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Gas Chromatography-Mass Spectrometric (GC-MS) Analysis of Ethanolic Extract of the Peel of *Dioscorea bulbifera* Linn (Air Potatoe)

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Abstract

The bioactive chemical compounds in the ethanolic extracts of the peel of *Dioscorea bulbifera* Linn, native to Nigeria was investigated and characterized using gas chromatography-mass spectrometric (GC-MS) analysis. The relatively high concentration of the phytochemicals in the peel of this tuber; saponin (32.28mg/g), terpenoid (22.90mg/g), cardiac glycoside (15.90mg/g), flavonoid (9.17mg/g), tannin (4.79mg/g) and phlobatannin (1.87mg/g), was the most attractive factors that triggered the present study. The GC-MS analysis of the ethanolic extract of the peel of *D. bulbifera* shows 4 prominent peaks as R-(-)-1,2-Propanediol ($C_3H_8O_2$) with retention time of 6.103min and the highest peak area of 86.05%; 1-Methylhexylhydroperoxide ($C_7H_{16}O_2$) with retention time of 42.567min and peak area of 5.57%; Cis-3-hexenyllactate ($C_9H_{16}O_3$) with retention time of 45.395min and peak area of 5.01%; and Pyrrole (C_4H_5N) showed peak of 3.37% with retention time of 45.508min. Other less prominent peaks at other retention times included; 2-Nitro-Dimethylnitromethane ($C_3H_7NO_2$) with retention time of 37.355min, Ethylenimine (C_2H_5N) with retention time of 42.665min, N-Formyl-N-Methyl-formamide ($C_3H_5NO_2$) with retention time of 44.225min, and Guanidine (CH_5N_3) with retention 44.225min. This work which is the first-time report on the bioactive compounds in the organic crude extracts of *Dioscorea bulbifera* native to Nigeria, using the GC-MS, has however established the presence of quite number of chemical compounds in the peel of the tuber, to which their pharmacological activities could be attributed. The presence of these secondary metabolites in the peel of *Dioscorea bulbifera*, is hence, the major contributing factors behind its antimicrobial potential.

Keywords: GC-MS, *Dioscorea bulbifera*, antimicrobial properties, bioactive compounds, ethanolic extract

1. Introduction

Despite that antibiotic has been widely used in the last decades because it was then proven to be the only effective therapeutic agent against microbial infections and have greatly benefited health-related quality of human life (Fransworth, 1993), the development of microbial resistance to them has however been increasingly alarming. To tackle and overcome this challenge, scientists have recently focused their research towards the antimicrobial components of medicinal plants as alternative solution (Thangavel *et al*., 2014).

The use of natural products such as plant materials as therapy for different pathologies can be dated from antiquity and continuously expanding, leaving its renaissance in recent time throughout the world (Mota *et al*., 2009). This is so, because nature and its compounds have been of great influence in the history of pharmacology, as they serve as source of invaluable therapeutic properties (Thangavel *et al*., 2014). Apparently, about 80% of the populace in developing countries, like Nigeria, has been estimated by the World Health Organization to rely on these medicinal plants for basic health care (Muruganantham *et al*., 2009). The upfront and increasing

reliance on these medicinal plants as remedy is due to their biological activities, higher safety compared to synthetic drugs and low cost (Grabley and Thiericke, 1999). This has therefore necessitated the understanding of the therapeutic properties, safety and efficiency of these natural entities.

Extract of different parts (root, leaves, peel and bulbil) of this plant, *Dioscorea bulbifera* Linn have been documented to be traditionally used in many part of the world to cure a number of diseases such as sore throat, gastric cancer, carcinoma of rectum, and goiter in China (Jiang, 1978; Gao *et al*., 2001); tumor and leprosy in Bangladesh (Czarapata, 2005). It has also been noted to be useful as; anorexiant (Jindal *et al*., 1969), antitumor (Gao *et al*., 2002), antihyperlipidemic (McKoy *et al*., 2003), antioxidant (Bhandari and Kawabata, 2004), plasmid curing agent (Shririam *et al*., 2008), antihyperglycemic (Ahmed *et al*., 2009), analgesic and anti-inflammatory agent (Nguelefack *et al*., 2011). The antimicrobial properties and potential of this tuber, native to Nigeria, against clinical pathogens were however first reported by Adeosun *et al*. (2016).

Furthermore, literature search revealed that there is still no work that has reported the actual bioactive chemical constituents in the organic crude extracts of *Dioscorea bulbifera* native to Nigeria, using the GC-MS. The present study is hence the first time report on the investigation and characterization of the bioactive chemical constituents (serving as the functional group) in the ethanolic extracts of the peel of *Dioscorea bulbifera* Linn, native to Nigeria.

2. Materials and Methods

2.1 Plant samples and authentication

Tubers of *Dioscorea bulbifera* Linn (Aerial yam) were obtained from the experimental garden of the Department of Microbiology at the back of Microbiology Laboratory, Obakekere, Federal University of Technology, Akure (FUTA), Ondo-State, Nigeria.

Authentication of the plant tubers was done in The Department of Crop, Soil and Pest, FUTA. Much tubers of *D. bulbifera* were harvested, packed in clean sterile manila papers, labelled with a voucher specimen and transported to the Laboratory of Department of Microbiology, FUTA, Ondo-State, Nigeria, for analysis.

2.2 Preparation of plant sample

The peel of the tuber was washed and sanitized in a 6% sodium hypochlorite solution (50 ppm), (Reckitt Benckiser, Nig. Ltd) (Ritenour *et al*., 2011). It was thinly sliced (approximately 25mm) and dried in an oven-drier (HME Global Laboratory Oven Model No. DHG-9101-1SA, England) set at 60^{o}C for 48hrs. The peel was then grounded using a 12-speed blender (Excella) for 5mins and stored in an air-tight container at 4^{o}C in the Laboratory Refrigerator, until used.

2.3 Extraction procedure

One hundred grams (100g) of dried powdered peel of *Dioscorea bulbifera* were macerated in a 3L flask using ethanol as solvent, making a ratio of 1 to 15 of the plant materials and solvents respectively; described by (Parish and Davidson, 1993). The mixture was placed in an orbital shaker (Stuart Orbital incubator, S1500) for 24hrs at a speed of about 100rpm at room temperature (25^{o}C) for extraction and filtered through a 90mm diameter filter paper (Whatman No. 1, Whatman® Schleicher and Schuel) (Adeniran and Sonibare, 2013). Extract was collected and concentrated under reduced pressure using rotary evaporator (SearchTech Instruments, RE52-1) at

40°C for 10mins and reconstituted with 50% dimethylsulphoxide (DMSO) to make a stock extract, which was stored at 4°C until needed.

2.4 Phytochemical screening

Standard techniques described by Patil and Paikrao (2012) for phytochemical analysis were respectively carried out on the crude extracts of the bulbils, peels and whole tuber of *D. bulbifera* to detect the presence and absence of alkaloids, saponins, tannins, coumarin, flavonoids and other bioactive compounds. Quantification of each bioactive compounds detected was done using different techniques described by (Sofowora, 1993).

2.5 Gas chromatography –mass spectrometry analysis (GC-MS)

The Gas Chromatography-Mass Spectrometry (GC-MS) analysis of the bioactive chemical and functional group constituent of the ethanolic extract of the peel of *Dioscorea bulbifera* Linn was performed using GC-MS SHIMADZU GC-MS QP2010 instrument equipped with AB innowax column (60 × 0.25mm i.d., film thickness 0.25µm). Initially, oven temperature was maintained at 50°C for 3 min and temperature was gradually increased up to 280°C at 30min before 0.2µL of the sample was injected for analysis, using helium gas (with a flow rate of 1.2ml/min) as the carrier gas. The sample injector and mass transfer line temperature were set at 270°C and 280°C and split ratio is 20 throughout the experimental periods. The ionization mass spectroscopic analysis was done with 70eV and the mass spectra were recorded across the range of 40 to 1000m/z for 35 min. the relative percentage of the chemical constituents in the crude extract from the peel of this tuber, *Dioscorea bulbifera* were expressed as percentage by peak area normalization (Verma *et al*., 2013; Al-Hashmi *et al*., 2013).

2.6 Identification of the chemical constituent

The chemical compounds serving as the functional group in the crude extract from the peel of *Dioscorea bulbifera* Linn were identified in accordance and comparison of their mass spectra with those of standard, contained in Wiley and NIST libraries and those described by Adams (1995) as well as on comparison of their retention indices with literature (Vanden and Kratz, 1963).

3. Results and Discussion

The results of the phytochemical analysis reported in a previous work by Adeosun *et al*. (2016) were actually adopted for this present report, because this research is not a fresh one per se, it rather corrects a blooper in the result and also compliments the preliminary results with the bioactive compounds in the tuber, *Dioscorea bulbifera* Linn. Hence, the preliminary phytochemical result presented in Table 1 shows that average constituent of saponin was 32.28mg/g, followed by terpenoid which was 22.90mg/g, cardiac glycoside was 15.90mg/g, flavonoid was 9.17mg/g, tannin followed with 4.79mg/g and phlobatannin with 1.87mg/g was the least among the phytochemical ingredients detected in the peel of *D. bulbifera*. The peel of this tuber comparatively recorded highest amount of the phyto-constituents, as against the bulbil of tuber with average concentration of active compounds as; saponin (21.37mg/g), terpenoid (20.40mg/g), cardiac glycosides (12.37mg/g), flavonoid (6.33mg/g), tannin (4.25mg/g) and phlobatannin (1.56mg/g), and even the whole tuber, *Dioscorea bulbifera*. Relatively high concentration of the phytochemicals in the peel of this tuber as reported by Adeosun *et al*. (2016), was the most attractive factors that triggered the present study. Adeosun *et al*. (2016) reported saponin which is consistent with the report by Eleazu *et al*. (2013); terpenoids, flavonoids and cardiac glycosides, all of which has been affiliated with one or other health promoting properties including, hypocholesterolemic effect of

saponin (Esenwah and Ikenebomeh, 2008) and as antibleeding agent (Okwu, 2004); antimicrobial and anti-inflammatory properties of terpenoids (Omojate *et al*., 2014) and flavonoids (Okwu, 2004), and antiprotozoal properties of cardiac glycosides (Omojate *et al*., 2014).

Notably, phytochemical evaluation has recently been seen as a facet tool for assessing and exploring the importance of any medicinal plants which starts with a preliminary phytochemical screening that unveil the real nature of compounds present in the plant (Shankar *et al*., 2016). Knowledge of these phytochemical constituents of plants is however desirable, not only for the discovery of therapeutic agents, but also because such information may be of value in disclosing new sources of economic materials such as tannins, oils, gums, flavonoids, saponins, essential oils precursors for the synthesis of complex chemical substances (Akrout *et al*., 2010; Thangavel *et al*., 2014).

In the Gas Chromatography Mass Spectrometric (GC-MS) analysis, four (4) compounds were identified in the ethanolic extract of *Dioscorea bulbifera* peel, based on the peak area (which depicts the percentage of each compounds), molecular weight and molecular formula, as depicted in Table 2. The chromatogram (Fig. 1) of the ethanolic extract of the peel of *D. bulbifera* shows 4 prominent peaks as R-(-)-1,2-Propanediol ($C_3H_8O_2$) with retention time of 6.103min and the highest peak area of 86.05%; 1-Methylhexylhydroperoxide ($C_7H_{16}O_2$) with retention time of 42.567min and peak area of 5.57%; Cis-3-hexenyllactate ($C_9H_{16}O_3$) with retention time of 45.395min and peak area of 5.01%; and Pyrrole (C_4H_5N) showed peak of 3.37% with retention time of 45.508min.

Other less prominent peaks at other retention times as observable on the chromatogram (Fig. 1) are shown in Table 2 as; 2-Nitro-Dimethylnitromethane ($C_3H_7NO_2$) with retention time of 37.355min, Ethylenimine (C_2H_5N) with retention time of 42.665min, N-Formyl-N-Methyl-formamide ($C_3H_5NO_2$) with retention time of 44.225min, and Guanidine (CH_5N_3) with retention 44.225min. The structure of these compounds detected in the ethanolic extract of the peel of *D. bulbifera* are presented in Table 3.

Plants have long proven to be a novel source of potentially useful compounds for the development of new chemotherapeutic agents, but searching for these biomolecules of plant origin and *in-vitro* evaluation for antimicrobial potency is essential towards achieving the goal for developing eco-friendly management of infectious disease of humans (Samy *et al*., 2008; Mohana *et al*., 2008; Thangavel *et al*., 2014). It is worthy of note that most of the major compounds detected in the peel of *Dioscorea bulbifera*, in this work, are biologically active molecules. Many of these compounds could be considered as part of plants defense systems, and as such have been included among large group of protective molecules found in plants called “phytoanticipins” or “phytoprotectants” (Hossain *et al*., 2011; Dobre *et al*., 2011; Al-Hashmi *et al*., 2013). Thus, identifying such good number of compounds in this tuber using GC-MS might have some ecological significance.

Table 1: Phytochemical composition of *Dioscorea bulbifera*

Bioactive compounds	Average concentration (mg/g)		
	Bulbils	Peel	Whole tuber
Saponin	21.37	32.28	24.00
Tannin	4.21	4.79	4.21
Phlobatannin	1.56	1.87	ND
Flavonoid	6.33	9.17	5.36
Steroid	ND	ND	ND
Terpenoid	20.40	22.90	8.48
Alkaloid	ND	ND	ND
Anthraquinone	ND	ND	ND
Cardiac glycoside	12.37	15.90	13.13

Key: ND- Not Detected

Table 2: Chemical constituents in the ethanolic extract of the peel of *D. bulbifera* using GC-MS

S/N	Rt (Min)	Peak area (%)	Name of Compound	Molecular formula	Molecular weight (≈)
1	6.103	76.01	R- (-) - 1, 2 - Propanediol	$C_3H_8O_2$	76
2	37.355	4.01	2-Nitro-Dimethylnitromethane	$C_3H_7NO_2$	89
3	42.567	5.57	1-Methylhexylhydroperoxide	$C_7H_{16}O_2$	132
4	42.665	1.98	Ethylenimine	C_2H_5N	43
5	44.225	2.04	N-Formyl-N-Methyl-formamide	$C_3H_5NO_2$	87
6	44.225	2.01	Guanidine	CH_5N_3	59
7	45.395	5.01	Cis-3-hexenyllactate	$C_9H_{16}O_3$	172
8	45.508	3.37	Pyrrole	C_4H_5N	67
Total	**-**	**100**	-	-	-

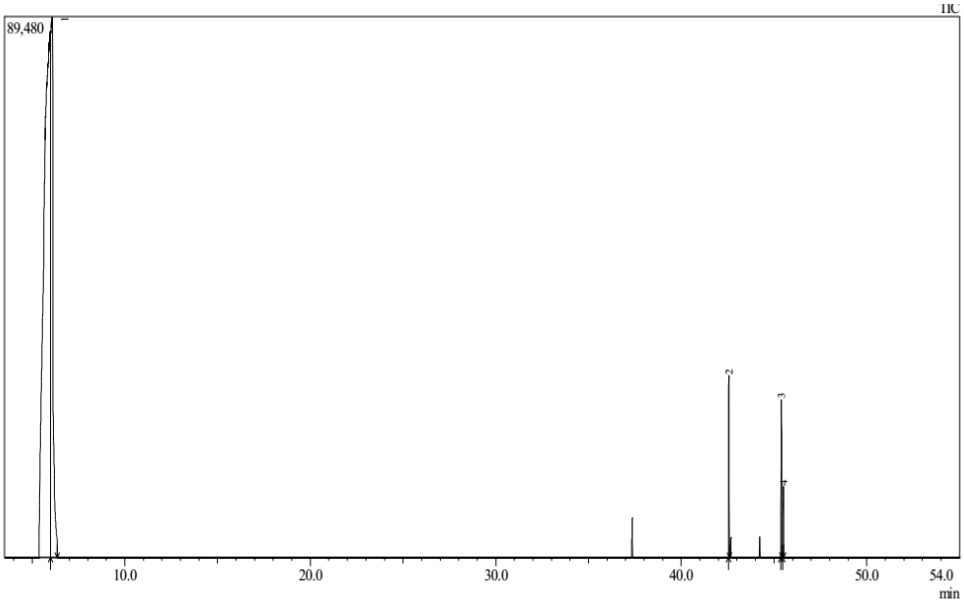


Figure 1: Chromatogram (GC-MS) of ethanolic extract of the peel of *D. bulbifera*

The potential biological activities of each of the chemical compounds found in the ethanolic extract of peel of *Dioscorea bulbifera*, in this work, has however been reported in many excellent reviews. R-(-)-1,2-Propanediol, a synthetic organic compound with chemical formula $C_3H_8O_2$, also known as propylene glycol, is a viscous colorless liquid which is nearly odorless with faintly sweet taste. It is an additive that is "generally recognized as

safe" for use in food, especially to absorb extra water (i.e. as Deicing agent) and maintain moisture in certain medicines, cosmetics and food products (FDA, 2016). Propylene glycol has been described by Rowe *et al*. (2009) as an antiseptic usable against mold, which is similar to ethanol and glycerin only slightly less effective as ethanol.

Pyrrole (C_4H_5N), a five-membered heterocyclic aromatic organic compound, is a colourless volatile liquid that darkens readily upon exposure to air. Pyrrole derivatives are the biosynthetic precursor to many natural products and more complex macrocycles, including the porphyrins of heme, the chlorins, bacteriochlorins, chlorophyll, porphyrinogens. Several biological activities of pyrrole derivatives have been reported, including anticoagulant (Idhavadhulla *et al*., 2012a), antimicrobial (Idhavadhulla *et al*., 2011), human tumor models (Evans *et al*., 2003), anticonvulsant (Carson *et al*., 1997), antiviral (Almerico *et al*., 2000) and anticonvulsant activities (Indumathi *et al*., 2015). It has gained diverse applications in the production of therapeutic compounds like antibiotics, fungicides anti-inflammatory drugs (Wilkerson *et al*., 1995), cholesterol reducing drugs (Wurz and Charette, 2005), antitumor agents (Lee *et al*., 2001) and many more. Pyrrole subunit has been reported by Artico *et al*. (1997) to inhibite reverse transcriptase human immunodeficiency virus type-1 (HIV-1) and cellular DNA polymerase protein kinases.

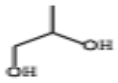
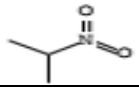
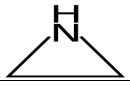
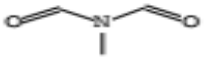
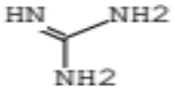
Ethylenimine is a derivative of aziridine, contains at least one three-membered ring which is very reactive because of the ring strain. It is produced by heating bromoethylamine hydrobromide or 2-aminoethyl hydrogen sulfate in the presence of sodium hydroxide. Bahnemann *et al*. (1975) have long reported ethylenimine to inactivate the foot-and-mouth disease virus in vaccines for cattle, as well as other viruses and mycoplasma in blood samples. The ability of ethylenimine to alter and/or modify nucleic acids and viral protein especially at higher pH values, has been reported as major inhibitor to viral particle from engulfing cell (Bahnemann *et al*., 1976). Another compound present in this tuber is guanidine (CH_5N_3); a colourless solid that is soluble in polar solvents and serve as strong base, which is found in urine as a normal product of protein metabolism. Guanidine is the functional group on the side chain of arginine, with several derivatives like guanidine Chloride with chaotropic properties that make it capable of denaturing proteins; and guanidine hydrochloride is known to denature proteins with a linear relationship between concentration and free energy of unfolding. Guanidine hydrochloride is used as an adjuvant in treatment of botulism, introduced in 1968 (Puggiari and Cherington, 1978; Kaplan *et al*., 1979).

Like the phytochemical constituents of plants, elucidating these bioactive chemical compounds will not only contribute to the discovery of new therapeutic agents, but also reveal information as regards some potential sources of economic chemical compounds. Hence, the peel of *Dioscorea bulbifera*, in this work, has shown to be a good source of such compounds, as cis-3-Hexenyllactate ($C_9H_{16}O_3$), which has been documented by Yannai (2004) to be useful as a flavouring ingredient in alcoholic beverages. The industrial applications of 2-Nitro-Dimethylnitromethane ($C_3H_7NO_2$) as a solvent, principally in blends, printing inks, paints, varnishes, adhesives and other coatings such as beverage container linings has long been reported (IARC, 1982; ACGIH, 1986). It has also been reported useful as a solvent to separate closely related substances such as fatty acids; as an intermediate in some chemical (such as 2, 2-Dinitropropane; 2-Nitro-2-methyl-1-propanol) syntheses; as fuel additive; and also for its carcinogenic properties (IARC, 1982). Kido *et al*. (1975) had long ago reported the antimicrobial properties of 2-Nitro-Dimethylnitromethane against bacteria (*Escherichia coli*, *Pseudomonas*

iodinum), yeasts (*Endomyces fibuliger*, *Hansenula anomala*, *H. octospora*, *H. sauveolens*, *H. matritensis*), and fungi (*Aspergillus niger*, *Penicillium oxalicum*, *Fusarium oxysporum*).

This work which is the first-time report on the bioactive compounds in the organic crude extracts of *Dioscorea bulbifera* native to Nigeria, using the GC-MS, has however provided an overview of different classes of molecules present, to which their pharmacological activities could be attributed. The presence of these secondary metabolites in the peel of *Dioscorea bulbifera*, is hence, the major contributing factors behind its antimicrobial potential reported by Adeosun *et al*. (2016).

Table 3: Chemical structures of compounds in the ethanolic extract of the peel of *D. bulbifera*

Name of the compound	Chemical structure of the compound
R- (-) - 1, 2 - Propanediol	
2-Nitro-Dimethylnitromethane	
1-Methylhexylhydroperoxide	
Ethylenimine	
N-Formyl-N-Methyl-formamide	
Guanidine	
Cis-3-hexenyllactate	
Pyrrole	

4. Conclusion

The present study has established the presence of quite number of chemical compounds in the peel of *Dioscorea bulbifera* Linn, which may be responsible for its pharmacological properties, like antibacterial activities. This work also showcased that this tuber can be as effective as modern medicine to combat pathogenic microorganisms and overwhelming the antibiotic resistance. Further studies are needed on these bioactive compounds including *in-vivo* toxic effects in experimental animals to formulate a new drug for regular practice.

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