts and isolated compounds **A** to **D** were evaluated their antiproliferative activity using the 3-(4,5-dimethyl-thiazol-2-yl)-2,5 diphenyltetrazolium bromide (MTT, Nacalai Tesque, Japan) assay, according to the published procedure. *α*-Minimum essential medium with l-glutamine and phenol red (*α*-MEM; Wako, Japan) were used to culture the HeLa, A549, MCF-7, and GSU cancer cell lines, and WI human fibroblast cell. All media were supplemented with 10% fetal bovine serum (FBS; Sigma, USA) and 1% antibiotic antimycotic solution (Sigma, USA). For the MCF-7 cells, the growth medium was supplemented with 1% 0.1 mM non-essential amino acids (NEAA; Gibco, USA) and 1% 1 mM sodium pyruvate (Gibco, USA). Each cancer cell line was seeded in 96-well plates (2×10^3 cells per well) and incubated in the respective medium at 37 °C, under 5% CO_2 and 95% air atmosphere, for 24 h. After the cells were washed with phosphate-buffered saline (PBS), different concentrations of the tested samples (1, 10, and 100 μg/mL) were added. After 72 h incubation, the cells were washed with PBS and 100 μL of medium containing 10 μL of MTT solution (5 mg/mL) was added to each well and incubated for 3 h. Subsequently, the absorbance of each well was measured at a 570 nm wavelength. 5-Fluorouracil (Wako, Japan) was used as a positive control. Cell viability was calculated from the mean values of data from three wells by using the following equation, and cytotoxicity was expressed as the IC_{50} (50% inhibitory concentration) value.

(%) Cell viability = 100 × [{Abs (test samples) – Abs (blank)}/ {Abs (control) – Abs (blank)}]

Antibacterial activity by MTT assay

Antibacterial assay was performed using the standard microdilution-MTT assay according to the published procedure with slight modifications. *B. subtilis* NBRC 13719, *S. aureus* NBRC 100910, *E. coli* NBRC 102203, and *K. pneumoniae* NBRC 14940 were utilized for this assay. The bacterial strains were inoculated on YP agar plates [1% polypeptone (Nihon Pharmaceutical Co., Ltd., Tokyo, Japan), 0.2% yeast extract (BD Difco™, USA), 0.1% $MgSO_4 \cdot 7H_2O$, and 2% agar (Nacalai Tesque Inc., Kyoto, Japan)] and incubated at 37°C for 12 h.

A stock sample solution (crude extracts and pure compounds) were prepared in DMSO with concentration of 10 mg/mL each and further diluted to varying concentrations in 96-well plates that contained microbial strains incubated in YP medium for the bacterial strains. The plate was incubated at 37°C overnight. Ampicillin and kanamycin (Nacalai Tesque) were used as positive controls for Gram-positive and Gram-negative bacterial strains. The MIC values were visually observed by addition of 10 µL of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT, Sigma-Aldrich, USA) solution (prepared 5 mg/mL in isopropanol-HCl) into each well, followed by 1 h incubation.

Results and Discussion

Compound **A** (4 mg, 0.0001% yield) was obtained as colourless needles. Its HRESIMS showed a quasi-molecular ion peak at *m/z* 421.3428 $[M+Na]^+$ compatible with the molecular formula of $C_{28}H_{46}O$ in conjunction with NMR data. The FT IR spectrum showed the absorption bands at 3100 and 1624 cm^{-1} due to the presence of alkene groups. The strong band appeared at 1742 cm^{-1} suggesting the presence of carbonyl groups. The ^1H NMR spectrum of compound **A** in $CDCl_3$ (Figure 1) showed the presence of ten methylene groups, seven methine protons, one olefinic proton at δ_H 5.71 (brs, H-4), and six methyl groups at δ_H 0.69 (s, H_3-18), 0.75 (dd, *J* = 1.7, 6.9 Hz, H_3-28), 0.79 (d, *J* = 6.9 Hz, H_3-27), 0.84 (dd, *J* = 1.7, 6.9 Hz, H_3-26), 0.90 (dd, *J* = 4.0, 6.9, H_3-21), and 1.18 (s, H_3-19). The ^{13}C NMR (Figure 2) spectroscopic data revealed 28 carbon resonances, including ten methylene carbons at δ_C 35.7 (C-1), 39.7(C-2), 32.5 (C-6), 32.1 (C-7), 38.7 (C-11), 21.1 (C-12), 20.3 (C-15), 24.0 (C-16), 33.7 (C-22), and 30.3 (C23), six methyl carbons at δ_C 12.0 (C-18), 15.4 (C-27), 15.5 (C-28), 17.5 (C-19), 18.3 (C-26), and 18.7 (C-21), one carbonyl carbon at δ_C 199.9 (C-3), two olefinic carbons at δ_C 123.8 (C-4) and 172.0 (C-5), two quaternary carbons at δ_C 38.7 (C-10) and 42.5 (C-13), seven methine carbons at δ_C 28.2 (C-25), 33.0 (C-24), 34.0 (C-20), 35.9 (C-8), 53.9 (C-9), 55.9 (C-14), and 56.1 (C-17). The ^1H and ^{13}C NMR spectroscopic data (Table 1) of compound **A** were similar to those of a campest-4-en-3-one (Seto *et al.*, 2000). Furthermore, the HRESIMS spectrum (Figure 3) of compound **A** shows the $[M+Na]^+$ peak at *m/z* 421 (100%) suggesting the molecular weight 398 consistent with the molecular formula, $C_{28}H_{46}O$, of the identified compound campest-4-en-3-one and its chemical structure was shown in Figure 4.

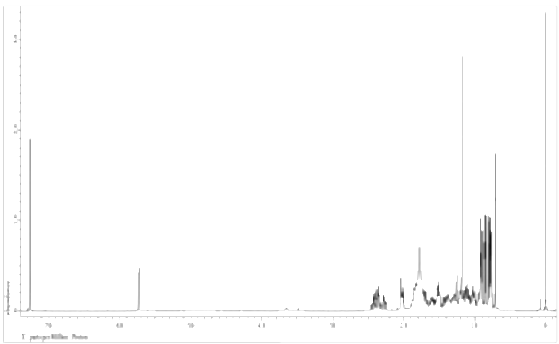

Figure 1. ^1H NMR spectrum of isolated compound A (500 MHz, $CDCl_3$)

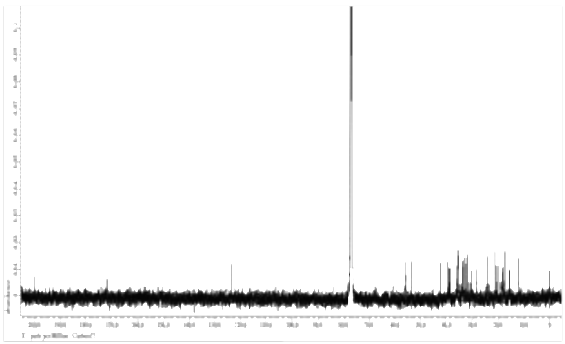

Figure 2. ^{13}C NMR spectrum of isolated compound A (125 MHz,$CDCl_3$)

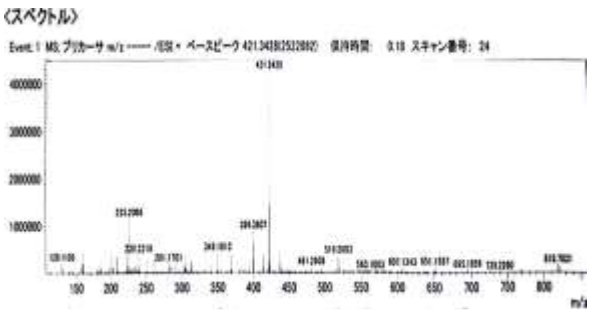

Figure 3. HRESIMS spectrum of compound A

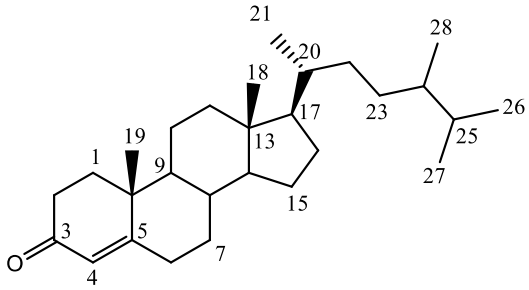

Figure 4. Chemical structure of campest-4-en-3-one ($C_{28}H_{46}O$)

Compound **B** (15 mg, 0.005 % yield) was obtained as colourless needles. Its molecular formula was deduced as $C_{29}H_{46}O$ from the NMR data and positive-ion HRESIMS (Figure 7) data (*m/z* 433.3425 $[M+Na]^+$), which was 12 amu higher than that of compound **A**. The ^1H and ^{13}C NMR spectroscopic data in $CDCl_3$ (Table 1 and Figures 5-6) of compound **B** showed a close structural resemblance to compound **A**, the presence of an additional olefinic group [δ_H 5.00 (dd, J = 15.5, 9.2 Hz, H-23) and 5.12 (dd, J = 15.5, 9.2 Hz, H-22); δ_C 129.5 (C-23) and 138.2 (C-22)], instead of two methylene group in compound **A**. Furthermore, the presence of one extra methylene carbon signal for C-28 (δ_C 25.5) in compound **B**, as compared to those of compound **A** (δ_C 15.5) was observed. From the above spectral data of (Figure 7) compound **B** was identified as stigmasta-4,22-dien-3-one (Rosandy *et al*., 2017) and its chemical structure was shown in Figure 8.

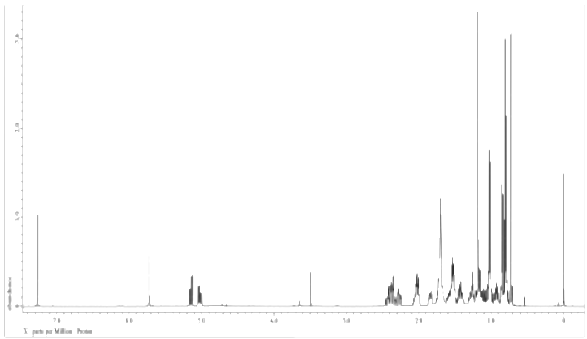

Figure 5. ^1H NMR spectrum of isolated compound B (500 MHz, $CDCl_3$)

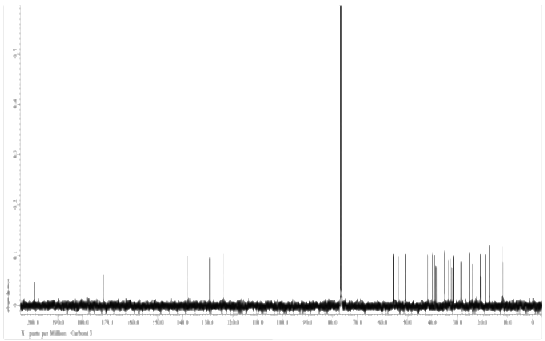

Figure 6. ^{13}C NMR spectrum of isolated compound B (125 MHz,$CDCl_3$)

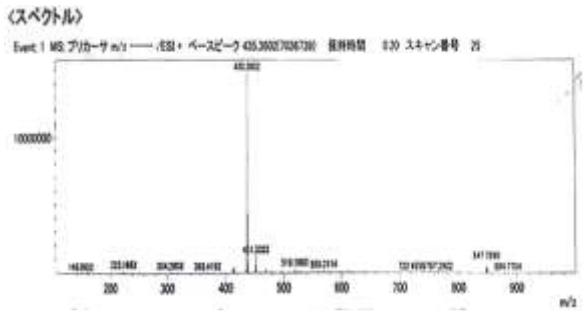

Figure 7. HRESIMS spectrum of isolated compound B (MeOH)

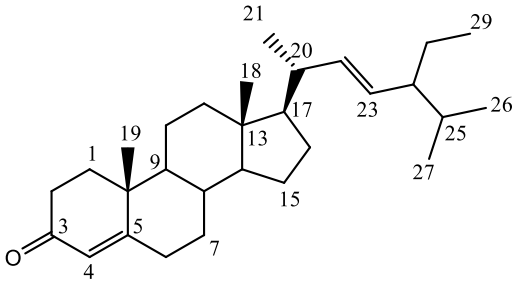

Figure 8. Chemical structure of stigmasta-4,22-dien-3-one ($C_{29}H_{46}O$)

Compound **C** (8 mg, 0.0002% yield) was obtained as colourless needles. The HRESIMS of compound **C** (Figure 11) showed a quasi-molecular ion peak at *m/z* 435.3602 $[M+Na]^+$ corresponding to the molecular formula $C_{29}H_{48}O$. The ^1H and ^{13}C NMR spectra of compound **C** (Figures 9 and 10) displayed the similar signals to compound **A**, except the presence of one more ethyl group at δ_C 23.1 (C-28) and 11.9 (C-29) in compound **C**, instead of methyl group at δ_C 15.5 (C-28) in compound **A**. The ^1H and ^{13}C NMR spectroscopic data of compound **C** (Table 1) were essentially identical to those of stigmast-4-en-3-one (Hoa *et al*., 2014) and it chemical structure of compound **C** was shown in Figures 11-12.

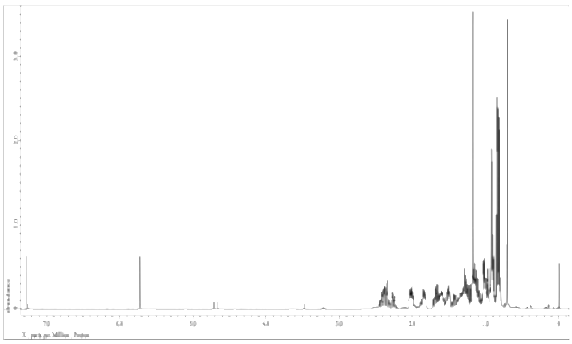

Figure 9. ^1H NMR spectrum of isolated compound C (500 MHz, $CDCl_3$)

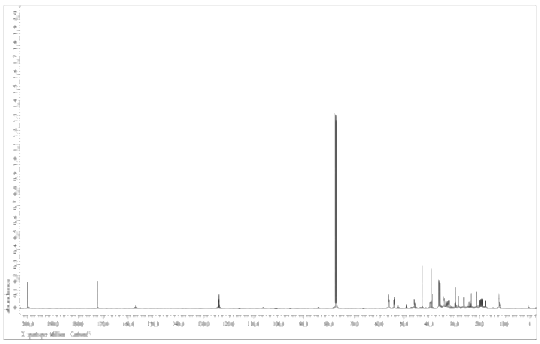

Figure 10. ^{13}C NMR spectrum of isolated compound C (125 MHz,$CDCl_3$)

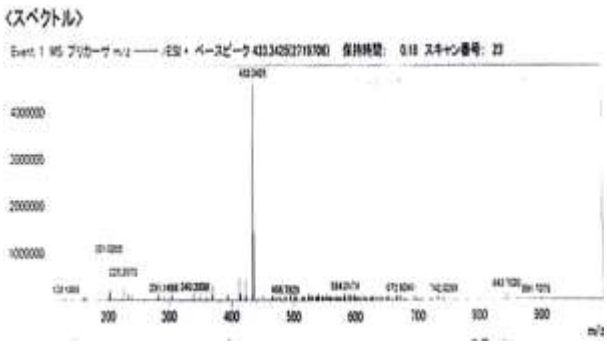

Figure 11. HRESIMS spectrum of isolated compound C (MeOH)

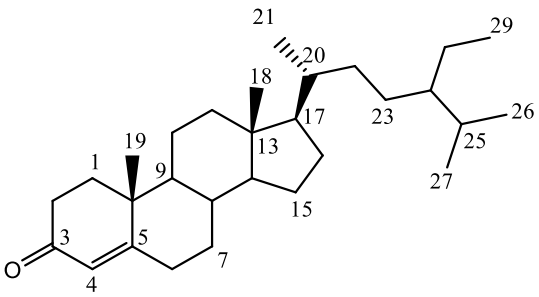

Figure 12. Chemical structure of stigmast-4- en-3-one ($C_{29}H_{48}O$)

Table 1. The ^{1}H and ^{13}C NMR Spectral Data of Compounds A to C (500 & 125 MHz, $CDCl_3$)

Position	A		B		C	
	^{1}H (mult, *J* in Hz)	^{13}C	^{1}H (mult, *J* in Hz)	^{13}C	^{1}H (mult, *J* in Hz)	^{13}C
1		35.7		35.7		35.7
2		39.7		34.0		34.0
3		199.9		199.9		199.9
4	5.71 (br.s)	123.8	5.71 (br.s)	123.8	5.71 (s)	123.8
5		172.0		171.9		172.1
6		32.5		33.0		34.0
7		32.1		32.1		33.0
8		35.9		35.7		35.7
9		53.9		55.9		53.9
10		38.7		38.7		38.7
11		21.1		21.1		21.1
12		38.9		39.6		39.8
13		42.5		42.3		42.5
14		55.9		53.9		55.9
15		20.3		24.3		24.3
16		24.0		28.9		28.3
17		56.1		56.1		56.1
18	0.69 (3H, s)	12.0	0.71 (3H, s)	12.2	0.69 (3H, s)	12.1
19	1.18 (3H, s)	17.5	1.17 (3H,s)	17.4	1.16 (3H, s)	17.5
20		34.0		40.5		36.2
21	0.90 (3H, dd, 4.0, 6.9)	18.7	1.00 (3H, d, 6.3)	21.2	0.91 (3H, d, 6.9)	18.7
22		33.7	5.12 (dd, 15.5, 9.2)	138.2		32.0
23		30.3	5.00 (dd, 15.5, 9.2)	129.5		26.1
24		33.0		51.3		45.9
25		28.2		31.9		29.2
26	0.84 (3H, dd, 1.7, 6.9)	18.3	0.84 (3H, d, 6.3)	19.1	0.82 (3H, d, 6.9)	19.9
27	0.79 (3H, d, 6.9)	15.4	0.80 (3H, d, 6.3)	21.1	0.80 (3H, d, 6.9)	19.1
28	0.75 (3H, dd, 1.7, 6.9)	15.5		25.5		23.1
29			0.79 (3H, d, 6.9)	12.3	0.84 (3H, d, 7.4)	11.9

Compound **D** (1 g, 0.0312% yield) was obtained as a white amorphous powder. Its HRESIMS showed the molecular ion peak at *m/z* 501.3915 $[M-H]^-$ corresponding to the molecular formula of $C_{32}H_{54}O_4$. The FT IR spectrum (Figure 13) showed the strong vibration bands at 3685 and 1288 cm^{-1} due to the stretching and bending vibrations showing a phenolic OH group. The stretching vibration of =CH and C=C groups appeared at 3032 and 1620 cm^{-1}. The stretching vibration of carbonyl peak appeared at 1712 cm^{-1}. The ^{1}H NMR spectrum of compound **D** in $CDCl_3$ (Figure 14 and Table 2) revealed the five aromatic proton signals at δ_H 6.29 (d, *J* = 15.9 Hz, H-2), 6.91 (d, *J* = 7.9 Hz, H-8), 7.03 (d, *J* = 7.9 Hz, H-5), 7.08 (d, *J* = 7.9 Hz, H-9), and 7.61 (d, *J* = 15.9, H-3), one oxygenated methylene signals at δ_H 4.18 (t, *J* = 6.7 Hz, H_2-1'), one methoxy group at δ_H 3.93 (s, H_3-10), twenty methylene group in the range between

δ_H 1.21- 1.71 (40 H, m, H-2' to H-21') and one methyl group at δ_H 0.88 (t, J = 7.3 Hz, H_3-22'). The ^{13}C NMR spectrum (Figure 15) exhibited a ester carbonyl carbon signal at δ_C 167.5 (C-1), three aromatic quaternary carbon signals at δ_C 127.1 (C-4), 146.8 (C-6) and 147.9 (C-7), five aromatic methine carbon signals at δ_C 109.4 (C-5), 115.8 (C-2), 144.7 (C-3), 114.8 (C-8), and 123.1 (C-9), one oxygenated methylene carbon at δ_C 64.7 (C-1'), one methoxy carbon at δ_C 56.0 (C-10), twenty methylene carbon in the range between δ_C 22.8 - 32.0 (C-2' to C-22') and one methyl carbon at δ_C 14.2 (C-22'). These NMR data of **D** were found to be similar those of docosyl ferulate which was isolated by Thongthoom *et al*. (2010) and it chemical structure was shown in Figure 16.

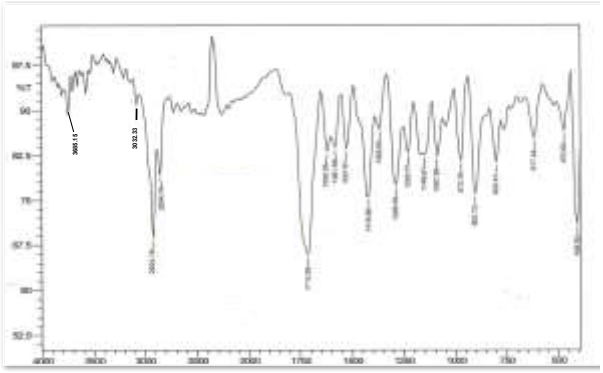

Figure 13. FT IR spectrum of isolated compound D (KBr)

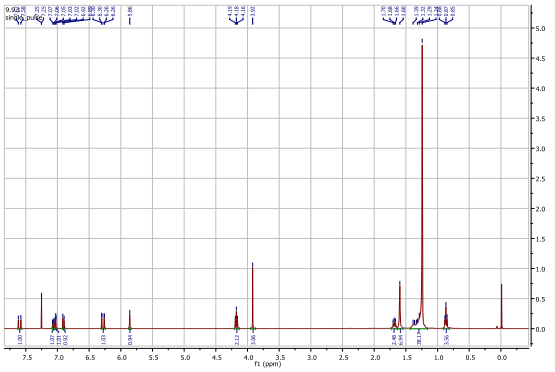

Figure 14. ^1H NMR spectrum of isolated compound D (500 MHz, $CDCl_3$)

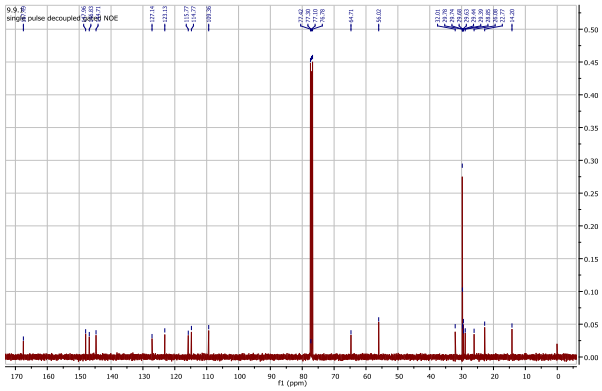

Figure 15. ^{13}C NMR spectrum of isolated compound D (125 MHz, $CDCl_3$)

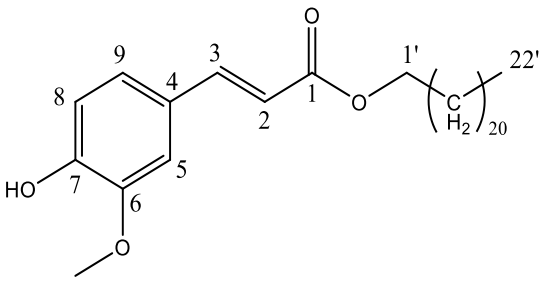

Figure 16. Chemical structure of docosyl ferulate ($C_{32}H_{54}O_4$)

Table 2. The ^{1}H and ^{13}C NMR Spectral Data of Compound D (500 & 125 MHz, $CDCl_3$)

Position	^{1}H (mult, *J* in Hz)	^{13}C
1	-	167.5
2	6.29 (d, *J* = 15.9)	115.8
3	7.61 (d, *J* = 15.9)	144.7
4	-	127.1
5	7. 03 (d, *J* = 7.9)	109.4
6	-	146.8
7	-	147.9
8	6.91 (d, *J* = 7.9)	114.8
9	7. 08 (d, *J* = 7.9)	123.1
10	3.93 (s)	56.0
1′	4.18 (t, *J* = 6.7)	64.7
2′ - 21′	1.21-1.71 (m)	22.8-32.0
22′	0.88 (t, *J* = 6.7)	14.2
OH	5.87 (s)	

Antiproliferative Activity of Crude Extracts and Isolated Compounds

The antiproliferative activity of crude extracts and all isolated compounds (**A-D)** were tested against human cervical cancer cell line HeLa, human lung cancer cell line A549, human breast cancer cell line MCF-7, human gastric cancer cell line GSU, and normal human fibroblast cell line WI at the concentrations of 1, 10, and 100 μg/mL, using MTT assay. All of the tested samples exhibited antiproliferative activity against the four cancer cell lines. $CHCl_3$ and MeOH extracts exhibited moderate activity against all tested cell lines with the IC_{50} values in the range of 19.3 to 93.7 μg/mL. On the other hand, isolated compounds **A** to **C** showed the most potent activity against GSU cancer cell line with IC_{50} values 14.4, 15.5, and 13.9 μg/mL. This is the first time that the antiproliferative activity of the crude extract of Padein-gno rhizomes and its constituents were screened. IC_{50} values of all the tested samples are shown in Figure 17. Furthermore, these results suggest that the combination of sterol moiety was crucial for increasing the cytotoxic activities against all tested cancer cell lines.

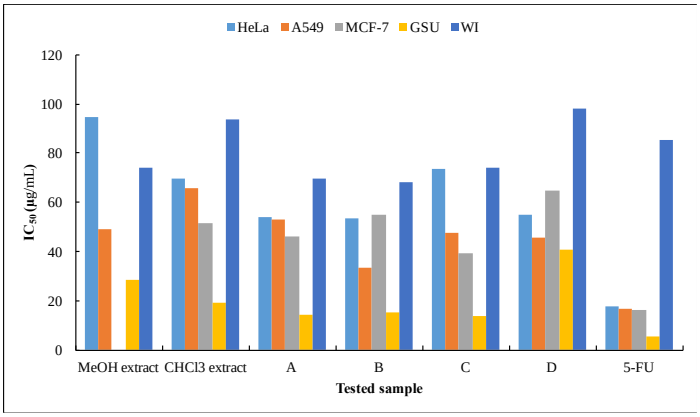

Figure 17. A bar graph of IC_{50} values of crude extract and isolated compounds

Antibacterial activity of Crude Extracts and Isolated Compounds

All tested were also evaluated for their antibacterial activities against the gram positive bacteria, *B. subtillis* and *S. aureus*, and the gram negative bacteria, *K. pneumonia* and *E. coli.* The MeOH and $CHCl_3$ extracts exhibited moderate antibacterial activities against the two Gram positive bacteria: *B. subtilis, S. aureus* with MIC values ranging between 25–100 μg/mL but did not show the any activity on the Gram-negative bacteria: *E-coli, K. pneumoniae*. Compounds **A**, **B**, and **C** possessed stronger antibacterial activities against *B. subtillis, S. aureus* with MIC values ranging from 3.12 to 25 μg/mL than compound **D**. Notably, the antibacterial activity was investigated for the first time with the crude extracts of Padein-gno rhizomes sample and its constituents.

Table 3. Antibacterial Activities of Crude Extracts and Isolated Compounds from Padein-gno

Samples	Minimum Inhibitory Concentration (MIC) (μg/mL)			
	Bacillus subtillis	***Staphylococcus aureus***	***Klebsiella pneumoniae***	***Escherichia coli***
MeOH extract	50	25	>100	>100
$CHCl_3$ extract	100	25	>100	>100
A	25	12.5	>100	>100
B	6.2	3.1	>100	>100
C	6.2	3.1	>100	>100
D	>100	>100	>100	>100
Ampicillin[a]	3.1	3.1	–	–
Kanamycin[b]	–	–	3.1	6.3

[a] positive control for Gram-positive bacteria. [b] positive control for Gram-negative bacteria.

Conclusion

By the silica gel column chromatographic separation, three steroid compounds and one phenolic compound were isolated from the $CHCl_3$ extract of the Padein-gno rhizomes. The isolated compounds were identified to be campest-4-en-3-one, stigmasta-4,22-dien-3-one, stigmast-4-en-3-one, and docosyl ferulate. The MeOH and $CHCl_3$ extracts of Padein-gno and its constituents were found to possess antiproliferative activity against all the tested cancer cell lines. Similarly, Padein-gno rhizomes could be applicable as broad-spectrum antibacterial agents especially on Gram positive bacteria. Thus, the present biological investigation contributed to the medicinal importance of the plant, Padein-gno, especially with respect to its potential use in treating diseases related to bacterial infection, and cancer.

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