ANTIOXIDANT AND ANTICANCER ACTIVITIES OF CHEMICAL CONSTITUENTS FROM THE RHIZOMES OF *Geodrum recurvum* (Roxb.)

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Abstract

Plants have been used for medicinally since prehistoric period. The rhizomes of *Geodrum recurvum* Roxb. (Orchidaceae) (Myanmar name – Thudar) are used by local people in Lashio Township for cancer treatment, however, it lacks scientific investigation. The purpose of this study is the isolation of bioactive compounds from the rhizomes of *Geodrum recurvum* Roxb. and evaluation of their anticancer and antioxidant activities. The three monophenanthrenes (**1**-**3**) were isolated, during the first investigation of the rhizomes of *Geodrum recurvum* collected in Myanmar by advanced separation techniques. Their structures were determined by ^{1}D and ^{2}D NMR, and FAB-MS spectral data. The anticancer activity of isolated compounds (**1**-**3**) was evaluated in vitro against Hela cells using cell counting kit 8. Compounds (**1**-**3**) showed the potent activity against Hela cancer cells. In addition, compounds (**1**-**3)** exhibited potent antioxidant activity according to DPPH radical-scavenging assay. The present study provides scientific evidence for the use of *Geodrum recurvum* in cancer treatment and as a source of antioxidants for pharmacological preparations by traditional healer.

Keywords: Antioxidant, anticancer, pharmacological, *Geodrum recurvum*, scavenging

Introduction

The phytochemicals with antioxidant and anticancer activities are widely isolated from many plant species. The compounds from the medicinal plants can be further developed into potent drugs against cancers. Recently, many researchers have been interested on bioactive compounds from plants to overcome the burden of chemotherapy related problems. The geneus Geodorum (family Orchidaceae) comprising about ten species is known to distribute in tropical Asia including Myanmar, as far North as South Japan, to Australia and the South-West Pacific Islands (Chen *et al*., 2009). The rhizome of this plant is used in Myanmar traditional medicine for tonics and tumor treatment. As a part of the investigations of the secondary metabolites from medicinal plants used in Myanmar, the constituents of *Geodrum recurvum* rhizomes were studied for the first time. In this paper, the isolation and characterization of three monophenanthrene compounds from the rhizome of *Geodrum recurvum* were described. The antioxidant and anticancer activities of chemically isolated compound were evaluated.

Materials and Methods

Plant Materials

The tubers of *Geodrum recurvum* Roxb. (Myanmar name – Thudar) were collected from Lashio, Eastern Shan State of Myanmar in September, 2015. The plant was identified by Dr Kazumi Fujikawa, Botanist from Makino Botanical Garden. A voucher sample has been deposited in the laboratory of Department of Chemistry, University of Mandalay, Myanmar.

Extraction and Isolation

The tubers of *Geodrum recurvum* (dried 800 g) were extracted with methanol. The methanolic extract was concentrated in vacuo, and dried extract was obtained (30.5 g). The extract was then partitioned with ethyl acetate and 1-butanol against water successively to give ethyl

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acetate (15.3 g), 1-butanol (2.7 g) and water-soluble fractions (12.1 g). The ethyl acetate fraction was partitioned again with acetonitrile and hexane to afford acetonitrile 13.4 g and hexane (1.5 g). The acetonitrile extract (2.67 g) was fractionated by using a silica gel column with mixed solvents of hexane and EtOAc (90:10 to 100 % EtOAc) to give 23 main fractions (A-W). Compound (**1**) (8.2 mg) and compound (**2**) (3 mg) were obtained by purification of fraction L (338.6 mg) using HPLC with octadecylsilanized silica gel (ODS, HG-5) column with methanol–water (1:1) as a mobile phase. Compound (**3**) (2.5 mg) were yielded by purification of sub-fraction K (177.g mg) using HPLC with octadecylsilanized silica gel (ODS, HG-5) column with methanol–water (55:45).

Anticancer Assay

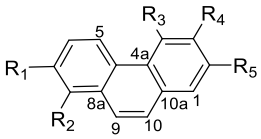
Anticancer effects were measured in vitro in a HeLa (cervix adenocarcinoma) cell line by the colorimetric method using a cell counting kit-8 that was based on the tetrazolium salt/formazan system (Ishiyama *et al*., 1993). HeLa cell (JCRB9004) was obtained from Japanese Collection of Research Bioresources (JCRB) cell bank. Cells were cultured in minimum essential media (MEM) supplemented with 10% fetal bovine serum. For the cytotoxic assay, cells were seeded at a density of 5×10^3 cells/well in 0.2 mL of medium in 96-multiwell plates and adhered. Samples were solved in saline containing 10% DMSO and sterilized by filtration. Series of diluted samples (0.2 mL) were then added to the cells. The plate was incubated at 37 °C under 5% CO_2 atmosphere for 48 h. Twenty microliters of cell counting kit-8 (based on the tetrazolium salt/formazan system) were added to each well, and the microplate was incubated for 1 h, after which cell densities were measured at 450 nm using Bio-RAD Model 550 Microplate Reader. Cisplatin was used as the cytotoxic reference compound.

Antioxidant Activity

The antioxidant activities of the isolated compounds were determined by DPPH scavenging activity assay (Yamaguchi *et al*., 1998). Its reaction principle was based on mechanism of free radicals inhibition by hydrogen transfer, the antioxidant activity of sample expressed in EC_{50}. A total of 500 μL of test solutions in various concentrations (1-100 μM), 500 μL of 0.2 M acetate buffer pH 5.5, and 1000 μL of ethanol are mixed in a test tube for water soluble compounds. For the ethanol soluble compounds, 1000 μL of test solutions in various concentrations (1-100 μM) and 1000 μL of 0.1 M acetate buffer pH 5.5 are mixed in a test tube. 500 μL of $5x10^{-4}$ M DPPH solution was added to the mixture. The mixture was homogenized using a vortex in a dark room (resistant to UV light) and was incubated for 30 minutes. After that, the mixture was measured by a spectrophotometer UV absorbance at this λ_{max} 517 nm. Vitamin C was used as a reference compound in the same concentration range as the test compounds. A control solution was prepared in the same manner as the assay mixture. The capability of scavenging DPPH radicals as a percentage of DPPH remaining in the resulting solution was determined using the following equation: $DPPH\ (\%) = (Abs_{EtOH} - Abs_{sample})/(Abs_{EtOH})$

Results and Discussion

The column chromatography over silica gel and HPLC separation of the MeOH extract of the rhizome of *Geodrum recurvum* afforded three compounds (**1**-**3**). The structures of them are elucidated by ^{1}D, ^{2}D NMR, FAB-MS spectral data and confirmed with previously reported data.



	R_1	R_2	R_3	R_4	R_5
1	OH	H	OMe	OH	OMe
2	OH	OMe	OMe	OH	OMe
3	OH	H	OMe	H	OH

Figure 1 Structures of compounds (**1-3**)

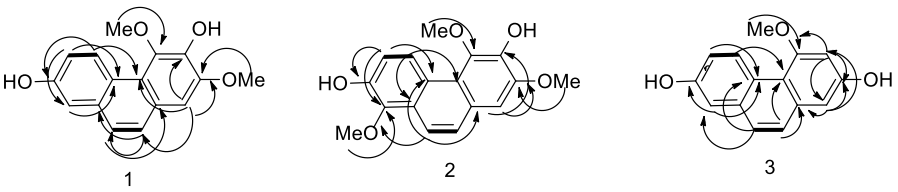


Figure 2 Key COSY (⟝) and HMBC (H↷C) correlations of compounds (**1-3**)

The first compound **1** was obtained as pale brown powder. FAB-MS at m/z 271 $[M+H]^+$, the molecular formula was established as $C_{16}H_{14}O_4$. The ^{1}H NMR and ^{13}C NMR had resonances for six aromatic CH groups [™$_H$ 7.16 (1H, *s*, H-1), ™$_C$ 106.2 (C-1); δ_H 9.33 (1H, *d*, H-5), δ_C 129.2 (C-5); δ_H 7.13 (1H, *dd*, H-6), δ_C 117.4 (C-6); δ_H 7.17 (1H, *d*, H-8), δ_C 112.3 (C-8); δ_H 7.42 (1H, *d*, H-9), δ_C 125.6 (C-9); δ_H 7.56 (1H, *d*, H-10), δ_C 128.2 (C-10)], two hydroxyl protons (3-OH and 7-OH), two methoxyl groups [δ_H 56.5 (3H, *s*, 2-OMe), δ_C 59.8 (3H, *s*, 4-OMe)] and eight tetra-substituted aromatic carbons which constitute a tricyclic system. In this case, the observed COSY, HMBC correlations (Figure 2) were again very valuable in the structure elucidation. Compound **1** was identified as **3,7**-dihydroxy-2,4-dimethoxyphenanthrene and nicely matched with previously reported data (Yuan *et al*., 1997).

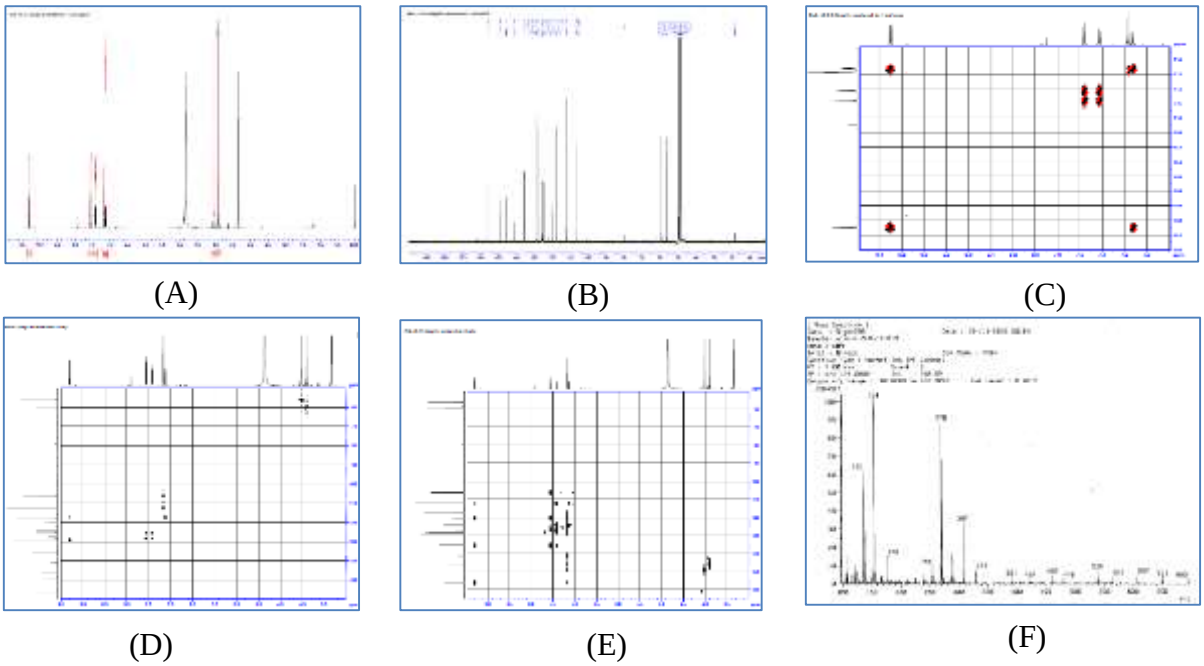


Figure 3 (A) ^{1}H NMR (600 MHz, $CDCl_3$) (B) ^{13}C NMR (150 MHz, $CDCl_3$)
(C) COSY (D) HSQC (E) HMBC (F) FAB-MS spectra of compound **1**

Compound **2** was obtained as pale brown powder. FAB-MS at m/z 301 $[M+H]^+$, the molecular formula was established as $C_{17}H_{16}O_5$. The ^1H NMR spectroscopic data of compound **2** were similar to those of **1** except methoxyl singlet, δ_H 4.01 (3H, *s*, 8-OMe) at C-8 (δ_C 59.8) (Figure 2). Compound **2** was identified as 3,7-dihydroxy-2,4,8-trimethoxyphenanthrene and well matched with previously published data (Sylvie and Roland, 2007).

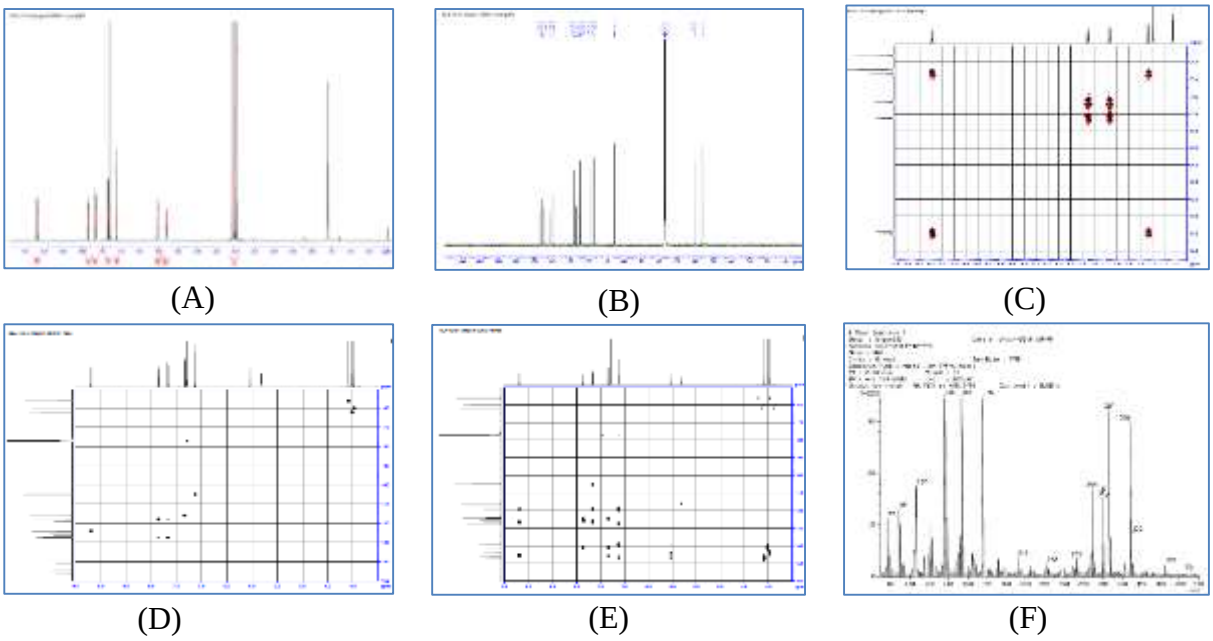


Figure 4 (A) ^1H NMR (600 MHz, $CDCl_3$) (B) ^{13}C NMR (150 MHz, $CDCl_3$)
(C) COSY (D) HSQC (E) HMBC (F) FAB-MS spectra of compound **2**

Compound **3** was obtained as pale brown powder. FAB-MS at m/z 241 $[M+H]^+$, the molecular formula was established as $C_{15}H_{12}O_3$. The ^1H NMR spectroscopic data of compound **3** were also similar to those of compound **1** except the lack of hydroxyl group at C-3 in compound **3** and methoxyl group at C-2 of compound **1** is replaced by a hydroxyl group (Figure 2). Compound **3** was identified as 2,7-dihydroxy-4-methoxyphenanthrene (Yuan *et al*., 1997).

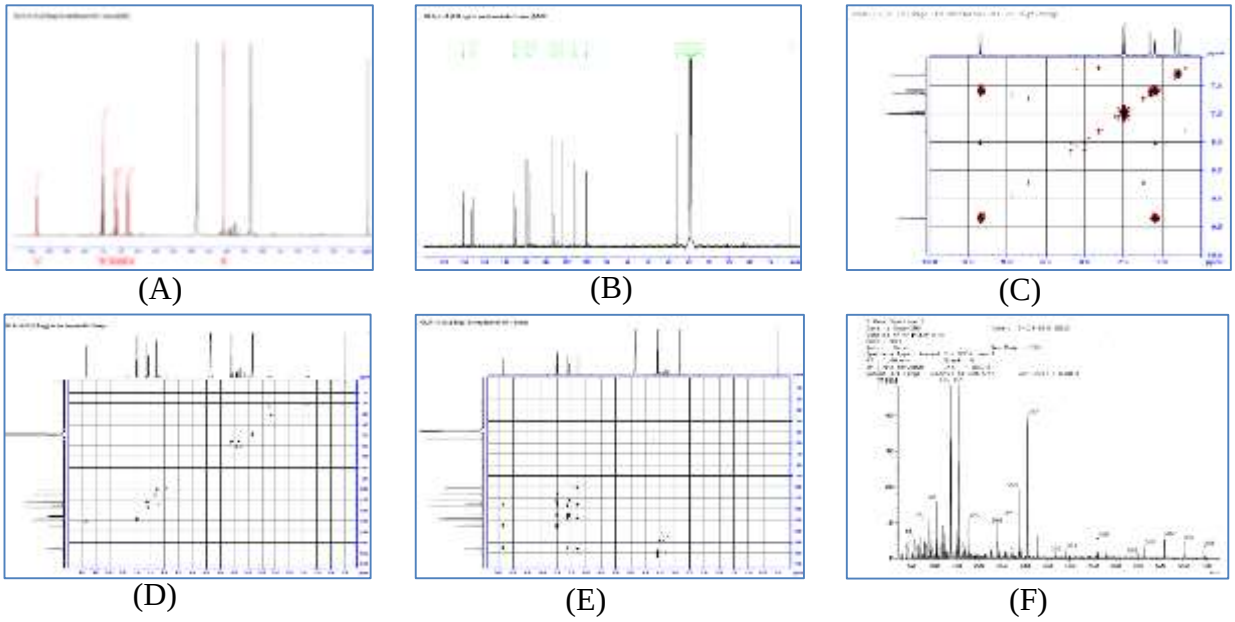


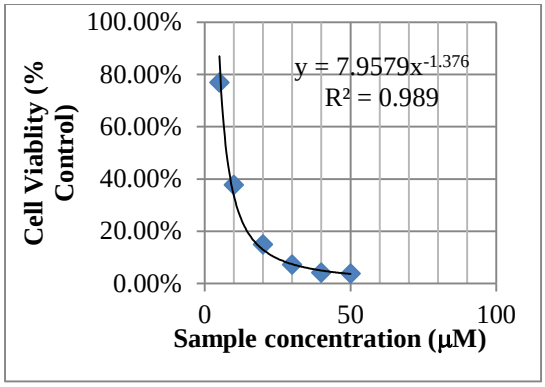
Figure 5 (A) ^1H NMR (600 MHz, $CDCl_3$) (B) ^{13}C NMR (150 MHz, $CDCl_3$)
(C) COSY (D) HSQC (E) HMBC (F) FAB-MS spectra of compound **3**

Table 1 ^{1}H NMR (δH; 600 MHz, $CDCl_3$) and ^{13}C NMR (δC; 150 MHz, $CDCl_3$) Signals of Compounds (1-3)

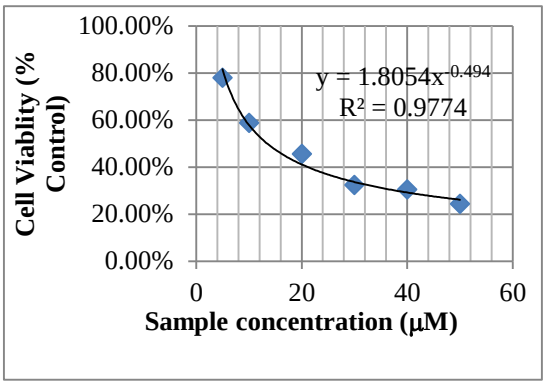
Position	3,7-dihydroxy-2,4-dimethoxy phenanthrene (1)		3,7-dihydroxy-2,4,8-trimethoxy phenanthrene (2)		2,7-dihydroxy-4-methoxy phenanthrene (3)	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1	7.16, s	106.2	7.10, s	105.0	6.83, d (*J* = 2.34 Hz)	105.8
2		148.9		146.8		156.3
3		141.2		139.3	6.77, d (*J* = 2.3 Hz)	100.4
4		145.8		144.0		160.7
4a		120.3		119.2		116.1
4b		124.2		124.2		125.5
5	9.33, d (*J* = 9.1 Hz)	129.2	9.18, d (J = 9.3 Hz)	123.9	9.34, d (*J* = 9.3 Hz)	130.2
6	7.13, dd (*J* = 2.8 and 9.1 Hz)	117.4	7.33, (J = 9.3Hz)	116.0	7.09, dd (*J* = 2.8 and 9.3 Hz)	117.3
7		156.0		145.6		155.3
8	7.17, d (*J* = 2.8 Hz)	112.3		140.8	7.15, d (*J* = 2.8 Hz)	112.3
8a		135.4		126.6		134.8
9	7.42 d (*J* = 8.8 Hz)	125.6	7.85, d (J = 9 Hz)	117.9	7.5, d (*J* = 8.8 Hz)	128.5
10	7.56, d (*J* = 8.8 Hz)	128.2	7.66, d (J = 9 Hz)	127.5	7.48, d (*J* = 8.8 Hz)	127.9
10a		126.9		125.7	6.83, d (*J* = 2.34 Hz)	135.9
OMe at C2	4.01, s	56.5	4.08, s	56.1		
OMe at C-4	3.94, s	59.8	3.98, s	61.9	4.1, s	55.9
OMe at C-8			4.01, s	59.8		

Anticancer Activity

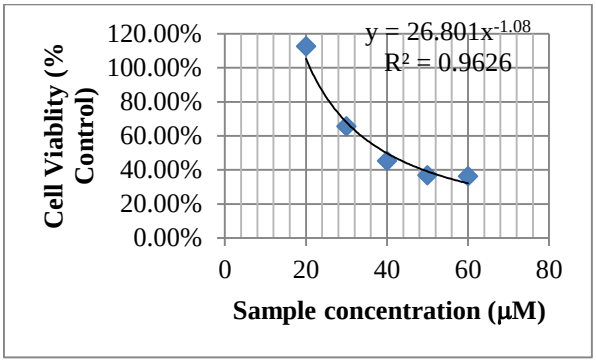
The isolated compounds (**1-3**) were tested for their anticancer activities on Hela cell lines using cell counting kit 8. The cell growth-inhibitory potencies of compounds (**1-3**), expressed as IC_{50} values, are shown in Table **2**. Among the tested compounds, compound **1** showed the very potent activity against Hela cancer cell (IC_{50} **7.5** μM respectively). In addition, compound **2** (IC_{50} **13.5** μM) and compounds **3** (IC_{50} 40μM) possessed high inhibitory activity on the proliferation of tested cancer cell lines.



Compound **1** (IC_{50} = 7.5 μM)



Compound **2** (IC_{50} = 13.5 μM)



Compound **3** (IC_{50} = 40 μM)

Figure 6 Anticancer activities of compounds (**1-3**) against Hela cell line

Table 2 Anticancer Activities of the Isolated Compounds (**1-3**) on Hela Cells.

Compound	IC_{50} (μM)
1	7.5
2	13.5
3	40

Antioxidant Activity

DPPH assay is used to determine free radical scavenging activity of isolated compounds (**1-3**) by hydrogen transfer mechanism. DPPH scavenging reaction was marked by changes in the solution color from purple to yellow after 30 minutes incubation. Measurements were performed at a maximum wavelength of 517 nm. It is observed that DPPH radical scavenging activities of compound **1** (EC_{50} 26.8 μM) exhibited stronger activity than ascrobic acid (EC_{50} 27.5 μM). In addition, compounds (**2-3)** showed high DPPH radical scavenging activities (EC_{50} 32.1 and 31.2 μM).

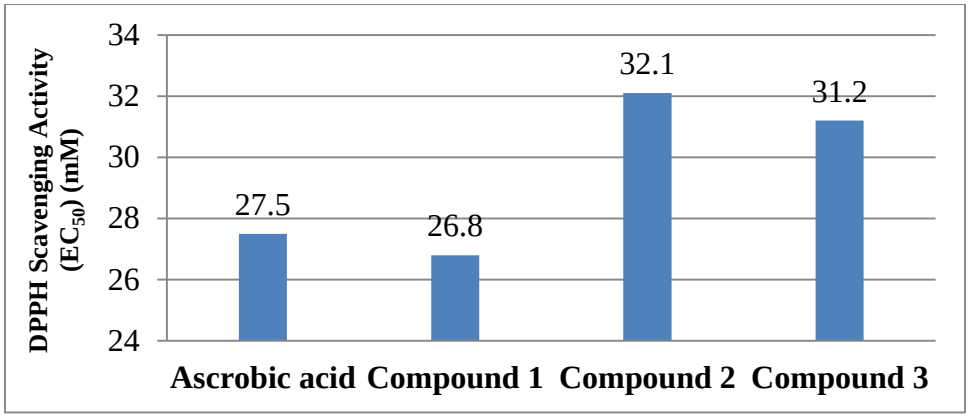


Figure 7 DPPH radical scavenging activity of ascrobic acid (positive control) and isolated compounds (**1-3**)

The isolated compounds (**1-3**) from the rhizomes of *Geodrum recurvum* had an ability to scavenge the free radicals by transferring proton to free radical. The antioxidant capacity of compounds **(1-3)** can be classified as good and potential antioxidant agents. The IC_{50} of pure compounds (**1-3)** on Hela cell lines less than 50 μg/mL is categorized as potential anticancer agents. Compounds (**1-3)** were found to be high antioxidant and anticancer activity. Among the isolated compounds, compound **1** exhibited both the highest antioxidant and anticancer activity in compare to compound **2** and **3**. The anticancer activity can be related to the antioxidant activity. Compounds (**1-3)** have been revealed as a scavenging radical that may be able to inhibit carcinogenesis.

Conclusion

In this study, three monophenanthrene compounds (**1-3**) were isolated from the rhizome of *Geodrum recurvum* for the first time by advanced separation techniques and their structures were elucidated by ^{1}D, ^{2}D NMR and FAB mass spectral data. The present research work suggests that *Geodrum recurvum* possess potent antioxidant and anticancer compounds and these compounds might be applicable for the pharmacological preparations as antioxidant and anticancer agents.

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References

Chen, X., J. C. Phillip and G. W. Stephan. (2009) "*Geodorum*. In: Flora of China." *Orchidaceae*, vol.25, pp. 258-260.

Ishiyama, M., M. Shiga, K. Sasamoto, M. Mizogucgi and P. He. (1993). "A new sulfonated tetrazolium salt that produces a highly water-soluble formazan dye." *Chem. Pharm. Bull*., vol.41, pp.1118–1122.

Sylvie, R. and B. Roland. (2007) "Total synthesis of two natural phenanthrenes: confusarin and a regioisomer." *Tetrahedron*, vol.63, pp.12379-12387.

Yuan, W. L., C. K. Chiang., J. H. Leslie and D. P. Andrew. (1997). "Phenanthrenes, dihydrophenanthrenes and bibenzyls from the orchid *Bulbophyllum vadinatum*." *Phytochemistry*, vol.44, pp.157-165.

Yamaguchi T, H. Takamura, T. Matoba and J. Terao. (1998). "HPLC method for evaluation of the free radical-scavenging activity of foods by using 1, 1diphenyl-2-picrylhydrazyl." Bioscience, Biotechnology, and Biochemistry, vol.62, pp.1201-1204.