ANTIMICROBIAL, ANTIOXIDANT PROPERTIES OF *DIOSCOREA BULBIFERA* (AIR POTATO) TUBER EXTRACT AND STRUCTURE ELUCIDATION OF ISOLATED BIBENZYL DERIVATIVE

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Abstract

Chemical investigation of tuber of *Dioscorea bulbifera* Linn. (Local name Put-sar-u) led to the isolation of bibenzyl derivative namely, 3-methoxy-4, 3', 5' -trihydroxybibenzyl (1). The structure of isolated metabolite was elucidated by spectroscopic techniques, particularly by 1D and 2D NMR spectroscopy and mass spectrometry. Antioxidant and antimicrobial properties of various solvent extracts was studied by using DPPH radical scavenging assay and agar-well diffusion method respectively.

Keywords: *Dioscorea bulbifera*, bibenzyl, NMR spectroscopy, DPPH

Introduction

Since ancient times, plants have been used as a source of medicine and a major resource for health care. Nowadays, the modern pharmaceutical industry is paying more and more attention to plants because plant is an almost infinite resource for medicine development (Thomson, 2007). Human beings have depended on nature for their simple requirements especially the sources for medicines, shelters, food stuffs, fragrances, clothing, flavours, fertilizers and means of transportation throughout the ages. In developing countries, medicinal plants show a dominant role in the healthcare system (Dar *et al*., 2017).

The active compounds in medicinal plants have direct or indirect therapeutic effects and are used as medicinal agents. In the body of these plants, certain materials are produced and stored that are referred to as active compounds (substances), which have physiological effects on the living organisms (Phillipson, 2001).

The objective of the present research is to investigate bioactive chemical constituents from *Dioscorea bulbifera*. To achieve this aim, preliminary phytochemical screening, antimicrobial test, antioxidant activity assay, isolation and structure elucidation of selected plant was performed. *Dioscorea bulbifera* tubers have therapeutic benefits as purgative, anthelmintic, diuretic, deflatulent, rejuvenating tonic, aphrodisiac and can also be used for treatment in scrofula, hematological disorders, diabetic disorders, worm infestations and skin disorders (Subasini *et al*., 2013).

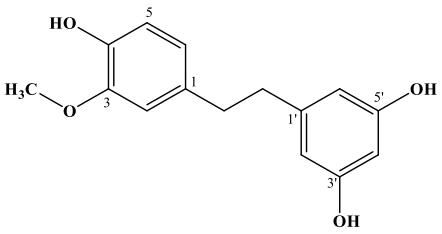

Figure 1 Structure of isolated compound

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Botanical Classification

Figure 2 Plant, flower and tuber of *Dioscorea bulbifera* Linn.

Botanical name	- *Dioscorea bulbifera* L.
English name	- Air potato (or) Yam
Myanmar name	- Put-sar-u
Family name	- Dioscoreaceae
Flowering period	- July-September
Part used	- Tuber

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). – ^{13}C NMR spectra: Varian Unity 300 (75.5 MHz), Varian Inova 500 (125.7 MHz). Chemical shifts were measured relatively to tetramethyl silane 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 Research Department, Insein, Yangon.

Plant Material

The tuber of *Dioscorea bulbifera* was collected from Kalay University Campus, Kalay Township, Sagaing Region, Myanmar and identified by Daw Myint Myint Khaing, Department of Botany, Kalay University. The plant material were cut into small pieces and dried at room temperature for about two weeks.

Preliminary Phytochemical Analysis

The preliminary phytochemical screening of *Dioscorea bulbifera* was determined using standard method.

Antimicrobial Assay

Antimicrobial tests were performed at Pharmaceutical Research Department (PRD), 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*, *Bacillius pumilus*, *Candida albicans* and *Escherichia coli*.

Antioxidant Activity

The antioxidant activities of the plant extracts were determined by DPPH radical scavenging assay (Yamaguchi *et al*., 1998). The antioxidant activity of sample was expressed in IC_{50}. 1000 µL of test solutions in various concentrations (100 µg/mL, 50 µg/mL, 25 µg/ml, 12.5 µg/ml and 6.25 µg/ml) and 1000 µL of 0.1 M acetate buffer pH 5.5 solutions were mixed in a test tube. 500 µL of $5x10^{-4}$ M DPPH solution was added to the mixture in dark. The mixture was homogenized using a vortex mixer in a dark room (resistant to UV light) and stand for 30 min at room temperature. After that, the absorbance of the solution was measured by a UV spectrophotometer at λ_{max} 517 nm. Vitamin C was used as a reference compound in the same concentration range as the test compound. A control solution was prepared by mixing 1000 µL of buffer (pH 5.5) solution, 1000 µL of ethanol and 500 µL of 5 x 10^{-4} M DPPH solution. Blank solution was prepared by mixing 1000 µL of buffer (pH 5.5) solution with 1500 µL of ethanol. The mean values were obtained from triplicate experiments.

The capability of scavenging DPPH radicals as a percentage of DPPH remaining in the resulting solution was determined using the following equation:

$$\% \text{ inhibition} = \frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}} \times 100$$

Where $Abs_{control}$ is absorbance of control and Abs_{sample} is absorbance of sample. 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.

Extraction and Isolation of Pure Compound

The air dried sample *of Dioscorea bulbifera* (1000 g) were percolated with methanol for two months. The methanol crude extracts were filtered and evaporated the solvent. The residue was extracted with ethyl acetate to attain 34.13 g of ethyl acetate crude extracts. The crude extract was dissolved in a mixture of n-hexane and EtOAc and 6 g of silica gel were added. The mixture was allowed to dryness under reduced pressure. The obtained crude powder extracts were subjected to silica gel by using various solvent systems of n-hexane and ethyl acetate. 3-methoxy-4,3',5'-trihydroxybibenzyl (1) was isolated as red solid from selected fraction V. It showed UV absorption band at 254 nm.

Results and Discussion

Preliminary Phytochemical Screening

According to preliminary phytochemical test, the crude extracts of *Dioscorea bulbifera* contained flavonoid, glycoside, phenolic compound, polyphenol, reducing sugar, saponin, steroid, tannin and terpenoid.

Antimicrobial Activities of the Tuber of *Dioscorea bulbifera*

The antimicrobial activities of the tuber of *Dioscorea bulbifera* were tested in various solvent systems by using agar-well diffusion method.

Table 1 Antimicrobial Activities of Tuber of Dioscorea bulbifera

Sample	Solvent	Inhibition zone (mm)					
		I	II	III	IV	V	VI
Dioscorea bulbifera	n-hexane	-	-	-	-	-	-
	EtOAc	17 (++)	15 (++)	21 (+++)	15 (++)	18 (++)	17 (++)
	MeOH	15 (++)	18 (++)	18 (++)	15 (++)	15 (++)	15 (++)

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

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

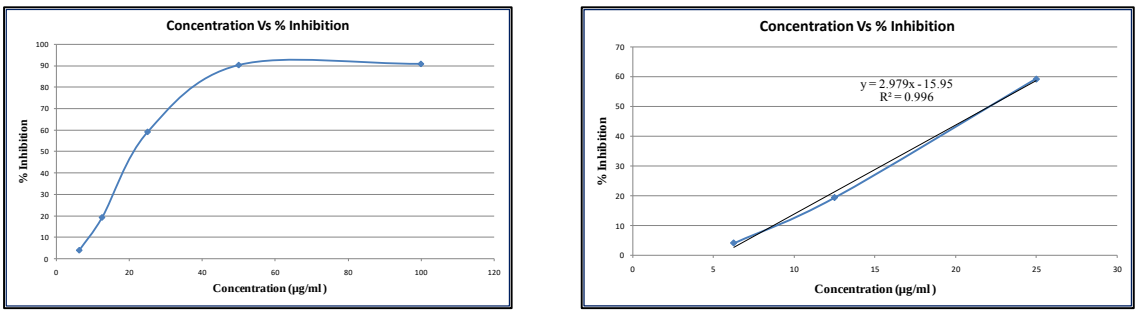
According to antimicrobial tests, the n-hexane extracts did not respond activity on all test microorganisms. The ethyl acetate extracts showed strong activities against *Pseudomonas aeruginosa* and medium activities against other five test microorganisms. The methanol extracts exhibited medium activities on all test microorganisms.

Antioxidant Activity by DPPH Radical Scavenging Assay

The percentage of inhibition in different concentrations of standard ascorbic acid and IC_{50} value was shown in Table 2.

Table 2 % Inhibition in Different Concentrations for Standard Ascorbic Acid and IC50 Value

Concentration (µg/mL)	% Inhibition	IC_{50} (µg/mL)
100	90.9	
50	90.4	
25	59.2	22.14
12.5	19.3	
6.25	4.0	

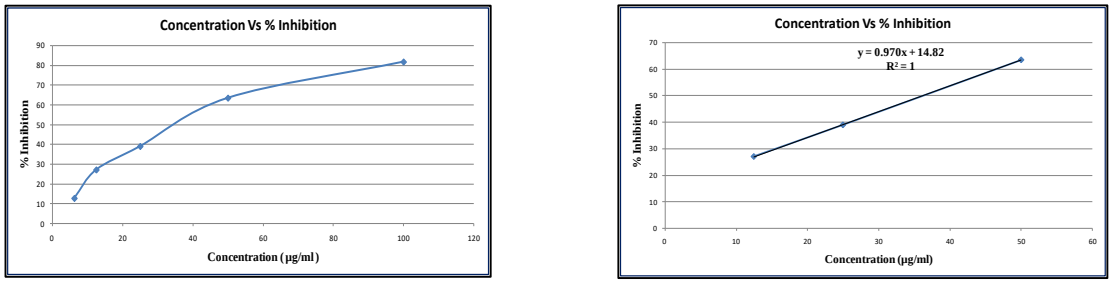


(a) (b)

Figure 3 (a) Standard calibration curve of ascorbic acid (b) Linear graph for calculation of IC_{50} value of standard ascorbic acid solution

Table 3 % Inhibition of Methanolic Extract in Different Concentrations and IC_{50} Value

Concentration (µg/mL)	% Inhibition	IC_{50} (µg/mL)
100.00	81.70	
50.00	63.41	
25.00	38.95	36.23
12.50	27.05	
6.25	12.65	



(a) (b)

Figure 4 (a) % Inhibition of methanolic extract of *Dioscorea bulbifera* (b) Linear graph for calculation of IC_{50} value of methanolic extracts

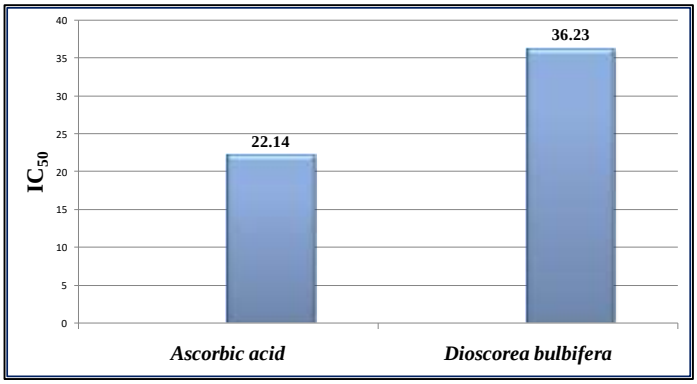


Figure 5 Bar graph of IC_{50} value of ascorbic acid and methanolic extracts of *Dioscorea bulbifera*

DPPH radical scavenging activities of the methanolic extract of *Dioscorea bulbifera* showed significant free radical scavenging activity with IC_{50} value of 36.23 µg/mL.

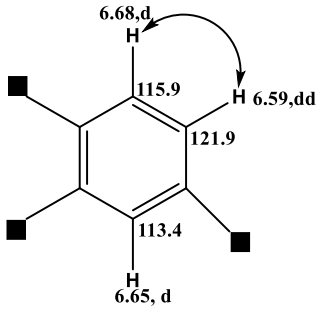
Structure Elucidation of 3-methoxy-4, 3',5'-trihydroxybibenzyl

Structural elucidation of isolated compound was determined by spectroscopic methods such as FT IR, ^{1}H NMR, ^{13}C NMR, DEPT, DQF-COSY, HSQC and HMBC spectra.

According to the ^{13}C NMR (Figure 8.2) and DEPT (Figure 8.3), total of 15 carbon signals were detected which comprised of six sp^2 quaternary carbons at δ 159.3 (two C_q), 148.6, 145.6, 145.5 and 134.8 ppm, six sp^2 methine carbons at δ 121.9, 115.9, 113.4, 108.2 (2 CH) and 107.1 ppm, one sp^3 methyl carbon at δ 56.3 ppm and two sp^3 methylene carbons at δ 39.5 and 38.4 ppm. Among them, four sp^2 quaternary carbons at δ 159.3, (two C_q), 148.6 and 145.6 ppm were probably attached to oxygen.

In the aromatic region of the ^{1}H NMR spectrum (Figure 8.1), one doublet of doublet methine proton at δ 6.59 ppm (J = 1.76, 7.98 Hz) showed ortho coupling with one doublet methine

proton at δ 6.68 ppm (J = 7.97 Hz) and meta coupling with another doublet methine proton at δ 6.65 ppm (J = 1.69 Hz). Thus, 1, 2, 4-trisubstituted benzene ring could be drawn. In the DQF-COSY spectrum (Figure 8.5), the methine proton at δ 6.59 ppm showed correlation with one methine proton at δ 6.68 ppm as expected. Therefore, fragment (a) could be assigned.



Fragment (a)

In the HMBC spectrum (Figure 8.6), the doublet of doublet methine proton at δ 6.59 ppm showed β-correlation with one sp^2 methine carbon at δ 113.4 ppm, one sp^2 quaternary carbon at δ 145.6 and one sp^3 methylene carbon at δ 38.4 ppm. Therefore, fragment (b) could be drawn. Moreover, one doublet methine proton at δ 6.65 ppm showed β-coupling with one sp^2 methine carbon at δ 121.9 ppm, one sp^2 quaternary carbon at δ 145.6 and one sp^3 methylene carbon at δ 38.4 ppm. Furthermore, in the HMBC spectrum (Figure 8.6), one doublet methine proton at δ 6.68 ppm showed strong correlation with two sp^2 quaternary carbons at δ 134.8 and 148.6 ppm. Therefore, the carbon atoms in the benzene ring could be assigned.

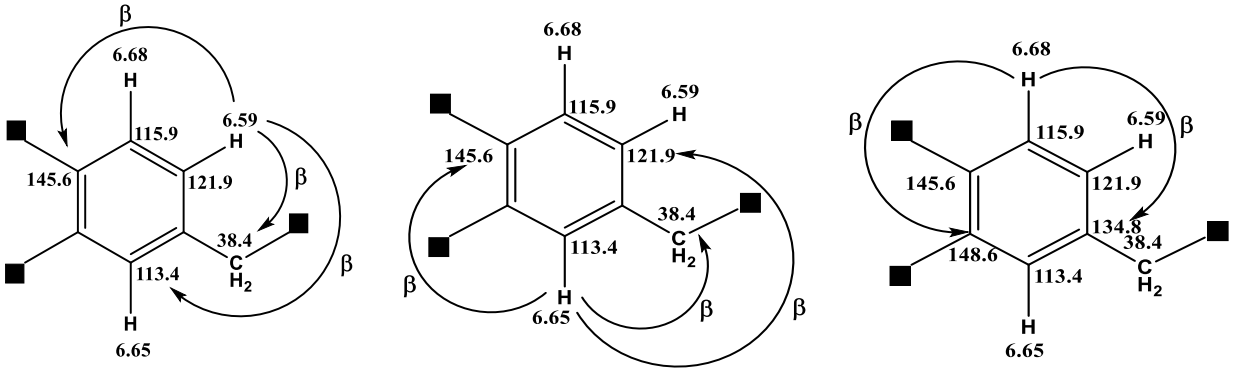
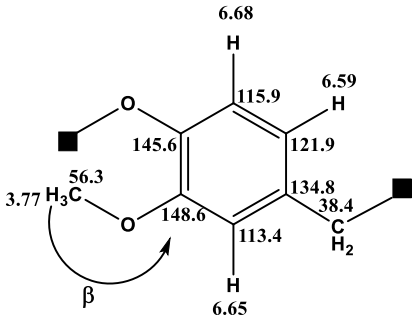


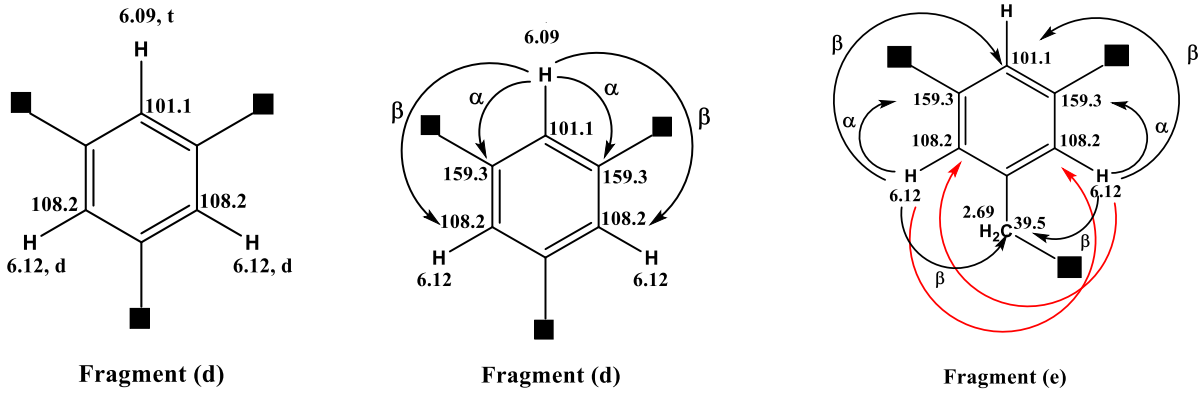
Figure 6 HMBC (→) correlations in fragment (b)

According to the chemical shift, the two quaternary carbons at δ 145.6 and 148.6 could be connected to oxygen. Furthermore, in the HMBC spectrum (Figure 8.6), there was the observation of β-coupling between singlet methoxy group at δ 3.77 ppm and aromatic sp^2 quaternary carbon at δ 148.6 ppm produced fragment (c).



Fragment (c)

In the aromatic region of the ^{1}H NMR spectrum (Figure 8.1), one triplet methine proton at δ 6.09 ppm (J = 2.09 Hz) showed meta coupling with two chemical shift equivalent methine protons at δ 6.12 ppm (J = 2.08 Hz). Thus, 1, 3, 5-trisubstituted benzene ring fragment (d) could be drawn. In the HMBC spectrum (Figure 8.6), the triplet methine proton at δ 6.09 ppm showed strong correlation with two equivalent sp^2 methine carbons at δ 108.2 ppm and two equivalent sp^2 quaternary carbons at δ 159.3 ppm. Moreover, the two chemical shift equivalent methine protons at δ 6.12 ppm showed HMBC correlation with each other. Furthermore, these two protons showed β-coupling with one sp^2 methine carbon at δ 101.1 ppm, two equivalent sp^2 quaternary carbons at δ 159.3 ppm and one sp^3 methylene carbon at δ 39.5 ppm. Therefore, fragment (e) could be assigned.

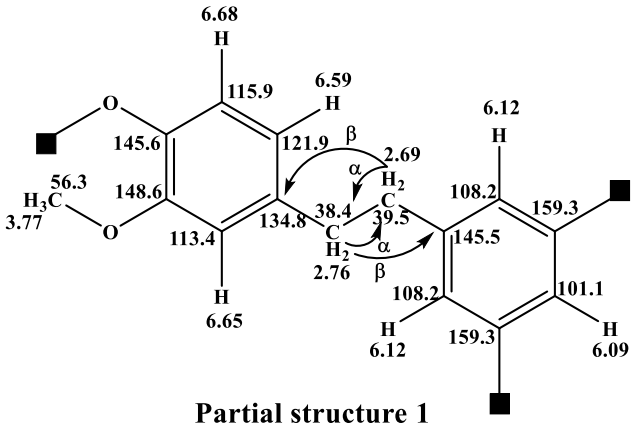


Fragment (d) **Fragment (d)** **Fragment (e)**

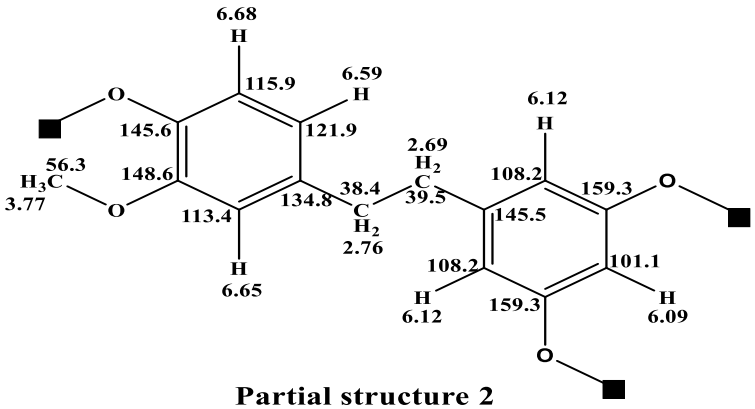
Figure 7 HMBC (→) correlations in fragment (d) and (e)

Furthermore, in the HMBC spectrum (Figure 8.6), the doublet of doublet methylene protons at δ 2.69 which is attached to carbon at δ 39.5 ppm showed strong correlation with one sp^2 quaternary carbon at δ 145.5 ppm and two equivalent sp^2 methine carbons at δ 108.2 ppm. Therefore, fragment (e) could be further confirmed.

Moreover, the doublet of doublet methylene protons at δ 2.69 ppm from fragment (e) showed β- correlation with one sp^2 quaternary carbon at δ 134.8 and a-correlation with one sp^3 methylene carbon at δ 38.4 ppm from fragment (c). Similarly, the doublet of doublet methylene proton at δ 2.76 ppm from fragment (c) showed β-correlation with one sp^2 quaternary carbon at δ 145.5 and a-correlation with one sp^3 methylene carbon at δ 39.5 ppm from fragment (e). So the fragment (c) and (e) could be connected as shown in partial structure (I).

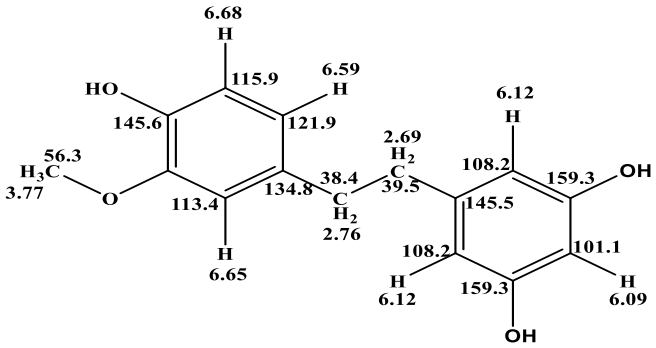


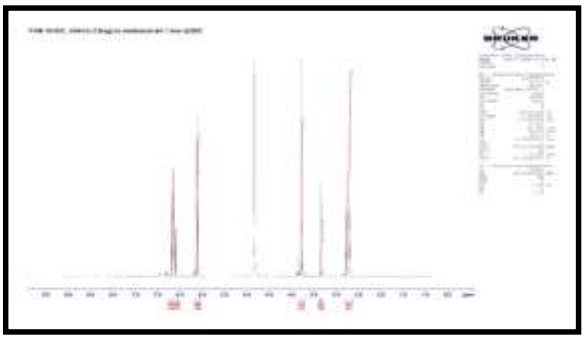
Partial structure 1



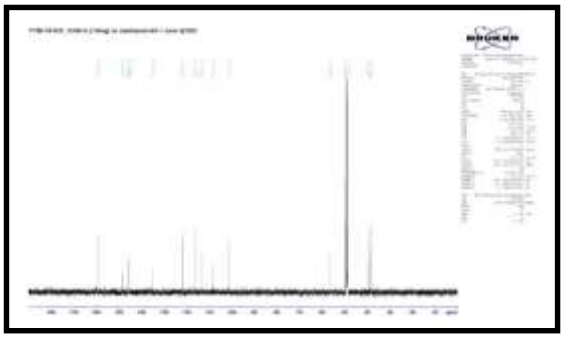
Partial structure 2

According to the chemical shift value, the two equivalent sp^2 quaternary carbons at δ 159.3 ppm could be connected to oxygen and partial structure 2 with the partial molecular mass of 257 could be assigned. Moreover, FT IR spectrum showed the presence of OH group. Therefore, the complete structure of isolated compound could be elucidated as 3-methoxy-4,3',5'-trihydroxybibenzyl. The isolated compound is bibenzyl derivative. Bibenzyl are naturally occurring fungicides. Both natural and synthetic bibenzyls show antifungal activity (Smriti *et al*., 2013). Bibenzyl derivatives constitute a class of stilbenoid compounds with interesting structural scaffolds and biological activities including antioxidant, cytotoxic, antibacterial and antifungal (Osei-Safo *et al*., 2017).





(8.1)



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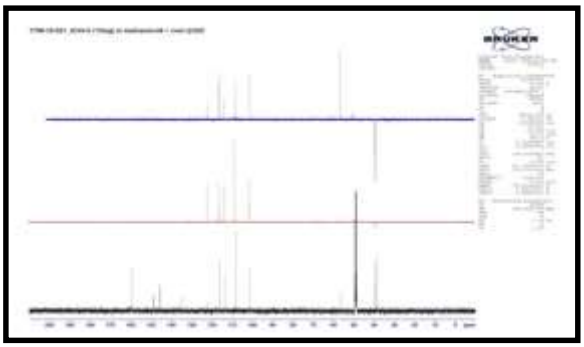

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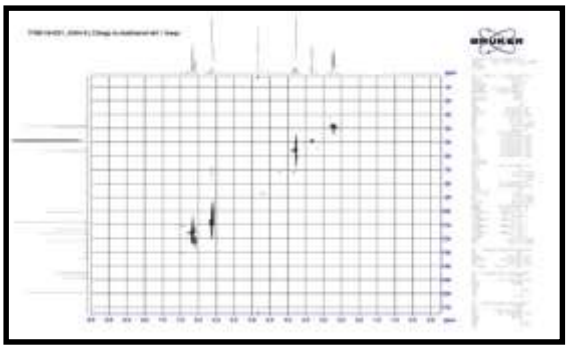

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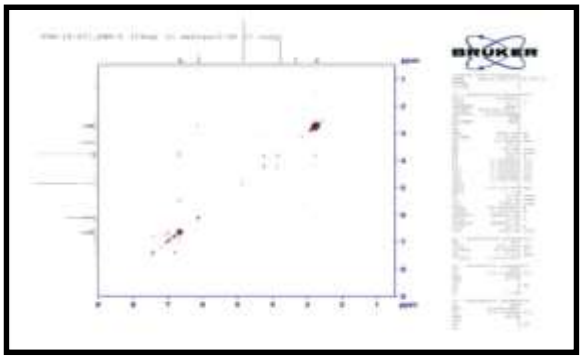

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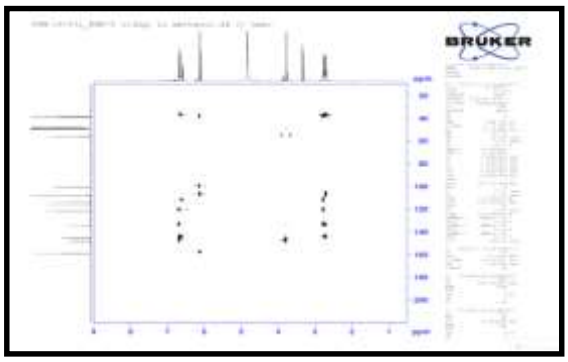
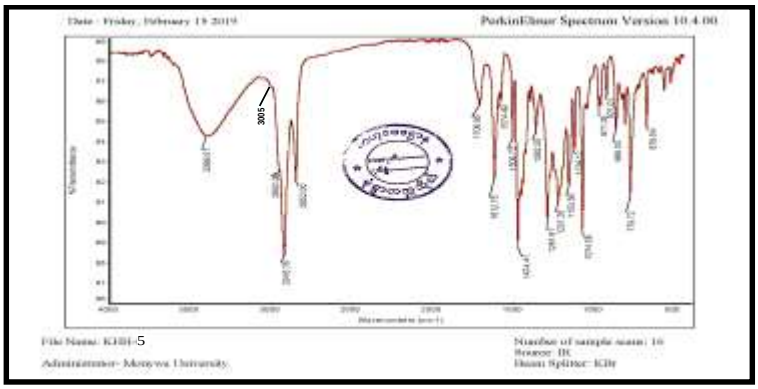


(8.6)



(8.7)

Figure 8 (8.1) ^{1}H NMR, (8.2) ^{13}C NMR, (8.3) DEPT, (8.4) HSQC, (8.5) DQF-COSY, (8.6) HMBC, (8.7) FT-IR of isolated compound

Conclusion

In this research work, *Dioscorea bulbifera* was selected for chemical screening due to its interesting medicinal uses. Preliminary photochemical screening of the crude sample revealed the presence of glycoside, phenolic, reducing sugar, tannin, saponin, flavonoid, steroid, terpenoids and polyphenol respectively. According to antimicrobial assay, the n-hexane extracts did not inhibit the growth of all test microorganisms. The ethyl acetate extracts showed strong activities against *Pseudomonas aeruginosa* and medium activities against other five test microorganisms. The methanol extracts exhibited medium activities on all test microorganisms. Moreover, methanolic extract showed antioxidant activity on DPPH with IC_{50} of 36.23 µg/mL. From ethyl acetate extract, 3-methoxy-4,3',5'-trihydroxybibenzyl was isolated and characterized by FT-IR and NMR studies. The result of the present study suggested that selected plant can be used as a source of antioxidant and antimicrobial for pharmacological preparations which is very well evidenced by the present work.

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References

Dar, R.A., Shahnawaz, M., and Qazi, P.H. (2017). "General Overview of Medicinal Plants". *The Journal of Phytopharmacology*, vol. 6, pp. 349-351.

Osei-Safo, D., Dziwornu, G. A., Salgado, A., Sunassee, S. N., and Chama, M.A. (2017). "Bi- and Bisbibenzyls from the Roots of *Dichapetalum heudelotii* and their Antiproliferative Activities". *Fitoterapia*, vol. 122, pp. 95-100.

Phillipson, J.D. (2001). "Phytochemistry and Medicinal plants". *Phytochemistry*, vol. 56, pp. 237-243.

Smriti, D., and Siddiqui, I.R. (2013). "Study of Fungicidal Activity of Synthesized Bio Active 4, 4'-Dihydroxy Bibenzyl Incorporating Various Heterocyclic Moieties". *International Journal of Engineering Science and Innovative Techanology (IJESIT)*, vol.2, pp.342-350.

Subasini, U., Thenmozhi, S., Sathyamurthy, D., Vetriselvan, S., Rajamanickam, G.V., and Dubey, G.P. (2013). "Pharmacognostic and Phytochemical Investigations of *Dioscorea bulbifera* L". *International Journal of Pharmacy & Life Sciences*, vol. 4, pp. 2693-2700.

Thomson, G.E. (2007). "The Health Benefits of Traditional Chinese Plant Medicines: Weighing the Scientific Evidence" Rural Industries Research and Development Corporation (RIRDC Publishing), pp. 1.

Yamaguchi, T., Takamura, H., Matoba, T., and Terao, J. **(**1998**).** "HPLC Method for Evaluation of the Free Radical-scavenging Activity of Foods by Using 1, 1-diphenyl-2-picrylhydrazyl". *Biosci., Biotechnol. Biochem*, vol. 62, pp. 1201-1204.