STUDIES ON BIOACTIVITY AND STRUCTURE ELUCIDATION OF ISOLATED BIBENZYL DERIVATIVES FROM *DENDROBIUM PULCHELLUM* ROOT EXTRACTS

Pann Yone[1], Hnin Yu Win[2]*, Thida Win[3]

Abstract

The objective of the present study is to investigate bioactive metabolites from *Dendrobium pulchellum* roots. Bibenzyl derivatives extracted by acetate ethyl namely, 4-(4-hydroxy-3-methoxyphenethyl)-2, 6-dimethoxyphenol (1) and 4-(3,4-dimethoxy-phenethyl)-2,6-dimethoxy phenol (2) were isolated by using separation techniques such as thin layer and column chromatography. The structure elucidation the isolated compounds was performed based on NMR and mass. Moreover, the antioxidant activity of crude extract was evaluated by using DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay. Furthermore, the acute toxicity and antimicrobial activities of crude extracts were also examined.

Keywords: *Dendrobium pulchellum*, bibenzyl, NMR, mass, DPPH

Introduction

Plants are used for variety of purpose and are also really important for the earth and for all living things. They provide us with food, fiber, shelter, medicine and fuel. Plant is an important source of medicine and plays a key role in world health. Medicinal herbs or plants have been known to an important potential source of therapeutic or curative aids. Man used medicinal plant or medicinal herbs since ancient times as they believed that medicinal plants can supply us with medical treatment and other effects. Since that time early man valued these medicinal plants. Nowadays, two-third of the people living in rural areas depends on medicinal herbs as primary health care. The term of medicinal plants involve a various types of plants used in herbalism and these contain a rich resource of active ingredients. Moreover, these plants play an important role in the development of human cultures around the whole world (Hassam, 2012). Medicinal plant consists of a wide range of secondary metabolites or compounds such as tannins, terpenoids, alkaloids, flavonoids that shows the curative effect of the plants most especially the antimicrobial activities (Oladej, 2016).

In Myanmar, there are many traditional plants which have been reputed for their various kinds of activities and usefulness in pharmacology. Therefore, the study of traditional plant and their usage in therapy play a very important role. In the present work, medicinal orchid *Dendrobium pulchellum* was selected for isolation of pure bioactive compounds. *Dendrobium pulchellum* is belonged to the family Orchidaceae and it is locally known as Kyaung-myet-lone (or) Sin-ma-myet-kwin (Fgure1). *Dendrobium* genus are used for therapeutic activities such as anticancer, hypoglycemic, antimicrobial, antidiabetic, anti-inflammatory, antiherpetic, antimalarial, antioxidant, immunomodulatory, hepatoprotective and neuroprotective activities (Singh *et al*., 2012). *Dendrobium pulchellum* was found to inhibit the lung cancer cell motility and invasion through suppression of endogenous reactive oxygen species (Kowitdamrong *et al*., 2013).

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Figure 1 Roots, plants and flowers of Dendrobium pulchellum Roxb.ex Lindl.

Materials and Methods

General Experimental Procedure

^{1}H NMR spectra: Varian Unity 300 (300.542 MHz), Bruker AMX 300 (300.542 MHz), Varian Inova 500 (499.8 MHz). ^{3}C NMR spectra: Varian Unity 300 (75.5 MHz), Varian Inova 500 (125.7 MHz). Chemical shifts were measured relatively to tetramethylsilane as internal standard. 2D NMR spectra: H, H COSY spectra (^{1}H,^{1}H-Correlated Spectroscopy), HMBC spectra (Heteronuclear Multiple Bond Connectivity) and HMQC spectra (Heteronuclear Multiple Quantum Coherence). Thin layer chromatography (TLC): DC-Folien Polygram SIL G/UV$_{254}$ (Macherey-Nagel & Co.). Column chromatography (CC): MN silica gel 60: 0.05-0.2 mm, 70-270 mesh (Macherey-Nagel & Co). Sephadex LH-20 (Pharmacia) was used for size exclusion chromatography. Commercial grade reagents and solvents were purchased from Super Shell Co. Ltd, Yangon. Common laboratory apparatus were used. PerkinElmer C93927 was used for FT IR spectra measurement. The antimicrobial activities of plant extracts were measured in Pharmaceutical and Food Research Department (PFRD), Insein, Yangon.

Plant Material

The roots of *Dendrobium pulchellum* were collected from Mawlu Township, Sagaing Region, Myanmar. The root materials were cut into small pieces and dried at room temperature for about one month.

Preliminary Phytochemical Analysis

The various solvent extracts of root sample were prepared to analyze the presence of certain phytochemicals such as alkaloids, flavonoids, phenolic compounds, polyphenols, saponins, steroids, tannins, terpenes, glycoside, lipophilic and reducing sugar by the standard method of Harborne (Harborne, 1998).

Biological Activities of Various Crude Extracts

Antimicrobial tests were performed at Pharmaceutical and Food Research Department (PFRD), Insein Township, Yangon Region. Antimicrobial activities of crude extracts were tested by agar-well diffusion method on six test microorganisms such as *Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Bacilius pumilus, Candida albicans* and *Escherichia coli.*

Measurement of DPPH Radical Scavenging Activity by Spectrophotometric Method

Antioxidant activity of ethyl acetate extract was determined by DPPH radical scavenging assay. The control solution was prepared by mixing 1.5 mL of 0.002% DPPH solution and 1.5 mL of ethanol. Moreover, the blank solution could be prepared by mixing 1.5 mL of test sample solution and 1.5 mL of ethanol. Furthermore, the test sample solutions were also prepared by gently

mixing 1.5 mL of 0.002 % DPPH solutions and 1.5 mL of test sample solution in various concentrations (0.78125, 1.5625, 3.125, 6.250, 12.5, 25, 50, 100, 200 and 400 mg/mL). Then, the resulting mixture was homogenized by applying vortex mixer. After that, the solutions were allowed to stand for 30 min at room temperature. Then, the absorbance value of each solution was measured at 517 nm by using UV-Vis spectrophotometer. The measured absorbance values were applied to calculate inhibition percentage by the equation:

$$\%\,\text{inhibition} = \frac{\text{Abs}_{\text{DPPH}} - [\text{Abs}_{\text{sample}} - \text{Abs}_{\text{Blank}}]}{\text{Abs}_{\text{DPPH}}} \times 100$$

Where, % inhibition = % inhibition of test sample, Abs_{DPPH} = absorbance of control solution, $\text{Abs}_{\text{sample}}$ = absorbance of test sample solution, $\text{Abs}_{\text{blank}}$ = absorbance of blank solution. The antioxidant power (IC_{50}) is expressed as the test substances concentration (μg/mL) that result in a 50% reduction of initial absorbance of DPPH solution and that allows to determine the concentration. IC_{50} (50% inhibition concentration) values were calculated by linear regressive excel program (Kiattsin *et al*., 2016). Ascorbic acid was used as a reference compound in the same concentration range as the test compound.

Acute Toxicity Test

Study on acute toxicity of ethyl acetate extract of the roots of *Dendrobium pulchellum* was performed at Department of Biotechnology, Mandalay Technological University, Pathein Gyi Township, Mandalay Region, Myanmar. The study was carried out to assess the acute toxicity on oral administration (Gallagpher, 2003).

Sample preparation for acute toxicity

About (100 g) of air dried roots of *Dendrobium pulchellum* were percolated with ethyl acetate (3 L) for two months. The extract was filtered and the filtrate was evaporated to dryness under reduced pressure to attain ethyl acetate extract (2.25 g).

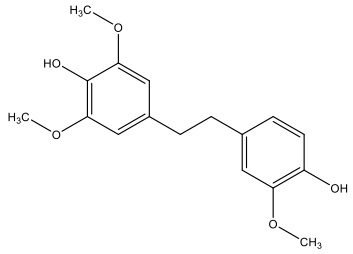
Method

Both sexes of healthy albino ICR (Institute of Cancer Research) strain mice (30 to 35 g) were randomly selected and kept in their cages for at least 5 days prior to the experiment for acclimatization of laboratory conditions. Before the experiment, the animals were kept fasting overnight for 18 h but were allowed with free access to water. Following period of fasting, mice were weighed and dose was calculated according to the body weight. Then, the test substance was dissolved in distilled water for required concentration and administered orally in a single dose by using disposable syringe. One group was served as the control and only distilled water was given orally. Five groups of mice (five mice in each group) were used for each dose level. Each dose of (500 mg/kg, 1000 mg/kg, 1500 mg/kg, 2000 mg/kg and 2500 mg/kg) were administrated orally to five groups of mice (Figure 2). Mice were observed after dosing at least one during the first 30 min, periodically during the first 24 h, with special attention given during the first 4 h and daily up to 10 days. Signs of toxicity and mortality of the mice were recorded. Changes in fur, eyes, mucous membranes, respiratory rate, autonomic central nervous systems and behavioral pattern were observed. At the end of the test (i.e 10 days) the mice were weighed (Litchfield and Wilcoxon, 1949).

Figure 2 Administration of ethyl acetate extract to the experimental mice

Extraction and Isolation of Pure Compounds

The air dried roots sample of *Dendrobium pulchellum* (700 g) were percolated with methanol (10L) for one month. The methanol crude extracts were filtered and evaporated the solvent under reduced pressure. The residue was extracted with ethyl acetate to attain 11.3 g of ethyl acetate crude extracts. The obtained crude extracts were subjected to silica gel by using various solvent systems of n-hexane and ethyl acetate. After purification on Sephadex LH-20 with methanol only, mixture of bibenzyl derivatives 1 and 2 were isolated from selected fraction II (Figure 3) as oily form.



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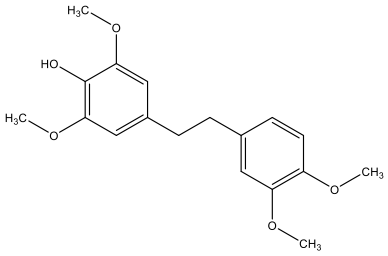

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Figure 3 Compounds isolated from the roots of *Dendrobium pulchellum*

Results and Discussion

Phytochemical Constituents

Preliminary phytochemical analysis was performed in order to know different types of organic compounds present in root of *Dendrobium pulchellum*. Analysis of the extract of root sample revealed the presence of phytochemicals such as alkaloids, flavonoids, phenolic compounds, polyphenols, saponins, steroids, tannins, terpenes, glycoside, carbohydrates, lipophilics and reducing sugars. These phytochemicals are known to exhibit medicinal as well as physiological activities.

Antimicrobial Activities

The antimicrobial activities of various solvent extracts of the roots of *Dendrobium pulchellum* were examined by using agar-well diffusion method as shown in Table 1.

Table 1 Antimicrobial Activities of Various Solvent Extracts on Different Microbial Strains

Sample	Solvent extracts	Inhibition zone (mm)					
		I	II	III	IV	V	VI
Dendrobium pulchellum roots	n-hexane	-	13	13	-	11	11
	EtOAc	15	-	13	14	14	14
	MeOH	14	11	12	12	12	13

Agar-well – 10 mm
10 mm ~ 14 mm (+)
15 mm ~ 19 mm (++)
20 mm above (+++)

I = *Bacillus subtilis*
II = *Staphylococcus aureus*
III = *Psudomonas aeruginosa*
IV = *Bacilius pumilus*
V = *Candida albicans*
VI = *Escherichia coli*

According to antimicrobial assay, n-hexane extract responded low activities on *Staphylococcus aureus, Pseudomonas aeruginosa, Candida albicans* and *Escherichia coli*, except *Bacillus subtilis* and *Bacilius pumilus*. The methanol extract showed low activities on all selected microorganisms. Ethyl acetate extract exhibited medium activities on *Bacillus subtilis* and low activities on *Psudomonas aeruginosa*, *Bacillus subtilis*, *Candida albicans* and *Escherichia coli* except *Staphylococcus aureus*.

Determination of Acute Toxicity

The mice (five per group) administered with 500 mg/kg, 1000 mg/kg, 1500 mg/kg, 2000 mg/kg and 2500 mg/kg doses of ethyl acetate extract of *Dendrobium pulchellum* roots were observed for 10 days (Table 2). At the end of observation period, all the mice were alive, did not show any toxic symptoms such as diarrhea, inactivity, restlessness, aggressiveness, eye-dullness, breathing, abnormalities, etc. and did not exhibit loss and obvious changes of body weight. Hence, the test substance can be considered relatively safe.

Table 2 Acute Toxicity Study of Ethyl Acetate Extract of Dendrobium pulchellum Roots Based on Mortality Record

Dose Sample (mg/kg)	No. of mice	Observation	Day 0	1	2	3	4	5	6	7	8	9	10
2500	5	Alive	5	5	5	5	5	5	5	5	5	5	5
		Dead	0	0	0	0	0	0	0	0	0	0	0
2000	5	Alive	5	5	5	5	5	5	5	5	5	5	5
		Dead	0	0	0	0	0	0	0	0	0	0	0
1500	5	Alive	5	5	5	5	5	5	5	5	5	5	5
		Dead	0	0	0	0	0	0	0	0	0	0	0
1000	5	Alive	5	5	5	5	5	5	5	5	5	5	5
		Dead	0	0	0	0	0	0	0	0	0	0	0
500	5	Alive	5	5	5	5	5	5	5	5	5	5	5
		Dead	0	0	0	0	0	0	0	0	0	0	0
Control (25 % EtOH)	5	Alive	5	5	5	5	5	5	5	5	5	5	5
		Dead	0	0	0	0	0	0	0	0	0	0	0

Determination of Antioxidant Activity

Antioxidant activities of crude extract were expressed as percentage of DPPH radical inhibition or IC_{50} values (μg/mL). The absorbance of standard ascorbic acid and ethyl acetate extract in different concentrations are described in Table 3.

Table 3 Absorbance of Standard Ascorbic Acid and Ethyl Acetate Extract

No.	Concentration (µg/mL)	Ascorbic acid		*Dendrobium pulchellum*	
		Abs_{sample}	Abs_{blank}	Abs_{sample}	Abs_{blank}
1	0.78125	0.200	0.001	0.219	0.001
2	1.5625	0.169	0.001	0.198	0.002
3	3.125	0.158	0.002	0.189	0.003
4	6.250	0.145	0.003	0.165	0.004
5	12.5	0.138	0.003	0.152	0.005
6	25	0.130	0.004	0.148	0.006
7	50	0.125	0.001	0.130	0.007
8	100	0.111	0.001	0.119	0.008
9	200	0.068	0.002	0.078	0.009
10	400	0.059	0.002	0.069	0.010

The inhibition percentage of standard ascorbic acid and ethyl acetate extract in different concentration are described in Table 4 and Figure 4.

Table 4 Percent Inhibition of Standard Ascorbic Acid and Ethyl Acetate Extract

No.	Concentration (µg/mL)	% Inhibition	
		Standard ascorbic acid	*Dendrobium pulchellum*
1	0.78125	48.71	43.81
2	1.562	56.70	49.23
3	3.125	59.79	52.06
4	6.25	63.40	58.51
5	12.5	65.20	62.11
6	25.0	67.53	63.40
7	50.0	68.04	68.30
8	100	71.65	71.39
9	200	82.98	82.22
10	400	85.31	84.79

* Absorbance of DPPH (Control) = 0.388

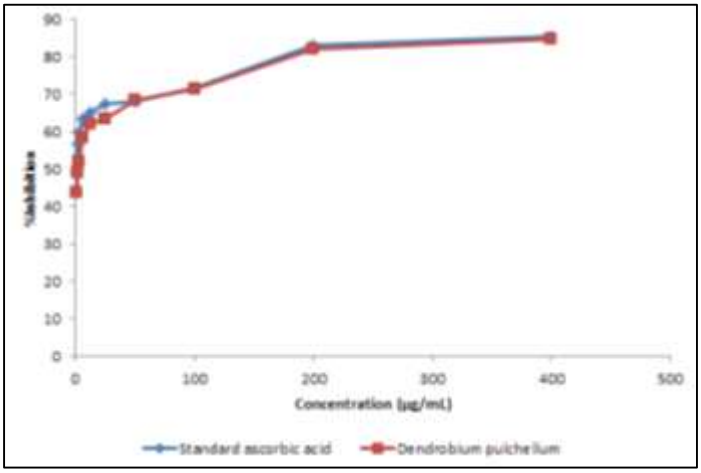
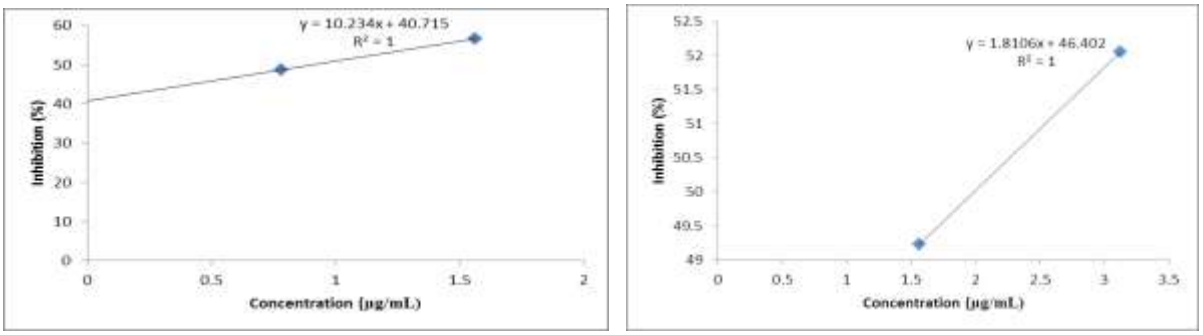


Figure 4 Percent Inhibition in Different Concentration of Standard Ascorbic Acid and Ethyl Acetate Extract of Dendrobium pulchellum



(a) (b)

Figure 5 (a) Linear regression analysis for IC_{50} value of standard ascorbic acid and (b) ethyl acetate extract of Dendrobium pulchellum

Table 5 The Linear Regression Equations and IC_{50} Values

No.	Test Solution	Regression Equations	IC_{50} (µg/mL)
1	Ascorbic acid	y = 10.234x + 40.715	0.9072
2	*D. pulchellum*	y = 1.8106x + 46.402	1.987

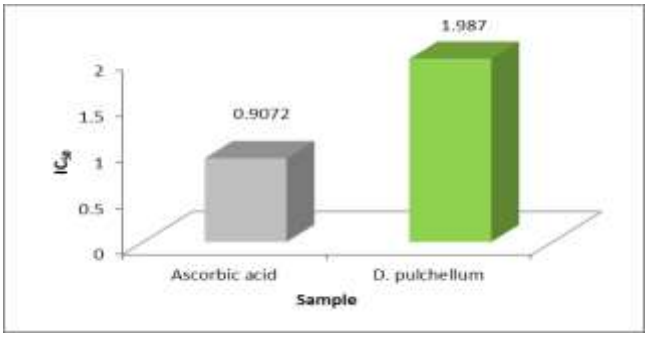


Figure 6 Histogram of IC_{50} values of standard ascorbic acid and Dendrobium pulchellum extract

The IC_{50} value is a parameter used to measure antioxidant activity and it is defined as the sample extract concentration required for 50 % scavenging of DPPH radicals under experiment condition employed. The smaller IC_{50} value corresponds to a higher antioxidant activity. The IC_{50} values was calculated by linear regressive excel program (Table 5, Figure 5). According to the results, the significant antioxidant property with IC_{50} value of 1.987 µg/mL which is comparable to ascorbic acid solution, standard antioxidant. The comparison of IC_{50} values of standard ascorbic acid with crude extracts are shown in Figure 6.

Structure Elucidation

In the aromatic region of ^{1}H NMR spectrum, Figure 8(a), one doublet methine proton at δ 6.83 ppm (δ_C = 114.1 ppm, J = 7.83 Hz) showed ortho coupling with another doublet methine proton at δ 6.67 (δ_C = 121.0 ppm, J = 7.82 Hz). In the DQF-COSY spectrum, Figure 8(d), these two methine protons showed correlation as expected. In the HMBC spectrum, Figure 8(f), doublet methine proton at δ 6.83 showed β-correlation with two sp^2 quaternary carbons at δ 133.5, 146.2 ppm and α-coupling with one sp^2 quaternary carbon at δ 143.7 ppm. Moreover, another methine proton at δ 6.67 ppm showed β-coupling with one sp^2 methine carbon at δ 111.2 ppm and one sp^2 quaternary carbon at δ 143.7 ppm. Furthermore in HMBC spectrum, Figure 8(f), one

methine proton at δ6.60 which is attached to carbon at δ111.2 ppm showed β correlation with one sp^2 quaternary carbon at δ143.7 ppm and one sp^2 methine carbon at δ121.0 ppm and α-coupling with one sp^2 quaternary carbon at δ146.2 ppm. Therefore, fragment (a) could be assigned as shown in Figure 7.

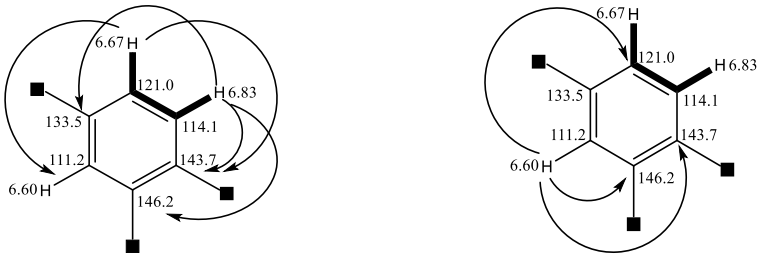
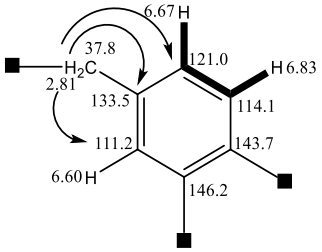
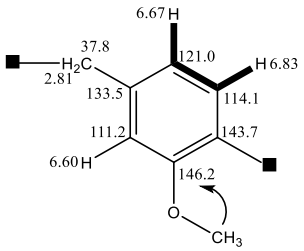


Figure 7 (—) COSY and (→) HMBC correlations in fragment (a)

Moreover, in the HMBC spectrum, Figure 8(f), methylene protons at δ 2.81 which is attached to carbon at δ37.8 ppm showed β-correlation with two sp^2 methine carbons at δ111.2 and 121.0 and α-coupling with δ 133.5 ppm. Similarly, two methine protons at δ 6.67 ppm (δ_C 121.0 ppm) and δ 6.60 ppm (δ_C 111.2 ppm) showed β-coupling with methylene carbon at δ37.8 ppm. Thus, the extended fragment (b) could be assigned.



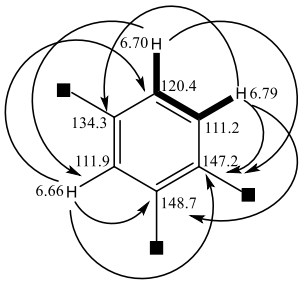
Fragment (b)



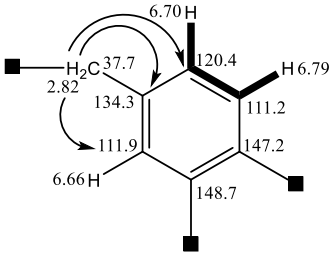
Fragment (c)

Moreover, singlet methoxy proton at δ 3.82 ppm showed HMBC correlation to sp^2 quaternary carbon at δ146.2 ppm and fragment (c) was elucidated.

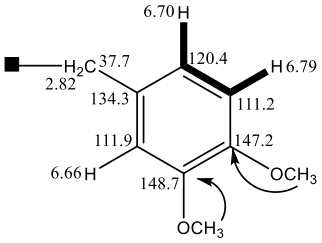
Similarly, in the aromatic region of the ^{1}H NMR spectrum, Figure 8(a), one doublet methine proton δ6.79 ppm (δ_C 111.2 ppm, J = 8.10 Hz) showed ortho coupling with another methine proton at δ 6.70 ppm (δ_C = 120.4 ppm, J = 8.08 Hz). In DQF-COSY spectrum, Figure 8(d), these two methine protons showed correlation as expected. In the HMBC spectrum, Figure 8(f), the methine proton at δ6.79 showed α-correlation with one sp^2 quaternary carbon at δ147.2 and one methine carbon at δ 120.4 ppm and β-correlation with two sp^2 quaternary carbons at δ 134.3 and 148.7 ppm. Moreover, one methine proton at δ6.70 ppm (δ_C 120.4 ppm) showed β-coupling with one sp^2 quaternary carbon at δ147.2 ppm and one sp^2 methine carbon at δ111.9 ppm. Furthermore, in the HMBC spectrum, Figure 8(f), one methine proton at δ 6.66 ppm (δ_C 111.9 ppm) showed β-correlation with one sp^2 quaternary carbon at δ 147.2 ppm and one methine carbon at δ 120.4 ppm. Therefore, fragment (a') could be assigned. In addition, the methylene protons δ 2.82 ppm showed β- correlation with two sp^2 methine carbons at δ 111.9 and 120.4 ppm and α-coupling with one sp^2 quaternary carbon at δ134.3 ppm which gave fragment (b'). On the other hand, the appearance of β-long range signals between two methoxy singlets δ3.82 and 3.83 ppm with two sp^2 quaternary carbons at δ147.2 and 148.7 ppm led to fragment (c').



Fragment (a')



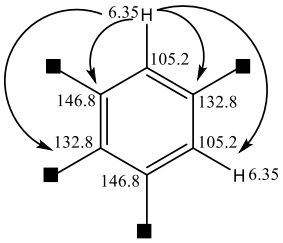
Fragment (b')



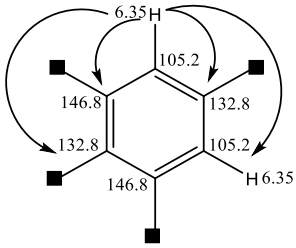
Fragment (c')

In the aromatic region of ^{1}H NMR spectrum, Figure 8(a), the signal at δ 6.35 ppm with the integration of four protons was detected. By the analysis of HMQC together with ^{13}C NMR spectra, the proton signal at δ 6.35 ppm was connected to methine carbon at δ 105.2 ppm. Therefore, the signal at δ 105.2 ppm must be four methine carbons. According to integration of the signals in ^{13}C NMR spectrum, Figure 8(b), the signals at δ 132.8 and 146.8 ppm were belonged to four carbons in each signal.

The four methine protons at δ 6.35 ppm in ^{1}H NMR spectrum, Figure 8(a), were ascribed to two 1, 2, 3, 5-tetrasubstituted benzene ring. In the HMBC spectrum, Figure 8(f), two equivalent protons at δ 6.35 ppm showed correlation with one sp^2 methine carbon at δ 105.2 and two sp^2 quaternary carbons at δ 132.8 and 146.8 ppm. Thus, fragments (1) and (1') could be assigned.

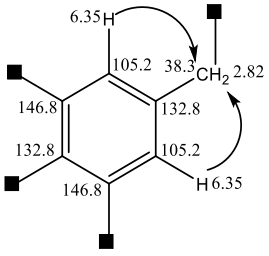


Fragment (1)

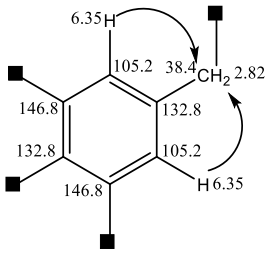


Fragment (1')

Furthermore, the two equivalent methine protons at δ 6.35 ppm from fragment (1) and (1') showed HMBC cross signals to two methylene carbons at δ 38.3 and 38.4 ppm respectively. Therefore, the fragment (2) and (2') could be assigned.



Fragment (2)



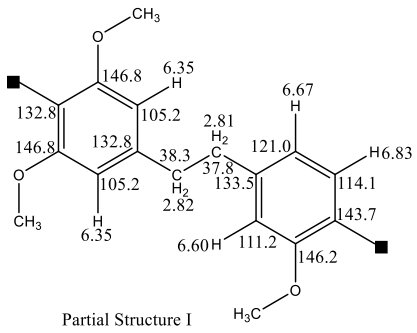
Fragment (2')

In the HMBC spectrum, Figure 8(f), the two methylene protons at δ 2.82 ppm (δ_C 38.3, 38.4 ppm) showed β-correlation with two equivalent sp^2 methine carbons at δ 105.2 ppm. Thus, the fragment (2) and (2') could be confirmed. Furthermore, in the HMBC spectrum, Figure 8(f), the methylene protons at δ 2.82 ppm (δ_C 38.3 ppm) from fragment (2) showed β-correlation with one sp^2 quaternary carbons at 133.5 ppm from fragment (c). The methylene protons at δ 2.82 ppm (δ_C 37.8 ppm) from fragment (c) showed β-correlation with one sp^2 quaternary carbon at δ 132.8 ppm from fragment (2). Therefore, the fragment (c) and (2) could be connected and partial structure I could be assigned.

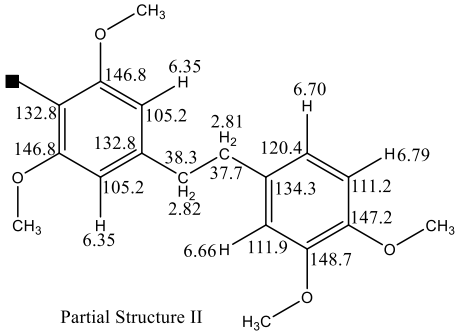
Similarly, the methylene proton at δ 2.82 ppm (δ_C 38.4 ppm) from fragment (2') showed β-correlation with one sp^2 quaternary carbon at δ 134.3 ppm from fragment(c'). The methylene

protons at δ 2.82 ppm (δ_C 37.7 ppm) from fragment (c') showed β-correlation with one sp^2 quaternary carbon at δ132.8 ppm from (2'). Therefore, the fragment (c') and (2') could be connected and partial structure II could be drawn.

The singlet methoxy signals at δ 3.84 ppm (δ_C 55.7, 55.8, 55.9, 56.2 ppm) showed correlation with four sp^2 quaternary carbons at δ146.8 ppm from partial structure I and II.

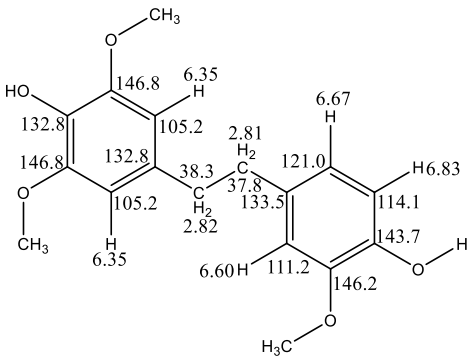


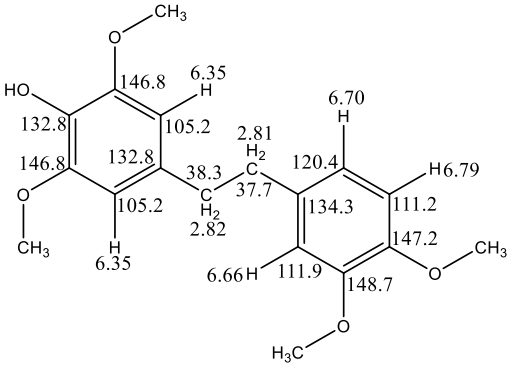
Partial Structure I



Partial Structure II

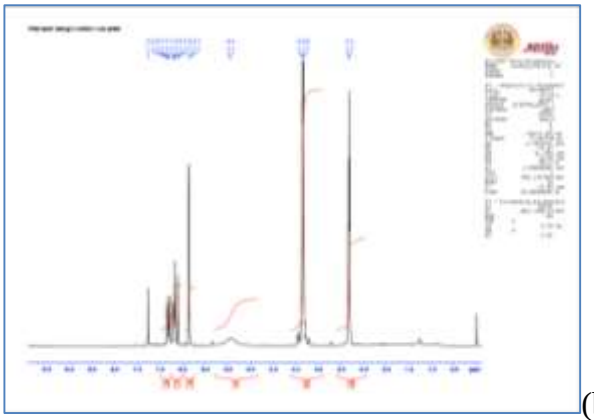
In addition, (+)-DART mass spectrum, (Figure 8g) revealed two pseudomolecular ion peaks $[M+H]^+$ at *m/z* 305.1362 and 319.1538 respectively. The two molecular mass were deduced as 304 and 318. Their molecular formula was $C_{17}H_{20}O_5$ and $C_{18}H_{22}O_5$. Therefore, the remaining two hydroxyl groups were attached to two sp^2 quaternary carbons at δ 143.7 and 132.8 ppm in partial structure I and one hydroxyl group was attached to one sp^2 quaternary carbons at δ 132.8 in partial structure II, and complete structures of two bibenzyl derivatives 1 and 2 were obtained.



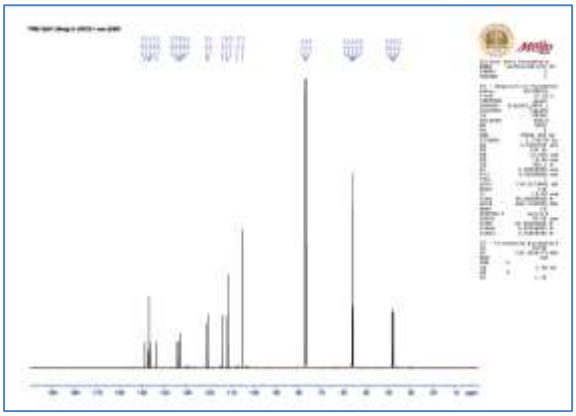


Structures of compound 1 and 2

(a)



(b)



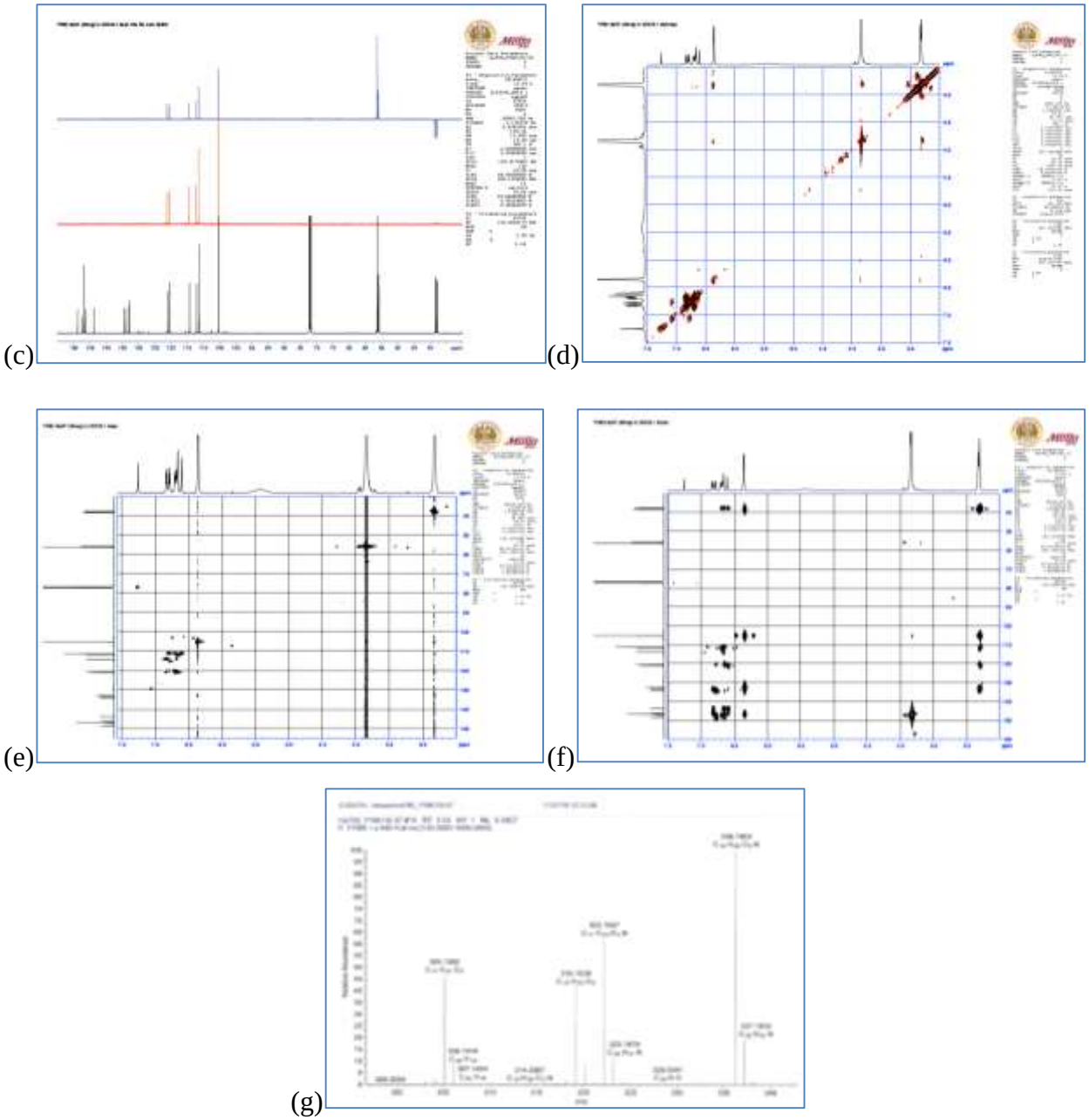


Figure 8 (a) ^{1}H NMR, (b) ^{13}C NMR, (c) DEPT, (d) COSY, (e) HMQC, (f) HMBC and (g) DART MS spectra of isolated compounds

Conclusion

In the present work, the antimicrobial activities of various extracts of *Dendrobium pulchellum* were investigated. According to antimicrobial assay, n-hexane extract showed low activities on *Staphylococcus aureus, Pseudomonas aeruginosa, Candida albicans* and *Escherichia coli*, except *Bacillus subtilis* and *Bacilius pumilus*. The methanol extract revealed low activities on all selected microorganisms. The ethyl acetate extract exhibited medium activities on *Bacillus subtilis* and low activities on *Psudomonas aeruginosa*, *Bacillus subtilis*, *Candida albicans* and *Escherichia coli* except *Staphylococcus aureus*. According to acute toxicity assay, the medium lethal dose LD_{50} was found to be more than 2500 mg/kg body weight. Thus, the ethyl acetate extract of this root is practically non-toxic and may be relatively harmless. Moreover, antioxidant activity of ethyl acetate extract was evaluated using DPPH radical scavenging assay. Ethyl acetate

extracts showed high antioxidant activity with IC_{50} of 1.987 μg/mL which is comparable to IC_{50} 0.9072 μg/mL of ascorbic acid. Finally, two bibenzyl derivatives from ethyl acetate extract were isolated and characterized by NMR and mass studies.

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