PHARMACOLOGICAL AND ANTIOXIDANT PROPERTIES OF A HYDROMETHANOL EXTRACT OF *DIOSCOREA BULBIFERA* (AIR POTATO)

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CERTIFICATION

We hereby certify that this thesis, "Pharmacological and Antioxidant properties of a Hydromethanol Extract of *Dioscorea bulbifera* (air potato)", is an original work of Nwoke, Uchenna D. with registration number 20134870588, and it has been accepted in partial fulfillment of the requirements for the award of Master of Science (M.Sc.) degree in Biochemistry of the Federal University of Technology Owerri.

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DEDICATION

This work is dedicated to God almighty who made everything possible and to my dear father Chief Francis Darby Nwoke who set the pace.

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ABSTRACT

Dioscorea bulbifera is one of the over 600 known species of yam and is distinguished from all other species by having specialized aerial bulbils on the base of petioles. This work investigated some pharmacological and antioxidant properties of this endemic but neglected yam specie. In the study, the extract was assessed for proximate content, phytochemical content, free-radical scavenging, antioxidant potentials and antisickling effects. The proximate and the phytochemical constitution were performed according to standard analytical procedures available in the literature. In vitro antioxidant activity was studied through nitric oxide radical, hydroxyl radical, DPPH radical scavenging ability and reducing power assay. Furthermore, the antisickling effects of the extract were also studied based on its ability to inhibit haemoglobin polymerization of sickle cell erythrocytes. The results of the proximate analysis showed, moisture content (12.10 ± 0.61%), ash (12.00 ± 0.68%), crude fibre (1.50 ± 0.08%), crude protein (10.10 ± 0.53%), crude fat (1.20 ± 0.06%) and carbohydrate (63.10 ± 4.12%). Quantitative phytochemical study showed the presence of alkaloids (3.30 ± 0.13%), saponins (0.50 ± 0.02%), total glycosides (0.80 ± 0.03%), tannins (0.17 ± 0.01%), flavonoids (0.05 ± 0.04%) and phenolics (0.03 ± 0.10%). Moreso, D. bulbifera extract was effective against nitric oxide radical, hydroxyl radical, DPPH radical scavenging with potential inhibitory concentration (IC50) values of 1257.01 ± 58.37 (µg/ml), 633.60 ± 54.52 (µg/ml), 2285.78 ± 245.20 (µg/ml) respectively. The O.D0.5 of the extract was 480.63 ± 33.82 (µg/ml). Moreso, the extract showed the highest level of inhibition of haemoglobin HbS polymerization at 15.84 ± 0.74%. The results of this work showed that D. bulbifera is a good source of essential nutrients and can be a reliable food source and energy security crops to both man and/or livestock.The phytochemicals present could be the reason behind its medicinal uses. Results obtained also showed the ability of the extract to scavenge free radicals in dose-dependent manner. It therefore had good antioxidant properties. The extract also inhibited haemoglobin polymerization to a varying degree with good inhibition percent polymerization when compared to standard HBA. The extract therefore could be used for the treatment of oxidative stress induced diseases, management of sickle cell disease and other related diseases.

KEYWORDS: D.bulbifera, free-radical scavenging activity, in vitro antioxidant activity, proximate analysis, antisickling.

CHAPTER ONE

INTRODUCTION

1.1 Background Information

Yams are the edible tubers of various species of the genus *Dioscorea* and are important staple foods of many tropical countries including Cộte d'Ivoire, Ghana, Togo, Burkina Faso and Nigeria (Kouakou *et al*., 2010; Amanze *et al*., 2011). It is a major contributor to food security in West Africa (Zannou, 2006), but out of the over 600 known yam species, only seven are mostly consumed (Jayakody *et al*., 2007). These include *Dioscorea rotundata Poir* (White yam), *Dioscorea cayenensis* (Yellow yam), *Dioscorea alata* (Water yam), *Dioscorea bulbifera* (Aerial yam), *Dioscorea esculenta, Dioscorea praehensalis* (Bush yam) and *Dioscorea dumetorum* (Bitter yam).

Yam is cultivated mainly in three areas of the world. West Africa and parts of East, Central and Southern Africa (FAO, 1999) are the primary cultivation areas, producing about 95% of the world yam production, followed by Southeast Asia including China, Japan and Oceania. The third area includes the Caribbean, Mexico, and parts of Central America (FAO, 1999). According to FAO statistics, 48.7 million tonnes of yams were produced on five million hectares in about 47 countries worldwide in 2005, and 97% of this was in sub-Saharan Africa (FAO, 2008). West and Central Africa account for 94% of world production. Nigeria is the leading producer with 34 million tonnes followed by Côte d'Ivoire (5 million tonnes), Ghana (3.9 million), and Bénin (2.1 million tonnes). Average yam consumption per capita per day is highest in Bénin (364 kcal) followed by Côte d'Ivoire (342 kcal), Ghana (296 kcal), and Nigeria (258 kcal) (IITA, 2009).

Dioscorea bulbifera is distinguished from all other species by having specialized aerial bulbils on the base of petioles (Martin, 1974).

The bulbelates of *D. bulbifera* have very high dry matter content; the flesh being very firm after cooking. *D. bulbifera* produces bulbilates 4-6 months after planting. *Dioscorea bulbifera* are also called aerial yam or air potato because the bubils are about the size of potatoes. It is found commonly in eastern part of Nigeria. *Dioscorea bulbifera* known as adu in Igbo in eastern part of Nigeria is a climber plant with tuberous root.

Dioscorea bulbifera, the "air potato", is found in both Africa and Asia, with slight differences between those found in each place. It is a large vine, 6 meters (20 ft) or more in length. It

produces tubers; however the bulbils which grow at the base of its leaves are the more important food product. They are about the size of potatoes, weighing from 0.5 to 2 kilograms (1.1 to 4.4 lb).

Some varieties can be eaten raw while some require soaking or boiling for detoxification before eating. It is not grown much commercially since the flavor of other yams is preferred by most people. However, it is popular in home vegetable gardens because it produces a crop after only four months of growth and continues producing for the life of the vine, as long as two years. Also the bulbils are easy to harvest and cook (Kay, 1987).

Tubers are toxic or edible according to the variety; they are renewed annually. *Dioscorea bulbifera* L. var sativa grows wild and has bitter tubers and bulbils (Murray *et al*., 1984). A steroidal saponin, spiroconazole A, a phenanthrene, 2,7-dihydroxy-4-methoxyphenanthrene, flavonoids as quercetin, quercetin-3-O-β-D-glucopyranoside, and quercetin-3-O-β-D-galactopyranoside, and seven clerodane diterpenoids namely, bafoudiosbulbins A, B, C, D, E, F, and G have been isolated from the methanol extract of the bulb of*Dioscorea bulbiferavar sativa*(Komori,1997). *Dioscorea bulbifera L. var* sativa is used in Bangladesh for the treatment of leprosy and tumours (Murray *et al*.,1984) and by the native people of the western highlands of Cameroon for the treatment of pig Cysticercosis.

The roots of *Dioscorea bulbifera L*, although considered poisonous because of cytotoxic activity, have been used in Chinese medicine as a remedy for sore throat and for struma (Cogne, 2002). In Zimbabwe, this plant is used as an infusion to apply on cuts and sores, both for humans and animals while in Cameroon and Madagascar, the pounded bulbs are applied to abscesses, boils, and wound infections (Gupta and Singh,1989). Its bulbs are used in India to treat piles, dysentery, syphilis and are applied to ulcers, pain, and inflammation (Richard, 1998). Although the plant material has been long used to treat pain and inflammation, no scientific work has been carried out to ascertain the claimed properties.

D. bulbifera Linn also have antinutritional factors. In Asia, detoxification methods, involving water extraction, fermentation and roasting of the grated tuber, are used for bitter cultivars of this yam. The bitter compounds in aerial yam include diosbulbin and possibly saponins. These substances are toxic, causing paralysis. Extracts are sometimes used in fishing to immobilize the fish and thus facilitate capture.

The community of Zulus use this yam as bait for monkeys, while hunters in Malaysia use it to poison wildlife such as tigers. In Indonesia, an extract of air potato is used in the preparation of arrow poison (Oke, 1990).

However, traditional food processing techniques were scientifically validated as most efficient for removal of bitter and toxic compounds from *D.bulbifera* tubers. Bitterness due to presence of saponins and sapogenins in Central American, South African and Indian species, tannins and polyphenols in Indo-Chinese varieties and furanoidnorditerpenes (diosbulbins) mostly in China, can be effectively removed by the traditional techniques like leaching, thereby, rendering palatability to the processed sliced tubers, otherwise described as "cheeky", meaning bitter or poisonous in aboriginal English. Treatment practices varying from baking, followed by overnight leaching of the sliced tubers for 12 h in running water, resulted in reduction of major bitter and toxic compound, diosbulbin D (0.07 mg/g) decreasing it to a very low level under the taste threshold rendering the final food palatable (Naik and Maheswarappa, 2007).

Under cultivation the plant loses its bitterness and is much grown for the tubers, which are roasted, cooked and eaten.

Free radicals are produced as a part of normal metabolic processes. They are extremely reactive, highly unstable and potentially damaging transient chemical species. Under physiological conditions, the cellular redox state is tightly controlled by antioxidant enzymatic systems and chemical scavengers such as endogenous enzymes, dietary antioxidants as well as some hormones (Dekker,1996). Antioxidants scavenge free radicals and quench the subsequent reactions, hence protecting the macromolecules and cellular environment from toxicity anddegeneration (Vichneveshaia and Roy, 2001).

However, oxidative stress, a key player in several diseases such as cancer, diabetes mellitus, atherosclerosis, cardiovascular diseases, ageing and inflammatory diseases, results from an imbalance between formation and neutralization of prooxidants (Mohamed *et al.,* 2013).

Oxidative stress is initiated by free radicals, which seek stability through electron pairing with biological macromolecules such as proteins, lipids and DNA in healthy human cells and cause protein and DNA damage along with lipid peroxidation (Sun *et al.,* 2012). Enzymes, particularly superoxide dismutase (SOD) and catalase as well as compounds like tocopherol, ascorbic acid and glutathione play a key role in protecting human cells from free radical mediated damage (Hazra *et al.,*2008). In conditions, where free radical production rate may

exceed the capacity of antioxidant defense mechanisms results in substantial tissues injury. Antioxidant principles from medicinally important plants possess enormous potential in correcting imbalance mediated oxidative stress and various degenerative diseases (Londhe *et al.,* 2009).Therefore to combat the free radical chain propagation effect, the body uses antioxidants (chemical electron sink) which quench the biochemical fire. The antioxidants include enzymes such as glutathione peroxidase, catalase and superoxide dismutase. Vitamin C, vitamin E, and A, beta carotene, coenzymeQ10 and some phytochemicals.

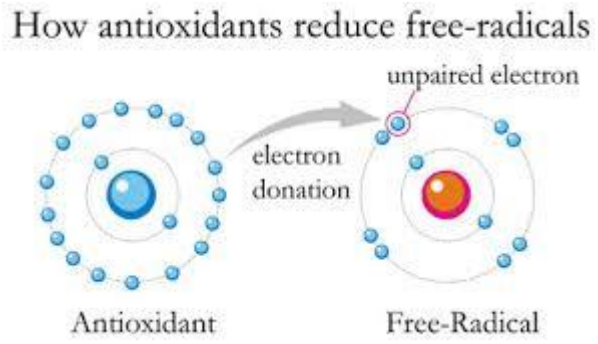


Fig1.0: How antioxidants reduce free-radicals (Dekker, 1996).

Recently, much attention has been directed towards development of ethnomedicines with strong antioxidant properties but low cytotoxicity. Additionally, it has been determined that antioxidant effect of plant products is mainly due to radical-scavenging activity of phenolic compounds such as flavonoids, polyphenols, tannins, and phenolic terpenes (Shetty *et al.*,2008).

Traditional medicinal plants have served to be efficient antidiabetic agents for ages due to their rich diversity of phytochemicals. Thus, there lies a profound scope of discovery of new molecules with pharmacological significance towards management of type II diabetes mellitus (T2DM). It was reported for the first time on detailed mechanism of antidiabetic potential of *D. bulbifera* as well as its applications in nanobiotechnology (Ghosh *et al.*, 2012).

Furthermore, the continuous emergence of Gram-negative MDR bacteria drastically reduces the efficacy of our antibiotic armory and, consequently, increases the frequency of therapeutic failure (Rice, 2006). On the other hand, the World Health Organization (WHO) estimates that there are nine million cases of tuberculosis (TB) currently, with 1.3 million reported deaths every year, 55 and 30% of the TB burden being shared by Asian and African countries respectively (WHO, 2010). Approximately 60% of world's population still relies on medicinal plants for their primary healthcare.

Medicinal plants have been used as a source of remedies since ancient times in Africa. *Dioscorea bulbifera L. var sativa* (*Dioscoreaceae*) is an African medicinal plant used to treat microbial infections and pig cysticercosis by the native people of western highlands of Cameroon. The plant is also used as a folk remedy to treat conjunctivitis, diarrhea and dysentery, among other ailments (Duke and Ducellierr, 1993). Previous phytochemical study on this medicinal plant led to the isolation and structural elucidation of seven new clerodane diterpenoids namely Bafoudiosbulbins A-G (Teponno *et al*., 2007) Furthermore, the extracts and Bafoudiosbulbins A and B were shown to possess anti-*Salmonella* activity (Teponno, 2006). In the study, the bioguided fractionation was undertaken in order to deeply evaluate the antimicrobial activity of *D. bulbifera.*

Sickle-cell disorder (SCD), or sickle-cell anaemia is an autosomal recessive genetic blood disorder with overdominance, characterized by red blood cells that assume an abnormal, rigid, sickle shape and known to be one of the diseases afflicting the population living mostly in Africa, South America and Asia. It also occurs in other ethnic groups, including people who are of Mediterranean and Middle Eastern descent. As a genetic hereditary disease, no specific drugs are yet available; however several treatments have been investigated: (1) medullar transplantation is not only expensive but also faces incompatibility problems; (2) various proposed drugs (hydroxyurea, piracetam, calcium antagonists) tested against this disease for inhibition of hemoglobin S polymerization inside erythrocytes in order to increase the foetal hemoglobin rate (HbF) or decrease the sickling are toxic especially for a long time of use. Considering all genetic disorders, the debilitating effect, the cost of managing the SCD and the greater quantities of O^{2-}, H_2O_2, •OH radicals produced from sickle RBCs than do normal RBCs, research has been on-going to determine the efficacy of the use of natural products such as medicinal plants, nutritional complement against sickle cell disorder.

In view of this background, there is a growing interest to investigate the unexplored potential of this endemic but neglected medicinal plantfound in Africa, India, Southeast Asia, Australia and tropical America.

1.2 AIMS AND OBJECTIVES

The aims and objectives of this study were to investigate the pharmacological and antioxidant properties of *D. bulbifera* extract.

1.3 SPECIFIC OBJECTIVES

The specific objectives of this study included the following:

1. To determine the Proximate composition of *D. bulbifera* extract
2. To determine the Phytochemical constituents of *D. bulbifera* extract
3. To determine the antioxidant properties of *D. bulbifera* extract
4. To determine the antisickling effect of *D. bulbifera* extract.

CHAPTER TWO

LITERATURE REVIEW

2.1: Phytochemical composition of *Dioscorea bulbifera*

Plants of genus *Dioscorea* have long been served as important carbohydrate foodstuff in the tropical and subtropical regions, and utilized as traditional herbal medicines to enhance digestive function, improve anorexia, and treat diarrhoea in oriental countries. It is known that around 600 species of *Dioscorea* are distributed in the world including 107 species in Asia, but actually utilized *Dioscorea* species are restricted to small numbers. Phytochemical investigations for *Dioscorea* species have revealed a number of chemical components such as sapogenins, saponins, phenanthrenes, stilbenes, diterpenes and purine derivatives. According to recent pharmacological studies, *Dioscorea* species possess significant antioxidant, antibacterial and anti-inflammatory activities as well as anticancer, antidiabetic, cholesterol-lowering and hypolipidemic effects. (Niyas, 2015). Also, the study carried out by Polycarp *et al.,* (2012) characterized the most cultivated and consumed yam (*Dioscorea*) cultivars within the Ghanaian yam germplasm based on their chemical composition and anti-nutritional factors. Samples were analyzed for proximate composition, mineral content and levels of tannins, phytates and oxalates using standard analytical methods. Significant differences existed between the means of the yam varieties based on their chemical characteristics.

Subasini *et al.,*(2013) carried out the pharmacognostic and phytochemical investigations of *D. bulbifera* in Namakkai, India and reported that *Dioscorea bulbifera* tubers possess significant activities like -purgative, deflatulent, aphrodisiac, rejuvenating and tonic, anthelmintic and is used in haematological disorders, scrofula, syphilis, haemorrhoids, flatulence, diarrhoea, dysentery, worm infestations, general debility, diabetic disorders, polyuric and skin disorders which comply with the claims made in the traditional medicinal texts. Moreso, according to Raphael *et al.,* (2016) in their study of the pharmacognostic, fluorescent, antibacterial and phytochemical analysis of tuber of *D. bulbifera* L from Jharkand ; Phytochemical screening of the tuber extracts exhibited the presence of alkaloids, starch, coumarin, flavonoids, steroids, terpenoids, cardiac glycosides, phenols, tannins and free amino acids in high concentrations. Analytical HPLC chromatogram revealed only a few bioactive compounds but in good concentrations. GC-MS analysis detected the presence of only five bioactive compounds. Fluorescent study exhibited characteristic colour data while the pharmacognostic evaluation indicated the storage of high amount of starch grains. The

study is supportive of the ethnomedicinal usage of tuber of *D. bulbifera* for the treatment of malaria, diarrhoea, aphrodisiac, rejuvenating and diabetes which may give lead to further the research in isolation and purification of noble drugs for the treatment of given diseases. Qualitative phytochemical analysis was also done in root tubers of six species of *Dioscorea* found in Meghalaya by Nilofer *et al* (2013). The test confirms the presence of various phytochemicals like flavonoids, saponins, steroids, cardiac glycosides and terpenoids in two aqueous extracts of methanol and ethyl acetate. Okwu and Ndu, (2006) evaluated the phytonutrients, minerals and vitamin contents of some varieties of yam (*Dioscorea sp*) in Umuahia, Abia State, Nigeria. All the species studied were found to contain bioactive compounds comprising saponins (2.98-19.4mg100-1g), alkaloids (0.38-1.68mg100-1g), flavonoids (1.10-19.94mg100-1g), tannins (4.4×10-2-9.0×10-2mg100-1g), and phenols (2.4×10-3-5.0×10-3mg100-1g). The yams contained vitamins such as ascorbic acid, niacin, riboflavin and thiamin. Appreciable quantities of calcium, magnesium, phosphorus, potassium and sodium were detected in the tubers. The importance of these chemical constituents is discussed with respect to the role of these *Dioscorea* species in herbal medicine in Nigeria.

2.2: Nutritional Significance of *D. bulbifera*

Yam is composed mainly of starch, with some proteins, lipids, vitamins and minerals (Lasztity *et al.*, 1998). Afoakwa and Sefa-Dedeh, (2001) reported that *D. dumetorum* is the most nutritious of the commonly consumed yam species, with fairly high protein content and a well balanced amino acid. Agbor-Egbe and Treche, (1995) reported a starch content of 15-38% (fresh/wet weight) and 70-80% (dry weight basis) in yams from Cameroon.They reported high moisture content of the fresh tubers and ranged between 58.18 to 77.79%. The varieties had low fat (<1.0%), protein (4.0-6.5%) and fibre (1.25-3.47%) with high carbohydrate (77.5-87.3%) and energy (1451.2-1574.7 kJ/100g). The most predominant minerals were potassium (475-1475 mg/100g), phosphorus (158-294.5 mg/100g) and sodium (62.5-102.5 mg/100g). All the studied varieties had low levels of oxalates, tannins and phytates (<15 mg/100g) and could all be safely recommended for food processing applications. *D. rotundata, D. praehensalis, D. cayenensis* and *D. bulbifera* differed from the rest by having higher levels of carbohydrate and energy with appreciable levels of minerals that make them nutritious and can be used as reliable food and energy security crops. *D.*

rotundata variety distinguishes itself because of low moisture content (high dry matter) that makes it suitable for high yield flour production.

Zhi-Gang *et al.,* (2016) also investigated and characterized diversity based on nutritional and bioactive compositions of yam germplasm (*Dioscorea* spp) commonly cultivated in China. In the study, a core germplasm containing 25 yam landraces was used to create an effective classification of usage by characterizing their nutritive and medicinal compositions. All studied landraces exhibited high contents of starch from 60.7% to 80.6% dry weight (DW), protein (6.3–12.2% DW), minerals (especially Mg 326.8–544.7 mg/kg DW), and essential amino acids. Allantoin and dioscin varied considerably, with values of 0.62–1.49% DW and 0.032–0.092% DW, respectively. However, *Dioscorea bulbifera* yam is used as food and it is a good source of calories and minerals such as iron, calcium and phosphorous (Tindall, 1983; Abara *et al.,* 2000). Data on proximate composition of yams including *Dioscorea bulbifera* in the available literature indicate that the proximate principles vary with species, level of maturity, between different parts of the tubers and cooking procedure (Coursey and Walker, 1960; Oyenuga, 1968; Martin, 1979; Ferguson *et al.,* 1980; Bell and Favier, 1981). Consequently, moisture, ash, protein, fat, fibre and carbohydrate content of yams vary widely among species and between cultivars. Available data indicate that the tuber peels are richer in ash, fat, protein and crude fibre than the tissue of the tubers (Oyenuga,1968; Martin, 1979; Ferguson *et al.,* 1980) although the availability of the nutrients from the peel may be limited by the high fibre content as this may affect digestibility (Eka, 1985). Data from available literature also indicate that yams are generally low in fat and protein but high in moisture and carbohydrate content (Oyenuga, 1968; Ferguson *et al.,* 1980). Chandra *et al.,* (2012) investigated the nutritional profile and phytochemical screening of Garhwal Himalaya medicinal plant *Dioscorea bulbifera* and revealed that *Dioscorea bulbifera* (Family *Dioscoreacea*) possess profound therapeutic potential. The study was undertaken to investigate the nutritional profile, successive value, thin layer chromatography and phytochemical screening of *Dioscorea bulbifera*. The ash value (total ash 2.94%), moisture 62.80%, crude fat 1.20% and crude fiber 7.50%. Extractive values were studied fresh weight. Preliminary phytochemical analysis test showed the presence of carbohydrate and glycosides, alkaloids, flavonoids, saponins, tannins, unsaturated triterpenoids and sterol, resins.

Shajeela *et al.,* (2011) studied the nutritional and antinutritional evaluation of wild yam (Dioscorea spp.) consumed by the tribes Kanikkars / Palliyars of South- Eastern slopes of Western Ghats, Tamil Nadu, India (*Dioscorea alata, D. bulbifera var vera, D. esculenta, D.*

oppositifolia var dukhumensis, D.oppositifolia var. oppositifolia, D. pentaphylla var. pentaphylla, D. spicata, D. tomentosa and D. wallichi) were evaluated for its nutritional quality. From the investigation, it is observed that most of the wild edible yams were found to be a good source of protein, lipid, crude fibre, starch, vitamins and minerals. Antinutritional substances like total free phenolics, tannins, hydrogen cyanide, total oxalate, amylase and trypsin inhibitor activities were quantified.

Abara, (2011) investigated the proximate and mineral elements composition of the tissue and peel of *Dioscorea bulbifera* tuber in Calabar, Nigeria. All the parameters analyzed apart from carbohydrate and energy value showed tissue/peel ratios of less than 1.0 which was consistent with the observed significantly lower levels of the mineral elements and proximate principles in the tissue than the peel excepting carbohydrate and energy value. Additionally, the about constant tissue/peel ratios observed for the mineral elements and some proximate principles such as ash, crude fibre, fat and protein for the wet and dry samples of *Dioscorea bulbifera* were indicative of the stability of these nutrients and in about the same proportions in the tissue and peel upon drying at 40^{o}C. The nutritional significance of the mineral elements in the tissue and peel of *Dioscorea bulbifera* was also highlighted. Ogbuagu, (2008) studied the determination of Nutritive and Anti-Nutritive Composition of the Wild (Inedible) Species of *Dioscorea bulbifera* (Potato Yam) and *Dioscorea dumentorum* (Bitter Yam) in Umuahia, Abia State. According to him the determination of the nutritive and anti-nutritive principles in the uncooked and cooked wild species of *Dioscorea bulbifera* and *Dioscorea dumentorum* showed that the wild yams contain all the food nutrients within the reported and acceptable values for root and tuber crops. The presence of alkaloids, oxalates, and saponins at high concentrations are responsible for the qualification of the yam species as wild (poisonous) and inedible. However, the cooking of these yam species reduced these principles to values that may earn the wild yams consideration as food for consumption. Princewill-Ogbonna, and Ibeji, (2015) carried out the comparative study on nutritional and anti nutritional composition of three cultivars (red, green and yellow) of aerial yam (*Discorea bulbifera*) in Umuahia, Abia State, Nigeria. The nutritional composition studied included; proximate composition, mineral and vitamin content of the three cultivars. The red cultivar had the highest moisture, carbohydrate and ash content (7.71%, 77.41%, and 3.97% respectively). The protein content (9.62%) of the green cultivar was significantly higher than that of the red (6.82%) and yellow (7.27%) cultivars. The green cultivar also had the least crude fibre (1.63%) and fat (0.37). The yellow cultivar had the highest crude fibre (2.45%) and crude fat (4.15%) content. The

Manganese (100.02mg/100g) and sodium (2403.0mg/100g) content of the red cultivar was significantly higher than that of the yellow and green cultivars. However, the yellow cultivar had the highest potassium 667.53mg/100mg) and the least sodium (87.25mg/100g) content. The vitamin content of the cultivars varied from 137.24 -700.88mg/100g). The red cultivar had the highest vitamin A content (700.88mg/100g) while the yellow cultivar had the least value (137.24mg/100g).The saponin (14.03mg/100 g), oxalate (12.60mg/100g) and tannin (0.22mg/100g) were highest in the green cultivar while the red cultivar had the least saponin and oxalate content, 5.46mg/100g and 9.00mg/100g, respectively. Furthermore, Uwaegbute, *et al*, (1998) reviewed the nutritional value of *Dioscorea* species and noted that the protein content and quality of roots and tubers are lower than other food staples. Of all roots and tubers, the protein content of yam and potato was the highest, being approximately 2 percent on a fresh weight basis. Yams, with cassava, provide a much greater proportion of the protein intake in Africa, ranging from 6 percent in East and southern Africa to about 16 percent in humid West Africa. Yam, like other root crops, is not a good source of essential amino acids. It is rich in phenylalanine and threonine but limiting in the sulphur amino-acids, cysteine, methionine and tryptophan. Yam consuming areas of Africa have a high incidence of kwashiorkor, a serious medical condition in children caused by protein deficiency. Experts emphasize the need to supplement a yam-driven diet with more protein-rich foods in order to support active and healthy growth in infants. Except for potassium, vitamin B and vitamin C, yam is a food with moderate nutrient density. Yam provides around 110 calories per 100 grams. It has good levels of potassium, manganese, thiamin and dietary fiber, while being low in saturated fat and sodium. Yam generally has a lower glycemic index, about 54% of glucose per 150 gram serving, compared to potato products (Harvard Medical School, 2008). United States Department of Agriculture, (2014) also compared the nutrient content of yam and major staple foods in a raw harvested form. Raw forms, however, are not edible and can not be digested. These must be sprouted, or prepared and cooked for human consumption. In sprouted or cooked form, the relative nutritional and anti-nutritional contents of each of these staples were remarkably different from that of raw form of these staples reported in the table below.

Table 2.1: Nutrient content of major staple foods(United States Department of Agriculture,2014)

STAPLE:	RDA	Maize / Corn[A]	Rice (white)[B]	Rice (brown)[I]	Wheat [C]	Potato [D]	Cassava[E]	Soybean (Green)[F]	Sweet potato[G]	Sorghum[H]	Yam[Y]	Plantain[Z]
Component (per 100g portion)	Amount	Amount	Amount	Amount	Amount	Amount	Amount	Amount	Amount	Amount	Amount	Amount
Water (g)	3000	10	12	10	13	79	60	68	77	9	70	65
Energy (kJ)		1528	1528	1549	1369	322	670	615	360	1419	494	511
Protein (g)	50	9.4	7.1	7.9	12.6	2.0	1.4	13.0	1.6	11.3	1.5	1.3
Fat (g)		4.74	0.66	2.92	1.54	0.09	0.28	6.8	0.05	3.3	0.17	0.37
Carbohydrates (g)	130	74	80	77	71	17	38	11	20	75	28	32
Fiber (g)	30	7.3	1.3	3.5	12.2	2.2	1.8	4.2	3	6.3	4.1	2.3
Sugar (g)		0.64	0.12	0.85	0.41	0.78	1.7	0	4.18	0	0.5	15
Calcium (mg)	1000	7	28	23	29	12	16	197	30	28	17	3
Iron (mg)	8	2.71	0.8	1.47	3.19	0.78	0.27	3.55	0.61	4.4	0.54	0.6
Magnesiu	400	127	25	143	126	23	21	65	25	0	21	37

m (mg)												
Phosphorus (mg)	700	210	115	333	288	57	27	194	47	287	55	34
Potassium (mg)	4700	287	115	223	363	421	271	620	337	350	816	499
Sodium (mg)	1500	35	5	7	2	6	14	15	55	6	9	4
Zinc (mg)	11	2.21	1.09	2.02	2.65	0.29	0.34	0.99	0.3	0	0.24	0.14
Copper (mg)	0.9	0.31	0.22		0.43	0.11	0.10	0.13	0.15	-	0.18	0.08
Manganese (mg)	2.3	0.49	1.09	3.74	3.99	0.15	0.38	0.55	0.26	-	0.40	-
Selenium (µg)	55	15.5	15.1		70.7	0.3	0.7	1.5	0.6	0	0.7	1.5
Vitamin C (mg)	90	0	0	0	0	19.7	20.6	29	2.4	0	17.1	18.4
Thiamin (B1)(mg)	1.2	0.39	0.07	0.40	0.30	0.08	0.09	0.44	0.08	0.24	0.11	0.05
Riboflavin (B2)(mg)	1.3	0.20	0.05	0.09	0.12	0.03	0.05	0.18	0.06	0.14	0.03	0.05
Niacin (B3) (mg)	16	3.63	1.6	5.09	5.46	1.05	0.85	1.65	0.56	2.93	0.55	0.69
Pantothenic acid(B5)	5	0.42	1.01	1.49	0.95	0.30	0.11	0.15	0.80	-	0.31	0.26

(mg)												
Vitamin B6 (mg)	1.3	0.62	0.16	0.51	0.3	0.30	0.09	0.07	0.21	-	0.29	0.30
Folate Total (B9) (µg)	400	19	8	20	38	16	27	165	11	0	23	22
Vitamin A (IU)	5000	214	0	0	9	2	13	180	14187	0	138	1127
Vitamin E, alpha-tocopherol (mg)	15	0.49	0.11	0.59	1.01	0.01	0.19	0	0.26	0	0.39	0.14
Vitamin K1 (µg)	120	0.3	0.1	1.9	1.9	1.9	1.9	0	1.8	0	2.6	0.7
Beta-carotene (µg)	10500	97	0		5	1	8	0	8509	0	83	457
Lutein+zeaxanthin (µg)		1355	0		220	8	0	0	0	0	0	30
Saturated fatty acids (g)		0.67	0.18	0.58	0.26	0.03	0.07	0.79	0.02	0.46	0.04	0.14
Monounsaturated fatty acids (g)		1.25	0.21	1.05	0.2	0.00	0.08	1.28	0.00	0.99	0.01	0.03

Polyunsaturated fatty acids (g)		2.16	0.18	1.04	0.63	0.04	0.05	3.20	0.01	1.37	0.08	0.07

A corn, yellow	B rice, white, long-grain, regular, raw, unenriched
C wheat, hard red winter	D potato, flesh and skin, raw
E cassava, raw	F soybeans, green, raw
G sweet potato, raw, unprepared	H sorghum, raw
Y yam, raw	Z plantains, raw
I rice, brown, long-grain, raw	

2.3: Antioxidant Properties of *D. bulbifera*

Suriyavathana and Indupriya, (2011) carried out the screening of antioxidant potentials in *Dioscorea bulbifera* at Salem, (Tamil Nadu) - India.They reported that *Dioscorea bulbifera* have been traditionally used to lower glycemic index, thus providing a more sustained form of energy and better protection against obesity and diabetes. It also has anti-cancer properties. However, the study was undertaken to investigate the antioxidant activity of *Dioscorea bulbifera*. The ethanolic extracts of tuber Dioscorea bulbifera were screened for their enzymatic and nonenzymatic antioxidant activity. The level of enzymatic antioxidant namely Glutathione peroxidase (GPx), Catalase (CAT), Superoxide dismutase (SOD), Glucose-6-phosphate dehydrogenase (G6PD) and glucose-s-transferase (GST) was found to be very impressive. *Dioscorea bulbifera* contains good and commendable store of non enzymatic antioxidants namely reduced glutathione (GSH), Vitamin – C and Vitamin – E. Their results have good significance, as this increase the innate antioxidant potential of *Dioscorea*

bulbifera, which is useful in providing the antioxidants needs in the diet and thereby *Dioscorea bulbifera* accomplishes high value nutritive and natural store of antioxidant. Moreover, Ghosh *et al.,* (2013) studied the phytochemical analysis and free radical scavenging activity of medicinal plants Gnidia glauca and *Dioscorea bulbifera* and found out that *Gnidia glauca* and *Dioscorea bulbifera* are traditional medicinal plants that can be considered as sources of natural antioxidants. They reported the phytochemical analysis and free radical scavenging activity of their sequential extracts. Phenolic and flavonoid content were determined. Scavenging activity was checked against pulse radiolysis generated ABTS•+ and OH radical, in addition to DPPH, superoxide and hydroxyl radicals by biochemical methods followed by principal component analysis. *G. glauca* leaf extracts were rich in phenolic and flavonoid content. Ethyl acetate extract of *D. bulbifera* bulbs and methanol extract of *G. glauca* stem exhibited excellent scavenging of pulse radiolysis generated ABTS•+ radical with a second order rate constant of 2.33×106 and 1.72×106, respectively. Similarly, methanol extract of *G. glauca* flower and ethyl acetate extract of *D. bulbifera* bulb with second order rate constants of 4.48×106 and 4.46×106 were found to be potent scavengers of pulse radiolysis generated OH radical. *G. glauca* leaf and stem showed excellent reducing activity and free radical scavenging activity. HPTLC fingerprinting, carried out in mobile phase, chloroform: toluene: ethanol (4: 4: 1, v/v) showed presence of florescent compound at 366 nm as well as UV active compound at 254 nm. GC-TOF-MS analysis revealed the predominance of diphenyl sulfone as major compound in G. glauca. Significant levels of n-hexadecanoic acid and octadecanoic acid were also present. Diosgenin (C27H42O3) and diosgenin (3á,25R) acetate were present as major phytoconstituents in the extracts of *D. bulbifera*. *G. glauca* and *D. bulbifera* contain significant amounts of phytochemicals with antioxidative properties that can be exploited as a potential source for herbal remedy for oxidative stress induced diseases. These results rationalize further investigation in the potential discovery of new natural bioactive principles from these two important medicinal plants. Abhishek *et al.,* (2012) in his comparative study between the aerial and underground tubers of *Dioscorea alata* L. for their antioxidant potentials by ABTS and DPPH methods, scavenging activities for various radicals including hydroxyl, nitric oxide, peroxynitrite, hypochlorous acid etc.; and iron chelating properties along with their total phenolic and flavonoid contents revealed that the aerial tuber showed considerable scavenging of hydroxyl and peroxynitrite radicals. The underground tuber showed a comparatively greater phenolic content than aerial tuber and a higher protection against hypochlorous acid damage at low doses and better reducing power. The underground tuber

showed a moderate activity in cases of other radicals like, DPPH, superoxide and singlet oxygen, when compared to aerial part. The scavenging activity for other radicals was found to be moderate with both the tubers. Javachandran *et al.*, (2012) studied the effect of flavonoid rich fraction of *Dioscorea bulbifera* Linn (yam) and revealed that it enhances mitochondrial enzymes and antioxidant status and thereby protects heart from isoproterenol induced myocardial infarction. According to him, with recent advances in nutrition sciences, natural products and health-promoting foods have received extensive attention from both health professionals and the common population. The flavonoid rich fraction (FRF) of *Dioscorea bulbifera* Linn. has a strong free radical scavenging activity. FRF (150 mg/kg) when intervened for a period of 35 days prior to isoproterenol (ISO) challenge to rats maintained the creatine kinase - MB (CK-MB) activity in serum without elevation. Alterations in the antioxidant status in the mitochondria were recognized in the heart tissue of ISO induced rats. ISO induced rats pretreated with FRF (150 mg/kg) ameliorated the lipid peroxidation and thereby enhanced the antioxidant status as evidenced by the increase in the reduced glutathione (GSH) content and the activity of antioxidant enzymes. Moreover, the tricarboxylic acid cycle enzymes such as isocitrate dehydrogenase (ICDH), succinate dehydrogenase (SDH), malate dehydrogenase (MDH) and α-ketoglutarate dehydrogenase (α-KGDH), which were found decreased in the ISO induced rats showed an enhanced activity in FRF (150 mg/kg) pretreated rats. The activity of NADH dehydrogenase and cytochrome-C-oxidase, the enzymes, which transfer the electron in the electron transport chain (ETC) was also increased significantly in FRF (150 mg/kg) pretreated rats, when compared with ISO induced rats. These results suggest the cardioprotective effect of FRF of *Dioscorea bulbifera* Linn. in ISO induced MI by attenuating the lipid peroxidation by scavenging free radicals and modulating the energy producing mitochondrial enzymes.

2.4: Medicinal applications and importance of *D. bulbifera*

Niyas, (2015) reviewed the medicinal uses of *Dioscorea bulbifera* and revealed that *Dioscorea bulbifera* possess potential therapeutic uses. According to the report, many tests prove phytochemically it contains flavnonoids, saponins, steroids, cardiac glycosides, terpenoids. Other aspects covered include anti-microbial, analgesic, anti-inflammatory and gastroprotective functions. Seetharam *et al.*,(2003) reported that the successive extracts of *Dioscorea bulbifera* (bulbils) has been investigated for *in vitro* antimicrobial activity against *Klebsiella pneumoniae, Escherichia coli, Bacillus aureus, Proteus vulgaris, Staphylococcus aureus, Aspergillus niger, Aspergillus flavus, Aspergillus fumigatus and Rhizopus nigricans.*

According to the report, the petroleum ether and chloroform extracts showed significant activity against *A. fumigatus and R. nigricans*. The petroleum ether and distilled water extract showed good activity against *K. pneumoniae*. The chloroform extract showed feeble activity against *S. aureus.* Also, Kuete *et al.*, (2012) investigated the antibacterial activities of the extracts, fractions and compounds of *Dioscorea bulbifera* and revealed that *Dioscorea bulbifera* is an African medicinal plant used to treat microbial infections. In the study, the methanol extract, fractions (DBB1 and DBB2) and six compounds isolated from the bulbils of *D. bulbifera*, namely bafoudiosbulbins A (1), B (2), C (3), F (4), G (5) and 2,7-dihydroxy-4-methoxyphenanthrene (6), were tested for their antimicrobial activities against Mycobacteria and Gram-negative bacteria involving multidrug resistant (MDR) phenotypes expressing active efflux pumps. Adeosun *et al.*, (2016) studied the antibacterial activities and the phytochemical properties of extracts *D.bulbifera* Linn tubers and peels against some pathogenic bacteria. According to them, the antibacterial activities and bioactive constituents of ethanolic and aqueous extract of parts of the tuber against ten (10) clinical pathogens were determined, using agar well diffusion and standard techniques respectively. The tuber was recorded to contain higher amount of saponin with the average of 24 mg/g, followed by cardiac glycosides with 13.13 mg/g, terpenoid with 8.48 mg/g, flavonoids followed with 5.36 mg/g and tannin with 4.21 mg/g was the least among the bioactive ingredients. Except for *Proteus vulgaris*, *Serratia liquefaciens*, *Micrococcus luteus*, *Bacillus cereus* and *Citrobacter freundii*, other test isolates were susceptible to the effect of the ethanolic extract of the peel of *D. bulbifera* at 500 µg/ml. High inhibition zones (between 17 and 22 mm) were recorded against 80% of the test organisms at 1000 µg/ml, except for 15 mm zone recorded against *Bacillus cereus*. The MIC and MBC of extract of *D. bulbifera* ranged in respect to the parts from 125 µg/ml to 500 µg/ml; and 250 µg/ml to 1000 µg/ml for peels and bulbils respectively. Antibacterial activity of the ethanolic and aqueous extracts of the bulbils of *D. bulbifera* was however, not profound in this present study compared to that of the peel. This study therefore, affirmed that *D. bulbifera* is a novel source of bioactive compounds which do not only enhance the antibacterial properties, but also ascertain its health promoting qualities.

Houston, (1973) revealed the anti-sickling effect of dietary thiocyanate in prophylactic control of sickle cell anemia. As a clinical entity, sickle cell anemia (SCA) is known to be relatively rarer in Africans than in the African-American population of the United States. Paradoxically, sickle cell trait (SCT), the non-anemic, heterozygous condition, is about three times more common among indigenous Africans than in African-Americans. The ratio of

SCA to SCT is 1:50 for African-Americans, and less than 1:1,000 for tropical Africans. This etiological disparity is attributed to an anti-sickling agent, thiocyanate, (SCN-) found abundantly in staple African foods, such as the African yam (*Dioscorea sp*) and cassava (*Manihot utilissima*). Staple American foods have negligible SCN-concentrations. Non staple foods in the American diet, such as carrots, cabbage, and radishes, have SCN- levels far below the African yam and cassava (Houston, 1973). This finding explains the high incidence of SCA among African-Americans and its rarity in Africans. The author concluded that SCA is a congenital deficiency anemia, ameliorable by prophylactic diets of foods with high SCN-contents. Thus, "thiocyanate deficiency anemia" is nutritionally a more correct clinical status for those born with the homozygous sickle haemoglobin genome.

Just as any iron undernourished person can suffer from iron deficiency anemia, sickle haemoglobin homozygotes suffer from "thiocyanate deficiency anemia" when they subsist on SCN-deficient foods. Furthermore, Nanfack Pualine *et al*., (2013) investigated the *in vitro* antisickling and antioxidant effects of aqueous extracts of *Zanthoxyllum heitzii* on sickle cell disorder. They reported that the result of induction of sickling with sodium metabisulphite (2%) shows an increase in sickling from 29.62 to 55.46%. Sodium metabisulphite (2%) induced sickling of erythrocytes with an average induction rate of 25.84% after 2 hr of incubation. After treatment of red blood cells with extracts at different concentrations (250, 500 and 1000 µg/mL), a significant decrease of the percentage of sickling cells was observed. This percentage of sickling varied from 38.78% at 1000 µg/mL to 44.05% at 250 µg/mL, depending on the part of the plant and the concentration of each extract used. The fruit extract demonstrated the highest decrease rate compared to others. The percentage of sickling cells increased with the extract concentration, although was non significant and non dose dependent for all extracts tested. The reversibility of the sickling cells was noted through its significant inhibition after 24 hr of SS-RBC incubation with the extracts of *Z. heitzii* at 250 µg/mL. The average rate of this inhibition varied between 19.31 and 16.62% depending on the extract. The fruit extract demonstrated the highest decrease rate while the lowest was that of the leaf. Regarding the erythrocyte osmotic fragility, this result showed a significant decrease of the percentage of hemolysis while increasing the concentration of salt solution at 250 µg/mL of extracts. All the extracts tested showed a lower hemolysis percentage compared to the control. Among the four extracts tested, that of the fruit presented the most significant reduction of heamolysis compared to extracts from other parts of the plant and then could have a better protective effect of the erythrocyte membrane. The anti-radical and antioxidant activities of the extracts using different methods showed that these activities vary

depending on the part of the plant. The IC_{50} of the extracts on DPPH and hydroxyl radicals varied from 6.60 to 32.25 μg/mL and from 9.2 to 3.5 μg/mL respectively. All the extracts have significantly inhibited the hydroxyl radicals with IC_{50} less than 3 μg/ml except the control. The noticeable reducing power activity and the antioxidant properties of this plant could serve as a significant indicator of the antioxidant potential. The results of reducing power activity showed that at 50 μg/mL all the extracts demonstrated a weak activity. The phytochemical screening of these extracts revealed the presence of alkaloids, phenols, mucilage and saponins. However, tannins and lipids were not detected in these extracts. Flavonoids are found in all extracts apart from that of the root.

Okigbo *et a.,l* (2009) investigated the Potential inhibitory effects of three African tuberous plant extracts of *Zingiber officinale* Rosc, *Curcuma longa* L. and *Dioscorea bulbifera* L. In their findings, ethanol and cold water were used as solvents for extraction. Three human pathogens, *Escherichia coli, Staphylococcus aureus* and *Candida albicans* were employed in this study. The potential inhibitory effects of the ethanol and aqueous extracts of *Zingiber officinale, Curcuma longa* and *Dioscorea bulbifera* on the test organisms were conducted using the disc-diffusion method of antimicrobial assay. Phytochemical screening of the plants was conducted using different standard methods. Standard antibiotics disc were used as positive control while discs impregnated in sterile distilled water were used as negative control. The ethanol extracts proved to be more potent than the aqueous extract. The potency of the extracts varied with solvent of extraction and concentration of the plant extracts. The minimum inhibitory concentration (MIC) of the ethanol extract ranged between 0.5-1.0 mg/ml on *E coli, Staphylococcus aureus* and *Candida albicans.* For the aqueous extract, MIC was 1.0 mg/ml on *E. coli* and ranged between 0.5-1.0 mg/ml on *S. aureus* and *C. albicans.* The solvent used for extraction varied significantly to the three test organisms. *Staphylococcus aureus* was more susceptible to the extract while *Candida albicans* was least inhibited. In all, Concentration of 1.5 mg/ml gave the maximum inhibition on the three test organisms. Phytochemical (qualitative) screening of the plants revealed the presence of biologically active chemical compounds such as tannins, Phenols, Saponins, alkaloids, flavonoids and Steroids/ triterpenes. The quantitative determination of the phytochemicals present revealed different levels of concentrations of the phytochemicals present. The significance of these findings are discussed in relation to phytochemicals as a means of disease control and the substitution of plant extracts as potential antimicrobial drug to the resistance of the human pathogens.

Okwu, *et al.,* (2016) carried out the comparative study of the phytochemical and antibacterial properties of two different varieties of *Dioscorea bulbifera* tubers and found out that thefour bacterial isolates were susceptible to the extracts from the wild type as compared to only two isolates that were susceptible to the edible variety. The methanol extract of the wild type indicated greater antibacterial activity against *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa* and *Streptococcus Pyogenes* with zones of inhibition values of 24.5mm, 18.5mm, 10.5mm and 7.5mm respectively. The minimum inhibitory concentration (MIC) of both methanol and ethanol extracts of the wild variety ranged between 25-50mg/ml. The bioactive compounds as revealed by phytochemical screening were saponin (11.5% and 6.80%), alkaloid (2.6% and 2.20%), Tannin (0.24% and 0.27%), Flavonoid (0.06% and 0.40%), steroid (0.1% and 0.03%) for the wild and edible varieties respectively. Methanol and ethanol extracts of the wild variety showed a more promising antibacterial with broad spectrum of activity than other extracts. The extracts of the types had better activity against all the test bacterial isolates when compared with the extracts of the edible variety.

Ghosh *et al.,* (2013) demonstrated the inhibitory activity of diosgenin from Dioscorea bulbifera against α-amylase and α-glucosidase as a novel hit for treatment of Type 11 diabetes mellitus. Diabetes mellitus is a multifactorial metabolic disease characterized by post-prandial hyperglycemia (PPHG). α- amylase and α-glucosidase inhibitors aim to explore novel therapeutic agents. They reported the promises of *Dioscorea bulbifera* and its bioactive principle, diosgenin as novel α-amylase and α-glucosidase inhibitor. Among petroleum ether, ethyl acetate, methanol and 70% ethanol (v/v) extracts of bulbs of *D. bulbifera*, ethyl acetate extract showed highest inhibition up to 72.06 ± 0.51% and 82.64 ± 2.32% against α-amylase and α-glucosidase respectively. GC-TOF-MS analysis of ethyl acetate extract indicated presence of high diosgenin content. Diosgenin was isolated and identified by FTIR, 1H NMR and 13C NMR and confirmed by HPLC which showed an α-amylase and α-glucosidase inhibition upto 70.94 ± 1.24% and 81.71 ± 3.39%, respectively. Kinetic studies confirmed the uncompetitive mode of binding of diosgenin to α-amylase indicated by lowering of both Km and Vm. Interaction studies revealed the quenching of intrinsic fluorescence of α-amylase in presence of diosgenin. Similarly, circular dichroism spectrometry showed diminished negative humped peaks at 208 nm and 222 nm. Molecular docking indicated hydrogen bonding between carboxyl group of Asp300, while hydrophobic interactions between Tyr62, Trp58, Trp59, Val163, His305 and Gln63 residues of α-amylase. Diosgenin interacted with two catalytic residues (Asp352 and Glu411) from α-glucosidase.

This is the first report of its kind that provides an intense scientific rationale for use of diosgenin as novel drug candidate for type II diabetes mellitus.

Mbiantcha *et al.,* (2011) studied the analgesic and anti-inflammatory properties of extracts from bulbils of *Dioscorea bulbifera* L in mice and rats in Dschang, Cameroon. The aqueous and methanol extracts from the dry bulbils of *Dioscorea bulbifera* L. var sativa (Dioscoreaceae)-evaluated orally at the doses of 300 and 600 mg/kg against pain induced by acetic acid, formalin, pressure and against inflammation induced by carrageenan, histamine, serotonin and formalin in mice and rats, showed a dose dependant inhibition of pain and inflammation with a maximum effect of 56.38%, 73.06% and 42.79% produced by the aqueous extract, respectively on pain induced by acetic acid, formalin and pressure while the methanol extract at the same dose respectively inhibited these models of pain by 62.70%, 84.54% and 47.70%. The oral administration of aqueous and methanol extracts caused significant anti-inflammatory activity on paw oedema induced by histamine, serotonin and formalin. The present results show that the bulbils of Dioscorea bulbifera var sativa possess potent analgesic and anti-inflammatory activities. These activities may results from the inhibition of inflammatory mediators such as histamine, serotonin and prostaglandins. Thus, the analgesic activity of the bulbils of *Dioscorea bulbifera* may be at least partially linked to its anti-inflammatory activity.

Furthermore, *D. bulbifera* has been used over the years for the treatment of the following diseases:

Respiratory disorders

Fresh tuber pieces are pounded with black pepper followed by mixing with curd is taken against cough and cold in India. Similarly, roasted, peeled and dried powder is known to cure respiratory problems when taken with honey. Bitter bulbils are useful against severe cough while edible normal tubers are used against asthmatic conditions. In China, it is considered as a styptic against lung bleeding and epistaxis (Williams *et al*., 2013).

Eye disorder

D. bulbifera serves as a well-known ophthalmic remedy. Purulent ophthalmia is treated with sap from stem as eye drops in Congo. Wakefulness is promoted in Ivory Coast by stillation of leaf sap into the eye. A portion made out of steamed leaf is used in east Africa for treating a type of conjunctivitis called „pink eye‟ (Williams *et al.,* 2013).

Goitre

Tubers of *D. bulbifera* are steeped in white wine for a week which is traditionally recommended as infused wine and taken daily to get benefits in treatment of goitre. Formulations have profound applications as clinical medicine to treat thyroid glands (Tang *et al.,* 2006).

Bacterial and fungal infections

D. bulbifera is known to be used for treatment of sexually transmitted diseases like gonorrhoea and syphilis in addition to sore throat in Chinese medicine (Williams *et al.,* 2013). In Congo it is used against parasitic and fungal infections (Williams *et al.,* 2013) Its aqueous extract showed superior activity against *Escherichia coli* while ethanol extract was found to be potent against *Staphylococcus aureus* and *Candida albicans* (Okigbo *et al.,* 2009). Bacterial pathogens have posed a threat to the human health by developing multidrug resistance leading to re-emergence of diseases once controlled. Genetic determinants conferring resistance to one or more antibiotics are mostly located on plasmids which being extra chromosomal DNA, can be effectively transferred to other bacteria, co-existing in the same environment. This underlying mechanism for spread of antimicrobial resistance pose an acute difficulty in treatment of infectious diseases which has led to the search of new drugs called plasmid-curing agents, although many of which such as acridine orange, ethidium bromide and sodium dodecyl sulphate, are toxic, mutagenic and carcinogenic. Hence, plasmid curing agents isolated from medicinal plants have gained more importance and are being investigated owing to their biocompatibility and minimum toxicity. Recently, norditerpene compound, 8-epidiosbulbin E acetate from *D. bulbifera* bulbs has exhibited significant broad spectrum potential to cure antibiotic resistance plasmids (R- plasmids) from clinical isolates although it showed low antimicrobial activity (MIC > 400 μg/mL) for all tested pathogens except *Escherichia coli* (pUC18) which showed an MIC = 200 μg/mL. However, R-plasmids in clinical strains of *Enterococcus faecalis*, *E. coli*, *Shigella sonnei*, *Pseudomonas aeruginosa* and *Bacillus subtilis* were effectively cured. Plasmid curing by 8-epidiosbulbin E acetate resulted in effective reversal of bacterial resistance to multiple antibiotics in an *E. coli* strain that was resistant to gentamicin, kanamycin, neomycin, streptomycin, tetracycline, novobiocin, ciprofloxacin, cefoperazone, oxacillin, ceftazidin, co-trimazine, imipenem, cefalexin, cefotaxime, oxytetracycline, cloxacillin, doxycycline,

levofloxacin, ofloxacin, gatifloxacin, moxifloxacin, norfloxacin, cefpirome and cotrimoxazole. Further, *S. sonnei* was sensitised to ampicillin, gentamicin, tetracycline, novobiocin, ciprofloxacin, cefoperazone, ceftazidime, oxacillin, ceftazidin, co - trimazine, imipenem, cefazolin, cefalexin, cefotaxime, levofloxacin, ofloxacin, norfloxacin, and cefpirome as a result of 8-epidiosbulbin E acetate mediated plasmid curing. Vancomycin resistant *Enterococcus faecalis*, resistant to roxithromycin, cloxacillin, cefalexin and clindamycin became sensitive to these antibiotics after plasmid curing by 8-epidiosbulbin E acetate.

Similarly, reference R-plasmids such as RP4, RMS163, RIP64 and pUB110 were also cured by 8-epidiosbulbin E acetate at curing efficiencies of 44%, 30%, 64% and 48%, respectively. Reduction of minimal inhibitory concentration (MIC) of antibiotics against MDR bacteria on curing of R-plasmid by 8-epidiosbulbin E acetate provided strong evidence for the novel mechanism behind the effective antibacterial treatment by *D. bulbifera* (Shriram *et al.,* 2008) Additionally, vanillic acid and isovanillic acid have shown antibacterial activity (Tang *et al*., 2006). In the western highlands of Cameroon, tubers of *D. bulbifera* are used against typhoid fever caused by *Salmonella typhi* and paratyphoid fever caused by *Salmonella* paratyphi A and *Salmonella* paratyphi B. Two clerodanediterpenoids, bafoudiosbulbins A and B were reported to have anti-bacterial activity when tested against Salmonella typhi, Salmonella paratyphi A, *Salmonella paratyphi B, Pseudomonas aeruginosa, Escherichia coli, Klebsiellapneumoniae and Staphylococcus aureus* by using both agar diffusion and broth dilution techniques (Teponno *et al.,* 2006).. Both bafoudiosbulbins A and B exhibited bactericidal activity selectively against *S. typhi, S. paratyphi A and S.paratyphi B* (Teponno *et al.,* 2006). Isolated bafoudiosbulbins B, C, F and G, as well as crude ethyl acetate (EtOAc) extract (DBB1) and fractionated DBB2 obtained from methanolic extract of bulbils from D. bulbifera L. var sativa from Bafou village near Dschang (West region of Cameroon), showed growth inhibition in fifteen test strains (both clinical MDR and ATCC) of bacterial pathogens, namely, *E. coli, E. aerogenes, K. pneumoniae, P. aeruginosa, M. smegmatis and M. tuberculosis*. Selective inhibition of ATCC strain of *E. coli, E.aerogenes, K. pneumoniae, M. smegmatis and M. tuberculosis* as well as *E. coli* AG100A and *M. tuberculosis* MTCS2 by crude extracts can be considered as significant. Bafoudiosbulbin C was found to be active against *M. smegmatis and M. tuberculosis* ATCC and MTCS2 strains at a value as low as 8 μg/mL. Inhibitory effect of the compounds isolated from *D. bulbifera* against MDR bacteria such as *E. aerogenes* EA289, CM64, *K. pneumoniae* KP63 and *P. aeruginosa* PA124 was

better than that of standard antibiotic chloramphenicol in absence as well as in presence of efflux pump inhibitor, phenylalanine arginine β- naphthylamide (Kuete *et al.*, 2012).

Inflammation and pain

D. bulbifera is used to treat inflammation associated dispersal of "lumps", hernia, sprain, injury, testicular inflammations, in China (Gao *et al.,* 2002). Rheumatic pain and breast problems are relieved in Congo and Gabon, respectively by an ointment prepared by incorporation of bulbils into palm oil (Williams *et al.,* 2013). It is also used as analgesic and antispasmodic. Vanillic acid and isovanillic acid present in *D. bulbifera* are reported to exhibit anti-inflammatory activity (Tang *et al.,* 2006). Recent *in vivo* studies of aqueous and methanol extracts from bulbils of *D. bulbifera* var sativa confirmed potent antinociceptive effect. A dose dependent reduction in the acetic acid induced abdominal constriction was observed in albino adult mice *Musmusculus* (weighing 25–30 g), on oral administration of aqueous and methanol extracts. Similarly, in case of formalin- induced paw licking test, intraplantar injection of formalin (2.5%) into the right hind paw of adult Wistar rats (weighing 180–200 g), generated a classical biphasic nociceptive response that was significantly inhibited by aqueous and methanol extracts as compared to indomethacin. These extracts also presented important anti-inflammatory effects on pressure induced pain sensitivity, acute oedema induced by carrageenan, histamine, serotonin, formalin and chronic oedema induced by formalin (Mbiatcha, 2011). Pronounced hypernociception induced by intraplantar injection of complete Freud's adjuvant (CFA) in Swiss mice, could be countered significantly (61 % inhibition) owing to the antinociceptive effect of methanol extract of *D. bulbifera* (MEDB) administered orally. Hypersensitivity in mice due to neuropathic pain induced by partial ligation sciatic nerve (PLSN) was significantly reduced (38 %) followed to oral administration of MEDB. Similarly, the extract even reduced LPS or PGE2 induced mechanical hypernociception and further study helped in partial prediction that the antinociceptive activities of *D. bulbifera* both in inflammatory and neuropathic models of pain were attributed to its ability to activate the NO–cGMP–ATP- sensitive potassium channels pathway (Gao *et al.,* 2002). Parasitic infections

D. bulbifera from Southwest Nigeria is reported to show anthelmintic activity as one of its significant ethnobotanical application when eaten after roasting. Scientific evidence to the fact was provided by a recent study confirming *in vitro* anthelmintic activity of methanol

extracts of the flesh and peel of *D. bulbifera* bulbils (Adeniran and Sonibare, 2013). The extracts rich in phenolics (tannins, flavonoids), saponins as well as other secondary metabolites could efficiently show anthelmintic activity against *Pheritimaposthuma* and *Fasciola gigantic*. Earthworms were paralysed in 5.6 min and death was observed in 10 min when treated with peel extract, while treatment with flesh extract showed paralysis in 8.4 min and death in 13.8 min at 100 mg/ml. Likewise, liverflukes were paralyzed after 10.2 min and died after 15.81 min at a concentration of 100 mg/ml of peel extract. The probable mechanism was explained to be the binding of phenolic and tannin compounds to glycoprotein present on the cuticle together with saponin mediated alteration of permeability and pore formation in the membrane of the parasite leading to paralysis and death (Adeniran and Sonibare, 2013).

Diabetes and digestive problems

Traditional use of *D. bulbifera* is known to lower glycaemic index in Diabetes mellitus. Diosgenin has shown to ameliorate diabetic neuropathy (Williams *et al.*, 2013). A study carried out to evaluate aqueous extract of *D. bulbifera* tubers (DBEA003) for antihyperglyceamic activity on streptozotocin (STZ) treated Wistar rats, provided scientific rationale behind the traditional use. A higher dose (1000 mg/kg p. o.) resulted in highly significant antihyperglycaemic effect in glucose tolerance test bringing down the blood glucose level from 129±8.81 mg/dL (30 min) to 97±5.83 mg/dL (90 min). Remarkably, a long lasting glycaemic control in diabetic condition was observed over administration of DBEA003, which might be attributed due to its action at the tissue or receptor level (Ahmed *et al.*, 2009). However, the actual mechanism of action by which *D. bulbifera* exerts the antidiabetic effect was furnished by the report that confirmed the inhibition of two key enzymes, α-amylase and α-glucosidase. Petroleum ether, ethyl acetate and methanol extracts of *D. bulbifera* bulbs were obtained by sequential hot Soxhlet extraction among which ethyl acetate extract showed most superior activity of 73.39 % against porcine pancreatic α-amylase. Strongest inhibition against α-glucosidase was found for ethyl acetate extract (99.6 %) of *D. bulbifera* bulb (Nguelefact *et al.*, 2009). Similarly, even among cold extracts of bulbs of *D. bulbifera*, ethyl acetate extract showed highest inhibition up to 72.06 ± 0.51% and 82.64 ± 2.32% against α-amylase and α-glucosidase, respectively.

The bioactive principle was identified as diosgenin which in its isolated form showed superior α-amylase and α-glucosidase inhibition up to 70.94 ± 1.24% and 81.71 ± 3.39%, respectively. The mechanism was established to be uncompetitive mode of binding to α-amylase as confirmed by decrease in both Km and Vm values. Further, hydrogen bonding between carboxyl group of Asp300 and hydrophobic interactions between Tyr62, Trp58, Trp59, Val163, His305 and Gln63 residues of α-amylase was indicated by molecular docking. Similarly, two catalytic residues (Asp352 and Glu411) from α-glucosidase were found to be the target of interaction with diosgenin (Wang *et al*., 2009). Inhibition of digestive enzymes like α-amylase (132 ± 2%) and trypsin (4.3 ± 0.2 %) were also reported for crude extract of *D. bulbifera* tubers collected from the central region (Narayani Zone) of Nepal which were found to be rich in oxalate (67 ± 9 mg/100g), phytate (184 ± 14 mg/100g) and cyanogens (3.3 ± 0.9 mg HCN/Kg FW) (Bhandari and Kawabata, 2004) Moreso, there is a global epidemic of diabetes mellitus, and the number of persons affected is expected to rise more than 430 million by 2030 (Shaw *et al.*, 2010).The wide spread of this chronic disorder (diabetes) adversely affects multiple organ systems including bones, a highly dynamic tissue that undergoes constant remodeling (Yan and Li, 2013). Besides, a worldwide prevalence of osteoporosis is estimated to be greater than 200 million people, with the majority being women (Sealand and Razavi, 2013). Recently, Teponno, 2006, demonstrated antidiabetic potential of *Dioscorea bulbifera* which is profusely used in Indian and Chinese system of traditional medicine owing to its anticancer, antioxidant, analgesic and anti-inflammatory properties. The earlier reports demonstrated that the excellent antioxidant property of the plant is attributed to its unique phytochemistry (Teponno, 2007). Another strong evidence of the diversified uses of this plant system is its application in nanobiotechnology for synthesis of gold and silver nanoparticles of exotic size and shapes (Nguelefact, 2009). Therefore, *D. bulbifera* offers a great scope for discovery of molecules with pharmacological activity.

Herbal medicine is the oldest form of health care known to humanity. The use of plants and plant foods to oppose hyperglycaemia has been practiced by herbalists for a long time (Kumar and Reddy, 2013). The World Health Organization (WHO) expert committee has recommended that plants possessing hypoglycaemic activity may provide a utilizable source of new oral anti diabetic drug, or may act as simple dietary adjuncts to the existing therapies (WHO, 1980).

The genus *Dioscorea* has been used widely in traditional Chinese medicine to promote human health. The Pharmacopoeia of the People's Republic of China describes its use for the treatment of several diseases including diabetes (Chinese pharmacopoeia Commission, 2015).

The physico-chemical properties and phytochemical investigations of starch foods compounds have been studied, but little attention has been given to the tubers of *Dioscoreaceas* (Sahore *et al.,* 2007).

Oxidative stress and degenerative diseases

Free radicals are key mediators for emergence of disease progression and its associated pathology, including diabetes, cancer and even AIDS. Antioxidants, namely, epicatechin, isovanillic acid, vanillic acid, myricetin are important bioactive principles in *D. bulbifera* that are responsible for protection against cardiovascular diseases and styptic activities (Song *et al.,* 2010). Tubers of *D. bulbifera* collected from Nepal showed superior DPPH radical scavenging, ferrous ion chelating, reducing power, and total antioxidant activity with high oxalic acid (67 ± 9 mg/100g), citric acid (282 ± 24 mg/100g), malic acid(266 ± 20 mg/100g), succinic acid(2510 ± 108 mg/100g) and polyphenols (166 ± 10 mg/100g). Thus these chemical constituents might be playing the key role behind its pronounced antioxidant activity (Song *et al.,* 2010). *D. bulbifera* from Guangzhou of China showed the highest phenolic content (59.43 mg GAE/g), that further exhibited most superior antioxidant property in terms of ABTS·+ radical scavenging activity (708.73 μmolTrolox/g) as well as ferric reducing antioxidant power (FRAP) (856.92 μmol Fe^{2+}/g) (Song *et al.,* 2010). Ethyl acetate fraction of hydro alcoholic extract of *D. bulbifera* from China, yielded a bibezyl compound, 2,5,2',5'-tetrahydroxy-3'-methoxybibenzyl and diobulbinone A amongst which Trolox-equivalent antioxidant capacity of the new bibenzyl 7 was found to be 0.52 ± 0.01 by FRAP at a concentration of 1mM. Similarly it showed an EC50 value of 2.57 ± 0.06 mM for DPPH radical scavenging. However, the new diarylheptanone, diobulbinone A did not show significant antioxidant activity at the same concentration by either of the methods (Liu *et al.,* 2011). Dried bulbs of *D. bulbifera* from India were reduced to fine powder and extracted with 70% (v/v) ethanol in distilled water which was further sequentially extracted with petroleum ether, ethyl acetate and methanol. Among the extracts, methanolic extract with maximum phenolic content (145.44 ± 3.29 μg/mL) showed most superior antioxidant activity. Percentage scavenging activity of methanolic extract against DPPH, hydroxyl, superoxide anion radical and nitric oxide was found to be 84.94 ± 0.62 %, 76.11 ± 1.26 %, 59.75 ± 0.98

% and 57.59 ± 0.64 %, respectively. Simultaneously, it scavenged pulse radiolysis generated ABTS·+ radical with a second order rate constant. Similarly, ethyl acetate extract also showed higher percentage scavenging activity of free radicals owing to its high phenolic content (98 ± 1.17 μg/mL) and 94.05 % diosgenin (Vasanthi *et al.,* 2010). Sprague– Dawley male rats administered with 1 ml of *D. bulbifera* extract dissolved in water (150 mg kg^{-1} of body weight) for 30 days produced significantly improved performance towards aortic flow (AF), left ventricular developed pressure (LVDP) and the first derivative of developed pressure (LVmaxdp/dt) in *D. bulbifera* treated hearts during post ischemic reperfusion. Additionally, marked reduction in myocardial infarct size (20 ± 2.64%) was observed in the treated group. Significant reduction of apoptotic cardiomyocytes (16.89 ± 1.7%) confirmed its anti-apoptotic activity. Increased BCl2 expression and decreased Bax expression leading to reduction in Bax/BCl2 ratio was evident. Furthermore, up regulation of procaspase 3 and down regulation of cleaved caspase 3 coupled with prevention of loss of phase II enzyme HO-1 with proven cardioprotective ability considerably, provided a strong scientific rationale that *D. bulbifera* has the potential to ameliorate myocardial ischemia and reperfusion injury by improving ventricular function and inhibition of necrosis and apoptosis in cardiomyocytes (Vasanthi *et al.,* 2010).

Tumor and cancer

Aborigines from Tully district in North Queensland use a decoction of *D. bulbifera* against skin cancer (Williams *et al.,* 2013). Similarly it is also used in traditional Chinese medicine against cancer (Tang *et al.,* 2014). Phytochemicals present in extracts (75 % ethanol, v/v) of chipped rhizomes exhibited potent antitumor promoting properties. Among the flavonols, kaempferol-3, 5-dimethyl ether (IC_5 = 0.64 μg/mL) exhibited strongest inhibition followed by caryatin (IC_{50} = 3.0 μg/mL), myricetin (IC_{50} = 3.7 μg/mL) and (+)-catechin (IC_{50} = 13.1 μg/mL) against tumor promotion in JB6 (Cl 22 and Cl 41) cells induced by a promoter, 12-O-tetradecanoylphorbol-13-acetate (TPA). In contrast to aglycones, flavonol glycosides, quercetin-3-O-galactopyranoside, myricetin-3-O-galactopyranoside, myricetin-3-O-glucopyranoside showed a considerably reduced activity due to the presence of sugar moieties (Gao *et al.,* 2002). However, diosbulbin B with demethylditerpenoid skeleton, selectively inhibited solid sarcoma 180 tumor growth in mice significantly, compared to A375-S2, Hela, L929 and JB6 cells as reported by (Kareru *et al.,* 2006). The study was conducted by administration of diosbulbin A (0.2 mg/head/day), diosbulbin B (0.2 mg/head/day) and diosbulbin A 2-O-β-D-glucopyranoside (0.2 and 0.1 mg/head/day) by i.p.

daily for 5 days to male ddy mice inoculated with total of 180 sarcoma cells. Sixteen days after inoculation, the tumors were removed and weighed which revealed that diosbulbin B resulted in reduction of tumor weight by 57.89 % while diosbulbin A reduced up to 50.19 %. Further studies revealed moderate inhibitory effect of ethyl-O-β-D- fructo-pyranoside (IC50 > 30 μg/mL) and butyl-O-β-D-fructopyranoside (IC_{50}> 30 μg/mL) from *D. bulbifera* on JB6 neoplastic transformation (Kareru *et al.,* 2006). In another study, norditerpene compound, 8-epidiosbulbin E acetate failed to show any cytotoxicity against variety of human cancer cells, namely MCF-7 (breast cancer), SiHa (cervical cancer) and A431 (epidermal carcinoma), ensuring its potential to be used in non-cancer drug discovery programmes (Ghosh *et al.,* 2012). Dried rhizomes of *D. bulbifera*, collected from Anhui Province of China containing pennogenin-3-O-α-L-rhamnopyranosyl-(1→3)-[α-L-rhamnopyranosyl-(1→2)]-β-D glucopyranoside and pennogenin- 3-O-α-L-rhamnopyranosyl-(1→4)-[α-L- rhamnopyranosyl-(1→2)]- β-D-glucopyranoside showed 99.1% and 92.6% inhibition against human hepatocellular carcinoma cells and Bel7402, respectively at a concentration of 10 μM. The compounds further, exhibited cell growth inhibition toward SMMC7721 human hepatocellular carcinoma cells (Liu *et al.,* 2009). Diosbulbins N, O, P showed lower activity (IC_{50}> 40μM) when tested against five cancer cell lines, namely, HL-60, SMMC7721, A549, MCF7 and SW480 for their cytotoxic activity (Tang *et al.,* 2014). Cytotoxicity studies on ECV304 cells (urinary bladder carcinoma cells), revealed that the isolated spirostanol derivatives, pennogenin 3-O-α-L-rhamnopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→4)- [α-L-rhamnopyranosyl-(1→2)]-β-D-glucopyranoside (IC50 = 8.5 μg/mL) and spiroconazol A (IC50 = 5.8 μg/mL) showed moderate inhibition due to membrane toxicity via LDH liberation. However, the furostanol derivative, 26-O-β-D-glucopyranosyl-(25R)-5-enfurost-3β,17α,22α,26-tetraol-3-O-α-L- rhamnopyranosyl-(1→4)- α-L-rhamnopyranosyl-(1→4)-[α-L-rhamnopyranosyl-(1→2)]-β-Dglucopyranoside (IC_{50} = 14.3 μg/mL), also showed a moderate activity, but by a direct influence of the mitochondrial metabolism, without liberation of lactate dehydrogenase (Tapondjou *et al.,* 2013).

Synthesis of Nanoparticles

In spite of having a treasure of diverse groups of phytochemicals, *D. bulbifera* were not explored in the field of nanobiotechnology, until recently. It was reported for the first time the potential of its tuber extract to synthesize AuNPs of exotic shapes, namely gold nanotriangles, nanoprisms, nanotrapezoid and spheres in a range from 50 to 300 nm. Maximum synthesis of AuNPs was achieved at 50ºC by complete reduction of 1 mM

chloroauric acid at 90 mins which was found to be faster as compared to maximum synthesis of AuNPs after 5 h by *Plumbagozeylanica* root extract (PZRE) (Salunke *et al.,* 2014). Rapid reduction by *D. bulbifera* tuber extract (DBTE) might be due to its high total reducing sugar content (3.41± 0.15 mg/mL) and high flavonoid content (4 ± 0.12 mg/mL) while PZRE contained 1.25 ±0.04 mg/mL and 0.95 ± 0.05 mg/mL of total reducing sugar and total flavonoids, respectively. Also, the aqueous tuber extract synthesized anisotropic AgNPs in the form of rare nanotriangles, nanorods, spheres and hexagons in the size range of 8–20 nm at 50°C with 0.7 mM $AgNO_3$ solution in 5 h. Bioreduced AgNPs possessed potent antibacterial activity against both Gram-negative bacteria, namely *Acinetobacter baumannii, Enterobacter cloacae, Escherichia coli, Haemophilus influenza, Klebsiella pneumonia Neisseria mucosa, Proteus mirabilis, Pseudomonas aeruginosa, Salmonella typhi, Serratiaodorifera, Vibrio parahaemolyticu*s and Gram-positive bacteria, namely, *Bacillus subtilis, Paenibacillus koreensis* and *Staphylococcus aureus*. A selective synergistic activity was observed where β- lactam, piperacillin exhibited 3.6 fold increase while macrolide, erythromycin showed 3 fold increase in potency against multidrug-resistant *A.baumannii* when combined with AgNPs. Likewise, a combination of AgNPs with chloramphenicol and vancomycin against *P. aeruginosa* resulted in 4.9-fold and 4.2- fold increase in zone diameter, respectively. Typically, 11.8-fold increase in zone diameter of streptomycin in combination with AgNPs against E. coli was found to be most significant. The unique phytochemistry of D. *bulbifera* with both reducing agents like ascorbic acid, citric acid and phenolics along with starch as capping agent and saponins help in reduction, stabilization and shape evolution of nanoparticles (Gao *et al.,* 2007).

Toxicity and Detoxification

Roots of *D. bulbifera* from China are reported to be toxic, indicating that it might have achieved limited focus on its use in traditional medicine. Dioscin and diosbulbin B, derived from *D. bulbifera* roots are responsible for liver toxicities, nausea, abdominal pain, coma and even death. Oral administration of decoction (400 g/kg) in male Sprague– Dawley rats for 72 h, exhibited elevated levels of taurine, creatine, betaine, dimethylglycine (DMG), acetate and glycine. On the contrary, a reduction in the levels of succinate, 2-oxoglutarate, citrate, hippurate and urea was observed (Javaregowda and Maheswarappa, 2007). Marked increase in the organ coefficients of liver and kidney of rats was noticed on exposure to extract. Histopathological alterations of liver included diffuse hepatocyte degeneration, apoptosis, necrosis in addition to ballooning degeneration of hepatocytes and vascular congestion of

hepatic sinusoidal and portal areas (Javaregowda and Maheswarappa, 2007). Impairment of mitochondrial energy metabolism indicated by reduction in citrate, 2- oxoglutarate and succinate levels coupled with inhibition of ornithine cycle and urea production confirmed oxidative injury of hepatic mitochondria leading to inhibition of ATP formation and inhibition of hippurate synthesis. Similarly, elevation in taurine levels due to glutathione depletion also indicated oxidative hepatic damage (Liu *et al.,* 2010). Another study, revealed that ethyl acetate fraction (EF) of hydroalcoholic extract of *D. bulbifera* rhizome from China exhibited dose dependent hepatotoxicity in ICR male and female mice when administered consecutively for fourteen days. Maximum elevation in the levels of biomarkers of liver injury, including, alanine transaminase (ALT) and aspartate transaminase (AST) were observed at a highest dose of 480 mg/kg. Reduction in the levels of glutathione- related enzymes including superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione-S-transferase (GST), glutathione reductase (GR) and glutamate-cysteine ligase (GCL) of hepatic tissues of EF treated mice indicated oxidative stress mediated liver injury in mice (Liu *et al.,* 2010). Hepatotoxicity against normal human liver cell line L-02 was reported by diosbulbin D isolated from the rhizome of *D. bulbifera* from China. A time and dose dependent manner of reduction in cell viability was shown by the benzene fraction, highest being 4.32% ± 1.87 at a dose of 800 µg/ml. AnnexinV and propidium iodide assay, Hoechst 33258 staining and the occurrence of a sub-G1 peak indicated diosbulbin D mediated apoptosis induction which was further confirmed to be caspase 3 dependent (Javaregowda and Maheswarappa, 2007)

CHAPTER THREE

MATERIALS AND METHODS

3.1 Materials

3.1.1 Chemicals and reagents

Reagents used were of analytical grade.

The following reagents were used

1 Petroleum ether
2 Tetraoxosulphate (iv) acid
3 Sodium hydroxide
4 Methanol
5 Ethanol
6 Copper sulphate
7 Ammonium sulphate
8 Hydrochloric acid
9 Folin-Dennis reagent
10 Sodium carbonate
11 Sodium chloride
12 Ammonium hydroxide
13 Ferrous sulphate
14 Ethylbenzthiazoline-6-sulfonic acid
15 Tricarboxylic acid (TCA)
16 Thiobarbituric acid (TBA)
17 Sodium nitrate
18 Quercetin
19 Diethyl ether
20 Aluminium chloride
21 N-butanol
22 Tannic acid
23 Acetic acid
24 Griess reagent
25 Deoxyribose
26 EDTA
27 Sodium dodecylsulphate

28 Tannic acid

29 Sodium metabisulphite

3.1.2 Equipments and apparata

The following equipement was used in this research

1. Bursen burner
2. Conical flasks
3. Centrifuge (Clay Adam USA)
4. Digital pH meter (Labtech India)
5. Incubator
6. Spectrophotometer (Turner Model 390)
7. Rotary shaker
8. Rotary evaporator
9. Weighing balance
10. Micropipette
11. Measuring cylinder
12. Volumetric flask
13. Crucibles
14. Ashing muffle furnace (Model 1184A Fisher Scientific, Houston, TX)
15. Dessicators
16. Markham distillation apparatus
17. Separatory funnel
18. Oven
19. Soxlet apparatus
20. Kjeldahl flask
21. Digester

3.1.3 Plant materials

D. bulbifera was bought from Eke Imoha market, Onueke in Ebonyi State, Nigeria. It was identified and authenticated by Prof. F N Mbagwu of the Department of plant science and biotechnology, Imo state university. It is deposited in the Imo state university Herbarium with Voucher number IMSUH 435

3.2 Methods

3.2.1 Preparation of sample

The bulbs were washed, sliced into pieces and shade dried. It was ground into fine powder in a milling machine and stored in an air tight container until use. Then, 100g of fine powder was extracted with 80% (v/v) methanol in distil water. The hydromethanol extract was evaporated to dryness under reduced pressure at 40 degrees centigrade in a rotary evaporator and stored at 4 degrees centigrade in an air tight bottle for further use.

3.2.2 Determination of the Proximate Composition of the *D. bulbifera*

Proximate composition of *D. bulbifera* was determined using the AOAC methods (1990) as follows:

3.2.2.1 Determination of Moisture Content:

Moisture was determined by Standard Official Methods of Analysis of the AOAC (1990). This involved drying to a constant weight at 100^0C and calculating moisture as the loss in weight of the dried samples. The crucible was thoroughly washed and dried in an oven at 100 ^{0}C for 30 mins and allowed to cool inside desiccators. After cooling, they were weighed using weighing balance and their various weights recorded as (W1). Then, 5.0 g of the finely ground samples were put into the crucibles and weighed to get W2. Thereafter, the sample plus crucible were placed inside the oven and dried at 100 ^{0}C for 4 hours, cooled and weighed at the same temperature for 30 min until constant weights were obtained to get W3. Then, the moisture content of the sample was calculated from the equation:

% moisture = (W2 – W3) / (W2 – W1) x 100; where, W1 = Initial weight of empty crucible, W2 = Weight of crucible + sample before drying and W3 = Final weight of crucible + sample after drying.

3.2.2.2 Determination of Ash Content:

Total ash of the sample was determined by Furnace Incineration described by AOAC (1990) based on the vaporization of water and volatiles with burning organic substances in the presence of oxygen in the air to CO_2 at a temperature of 600^0C (dry ashing). About 2.0 g of finely ground dried sample was weighed into a 277 tared porcelain crucible and incinerated at 600^0C for 6 hr in an ashing muffle furnace (Model 1184A Fisher Scientific, Houston, TX)

until ash was obtained. The ash was cooled in a dessicator and reweighed. The % ash content in the sample was calculated as:

% Ash = Weight of Ash x 100/ Weight of original sample

3.2.2.3 Determination of Crude Fibre:

Crude fibre was determined using the method of AOAC (1990). About 2.0 g of the ground sample was hydrolyzed in a beaker with petroleum ether after which it was boiled under reflex for 30 min with 200 ml of a solution containing 1.25% H_2SO_4 per 100 ml of solution. The solution was filtered through a filter paper onto a fluted funnel. After filtration, the sample was washed with boiled water until they were no longer acidic. Then, the residue was transferred onto a beaker and boiled for another 30 min with 200 ml of solution containing 1.25 % NaOH per 100 ml. The boiled samples were washed with boiled distilled water. The residues were filtered through Gooch filter crucible, dried at 100^0C for 2 hours in an oven, cooled and washed. The percentage crude fibre in the sample was calculated as per the formula:

% Crude fiber = (Wt. after drying) / (Wt. of sample) x 100.

3.2.2.4 Determination of Fat:

Total fat in the sample was determined using Soxhlet extraction for 4 hr starting with methanol and ethanol, respectively. About 250 ml clean boiling flasks were dried in an oven at 105 – 110^0C for about 30 min and cooled in a dessicator. Approximately, 5.0 g of samples were weighed accurately into labeled thimbles. The dried boiling flasks were weighed correspondingly and filled with about 300 ml of petroleum ether (boiling point 40 -60^0C). The extraction thimbles were plugged tightly with cotton wool. After that, the Soxhlet apparatus was assembled and allowed to reflux for 6 hrs. The thimble was removed with care and petroleum ether collected from the top container and drained into another container for re-use. After that, the flask was dried at 105 – 110^0C for 1 hour when it was almost free of petroleum ether. After drying, it was cooled in a dessicator and weighed. Then, % fat in the sample was computed using the formula below:

% fat = Weight of fat x 100/ Weight of sample

3.2.2.5 Determination of Protein:

The crude protein content of the sample was determined using the Microkjeldahl method of AOAC (1990), which involved protein digestion and distillation.

a. Protein Digestion: About 0.5 g of the sample was weighed into a Kjeldahl flask and 4 tablets of Kjeldahl Catalyst were added. This was followed up with the addition of 1.0 g copper sulphate and a speck of selenium catalyst into the mixture, and 25 ml concentrated sulphuric acid was introduced. The whole mixture was subjected to heating in the fume cupboard. The heating was done gently at first and increased with occasional shaking till the solution assumed a green color. The temperature of digester was above 420℃ for about 30min. The solution was cooled and black particles showing at the neck of the flask were washed down with distilled water. The solution was re-heated gently at first until the green color disappeared. Then, it was allowed to cool. After cooling, the digest was transferred into a 250 ml volumetric flask with several washings and made up to the mark with distilled water and then distilled using Markham distillation apparatus.

b. Protein Distillation: Before use, the Markham distillation apparatus was steamed through for 15 min after which a 100 ml conical flask containing 5 ml boric acid /indicator was placed under the condenser such that the condenser tip was under the liquid. About 5.0 ml of the digest was pipetted into the body of the apparatus via a small funnel aperture. The digest was washed down with distilled water followed by addition of 50 ml of 60 % NaOH solution. The digest in the condenser was steamed through for about 5-1 minutes after which enough ammonium sulphate was collected. The receiving flask was removed and the tip of the condenser washed down into the flask after which the condensed water was removed. The solution in the receiving flask was treated with 0.1M hydrochloric acid. Also, a blank was run through along with the sample. After titration, the % nitrogen was calculated using the formulae below:

% Nitrogen = Vs–VB x M acid x 0.01401 x 100 / W

Where, Vs = Volume (ml) of acid required to titrate sample; VB = Volume (ml) of acid required to titrate the blank; M acid = Molarity of acid; W=Weight of sample (g). Then, percentage crude protein in the sample was calculated from the % Nitrogen as:

% crude protein = % N x F, where, F (conversion factor), is equivalent to 6.25.

3.2.2.6 Determination of Carbohydrate: The total percentage carbohydrate content in the sample was determined by the difference method as reported by Onyeike *et al.,* (1995). This method involved adding the total values of crude protein, lipid, crude fibre, moisture and ash constituents of the sample and subtracting it from 100. The value obtained is the percentage carbohydrate constituent of the sample. Thus:

% carbohydrate = 100 – (% moisture + % crude fibre +% protein + %lipid + % ash).

3.2.3 Total Phenolic Content

Principle: A colorimetric assay using the Folin-Dennis reagent for the determination of phenolic compounds is based on the reaction between the Folin-Dennis reagent and phenolic compounds, which results in the formation of a blue colour complex that absorbs radiation and allows quantification.

Procedure:

The total phenolic content was determined using the method of Swian and Hillis as described by (Wattashinghe and Shaidi, 2000). In this method, 50mg of aqueous extract was diluted with 100ml of distilled water to obtain a concentration of 0.5mg/ml (Solution A). Folin-Denis reagent (0.5ml) was added to centrifuge tube containing 0.5ml of Solution A. Tubes were shaken and 1ml of saturated Sodium Carbonate solution was added into each tube. The volume for each tube was then adjusted to 10ml by the addition of 8ml of deionised water and the content was mixed vigorously. Tubes were allowed to stand at ambient temperature for 25mins and then centrifuged for 5mins at 4000xg. Absorbance of the supernatants was measured at 725nm. Content of total phenolics in the extract was determined using a standard curve prepared for (+) Tannic acid (y=0.0039x) R^2 = 0.989. Total extracted phenolic compound was expressed as mg (+) Tannic acid equivalent/g extract.

3.2.4 Total Flavonoid content

Principle: This method uses aluminum chloride and is based on the formation of a complex between the aluminum ion (Al^{3+}), and the carbonyl and hydroxyl groups of flavones and flavonols that produce a pink colour, and the absorbance is determined colorimetrically (Popova *et al.*,2004).

Procedure:

The total flavonoid content of the aqueous plant extract was determined colorimetrically as described by (Zuo *et al.,* 2004). In brief, 0.5ml of *D. bulbifera* extract was mixed with 2ml of distilled water and subsequently 0.15ml of 5% of $NaNO_3$ solution. After 6 mins of

incubation, 0.15ml of 10% of $AlCl_3$ solution was added and then allowed to stand for 6 mins, followed by addition of 2ml of 4% NaOH solution to the mixture. Immediately, water was added to the sample to bring the final volume to 5ml. The mixture was thoroughly mixed and allowed to stand for another 15 mins. The absorbance was read at 510nm. The total flavonoid content was expressed in milligrams of Quercetin equivalent per gram of the plant extract. Content of total flavonoid in each extract was determined using standard curve prepared for (+) Quercetin ($y = 0.0024x$) $R^2 = 0.9816$

Total extracted flavonoid was expressed as mg (+) Quercentin equivalent /g extract.

3.2.5. Saponin determination

Saponin was determined following the methods reported by Obadoni and Ochuko (2001). In this method, 20 g of ground sample was put into a conical flask and 100cm3 of 20% aqueous ethanol was added. Then the flask was heated on a hot water bath for 4 h. with constant stirring at about 55°C. The mixture was then filtered and the residue was again extracted with another 200 ml 20% ethanol. The combined extract was reduced to 40 ml on a hot water bath at about 90°C. The concentrate was transferred into a 250 ml separatory funnel, added 20 ml diethyl ether in it followed by vigorous shaking. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated. Then 60 ml of n-butanol was added.The combined n-butanol extracts were washed twice with 10 ml of5% aqueous sodium chloride. The remaining solution was heated in a water bath for 30mins. After evaporation the samples were dried in oven, weighed and saponin content was calculated as percentage:

% Saponin = Weigt of saponin / Weight of sample x 100

3.2.6 Determination of Tannins

Principle

Tannin-like compounds reduce phosphotungstomolybdic acid in alkaline solution to produce a highly coloured blue solution, the intensity of which is proportional to the amount of tannins. The intensity is measured in a spectrophotometer at 760 nm.

Procedure:

The quantitative estimation of tannins was performed by the method of Swain (1979) with minor modifications. Finely powdered bulbils of *D. bulbifera* were kept in a beaker

containing 20 mL of 50% methanol covered with parafilm and then heated at 80°C in water bath for 1 hr with continuous stirring. The extract was quantitatively filtered using a double layered Whatman Number 1 filter paper and rinsed with 50% methanol. Thereafter, 1 mL of sample extract was treated with 20 mL distilled water, 2.5 mL Folin-Denis reagent, and 10 mL of 17% Na_2CO_3 for the development of a bluish-green colour and was allowed to stand for 20 minutes. The absorbance was measured at 760 nm and amount of tannin in the extract was determined using a standard curve prepared for (+) Tannic acid ($y = 0.0039x$) $R^2 = 0.989$.

3.2.7 Determination of alkaloid:

The gravimetric method as described by Harbone, (1973) was used to determine the total alkaloid content of the samples. Five (5) grams of the powdered sample was dispensed into 50ml of 10% acetic acid solution in ethanol. The mixture was shaken and allowed to stand for 4hrs. Drops of ammonium hydroxide were added to the extract until precipitation was complete, thereafter the residue was filtered with whatman filter paper No 1, and oven-dried at 60°C for 30 minutes. Finally the residue was weighed and the percentage alkaloid content calculated thus:

% alkaloid = weight of alkaloid residue / weight of sample x 100

3.2.8 Cardiac glycosides quantitative determination

To determine cardiac glycoside, 25 g of ground sample was weighed into 250 ml sterile bottles and 200 ml of 70 % ethanol was added into the bottle. Bottles were covered and placed on Stuart orbital shaker SSL1 set at 300 rmp and were shaken vigorously for 6 hours at room temperature. They were separately filtered through Whatman No. 1 filter papers, and were transferred into 1 Litre volumetric flask. Thereafter, 500 ml of distilled water was added into each flask followed by 100 ml of 12.5 % lead acetate (to precipitate tannins, resins and pigments). Volumes were made to 800 ml with distilled water and vigorously shaken on Stuart orbital shaker SSL1 set at 300 rmp for 10 minutes. To the 800 ml, 200 ml of 4.77 % disodium hydrogen phosphate (Na_2HPO_4) solution was added (to precipitate the excess Pb++ ions). Resultant solution was filtered through Whatman No. 1 filter papers to give a clear filtrate. The filtrates were then individually evaporated to dryness. Then, 50 mg of dried extract was dissolved in 2 ml of glacial acetic acid containing 2 drops of 2% solution of $FeCl_3$ and assayed cardiac glycosides as described previously in order to establish its presence.

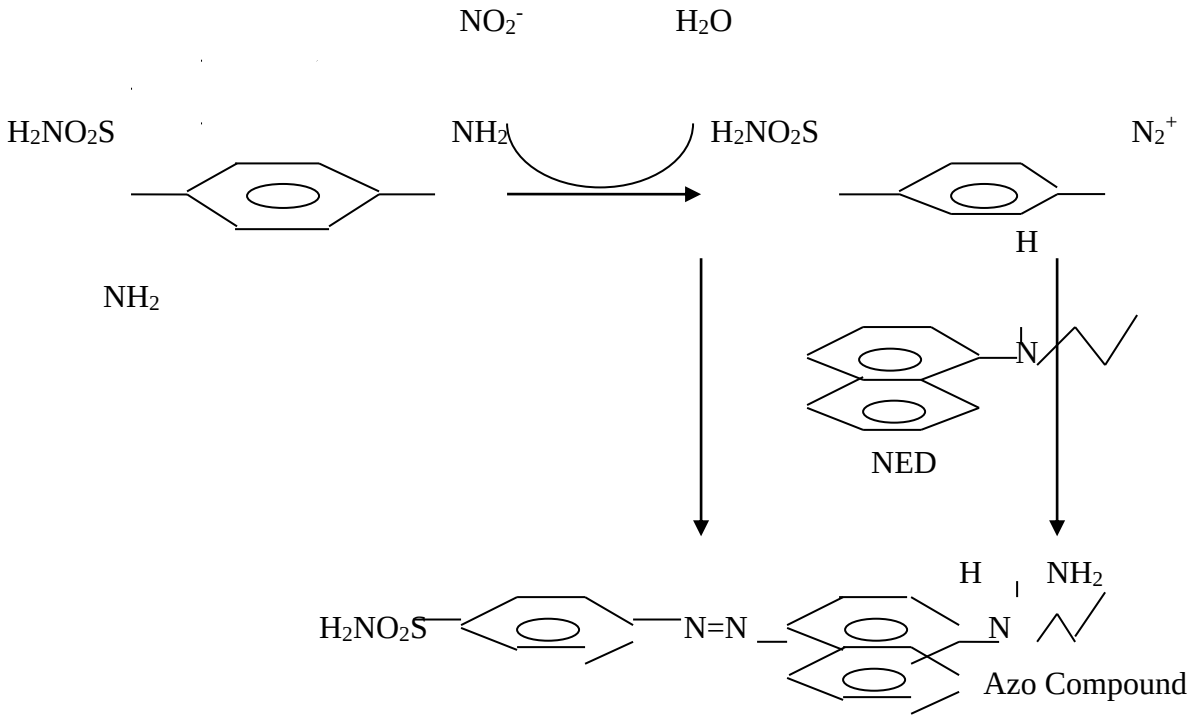
The percent cardiac glycosides content was calculated as:

$\%\ Cardiacglycosides = weight\ of\ dried\ extract\ /\ weight\ of\ dried\ ground\ plant\ sample\ X\ 100.$

3.2.9 Determination of Radical Scavenging Ability

3.2.9.1 Determination of Nitric Oxide Radical Scavenging Ability of the Extract

Principle: The compound Sodium Nitro Prusside (SNP) is known to decompose in aqueous solution at physiological pH (7.2) producing NO^-. Under aerobic condition, NO^- reacts with oxygen to produce stable products: nitrate and nitrite, the quantities which can be determine using Griess reagent (Marcocci *et al.*, 1994). The Griess reagent system is based on the chemical reaction shown below which uses sulfanilamide and N-1-napthyethylenediamine dihydrochloride (NED) under acidic (phosphoric acid) conditions.



Procedure: The scavenging effect of the extract on nitric acid was measured according to the method of Marcocci *et al.,* (1994) with little modification (Alisi and Onyeze, 2008). In the modification, 4ml of extract solution at three different concentrations (1000(µg/ml), 2000(µg/ml) and 3000(µg/ml)) were added to 1ml of sodium nitro prusside (SNP) solution (10mM) and the tubes were incubated at 29°C for 2 hours. An aliquot (2ml) of incubated solution was removed and diluted with 1.2ml of Griess reagent (1% sulfanilamide in 5% H_3PO_4 and 0.1% Naphthyl ethylenediamine dihydrochloride). The absorbance of the chromophore that formed during diazotization of nitrite with sulfanilamide and subsequent coupling with Naphthylethylenediamine dihydrochloride was immediately read at 550nm and compared with the absorbance of standard solution of sodium nitrate salt treated in the same way as Griess reagent. Inhibition of nitrate formation by the extract or the standard antioxidant (Quercetin) was calculated relative to the control.

$$\%\ Inhibition = \frac{Absorbance\ control - Absorbance\ test}{Absorbance\ control} \times 100$$

3.2.9.2 Determination of Hydroxyl Radical Scavenging Ability of the Extract

Principle: Free radical dependent 2-deoxyribose degradation was studied using fenton oxidant reaction mixture of F^{3+}/ ascorbic acid and H_2O_2 as described by Halliwell, *et al.*, (1987).

Fenton oxidant reaction mixture releases hydroxyl radicals which attack and degrade 2-deoxyribose into fragments. These fragments formed from 2-deoxyribose degradation react with Thiobarbituric acid on heating at low pH to form a pink product (Thiobarbituric Acid Reacting Substances {TBARs}), whose optical density was read at 532nm.

Procedure: Hydroxyl radical scavenging ability of extract was measured by studying the competition between deoxyribose and the test extract for hydroxyl radicals generated from the Fe^{3+}/ ascrbate/ EDTA/H_2O_2 system. The reaction mixture contained: Deoxyribose (2.8mM), $FeCl_3$ (0.1mM), EDTA (0.1mM), H_2O_2 (1mM), Ascorbic acid (0.1mM), NaH_2PO_4-NaOH buffer (20Mm, pH 7.4) and the extract (0-200µg/ml) in a final volume of 1.0ml. After incubation for 1hr at 37°C, the deoxyribose degradation was measured as TBARs by the method of Ohkawa, *et al.*, (1979), as modified by Lui, *et al.*, (1990). Briefly, 1.5ml of 20% acetic acid (PH 3.5), 1.5ml of 0.8% thiobarbituric acid (TBA), 0.2ml of 8.1% sodium dodecyl sulphate (SDS) and the incubated mixtures were heated at 100°C for 1hr, cooled and 2ml of trichloroacetic acid added. The mixture was vortexed vigorously and centrifuged at 3000 xg for 10mins and the absorbance of the mixture was read at 523nm. Concentration of thiobarbituric acid reactive substances (TBARs) was determined using the molar extinction coefficient of Malondialdehyde. Inhibition of deoxyribose degradation which gives an indication of hydroxyl radical scavenging action was calculated by:

$$\%\ \text{OH Radical Scavenging} = \frac{Absorbance\ MDA\ con\ 捴rol - Absorbance\ MDA\ test}{Absorbance\ MDA\ control} \times 100$$

3.2.9.3 Determination of Free Radical (2, 2-Diphenyl-1-Picrylhydrazyl (DPPH) Scavenging Activity

The DPPH radical is a stable organic free radical with an absorption maximum band around 515–528 nm. It is a useful reagent for evaluation of antioxidant activity of compounds. In the DPPH test, the antioxidants reduce the DPPH radical to a yellow-coloured compound, diphenylpicrylhydrazin, and the extent of the reaction will depend on the hydrogen donating ability of the antioxidant (Bondent *et al.,* 1997).

Procedure:

The scavenging activity of extract for the radical 2, 2-diphenyl-1-picrylhydrazyl (DPPH) was measured by the method described by Velázquez et al. (2003). Extract was dissolved in methanol at concentrations of 2.65–170mg/ml, and 0.75ml of each sample was mixed with1.5 ml of DPPH (Fluka-chemie, Switzerland) (0.02mg/ml) in methanol, with methanol serving as the blank sample. The mixtures were left for 15 min at room temperature and its absorbance then measured at 517nm. Catechin (0–50mg/l) was used as positive control. The radical-scavenging activity was calculated as follows:

%DPPH radical Scavenging = Blank absorbance - Sample absorbance /Blank absorbance X 100

The IC_{50} (concentration causing 50% inhibition) values of the extracts and Tannic acid (standard antioxidant substance) were determined graphically.

3.2.9.4 Reducing Power Determination

The reducing power of the prepared extract was determined according to method of Oyaizu, (1986). This method is used to investigate the Fe^{3+}/Fe^{2+} transformation in the presence of the test compound. The reducing capacity of a compound may serve as an indicator of its potential antioxidant activity (Hsu, *et al*., 2006).

Procedure:

Briefly, different amounts (0 - 5.0 mg) of the plant extract were dissolved in a buffered medium to give final concentrations of 0-1000µg/ml in a reaction mixture equilibrated thoroughly with 2.5ml of 0.2M phosphate buffer at pH 6.6 and 2.5 ml of 1% $K_3Fe(CN)_6$. The mixture was incubated at 50^0C for 20 minutes, 2.5 ml of 1% TCA was added to the above mixture and centrifuged at 3000rpm for 5

minutes. Thereafter, 2.5 ml of the upper layer of the mixture was mixed with 2.5 ml of distilled water and 0.5ml of 0.1% $FeCl_3$. The absorbance was read at wavelength of 700nm. Reducing power was determined from the plot of optical density against concentration of extract. Reducing power (RP 0.5_{AU}) was taken as the concentration of extract able to give 0.5 absorbance reading.

3.2.10 Determination of Antisickling Effects of the Extracts

3.2.10.1 Preparation of erythrocyte haemolysate

Three milliliters (3ml) of blood sample was collected by venepuncture into a citrate containerfrom a confirmed homozygous sickle cell disease patient. About 0.2ml portions of the HbSS blood sample was centrifuged (Nickel-Electro Centrifuge) at 1500 x g for 15 minutes to sediment the erythrocytes. After careful siphoning of the plasma with a pasteur pipette, the erythrocytes were washed thrice with isotonic saline (0.9% NaCl) solution, and then re-suspended in a volume of the saline solution equivalent to the siphoned plasma volume. The erythrocyte suspension was then frozen at 0°C and subsequently thawed to produce a haemolyzate for the haemoglobin polymerization study.

3.2.10.2 Determination of *D. bulbifera* extract haemoglobin polymerization inhibition potential

The determination of *D. bulbifera* extract haemoglobin polymerization inhibition potential was done using the method of (Iwu *et al*., 1988) with little modification from (Nwaoguikpe *et al*., 1999). The rate of inhibition of sickle cell haemoglobin (HbSS) polymerization by the extract were carried out by monitoring with time the turbidity of the polymerizing mixture at 700nm using 2% solution of sodium metabisulphite as a deoxygenating agent. In this method, 4.4 ml of 2% Sodium metabisulphite, 0.5 ml normal saline (0.9 %NaCl) and 0.1 ml of haemoglobin were pipetted into a cuvette shaken and absorbance read in a Spectrophotometer, (Spectronic 20) at 700 nm for 30 minutes at 2 minutes Intervals. This served as blank for all assays. For the test assay, 0.5 ml normal saline was replaced with 0.5 ml antisickling agent or sample and readings taken as usual .The rates of polymerization were calculated from the formula of average change in absorbance against time in minutes.

Rp= ODf - ODI /t

Rp = ΔOD/t, where Rp = rate of polymerization

ODf = final absorbance, ODi = initial absorbance

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1: Results

Table 4.1. Shows the results of the proximate composition of *D. bulbifera* extract. The proximate analysis showed that *D. bulbifera* has low content of crude fat and crude fibre (1.20 ± 0.06 and 1.50 ± 0.08 % respectively). It also has a low moisture content of 12.10 ± 0.61%. The protein content was 10.10 ± 0.53%. Carbohydrate content has the highest value (63.10 ± 4.12%).

Table 4.1: Proximate Composition of *D. bulbifera* extract

Proximate	Composition (%)
Moisture	12.10 ± 0.61
Ash	12.00 ± 0.68
Crude Fibre	1.50 ± 0.08
Crude Protein	10.10 ± 0.53
Crude Fat	1.20 ± 0.06
Carbohydrate	63.10 ± 4.12

Values are means ± standard deviations of triplicate determinations.

Table 4.2 below shows the results of the phytochemical analysis of D. *bulbifera* extract. The phytochemical content showed that alkaloid had the highest value (3.30 ± 0.13%) and the phenolics had the lowest value (0.03 ± 0.10%). Total glycosides, tannins, flavonoids and saponins were present in appreciable amounts.

Table 4.2: Phytochemical Content of *D. bulbifera* extract

Phytochemical Content	(%)
Alkaloids	3.30 ± 0.13
Saponins	0.50 ± 0.02
Total Glycosides	0.80 ± 0.03
Tannins	0.17 ± 0.01
Flavonoids	0.05 ± 0.04
Phenolics	0.03 ± 0.10

Values are means ± standard deviations of triplicate determinations.

4.2 Antioxidant and free radical scavenging ability of *D. bulbifera.*

Table 4.3 below shows the spectrophotometric measurement of the total phenolic and flavonoid contents of D. *bulbifera*. Total phenolic content (TPC) was 1.33 ± 0.06/100g Tannic acid equivalent, while total flavonoid content (TFC) was 1.79 ± 0.09g quercetin equivalent.

Table 4.3: Quantitative Phytochemical Content of *D. bulbifera* Ethanol Extract

Quantitative Phytochemical Content of *D. bulbifera* Ethanol Extract	
Total Phenolic Content (mg Tannic acid/g Extract)	1.33 ± 0.06
Total Flavonoid Content (mg Quercetin/g Extract)	1.79 ± 0.09

Values are means ± standard deviation of triplicate determinations.

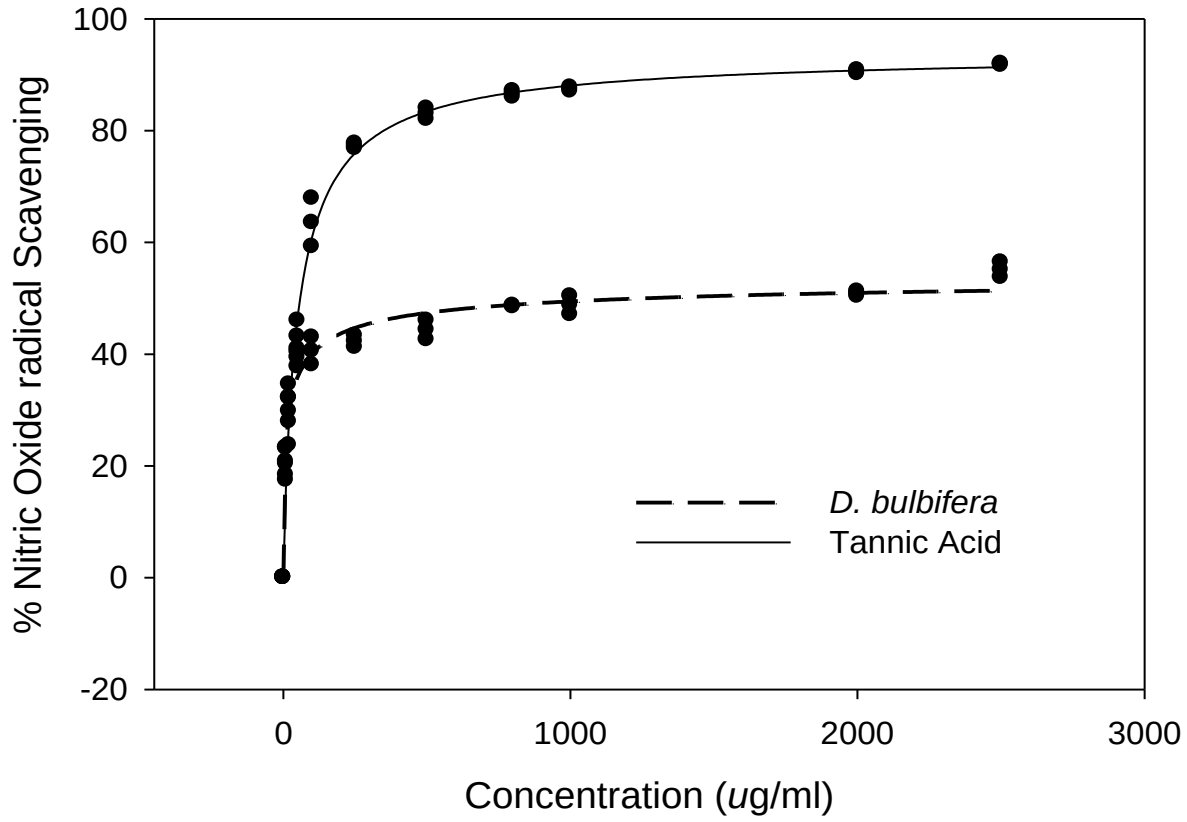


Fig 4.1: Nitric Oxide Radical Scavenging abilities of D. *bulbifera* extract and Tannic acid

Fig 4.1 above represent % Nitric Oxide Radical scavenging of *D. bulbifera* extract and % Nitric Oxide Radical Scavenging of Tannic Acid. Results showed that the extract of *D. bulbifera* caused a dose-dependent scavenging of Nitric Oxide radicals generated by Sodium nitroprusside. The IC_{50} of *D. bulbifera* extract and Tannic Acid were 1257.01 ± 58.37(µg/ml) and 58.37 ± 8.09 (µg/ml), respectively. The extract showed a significant antioxidant ability to scavenge $NO^{\cdot}$ and or inhibit nitrite formation as it compared well with standard antioxidant (Tannic acid), though, the standard tannic acid has more antioxidant activity.

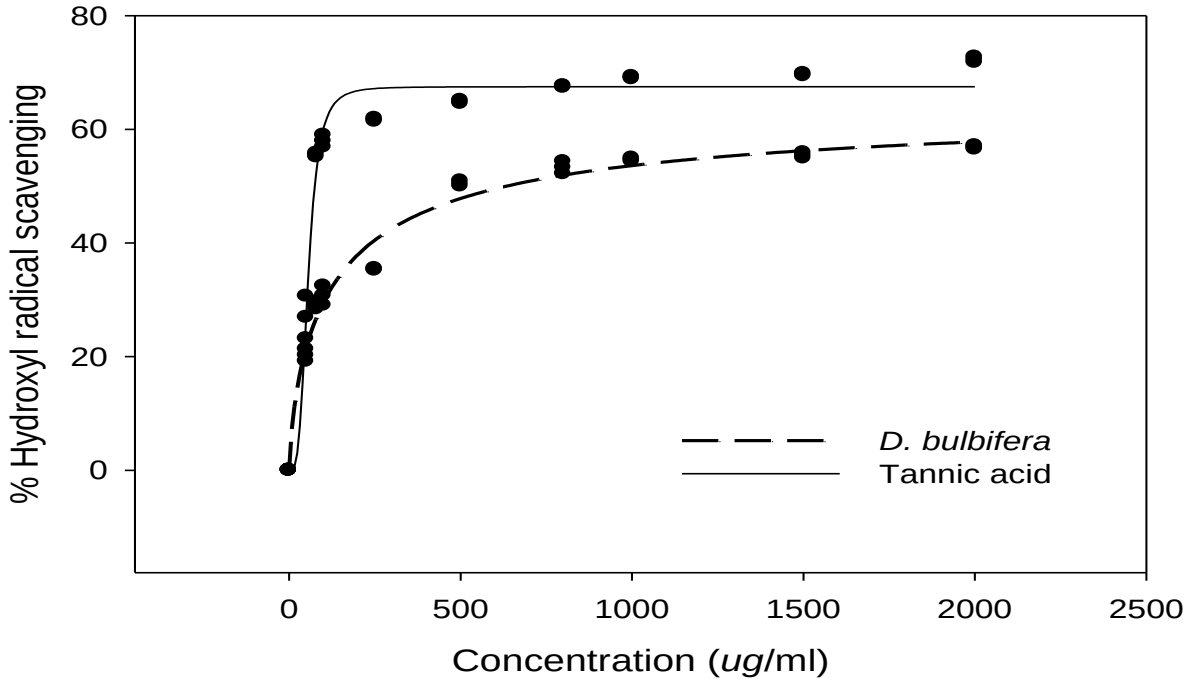


Fig 4.2: % Hydroxyl Radical Scavenging ability of *D. bulbifera* extract and Tannic Acid.

The results presented in fig 4.2 above shows the percentage hydroxyl radical scavenging abilities of *D. bulbifera* extract and Tannic acid which was used as the reference compound. The % Hydroxyl radical scavenging of *D. bulbifera* increases with increase in extract concentration. The IC_{50} values for *D. bulbifera* and Tannic Acid were found to be 633.60 ± 54.52 (µg/ml) and 74.56 ± 5.25 (µg/ml), respectively. This indicated a significant free radical scavenging activities of the extract.

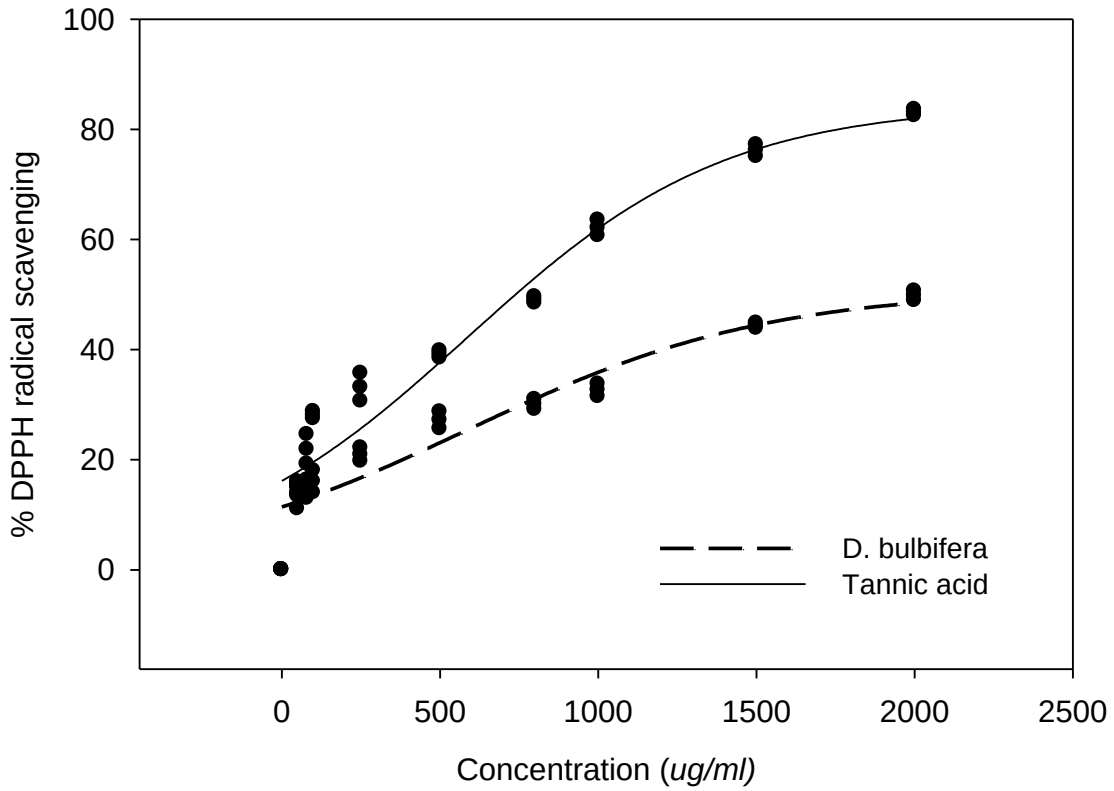


Fig. 4.3: DPPH Radical Scavenging abilities of *D. bulbifera* and Tannic acid

The results shown in fig. 4.3 are comparison of the DPPH radical scavenging activities of *D. bulbifera* extract and the standard Tannic acid. The IC_{50} values of *D. bulbifera* extract and Standard Tannic Acid were 2285.78 ± 245.20 µg/ml and 651.01 ± 33.73 µg/ml, respectively. According to the data, it indicated that the extract favourably compared with the standard (Tannic Acid) and hence a good DPPH radical scavenger. The results above showed that free radical scavenging by *D. bulbifera* followed a logistic dose response model.

Reducing Power of D. *bulbifera* and Ascorbic Acid

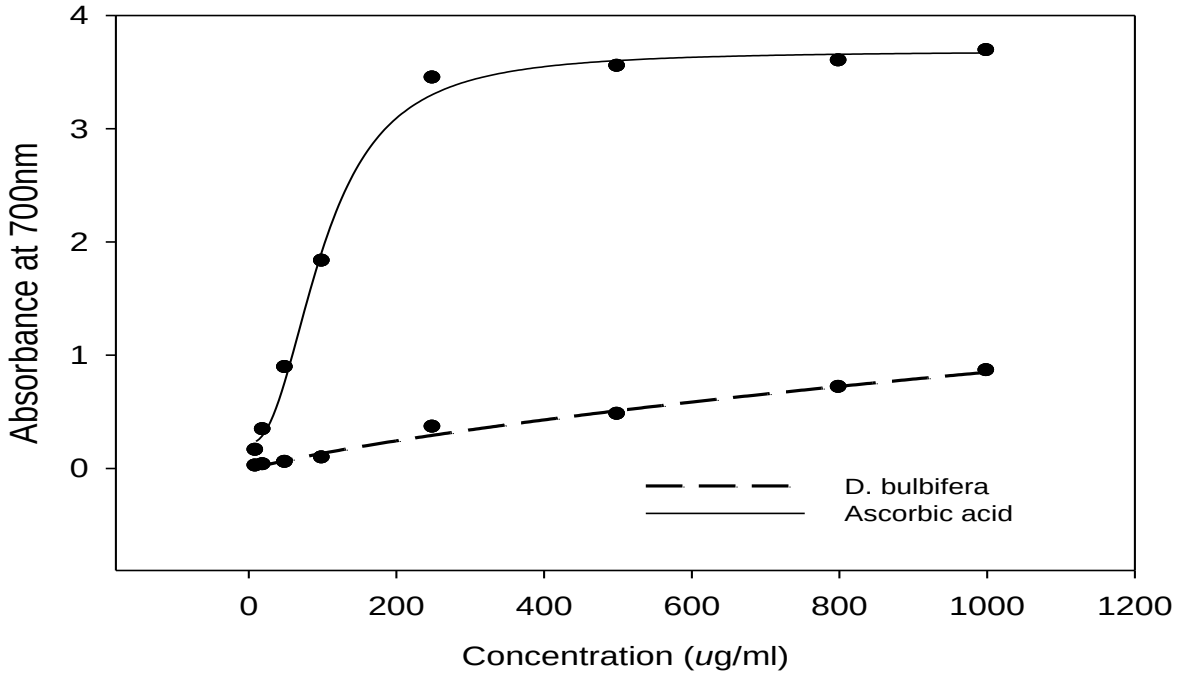


Fig. 4.4: Half Maximum Optical Density of *D. bulbifera* and Ascorbic Acid (µg/ml)

The result showed the optical density of *D. bulbifera* extract and standard ascorbic acid respectively. The extract showed a dose dependent response when compared with the standard ascorbic acid. This indicated that the the reducing power of *D. bulbifera* extract is significant as it compared well with the standard ascorbic acid.

4.3 Inhibition of Haemoglobin Polymerization of HBSS by the Extract

The table below shows the effects of *D. bulbifera* extract on the rate of polymerization (ROP), relative percent polymerization (RPP), relative percent inhibition (RPI).

Table 4.4: The effects of *D. bulbifera* ethanol extract on the rate of polymerization (ROP), relative percent polymerization (RPP), and Relative percent inhibition (RPI) of HbSS.

Concentration (µg/ml)	0	20	50	100	200	500
ROP	3.37E-03 ± 1.68E-04	3.23E-03 ± 1.62E-04	3.43E-03 ± 1.72E-04	3.70E-03 ± 1.85E-04	3.30E-03 ± 1.65E-04	2.83E-03 ± 1.44E-05
RPP	100 ± 6.20	96.04 ± 4.80	101.98 ± 5.10	109.90 ± 5.50	98.02 ± 4.90	84.16 ± 4.21
RPI	0.00 ± 0.00[a]	3.96 ± 0.2[b]	-1.98 ± -0.10[c]	-9.90 ± -0.5[d]	1.98 ± -0.10[e]	15.84 ± 0.74[f]

Values are Mean ± standard deviation of 3 determinations

Mean with different superscript are significantly different ($P<0.05$).

4.4 Inhibition of Hemoglobin Polymerization of HBSS by the standard Hydrobenzoic acid (HBA)

The table below shows the effects of HBA on the rate of polymerization (ROP), relative percent polymerization (RPP), relative percent inhibition (RPI).

Table 4.5: The effects of Hydrobenzoic acid standard on the rate of polymerization (ROP), relative percent polymerization (RPP), and relative percent inhibition (RPI) of HbSS

Concentration (µg/ml)	0	20	50	100	200	500
ROP	3.37E-03 ± 1.68E-04	2.33E-03 ± 1.17E-04	4.23E-03 ± 2.12E-04	2.97E-03 ± 1.48E-04	4.80E-03 ± 2.40E-04	4.00E-04 ± 1.84E-04
RPP	100.00 ± 5.00	69.31 ± 3.47	125.74 ± 6.29	88.12 ± 4.41	142.57 ± 7.13	11.88 ± 0.59
RPI	0.00 ± 0.00[a]	30.69 ± 1.53[b]	-25.74 ± -1.29[c]	11.88 ± 0.65[d]	-42.57 ± -2.13[e]	88.12 ± 4.49[f]

Values are Mean ± standard deviation of 3 determinations

Mean with different superscript are significantly different ($P<0.05$).

Nitric Oxide Threshold Inhibitory concentration of D. *bulbifera* and Tannic Acid.

Table 4.6: Threshold inhibitory concentration of *D. bulbifera* and Tannic acid against Nitric

Threshold Inhibitory Concentration (IC)					
% Inhibition	5	10	20	50	80
D.bulbifera Ethanol extract (µg/ml)	0.21 ± 0.03[a]	0.90 ± 0.08[a]	5.29 ± 0.16[a]	1257.01± 58.37[a]	ND
Tannic Acid (µg/ml)	1.81 ± 0.115[b]	4.51 ± 0.09[b]	11.64 ± 0.39[b]	58.37 ± 8.09[b]	349.93 ± 49.31

oxide radical

Values are Mean ± standard deviation of 3 determinations

Mean with different superscript down columns are significantly different (P<0.05).

ND = Non determinable

The results showed Nitric Oxide radical Scavenging Threshold Inhibitory Concentrations of *D. bulbifera* and Tannic acid. The IC_{50} of the ethanol extract of *D. bulbifera* (1257.01 ± 58.37 µg/ml) showed that it is a good antioxidant when compared with Tannic acid which was used as the standard antioxidant with an IC_{50} of 58.37 ± 8.09 µg/ml.

Hydroxyl Radical Inhibitory Concentration of *D. bulbifera* and Tannic acid

Table 4.7: Threshold inhibitory concentration of *D. bulbifera* and Tannic acid against Hydroxyl Radical

Threshold Inhibitory Concentration (IC)					
% Inhibition	5	10	20	50	80
D.bulbifera Ethanol extract (µg/ml)	4.59 ± 0.60[a]	13.10 ± 2.02[a]	43.35 ± 1.70[a]	633.60± 54.52[a]	ND
Tannic Acid (µg/ml)	27.45 ± 2.36[b]	33.98 ± 3.17[b]	43.51 ± 2.15[a]	74.56 ± 5.25[b]	ND

Values are Mean ± standard deviation of 3 determinations

Mean with different superscript down columns are significantly different (P<0.05).

ND = Non determinable.

Table 4.7 shows the Hydroxyl radical Scavenging Threshold Inhibitory Concentrations of D. *bulbifera* and Tannic acid. Comparing the extract ability with the standard antioxidant (Tannic acid) using IC_{50} as an index, *D. bulbifera* extract and Tannic acid had IC_{50} 633.60 ± 54.52 µg/ml and 74.56 ± 5.25 µg/ml respectively. This showed that *D.bulbifera* had significant Hydroxyl radical scavenging ability as it compared well with the standard.

DPPH Radical Threshold Inhibitory Concentrations of D. *bulbifera* and Tannic

Table 4.8: Threshold Inhibitory concentrations of *D. bulbifera* and Tannic acid on DPPH Radical

Threshold Inhibitory Concentration (IC)					
% Inhibition	5	10	20	50	80
D. bulbifera Ethanol extract (µg/ml)	4.46 ± 0.27[a]	35.65 ± 3.58[a]	222.43± 15.65[a]	2285.78± 245.20[a]	7774.04± 765.10[a]
Tannic Acid (µg/ml)	1.95 ±0.02[b]	14.97 ± 1.76[b]	81.63 ± 7.09[b]	651.01± 33.73[b]	1856.85± 148.65[b]

Values are Mean ± standard deviation of 3 determinations

Mean with different superscript down columns are significantly different (P<0.05).

Table 4.8. Shows the DPPH Radical Scavenging Threshold Inhibitory Concentrations of *D. bulbifera* and Tannic acid which is a standard antioxidant. With IC_{50} as an index, the results indicated a significant antioxidant activity of the *D. bulbifera* extract as it compared well with the standard and therefore a good scavenger of DPPH radical.

Half Maximum Optical Density of *D. bulbifera* and Ascorbic Acid (µg/ml)

Table 4.9: Half Maximum Optical Density of *D. bulbifera* and Ascorbic Acid (µg/ml)

Extract	**Reducing Power (*µ*g/ml)**
***D. bulbifera* ethanol extract**	480.63 ± 33.82^{a}
Ascorbic acid	30.07 ± 2.17^{b}

Values are Mean ± standard deviation of 3 determinations

Mean with different superscript are significantly different ($P<0.05$).

The result above showed the $O.D_{0.5}$ of *D. bulbifera* extract and Ascorbic acid which was used as standard. On comparison, this indicated a significant amount of reducing power of *D. bulbifera* extract as it compared well with the standard Ascorbic acid. Hence, it's strong free radical scavenging ability.

4.5 Discussion

Medicinal importance of plants have assumed an important dimension in the past few years. Plants, for instance *D. bulbifera* from this study have been found besides its nutritive value, to possess a very diverse group of bioactive agents with both pharmacological and free radical scavenging abilities.

The results of this study showed that the moisture content is similar to the values reported by Ogbuagu, (2008), Adeosun, *et al*., (2016), Princewill and Ibeji, (2015) and Abara, (2011). They reported a value of 7.02%, 4.60%, 7.16%and 9.20% respectively. The low percentage of moisture content obtained from this study is however contrary to results in previous literatures. Polycarp D. A., (2012) reported a range of 61.55% and 71.09%. Shajeela *et al.*, (2011) also reported a value of 86.70% for same *D. bulbifera*. The differences observed between the result of this study and the report of other researchers could be associated with level of maturity of the bulbils, experimental method of analysis, environmental factors and the type of cultivar used (Coursey and walker, (1960), Abara *et al.,* (2013), Adeosun *et al.,* (2016) and Shajeela *et al., (*2011)). High moisture content reduce storage value (Shajeela *et al.,* (2011)). The low moisture content of the bulbil could enhance the quality and shelf life. It implies that the *D. bulbifera* may be suitable for prolonged storage and industrial food processing.

The ash content of *D. bulbifera* in this study was found to be higher when compared with earlier reports of Polycarp *at al.,* (2012), Chandra *et al.,* (2012), Ogbuagu, (2008), Princewill-Ogbonna, (2015) and Abara *et al.,* (2003). The high value of ash content in the *D. bulbifera* from this study could be due to genetic factors and or the mineral content of the soil (Edeogu *et al.,* (2007); Rivero *et al.,* (2007)). The ash content of a food sample gives an idea of the mineral elements present in the food sample (Edeogu *et al.,* (2007)). Minerals generally in the diet are required for metabolic reactions, transmission of nerve impulses, rigid bone formation and regulation of water and salt balance among others.

Crude fibre content noted in this study were found between the range of 0.6-2.44% reported by earlier reseachers Afoakwa and Sefa-Dedeh, (2001), Bhandari *et al.,*(2003), Alinnor and Akalezi, (2010) and Polycarp *et al.,* (2012). High crude fibre in diet is known to enhance the digestibility, decrease blood cholesterol and reduce the risk of large bowel cancers (Ujowundu, C. U *et al.,* (2010)). Crude fibre increases stoolbulk and decrease the time that

intestinal contents spend in the gastrointestinal tract. Therefore, *D. bulbifera* could be recommended as a good source of crude fibre in the diet as a result of its relatively high fibre content as reported in this study.

Crude protein content recorded in this study is within the range reported by Agbor-Egbe and Treche, (1995) on Cameroonian yams and Shanthakumari *et al*., (2008). The value reported in this work is higher than that obtained by Polycarp *et al.,* (2012) and Charles *et al.,* (2005). Proteins are for growth and repair of worn-out tissues and also for alternative energy source in the absence of carbohydrate and lipids.

The crude fat content (ether extract) of the *D. bulbifera* obtained in this study was slightly higher than those obtained by earlier researchers (Agbor-Egbe and Treche, 1995) for Cameroonian yams, Polycarp *et al*., *(2*012**)** and Chandra *et al*.,(2012). The little value obtained in this study is quite reasonable as all root crops exhibits very low value of lipid content (Ekpeyong, 1984). Lipids contribute to the palatability of the crops and it is also a source of energy. Since *D. bulbifera* has low fat content, they could be recommended as a good source of food supplement for patient with cardiac problems or at risk with lipid induced disorders.

The carbohydrate content obtained from this work is comparable to literature values (Ogbuagu, (2008), Polycarp *et al.,* (2012), Udensi *et al.,* (2008)). The high carbohydrate content obtained from this work make them reliable food security crops. Carbohydrates and lipids are the major energy sources in human and animal.

However, the medicinal value of plants lies in some bioactive agents that have a definite physiological action on the human body. Different phytochemicals have been found to possess a wide range of activities, which may provide protection against chronic diseases (Mir *et al.*, 2013). The presence of phytochemicals in the extract of *D. bulbifera* suggests possible medicinal relevance.

In this study, the level of alkaloid obtained was higher than the levels obtained by Ogbuagu, (2008), Subasini *et al.,* (2013), Okwu *et al.,* (2016) and Ezeocha and Ojimelukwe.,(2012). Alkaloids have amazing effects on humans and have been exploited in numerous pharmaceutical applications as anaesthetics, analgesic, antibiotics, disinfectants, central

nervous system (CNS) stimulants and sedatives (Ibraheem and Maimako, 2014; Ahmed and Mohamed, 2014.). Despite the medicinal value of alkaloids, they cause gastro-intestinal upsets and neurological disorders. Alkaloids have also been reported to contain dihydrodioscorine-a compound which causes paralysis in the CNS in animals. (Okwu *et al.,* 2016; Oliver-Bever, 1989).

Saponins possess a carbohydrate moiety attached to a triterpenoid or a steroidal aglycone (Sridhar and Bhat, 2007). The level of saponin obtained in this work was lower than the values reported by Ezeocha and Ojimelukwe, (2012) and Okwu *et al.,* (2016). Also, a similar value was reported by Ahmed and Mohammad, (2014) for Loranthus bengwensis leaf. Saponins have been reported to possess antimicrobial properties which make them good for preventing infections and microbial invasions. (Okwu, 2004; Okwu and Emenike, 2006; Okwu and Ndu 2006; Princewill-Ogbonna and Ibeji, 2015). Saponin also have cholesterol lowering effects which could give some chemoprotection against heart diseases to human consumers (Ujowundu *et al.,* 2010; Haborne, 1973). Also, saponin are known to possess the ability to inhibit Na ion efflux by blockage of influx of concentration in the cells, activating a Na^+ - Ca^+ antiporter in cardiac muscles. The increase in Ca^{2+} influx through this antiporter strengthens the contraction of heart muscles (Egwim, *et al.,* 2011). It is believed that saponin possess the properties of precipitating and coagulating red blood cells and therefore could be used as antibleeding agent to arrest lost of blood in case of injury (Adeosun *et al.,* 2016).

In spite of the numerous beneficial properties, saponins also possess deleterious (cytotoxic permeabilization of the intestine) and poisonous effects in animals and humans (Ahmed and Mohammad, 2014; Soladoye and Chukwuma, 2012).

The result of this study indicated that the extract of *D. bulbifera* contained an appreciable amount of Cardiac glycosides which is comparable with what was reported by Ujowundu *et al* (2010). The cardiac (heart) glycosides are used to treat heart failure and they also have potential in cancer therapy. It's mechanism of action is by reducing atrial fibrillation mainly by increasing vasovagal activity on atrial-ventricular conduction and inhibition of sarcolemmal Na^+/K^+-ATPase, increasing influx, and thereby the force of heart muscle contraction. A decrease in sympathetic nervous system activity due to heart glycosides is beneficial in chronic heart failure (Elisabet *et al.,* 2009 and Ujowundu *et al.,* 2010).

In this work, the level of tannin obtained was in agreement with that obtained by Princewill-Ogbonna, (2015). Tannins are polyphenols and have been reported to have wound healing,

antiviral, antibacterial and antiparasitic effects (Okwu, 2004). They also have the ability for digestibility and palatability of proteins because they can form insoluble complexes with them. (Osagie, 1998). The bitter principle of *D. bulbifera* may be due to the presence of tannins (Princewill-Ogbonna, 2015).

Flavonoids in this study recorded a low value which was similar to the values reported by Okwu *et al.,* (2016). Flavonoids are important class of polyphenols found in plants. Alkaloids and flavonoids are responsible for antimicrobial properties found in higher plants (Soladoye and Chukwuma, 2012). The biological functions of flavonoids besides its antioxidant properties include protection against allergies, inflammation, free radicals, platelet aggregation, ulcers, hepatoxins, viruses and tumours (Ujonwundu *et al.,* 2010; Alisi *et al.,* 2012; Soladoye and Chukwuma, 2012; Nilofer *et al.,* 2013). The presence of flavonoids in *D. bulbifera* may confer anticarcinogenic potential to consumers.

Phenolics have been suggested to exhibit health related functional properties such as anticarcinogenic, antiviral, antimicrobial, anti-inflammatory, hypotensive and antioxidant activity (Shetty, 1997; Shejeela *et al.,* 2011). However, phenolic compounds inhibit the activity of digestive as well as hydrolytic enzymes such as amylase, trypsin, chymotrypsin and lipase. The phenolics and tannins are water soluble compounds and as such can be eliminated by soaking followed by cooking (Uzogara *et al.,* 1990; Singh and Singh., 1992; Shanthakumari *et al.,* 2008). Plants produce an astonishing diversity of phenolic metabolites which contribute as one of the principal agents acting as primary antioxidants or free radical terminators. The phenolic content therefore, can be correlated directly with the antioxidant capacity, the flavonoids mostly facilitate scavenging or chelation activities

Apart from the presence of phytochemicals with well known antioxidant properties, free radical scavenging abilities of *D. bulbifera* was also investigated in this study against nitric oxide radicals, hydroxyl radicals, DPPH radicals and reducing power. Antioxidants reduce free radicals by donating electron to the unpaired electron of the free radical perhaps to enable it achieve stability. Antioxidants inhibit the action of free radicals which have been implicated in the pathogenesis of many diseases and in the ageing process (Alisi and Onyeze, 2008).

Results from this study showed that the extract of *D. bulbifera* caused a dose-dependent scavenging of Nitric Oxide radicals generated by Sodium nitroprusside (SNP). The extract showed a significant antioxidant ability to scavenge NO• and or inhibit nitrite formation as it compared well with standard antioxidant (Tannic acid). The standard tannic acid has more antioxidant activity than the extract. Direct tissue toxicity and vascular collapse associated with septic shock, may result from a sustained production of the nitric oxide radical; moreover, chronic expression of the radical contributes in many carcinomas and inflammatory conditions including juvenile diabetes, multiple sclerosis, arthritis and ulcerative colitis (Anjali and Sheetel ., 2013). Results of this work showed that the extracts showed a moderate NO• scavenging activity. It has been established that many phenolic and flavonoid compounds show antioxidant and free radical scavenging abilities (Robert *et al.*, 2003; Wozniak *et al.*, 2004; Alisi and Onyeze, 2008). The results showed that *D. bulbifera* extract has significant phenolic and flavonoid contents. The antioxidant and the free radical scavenging activity of the extract may be due to these diverse phytochemicals.

Furthermore, the results of this work showed that the percentage Hydroxyl radical scavenging activity of *D. bulbifera* extract and the tannic acid which is used as standard increases with increase in concentration. One of the most damaging of all the free radicals formed in the biological systems is the hydroxyl radical that has the potential to bring enormous damage to biomolecules. The addition of the *D. bulbifera* extracts and standard tannic acid to the Fenton's reaction has shown that they effectively scavenge the resulting hydroxyl radicals, which if implemented in biological system, may block the deoxyribose damage. (Chatterjee *et al.*, 2012; Valko *et al.*, 2007; Alisi *et al.*, 2012). The extract compared favourably to the standard though the standard has higher scavenging ability. The hydroxyl radical scavenging activity of *D. bulbifera* may also be partly due to the presence of flavonoid and phenolic compounds.

According to the data, it indicated that the extract favourably compared with the standard (Tannic Acid) and hence a good DPPH radical scavenger. The results showed that free radical scavenging by *D. bulbifera* followed a logistic dose response manner. The IC_{50} values of the extract and the standard showed that the extract is a good scavenger though not as potent as the standard. The high free radical scavenging properties of this extract is indicative of a high antioxidant activity mediated through electron or hydrogen ions donation, which is attributable to their rich phenolic and flavonoids contents. Earlier studies have indicated that flavonoids exert their biological effects at the cellular level by specific interaction, with

molecular targets such as nucleic acid, polysaccharides, protein and nerve cells. Flavonoids and generally plant derived phenolic compounds are electron rich and therefore could act as sink for protons and free radicals (Oboh *et al.,* 2007; Narayara *et al.,* 2001; Alisi *et al.,* 2014). *D. bulbifera* is rich in phenolic compounds as shown in the results.

The extract also showed a dose dependent response when compared with the standard ascorbic acid. This indicates that *D. bulbifera* has a good reducing power. Reducing power is associated with antioxidant activity and may serve as a significant reflection of the antioxidant activity. Most antioxidants and pharmacologically therapeutic agents used in the treatment of oxidative stress related diseases have been shown to have strong reducing power (Amin and Razieh, 2007). Compounds with reducing power indicate that they are electron donors and can reduce the oxidized intermediates of lipid peroxidation processes, so they can act as primary and secondary antioxidants. Reducing power of a compound is determined by its ability to facilitate the transformation of $Fe3^{+}$ to $Fe2^{+}$. Reducing power provides effectiveness in the conversion of free radicals to more stable products and thus brings about the termination of free radical initiated chain reaction. (Alisi *et al*., 2011).

This study also showed that polymerization of HbS molecules was inhibited upon introduction of the extract in a pattern similar to that caused by hydroxybenzoic acid (HBA). It has been hypothesized that an ideal antisickling drug or agent should significantly inhibit polymerization of the abnormal sickle haemoglobin HbS (Nwaoguikpe and Ejele, 2010; Ojiako, O. A *et al.,* 2012). The extract exhibited the highest level of inhibition of haemoglobin HbS polymerization at 15.84±0.74%, which is significant when compared with HBA - a standard antisickling agent which showed a highest inhibition of 88.12±4.49. At a certain concentration the extract and the HBA enhanced sickle cell hemoglobin polymerization with relative % inhibition of -9.90±-0.5% and -25.74±-1.29% respectively thus exhibiting sickling which is in contrary to earlier reports. Although, Nwoguikpe and Braide, (2012), reported enhanced sickle cell hemoglobin polymerization of copper, a micronutrient. These results suggest that the extract might have the ability to diffuse into the haemoglobin molecule to bind at the heme pocket, thereby obstructing the 'sticky patches' of the sickle cell Hb molecules (Noguchi *et al.,* 1982). This will prevent polymerization of Hb molecules into long fibers that would have caused deformation into sickle shapes of the normal disc biconcave shape of RBCs (Iyamu *et al.,* 2003). Furthermore, the results of the proximate, phytochemicals and free-radical scavenging ability from this study suggest that

the extracts must have offered protection to the erythrocytes membranes of HbSS blood from oxidative injury by reactive oxygen species (ROS), thus preventing membrane deformation, haemolysis and the formation of dense cells (Ekeke *et al.*, 200
0; Ekeke *et al.*, 2001; Iwu *et al.*, 1988; Noguchi and Schetcher, 1978).

CHAPTER FIVE
CONCLUSION AND RECOMENDATION

5.1 Conclusion

The results obtained in this study have shown that *D. bulbifera* has significant concentrations of proximate contents, phytochemical contents, free-radical scavenging ability, antioxidant potential and *in-vitro* antisickling effects.

The proximate study showed that *D. bulbifera* is an important source of nutrients and therefore could be used as a good source of protein, minerals, fibre and energy for both man and / or livestock.

Moreover, the presence of important and active phytochemicals may be related to its traditional medicinal value and thus confirmed that it serves as a potent source for modern drugs.

Moreso, the extract has also shown a good antioxidant and free-radical scavenging abilities against nitric oxide radicals, hydroxyl radicals, DPPH radicals and a good reducing potential. The results also showed the ability of the extract to inhibit haemoglobin polymerization of sickle cell erythrocytes. Therefore, they can be recommended for management of sickle cell disease. The phytochemicals found in the extract are thought to be responsible for the antioxidant and free radical scavenging properties observed. These findings justified the medicinal use of this extract in treatment and management of diseases.

5.2 Recommendation

In line with increasing global demand for food, *D. bulbifera* finds use as a potential source of functional foods. I, therefore recommend its usage as a food source. I also recommend increased awareness on the health benefits and use of *D. bulbifera*. Moreso, further pharmacological studies and investigation into the mechanism of action of the extract is also recommended.

REFERENCES

Abara, A.E. (2011). Proximate and mineral elements composition of the tissue and peel of *D. bulbifera* tuber; *Pakistan Journal of Nutrition*. **10**(6): 543-551.

Abara, A.E., Udosen E.O., and Eka, O.U. (2000). Estimation of calcium, zinc, hydrocyanate oxalate and phytate in *Dioscorea bulbifera* tuber. *Global Journal Pure Applied Sci*. 6: 449-453.

Abara, A.E., Udosen, E.O., Eka, O.U. (2013). Moisture content and polyphenol oxidase activity of growing *Dioscorea bulbifera* as indicators of tuber maturation. *Global Journal Of Pure Applied Science*. 9: 113-115.

Abhishek, D., Dipankar, C., Nripendranath, M., and Anupam, C. (2012). Study of antioxidant and reactive oxygen species scavenging activity of the edible tuber of greater yam (Dioscorea alata L) from North-East India. *Asian Journal of Pharmaceutical and Clinical Research.* **5**(3):74- 84.

Adeniran, A.A and Sonibare, M .A (2013). In vitro potential anthelmintic activity of bulbils of *Dioscorea bulbifera* L. on earthworms and liverflukes. *Journal Pharmacognosy Phytotherapy.* 5: 196-203.

Adeosun, M.A., Daniel, J. A., Odeyemi, A.T., and Alaba, A.A. (2016). Antibacterial activities and phytochemical properties of extracts of *D. bulbifera* (air potatoe) tubers and peels against some pathogenic bacteria. *The Journal of Phytopharmacology*. **5**(1):20-26.

Afoakwa, E.O. and Sefa-Dedeh, S. (2001) Chemical composition and quality changes occurring in *Dioscorea dumetorum* pax tubers after harvest. *Food Chemistry***75**: 85–91.

Agbor-Egbe, T. and Treche, S. (1995). Evaluation of the chemical composition of Cameroonian Yam Germplasm. *Journal of Food Composition and Analysis* **8**: 274-283.

Ahmed, Z. Chishti, M.Z., Johri, R.K., Bhagat, A. Gupta, K.K., and Ram, G. (2009). Antihyperglycemic and antidyslipidemic activity of aqueous extract of *D. bulbifera* tubers. *Diabetologia Croatica.* **38**: 63–72.

Ahmed, O. and Mohammad, A.D. (2014). Qualitative and quantitative analysis of phytochemicals of *Loranthus bengwensis* leaf. *International Journal of Pharmaceutical Science*. 05(01)

Alinnor, I.J. and Akalezi, E.O. (2010). Proximate and Mineral Compositions of *Dioscorea rotundata* (White Yam) and *Colocasia esculenta* (White Cocoyam). *Pakistan Journal of Nutrition* **9** (10): 998-1001.

Alisi, C. S., Ojiako, O.A., Igwe, C. U., Ujonwundu, C. O., Anugweje, K. and Okwu, G. N (2012). Antioxidant content and free radical scavenging activity of Honey of Apis mellifera of Obudu Cattle Ranch. International, *Journal of Biochemistry Research*.**2** (4):164-175.

Alisi, C.S and Onyeze, G. O. C (2008). Nitric Oxide scavenging ability of ethel acetate fraction of methanolic leaf extracts of chromolaena Odorata (linn). *African Journal of Biochemistry Research*. **2**(7):145-150.

Alisi, C.S., Asiwe, E.S., Emejulu, A.A., ENE, A. C. and Nwoguikpe, R.N. (2014). Neuroprotective and free radicals scavenging potentials of some common leafy vegetables consumed in South- East Nigeria. *Annual Research and Review in Biology*. **4** (22):3345-3358.

Alisi, C.S., Ojiako, O. A., Osuagwu, C. G., and Onyeze, G. O. C (2011). Free radical scavenging and in-vitro antioxidant effects of ethanol extract of medicinal Herb *Chromolaena odoratalinn*. *British Journal of Pharmaceutical Research*. 1(4):141-155.

Amanze, N.J., Agbo, N.J., Eke-Okoro, O.N. and Njoku, D.N. (2011). Selection of yam seeds from open pollination for adoption in yam (*Dioscorea rotundata Poir*) production 'zones in Nigeria. *Journal of Plant Breeding and Crop Science* **3**(4): 68-73.

Amin, A., and Razieh, Y. (2007) Antioxidant and free radical scavenging potential of *Achillea* and antiradical properties of flavones from the roots of *scutellaria baicalensis Georgi. Nahrung* 48: 9 –12.

Anjali, S and Sheetal, S (2013). Phytochemical analysis and free radical scavenging potential of herbal and medicinal plant extracts. *Journal of Pharmacognosy and Phytochemistry*. 2(4):22-29.

Bell, A. and Favier, J.C. (1981). Effect of traditional food processing methods on the nutritional value of yam in Cameroon. IDRC: tropical root crops: *Proc 1Triennn Root Crops Symp* Ibadan. **163**: 214–224.

Bhandari, M.J. and Kawabata, J. (2004). Organic acid, phenolic content and antioxidant activity of wild yam (*Dioscorea* spp.) tubers of Nepal. *Food Chemistry* 88: 163-168.

Bhandari, M.R., Kasai, T. and Kawabata, J. (2003). Nutritional evaluation of wild edible yam (*Dioscorea* sp.) tubers of Nepal. *Food Chemistry* 82 (4): 619–623.

Bondent, V. Brand-Williams, W., and Bereset, C. (1997). Kinetic and mechanism of antioxidant activity using the DPPH free radical methods. Lebensmittel Wissenschaft and Technologie.;30:609–615.

Edeogu, F. C., Ezeonu, A.. Okaka, N. C., Ekuma, C. E. and Elom, S. O. (2007). Proximate composition of staple food crops in Ebonyi State (South Eastern Nigeria), *International Journal of Biotechnology and Biochemistry*, **3**(1), 1-8.

Chandra, S., Saklami, S., Mishra, P., Abhay, and Bamrara, A. (2012). Nutritional profile and phytochemical screening of Garhwal Himalaya Medicinal plant *D.bulbifera*. *International. Research Journal of Pharmacy*. **3**(5):289-294.

Charles, .L., Sriroth, K., and Huang, T.C. (2005). Proximate composition, mineral contents, hydrogen cyanide and phytic acid of 5 cassava genotypes. *Food Chemistry* 92: 615–620.

Chatterjee, M., Roy, K., Janarthan, M., Das, S and Chatterjee, M. (2012). Biological activity of carotenoids: its implications in cancer risk and prevention. *Current Pharmaceutical Biotechnology.* 13(1):180-90.

Chinese Pharmacopoeia Commission. (2015). Pharmacopoeia of the People's Republic of China. People's Medical Publishing House, Beijing. China. *Journal of food and drug analysis.* **24**(2):367-375.

Cogne, A.L. (2002). Phytochemical investigation of plants used in African medicine: *Dioscorea sylvatica (Dioscoreaceae), Urginea altissima (Liliaceae), Jamesbrittenia fodina and Jamesbrittenia elegantissima (Scrophulariaceae),* M.S. thesis,University of Lausanne, Lausanne, Switzerland

Coursey, D.G. and Walker, H.M (1960). A study of the origins of the weight losses in stored yams. Report of West African Stored Products Research Unit. pp: 61-64.

Dekker, M (1996). Toxicological, Health Perspective, 1st ed.; New York, USA, 1-32.

Duke J. A, and DuCellier, J.L. (1993). Handbook of Alternative Cash Crops. *Boca Raton, CRC Press, Florida*

Egwim, E.C., Eleme, R. C. and Egwuche, R. U (2011). Proximate composition, phytochemical screening and antioxidant activity of ten selected wild edible Nigeria mushrooms. *American Journal of Food and Nutrition*. 1(2):89-94.

Eka, O.U. (1985). "The Chemical Composition of Yam Tubers". In: Advances in Yam Research. The Biochemistry and Technology of Yam Tubers. Osuji, G. (ed). Biochemical Society of Nigeria: Enugu, Nigeria. **1**:51 – 75.

Ekpeyong, T. E. (1984). "Composition of Some Tropical Tuberous Foods".*Food Chemistry*. **15**:31 – 36.

Elisabeth, L.Ø, Unni, J., Mimi, S.O., Stein, B., and Asbjørg, S. C. (2009). Determination of Digoxin and Digitoxin in whole blood. *Journal of Analytical Toxicology*, Vol. 33,

Ezeocha, V.C and Ojimelukwe, P.C (2012). The impact of cooking on the proximate composition and antinutrional factors of water yam (*Dioscorea alata*). *Journal of Stored Products and Postharvest Research*. 3(13):172-176.

FAO. (1999). Food and Agriculture Organization of the United Nations. Production Yearbook vol 53. FAO Statistics 1999. FAO, Rome, Italy.

Ferguson, T. U., Haynes P.H and Spence J.A (1980). Distribution of dry matter and mineral nutrients in tubers of two cultivars of *Dioscorea alata L. Tropical. Agriculture, Trinidad.* **57**:61-67.

Gao, H., Hou, B., Kuroyanagi, M., and Wu, L. (2007). Constituents from anti-tumor-promoting active part of *Dioscorea bulbifera L*. in JB6 mouse epidermal cells. *Asian Journal of Traditional Medicine.* 2: 104-109.

Gao, H., Kuroyanagi, M., Wu, L., Kawahara, N., Yasuno, T., and Nakamura, Y. (2002) Antitumor-promoting constituents from *Dioscorea bulbifera L*. in JB6 mouse epidermal cells. *Biological and Pharmaceutical Bulletin* 25: 1241-1243.

Ghosh, S., Ahire, M., Patil, S., Jabgunde, A., BhatDusane, M., Joshi, B.N., Pardesi, K., Jachak, S., Dhavale, D.D., and Chopade, B.A (2012) Antidiabetic Activity of *Gnidia glauca* and *Dioscorea bulbifera*: Potent Amylase and Glucosidase Inhibitors. *Evidence-Based Complementary and Alternative Medicine*: 92:90-51.

Ghosh, S., Derle, A., Ahire, M., More, P., and Jagtap S, (2013). Phytochemical analysis and free radical scavenging activity of medicinal plants *Gnidia glauca and Dioscorea bulbifera.* PLoS One 8: e82529.

Ghosh, S., Patil, S., Ahire, M., Kitture, R., and Kale, S, (2012). Synthesis of silvernanoparticles using *Dioscoreabulbifera* tuber extract and evaluation of its synergistic potential in combination with antimicrobial agents. *International Journal of Nanomedicine* 7: 483-496.

Gupta, D. and Singh, J. (1989) "p-Hydroxy acetophenone derivatives from *Dioscorea bulbifera*," *Phytochemistry*,**28** (3):947–949.

Halliwell, B. (1987). Oxidants and human disease: some new concepts. FASEB J., 1, 358.

Harbone, J.B. 1973. *Phytochemical Methods.* A Guide to Modern Techniques of Plant Analysis. Chapman and Hall Ltd., London. 49-188.

Harvard Medical School. (2008)."Glycemic index and glycemic load for 100+ foods". *Harvard Health Publications*. 13(12):2281-2283

Hazra B, Biswas S, Mandal N (2008). Antioxidant and free radical scavenging activity of *Spondias pinnata. BMC Complementary and Alternative Medicine* 8: 63.

He K, Li X, Chen X, Ye X, Huang J, Jin Y, Li P, Deng Y, Jin Q, Shi, Q, Shu H. (2011) Evaluation of antidiabetic potential of selected traditional Chinese medicines in STZ-induced diabetic mice. *J Ethnopharmacol*; **137**: 1135- 42.

Houston, R.G. (1973). Sickle cell anemia and dietary precursors of cyanate. *American Journal of Clinical Nutrition.* **26**(11):1261–1264.

Houston, R.G., (1974): Dietary nitriloside and sickle cell anemia in Africa. *American Journal of Clinical Nutrition*. **27**(8):766–769.

Hutchinson, J. and Dalziel, J. M. (1968) Flora of West Tropical Africa, vol. 3, Millbank, London, UK, 2nd edition.

Hsu, B., Coupar, I.M., and Ng, K. (2006). Antioxidant activity of hot water extract from the fruit of the Doum palm, Hyphaene thebaica. *Food Chemistry*., 98, 31-328.

Ibraheem, N.K., Ahmed, J.H. and Hassan, M.K. (2010). The effect of fixed oil and water extracts of *Nigella sativa* on sickle cells: an *in vitro* study. *Singapore Medical Journal*. ; 51(3):230–234.

Ibraheem, O and Maimako, R.F (2014). Evaluation of alkaloids and cardiac glycoside contents of Ricimus communis Linn (Castor).*International Journal of Toxicology and Pharmacological Research*.6 (3):34-42.

IITA. (2009). Yam production in Africa. International Institute of Tropical Agriculture (IITA). Pp 1-5

Iwu, M.N., Igboko, A.O., Onwubiko. H., and Ndu, U.E. (1988). Effect of Cajanus cajan on gelation and oxygen affinity of sickle cell heamoglobin *Journal of Ethnopharmacology.* 22: 99-104.

Iyamu, E.W, Turner, E.A, Asakura, T. (2003). Niprisan (Nix-0699) improves the survival rates of transgenic sickle cell mice under acute severe hypoxic conditions. *British Journal of Haematology*., 122(6), 1001-1008.

Izekor and Olumese (December 2010). "Determinants of yam production and profitability in Edo State, Nigeria" . *African Journal of General Agriculture* **6** (4).

Javaregowda, K. and Maheswarappa V. (2007) Ovicidal properties of plant extracts against the eggs of teak defoliator, *Hyblaea puera* Cramer. *Karnataka Journal of Agricultural Science* **20**: 291–293.

Javanchandran, K.S., Vasanthi, H.R., and Rajamanickama, G .V. (2012). Flavonoid rich fraction of *D. bulbifera* linn. enhances mitochondrial enzymes and antioxidant status. *Current pharmaceutical biotechnology*:11(8):887-894.

Jayakody, L., Hoover, R., Liu, Q. and Donner, E. (2007). Studies on tuber starches.II. Molecular structure, composition and physicochemical properties of yam (*Dioscorea* sp.) starches grown in Sri Lanka. *Carbohydrate Polymers* 69: 148–163.

Jiao, Z., Liu, J. and Wang, S. (2005) Antioxidant activities of total pigment extract from blackberries. *Food Technology and Biotechnology* **43**: 97–102.

Jindal, M.N., Kelkar, V.V. and Doctor, R.B. (1969). The anorexient activity of Kalio-kund (*Dioscoreabulbifera* Linn.), methylphenidate and cocaine in rats: a preliminary study. *Indian Journal Medical Research* 57: 1075-1080.

Judd, W. S., Campbell, C. S., Kellogg, E. A. and Stevens, P. F. (1999). Plant Systematics: A Phylogenetic Approach, Sinauer Associates, Sunderland, Mass, USA.

Kareru, P.G., Kenji, G.M., Gachanja, A.N., Keriko, J.M. and Mungai, G. (2006) Traditional Medicines among the Embu and Mbeere peoples of Kenya. *African Journal of Traditional, Complementary and Alternative Medicine* **4**: 75–86.

Kay, D. E. (1987). Root crops. Tropical development and research institute: London.**66** (4):86-92.

Khare, C.P. (2007). Indian medicinal plants an illustrated dictionary. Springer-Verlag, New York 215-216.

Kitture, R., Ghosh. S., Kulkarni, P., Liu, X.L. and Maity, D. (2012). Fe_3O_4-citrate-curcumin: Promising conjugates for superoxide scavenging, tumor suppression and cancer hyperthermia. *Journal of Applied Physiology* **111**: 064702–064707.

Komori, T. (1997). Glycosides from *Dioscorea bulbifera*. *Toxicon* 35: 1531-1536.

Kouakou, M.D., Dabonne, S., Guehi, T. and Kuoame, L.P. (2010). Effects of post-harvest storage on some biochemical parameters of different parts of two yams species (*Dioscorea* spp). *African Journal of Food Science and Technology* 1: 1-9.

Kuete, V., Betrandteponno, R., Mbaveng, A.T, Tapondjou, L.A. and Meyer J.J. (2012). Antibacterial activities of the extracts, fractions and compounds from *Dioscore abulbifera. BMC Complementary and Alternative Medicine* 12: 228.

Kumar, and Reddy. (2012). Importance of traditional system of medicine -a review. *International Journal of Phytotherapy*; **2**: 1-6.

Lasztity, R., Hidvegi, M. and Bata, A. (1998). Saponins in food. *Food Reviews International***14** (4): 371–390.

Lather, A., Gupta, V., Garg, S., Singh, A. and Sachdeva, K. (2011). Pharmacological potentialof the plants used in treatment of piles -A review. *Journal of Natura Conscintia* **2**: 255-265.

Lewu, M.N., Adebola, P.O. and Afolayan, A.J. (2010). Effect of cooking on the mineral contents and anti-nutritional factors in seven accessions of *Colocasia esculenta (L.)* Schott growing in South Africa. *Journal of Food Composition and Analysis* **23**:389–393.

Liu, H., Chou, G.X., Guo, Y.L., Ji, L.L., Wang, J.M. and Wang, Z.T. (2010) Norclerodanediterpenoids from rhizomes of *Dioscorea bulbifera. Phytochemistry* 71: 1174-1180.

Liu, H., Chou, G.X., Wu, T., Guo, Y.L., Wang, S.C., Wang, C.H and Wang, Z.T. (2009) Steroidal sapogenins and glycosides from the rhizomes of *Dioscorea bulbifera* . *Journal of Natural Products* 72: 1964-1968.

Liu, J., Edamatsu, R., Kabuto, H. and Mori A, (1990). Antioxidant action of Guilingji in the brain of rats with FeCl3 induced epilepsy. *Free Radical Biology and Medicine*;9:451–454.

Liu, H., Tsim, K.W., Chou, G.X., Wang, J.M., Ji, L.L and Wang, Z.T. (2011) Phenolic compounds from the rhizomes of *Dioscorea bulbifera*. *Chemistry and Biodiversity* 8: 2110-2116.

Liu, M.J., Wang, Z., Ju, Y., Wong, R.N. and Wu, Q.Y. (2005). "Diosgenin induces cell cycle arrest and apoptosis in human leukemia K562 cells with the disruption of Ca^{2+} homeostasis". *Cancer Chemotherapy. Pharmacology*.**55** (1): 79–90.

Liu, Y., Huang, R., Liu, L., Peng, J., Xiao, B., Yang, J., Miao, Z. and Haung, H. (2010) Metabonomics study of urine from Sprague-Dawley rats exposed to Huang-yao-zi using (1)H NMR spectroscopy. *Journal of Pharmaceutical and Biomedical Analysis* 52(1): 136-141.

Londhe, J.S., Devasagayam, T.P., Foo, L.Y and Ghaskadbi, S.S. (2009) Radioprotective properties of polyphenols from *Phyllanthus amarus* Linn. *Journal of Radiation Research* **50**: 303–309.

Longe, O.G. (1986). "Energy Content of Some Tropical Starchy Crops". *Nigerian Agricultural Journal*. **21**:136.

Ma, M. Jiang, Z., Ruan, J., Tan, X. and Liu, J.(2012).The furanonorclerodanediterpenoiddis obulbin-D induces apoptosis in normal human liver L-02 cells. *Experimental and Toxicologic Patholology* 64: 611-618.

Marcocci, L., Packer, L., Droy-Lefaix, M.T., Sekaki, A. and Gardes-Albert, M. (1994) Antioxidant action of *Ginkgo biloba* extract EGB 761. Methods Enzymology 234: 462–475. doi: 10.1016/0076-6879(94)34117-6

Manjula, R.T. (2015). Depletion of glutathione during oxidative stress and efficacy of n-acetyl cysteine: An old drug with new approaches. *Medicinal Chemistry* 5: 037-039.

Marker, R.E. and Krueger, J. (1940). "Sterols. CXII. Sapogenins. XLI. The Preparation of Trillin and its Conversion to Progesterone". *Journal of the American Chemical Society*. **62** (12): 3349–3350.

Martin, F.N. (1974). "Tropical Yam and their Potentials. Part 2. *Dioscorea bulbifera*". *Agric. Handbook*, No. 466. Pp 18 USDA: Washington, DC.

Martin, F.W. (1979). Composition, nutritional value and toxic substances of the tropical yams. In Tropical Foods: Chemistry and Nutrition (Inglett and Charalambous, Eds.) Academic Press, New York, Vol. 1, pp: 249-264.

Mbiantcha, M., Kamanyi, A., Teponno, R.B., Tapondjou, A.L. and Watcho P. (2011).Analgesic and Anti-Inflammatory Properties of Extracts from the Bulbils of Dioscorea bulbifera L. var sativa (Dioscoreaceae) in Mice and Rats. *Evidenced-Based Complementary and Alternative Medicine*. doi:10.1155/2011/912935

McKoy, M.L., Omoruyi, F., Simon, O. and Asemota, H. (2003). Investigation of the effects of a sapogenin-rich preparation from a Jamaican yam (*Dioscorea* sp.) onblood cholesterol levels in rats. *Proceedings of the Western Pharmacology Society* 46: 156-159.

Meneely, G.B. and Barttablee, H.D. (1976). "Sodium and Potassium". *Nutrition Reviews.* 34:225 – 235.

Mir, M.A., Sawhney, S.S., and Jassal, M.M.S. (2013); Qualitative and quantitative analysis of phytochemicals of *Taraxacum officinale*. *WudpeckerJournal of Pharmacy and Pharmocology*. 2(1): 001-005.

Mohamed, A.A., Ali, S.I. and El-Baz, F.K. (2013). Antioxidant and antibacterial activities of crude extracts and essential oils of *Syzygium cumini* Leaves. *Public Library of Science One* **8**(4): e60269.

Mpiana, P.T., Mudogo, V., Tshibangu, D.S.T., Kitwa, E.K., Kanangila, A.B., Lumbu, J.B.S. Ngbolua, K.N., Atibu, E.K. and Kakule, M.K. (2008). Antisickling activity of anthocyanins from *Bombax pentadrum*, *Ficus capensis* and *Ziziphus mucronata*: Photodegradation effect. *Journal of Ethnopharmacology*. 120(3):413–418.

Munro, A. and Bassir, D. (1969). "Oxalates in Nigerian Vegetables". *West. African. Journal of Biological and Applied Chemistry*. 12(1): 4 – 18.

Murray, R.D.H, Jorge, Z.D., Khan, N.H., Shahjahan, M. and Quaisuddin M (1984). Diosbulbind and 8-epidiosbulbine acetate, norclerodanediterpenoids from *Dioscorea bulbifera* tubers. *Phytochemistry* 23: 623–625.

Naik, S.T. and Maheswarappa, V. (2007) Prospects of using plant extracts in management of pineapple heart rot. *Karnataka Journal of Agricultual Science* 20: 180–182.

Nanfack, P., Biapa, N., Prosper, C. P., Constant, A, A., Vicky, J., Moukette, B. and Ngogang Y, J. (2013).The *in vitro* antisickling and antioxidant effects of aqueos extract Zanthoxyllum heitzii on sickle cell disorder. *BMC Complementary and Alternative Medicine*. 2013; 13:162.

Narayana, K.R., Sripal, R.M., Chaluvadi, M.R. and Krishna, D.R. (2001). Bioflavonoids classification, pharmacological, biochemical effects and therapeutic potential. *Indian Journal of Pharmacy*.33:2-16

Nguelefack, T.B., Mekhfib, H., Dongmoc, A.B., Dimod, T., Watchoa, P., Zohere, I., Legssyerb, A., Kamanya, A., Ziyyat, A. (2009). Hypertensive effects of oral administration of aqueos extract of *Solanum torvum* fruits in L-NAME treated rats: Evidence from *in vivo* and *in vitro* studies. Journal of Ethnopharmacology, 124: 592-599

Nguelefack, T.B., Dutra, R.C., Paszcuk, A.F., Andrade, E.L., Tapondjou, L.A. And Calixto, J.B. (2010). Antinociceptive activities of the methanol extract of the bulbs of *Dioscorea bulbifera* L. var sativa in mice is dependent of NO-cGMP-ATP- sensitive-K (+) channel activation. *Journal of Ethnopharmacology*, 128: 567-574.

Nilofer, S., Yogenda, K., Misra, A. K. and Lokho, P. (2013). Phytochemical screening to validate the ethnobotanical importance of root tubers of *Dioscorea* species of Meghalan, North East India. *Journal of Medicinal Plants studies*. **1** (6).

Niyas, F. M. (2015). Medicinal Uses of *Dioscorea bulbifera*-A Review; *Research Journal of Pharmacy and Technology*. **8**(8):1059-1062.

Noguchi, C.T., Ackermann, S., Dimai, J., Sciller, P.W. and Schechter, A.N. (1982). The effect of phenylalanine analogs on the solubility of deoxyHbS. A model class of gelation inhibitors. *Molecular Pharmaceuticals*. 23, 106-110.

Noguchi, C.T, and Schetcher, A.N. (1978). The inhibition of sickle cell heamoglobin gelation by amino acids and related compounds. *New England Journal of Medicine*. 17: 5455-59.

Nwaoguikpe, R.N. and Ejele, E.A. (2010). Amino acid profile of some anti-sickling plant extracts and their haemoglobin polymerization inhibition. *Nigerian Journal of Biochemistry and Molecular Biology*. 25(2), 53-59.

Nwaoguikpe, R.N and Braide W., (2012). The antisickling effects of some micronutrients and antioxidant vitamins in sickle cell disease management. *Journal of Medicine and Medicinal Sciences*; 2(5); 334-340.

Nwaoguikpe, R.N., Ekeke, G.I., and Uwakwe, A.A. (1999). The effects of extracts of some foodstuffs on Lactate dehydrogenase activity and haemoglobin polymerization of sickle cell blood. Ph.D thesis University of Portharcourt, Nigeria.

Oboh, G., Puntel, R.L. and Rocha, J.B.T (2007). Hot peper (*Capsicum annuum, Tepin* and *Capsicum* Chinese, Habanero) prevents Fe (ii) ion induced lipid peroxidation in brain –*in vitro. Food chemistry* 102(1):178-185

Ogbuagu, M.N. (2008). "Nutritive and Anti-Nutritive Composition of the Wild (Inedible) Species of *Dioscorea bulbifera* (Potato Yam) and *Dioscorea dumentorum* (Bitter Yam)". *Pacific Journal of Science and Technology*. **9**(1):203-207.

Ojiako, O. A., Anugweje, K., Igwe, C.U and Alisi, C.S (2012). Evaluation of the amino acid profile and haemoglobin polymerization inhibition potential of some Nigeria legumes. *British Journal of Pharmaceutical Research*; **2**(2):80-88.

Oke, O.L. (1990).The role of hydrocyanic acid in nutrition. *World Review of Nutrition and Dietetics*. **11**:170–198.

Okigbo, R. N., Anuagasi, C.L., Amadi, J.E. and Ukpabi,U.J. (2009). Potential inhibitory effects of some African tuberous plant extracts on *Escherichia coli, Staphylococcus aureus and Candida albicans*. *International Journal of Integrative Biology* 6: 91-98.

Okwu, D.E. and Emenike, I.N. (2007). Nutirtive value and mineral content of different varieties of indigenous spices of Southeastern Nigeria. *Journal of Sustainable Agriculture and the .Environment*. **6**(1): 30 – 37.

Okwu, D.E. and Emenike, I.N. (2006). Evaluation of the phytonutrients and vitamin contents of citrus fruits. *International Journal of Molecular Medicine and Advanced Science*., **2**(1): 1-6.

Okwu, D. E. and Ndu, C.U. (2006). Evaluation of the Phytonutrients, Mineral and Vitamin Contents of Some Varieties of Yam (*Dioscorea sp*.). *International Journal of Molecular Medicine and Advanced Sciences*, (292):199 – 203.

Okwu, D.E. (2004); Phytochemicals and vitamin content of indigenous species of South Eastern Nigeria. *Journal of Sustainable Agriculture and the Environment* 6: 30-34.

Okwu, G. I., Enweani, I. B., Amengialue O. O., and Obiazi H. A. K. (2016) Comparative study of the phytochemical and antibacterial properties of two different varieties of *Dioscorea bulbifera* tubers. *American Journal of Research Communication*, **4**(7): 46-59.

Olatundi, A and Mohammad, A.D (2014). Qualitative and quantitative analysis of phytochemicals of Loranthus bengwensis leaf. *International, Journal of pharmaceutical Science*. **05**(01):0010.

Oliver-Bever, B. (1989). Medicinal plants in Tropical West Africa Cambridge Uni Cambridge.doi.org/10.1017

Onyenuga, V.A. (1968). Nigeria's Food and Feeding Stuffs, their Chemistry and Nutritive Value. 3rd edition. Ibadan University Press: Nigeria. Pp 1-90

Onyeike, E.N Olungwe, T. and Uwakwe, A. A (1995). Effect of heat treatment and defatting on the proximate composition of some Nigerian local soup thickeners, *Food Chemistry*, 53, 173-175

Osagie, A.U and Eka, O.U. (1998); Nutritional quality of Plant foods. Postharvest Research Unit, University of Benin, Benin City, Nigeria. Ambik press, Benin City, Edo State, Nigeria. 8, 14, 43 and 86.

Ohkawa, H., Ohishi, N., and Yagi, K. (1979). Assay of lipid peroxides in animal tissues bythiobarbituric acid reaction. *Anal. Biochem*. 95, 351–358.

Oyaizu, M. (1986). Studies on products of browning reaction prepared from glucoseamine.Japanese *Journal of Nutrition*., 44, 307–315.

Polycarp, D., Afoakwa, E. O., Budu, A. S. and Otoo, E. (2012). Characterization of chemical composition and anti-nutrient factors in seven species within the Ghanian yam (Dioscorea) germplasm.*International Food Research Journal* **19** (3): 985-992

Popova, M., Bankova, V.S., Butovska, D., Petkov, V., Nikolova Damyanova, B., Sabatini, A.G., Marcazzan, G.L., and Bogdanov, S. (2004). Validated methods for the quantification of biologically active constituents of poplar type propolis. *Phytochemistry Anal*. 15:235-240

Princewill-Ogbonna, I.L. and Ibeji, C.C (2015). Comparative study on nutritional and anti-nutritional composition of three cultivars of aerial yam (*D. bulbifera*). *Journal of Environmental Science, Toxicology and Food Technology.9* (5): 79-86.

Princewill-ogbonna, I.L, (2015). Effect of various processing methods on pasting and functional properties of Aerial Yam (*D. bulbifera*) flour. Current Journal of Applied Science and Technology. 9(5):517-526.

Raphael, R.M., John, B.S., Mariat, G and Emasushan, M, (2016). Pharmacognostic, fluorescent,antibacterial and phytochemical analysis of tuber of *Dioscorea bulbifera L* from Jaharkhand. *Journal of Pharmacognosy and Phytochemistry*.5 (1):8-14.

Reddy, N.R., Sathe, S.K. and Salumkhe, D.K. (1982). Phytates in legumes and cereals. *Advances in Food Research* **28**:89-92.

Rice, L.B. (2006).Unmet medical needs in antibacterial therapy. *Biochemical Pharmacology.* **71**: 991-995.

Richard, J.P. (1998) Natural Product Isolation, Humana Press, Totowa, NJ, USA, pp 1-51

Rivero, H., Mario, J., Raquel, H., Lorena, F., Liliana, V and Elena, D. (2007). Concentration of As, Ca, Cd, Cr, Cu, Fe, Hg, K, Mg, Mo, Na, N, Pb and Zn in Uruguayan rice, determined by AAS, *Atomic Spectroscopy*, 27(2), 48-55.

Robert, K.M., Daryl, K.G., Peter, A.M. and Victor, W.R. (2003). Harper's Illustrated

Biochemistry. 26th Edition, International Edition. pp. 482-484.

Sahore, D.A., Amani, N.G. and Kamenan A. (2007). Functional properties of wild yam (*Dioscorea* spp.) starches. *Tropical Science*; **47** (1): 33-7.

Salunke, G.R., Ghosh, S., Kumar, R.J.S., Khade, S., Vashisth, P., Kale, T., Chopade, S., Pruthi, V., Kundu, G., Bellare, J.R and Chopade, B. A. (2014) Rapid efficient synthesis and characterization of silver, gold, and bimetallic nanoparticles from the medicinal plant Plumbago zeylanica and their application in biofilm control. *International Journal of Nanomedicine* 9: 2635–2653.

Saravanan, M. and Ignacimuthu, S. (2015) Hypocholesterolemic effect of Indian medicinal plants - A review of *Medicinal Chemistry* 5: 040-049.

Sautour, M., Miyamoto, T. and Lacaille-Dubois, M-A. (2006) "Steroidal saponins and flavan-3-ol glycosides from Dioscorea villosa," *Biochemical Systematics and Ecology*. **34** (1) 60–63.

Sealand, R, and Razavi, C. (2013). Diabetes Mellitus and Osteoporosis. Current Diabetes Report March. p. 8.

Seetharam, Y.N., Jvothishwaran, G., Sujeeth, H., Barad, A., Sharanabasappa, G. and Shivkumar, D. (2003). Antimicrobial activity of *Dioscorea bulbifera* bulbils. *Indian Journal of Pharmaceutical Science* **65**(2):195-196.

Sefa-Dedeh, S. and Agyir-Sackey, E.K. (2004). Chemical composition andthe effect of processing on oxalate content of cocoyam*Xanthosoma sagittifolium* and *Colocasia esculenta* cormels. *Food Chemistry***85**: 479– 487.

Shajeela, P.S., Mohan, V.R., Louis, J,L and Tresina, S. P. (2011). Nutritional and anti-nutritional evaluation of wild yam (*Dioscorea Spp*): *Tropical and Subtropical Agroecosystems.* **14** (2011):723-730.

Shanthakumari, S., Mohan, V.R. and John de Britto. (2008). Nutritional Evaluation and Elimination of Toxic Principles in Wild Yam (*Dioscorea* spp.), *Tropical and Subtropical Agroecosystems***8**: 319-325.

Shaw, J.E., Sicree, R.A. and Zimmet PZ. (2010) Global estimates of the prevalence of diabetes for 2010 and 2030. *Diabetes Research and Clinical Practice*. **87**: 4-41.

Shetty, S., Yazdanparast, R., Bahramikia, S. and Ardestani, A. (2008). *Nasturtium oficinale* reduces oxidative stress and enhances antioxidant capacity in hypercholesterolaemic rats. *Chemico Biological Interactions* 172: 176–184.

Shetty, K. (1997). Biotechnology to harness the benefits of dietary phenolics focus on Lamiaceae. *Asia Pacific Journal of Clinical Nutrition.* 6: 162-171.

Shriram, V., Jahagirdar, S., Latha, C., Kumar, V., Puranik, V., Rojatkar, S., Dhakephalker, P.K. and Shitole, M.G. (2008) A potential plasmid-curing agent, 8-epidiosbulbin E acetate, from Dioscoreabulbifera L. against multidrug-resistant bacteria. *International Journal of Antimicrobial Agents* 32: 405-410.

Soladoye, M. O and Chukwuma, E, C. (2012). Phytochemical analysis of the stem and root of cissus populnea (Vitaceae) - an important medicinal plant in central Nigeria.Species of South Eastern Nigeria. *Journal of Sustainable Agriculture and the Environment* 6:30-34.

Song FL, Gan RY, Zhang Y, Xiao Q, Kuang L, and Li, H.B. (2010) Total phenolic contents and antioxidant capacities of selected chinese medicinal plants. *International Journal of Molecular Sciences*11: 2362-2372.

Sridhar, K.R and Bhat R. 2007. Agrobotanical, nutritional and bioactive potential of unconventional legume-Mucuna. *Livestock Research for Rural Development*.19(126)

Subasini, U., Thenmozhi, S., Sathyamurthy, D., Vetriselvan, S. and Rajamanickam, G.V. (2013). Pharmacognostic and phytochemical investigations of *Dioscoreabulbifera* L. *International Journal of Pharmacy and Life Sciences* 4: 2693-2700.

Singh, U. and Singh, B. 1992. Tropical grain legume as important human foods. *Economic Botany.* 46: 310-361.

Sundaresan, S., Babu, L. and Nambisan, B. 1990. Biochemical changes in yam tubersduring storage. *Journal of Root Crops.* 17: 265-269.

Sun, J., Liu, S-f., Zhang, C-s., Yu, L-n. and Bi, J. (2012). Chemical composition and antioxidant activities of *Broussonetia papyrifera* fruits. *Public Library of Science* One **7**(2): e32021.

Suriyavathana, M. and Indupriya, S. (2011). Screening of antioxidantpotentials in *Dioscorea bulbifera*. *International Journal. of Pharmaceutical and Life Sciences. (IJPLS).***2**(4): 661-664.

Swain, T. (1979). Tannins and lignins, herbivores: Their interaction with secondary plant metabolites. Academic Press, New York. pp.657-682, in G. A Rosenthal and D.H. Janzen (eds).

Tang, Y., Xue, Y.B., Zhou, L., Zhang, J.W, and Yao, GM. (2014).Newnorclerodanediterpenoids from the tubers of *Dioscorea bulbifera*. *Chemical Pharmaceutical Bulletin* (Tokyo) 62: 719-724.

Tang, Z., Zhou, Y., Zeng, Y., Zang, S. and He P. (2006). Capillary electrophoresis of the active ingredients of *Dioscoreabulbifera L*. and its medicinal preparations. *Chromatographia* 63(11-12): 617-622.

Tapondjou, L.A., Jenett-Siems, K., Bottger, S. and Melzig, M.F. (2013) Steroidal saponins from the flowers of *Dioscorea bulbifera var. sativa. Phytochemistry* 95: 341-350.

Teponno, R.B., Tapondjou, A.L., Gatsing, D., Djoukeng, J.D. and Abou-Mansour, E (2006). Bafoudiosbulbins A, and B, two anti-salmonellalclerodanediterpenoids from *Dioscorea bulbifera L. var sativa*. *Phytochemistry* 67: 1957-1963.

Teponno, R.B., Tapondjou, A. L., Hyun, J.-J., Nam, J.-H., Tane, P. and Park, H.-J. (2007). "Three new clerodane diterpenoids from the bulbils of *Dioscorea bulbifera L. var*. sativa," *Helvetica Chimica Acta*. **90** (8). 1599–1605.

Tindall, H.D., (1983). Vegetables in the Tropics. 1st Edn. Macmillan Education LTD. Houndmills,Hampshire, pp: 207-221.

Udensi, E.A., Oselebe, H.O. and Iweala, O.O. (2008). The Investigation of Chemical Composition and Functional Properties of Water Yam (*Dioscorea alata*): Effect of Varietal Differences. *Pakistan Journal of Nutrition***7** (2): 342-344.

Ujowundu, C.O., Kalu, F. N., Okafor, O.E., Agha, N.C., Alisi, C.S. and Nwoguikpe, R. N (2010). Evaluation of chemical composition of *Dacryodes edulis* (G. Don) seeds.International Journal of Biological and Chemical Sciences . 4(4):1225-1233.

Unconventional legume–*Mucuna*.*Livestock Research for RuralDevelopment*, **19**(9): 223 – 230.

United States Department of Agriculture."Nutrient data laboratory". Retrieved June 2014.

Uwaegbute, A.C., Osho, S.M and Obatolu, V. A (1998). Postharvest technology and commodity marketing: Proceedings of a postharvest conference. *International Institute of Tropical Agriculture.* p. 172.

Uzogara, S.G. Morton, I.D. and Daniel, J.W. (1990). Changes in some antinutrients of cow peas (*Vigna unguiculata*) processed with 'Kanwa' alkaline salt. *Plant Foods for Human Nutrition.* 40: 249-25.

Valko, M., Leibfritz, D., Moncol, J., Cronin, M.T.D., Mazur M, and Telser, J. (2007). Free radicals and antioxidants in normal physiological functions and human disease. *International Journal of Biochemistry and Cell Biology*. 7: 560-577.

Vasanthi, H.R., Mukherjee, S., Ray, D., PandianJayachandran, K.S. and Lekli, I. (2010). Protective role of air potato (*Dioscorea bulbifera*) of yam family in myocardial ischemic reperfusion injury. *Food and Function* 1: 278-283.

Velazquez, E., Tournier, H.A., Mordujovich de Buschiazzo, P., Saavedr, G. and Schinella, G.R. (2003). Antioxidant activity of Paraguayan plant extract. *Fitoterapia*. 74(1-2):91-7

Vichneveshaia, K.D. and Roy, D.N. (2001). Oxidative stress and antioxidative depense with an emphasis on plants antioxidants. *Environmental Reviews*., **7**: 31-35.

Vogel, H.G and Vogel, W. H (1997). Drug Discovery and Evaluation, Pharmacological Assays, Springer, Berlin, Germany.

Wanasundera, J.P.D. and Ravindran, G. (1994). Nutritional assessment of yam (*Dioscorea alata*) tubers. *Plant Foods for Human Nutrition* 46: 33-39.

Wang, G., Lin, B., Liu, J., Wang, G., Wang, F. and Liu, J. (2009) Chemical constituents from tubers of *Dioscorea bulbifera*. *ZhongguoZhong Yao ZaZhi* **13**: 1679-1682.

Wang, G., Liu, J.S., Lin, B.B., Wang, G.K. and Liu JK (2009) Two new furanoidnorditerpenes from *Dioscorea bulbifera*. *Chemical Pharmaceutical Bulletin* (Tokyo) **57**: 625-627.

Wang, J., Ji, L., Liu, H, and Wang, Z. (2010). *Study of the hepatotoxicity induced by Dioscorea bulbifera L*. rhizome in mice. *Bioscience Trends***4**: 79-85.

Wettasinghe, M. and Shahidi, F. (2000). Scavenging of reactive-oxygen species and DPPH free radicals by extracts of borage and evening primrose meals. *Food Chemistry*.;70:17-26.

Webster, J., Beck, W. and Ternai B (1984) Toxicity and bitterness in Australian *Dioscorea bulbifera L. and Dioscorea hispida Dennst*. from Thailand. *Journal of Agriculture and Food Chemistry* **32**: 1087–1090.

Wheeler, E.L. and Ferrel, R.E. (1979). A method for phytic acid determination in wheat and wheat fractions. *Cereal Chemistry* **48**: 312-320.

WHO (2007). Managment of haemoglobin disorders. Nicosia, Cyprus; 2007. pp. 16–18.

Wilkin, P. (1998). Morphometric study of *Dioscorea quartiniana*, A. Rich (*Dioscoreaceae*). *Kew Bulletin*, **54**:1–18.

Williams, C. (2013). Medicinal plants in Australia volume 4: an antipodean apothecary, *Rosenberg Publishing Science* 552: 437-444.

World Health Organization (1997). Medicinal plants in China, A selection and 150 commonly used species, WHO Regional Publications, *Western Pacific Series* No.2, 110-1.

World Health Organization (2010). Tuberculosis, World Health Organization, Geneva. Fact sheet No. 104. 2010

World Health Organization, (1980). The WHO Expert committee on diabetes mellitus, Technical Report Series. No 646

Wozniak, D., Lamer – Zarawska, E. and Matkowski, A. (2004). Antimutagenic and antiradical properties of flavones from the roots of *scutellaria buicalensis Georgi*. *Nahrung* 48: 9 –12.

Yan W, and Li X. (2013). Impact of diabetes and its treatments on skeletal diseases.*Frontiers of Medicine* **7** (1): 81-90.

Yang, D.J. and Lin, J.T. (2008). Effects of different storage conditions on steroidal saponins in yam (*Dioscorea pseudojaponica Yamamoto*) tubers. *Food Chemistry* **110**: 670–677.

Zannou, A. (2006). Socio-economic, agronomic and molecular analysis of yam and cowpea diversity in the Guinea-Sudan transition zone of Benin. PhD thesis, Wageningen University.

Zhi-Gang, Wu., Jiang, Wu., Mantri, N., Xiao-Qing, B., Song-Lin, C. and Zheng- Ming, T. (2016). Characterising diversity based on nutritional and bioactive composition of yam germplasm (Dioscorea Spp) commonly cultivated in China. *Journal of Food and Drug Analysis*. **24**(2):367-375.

Zou, Y.P., Lu, Y.H. and Wei, D.Z. (2004). Antioxidant activity of a flavonoid-rich extract of *Hypericumperforatum* L. *In vitro*. *Journal of Agricultural and Food Chemistry*.52:5032–5039.

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APPENDIX

Nitric Oxide radical Scavenging ability of *D. bulbifera*

"Evaluation"

"X" "Fn" "95 Pred -" "95 Pred +"

0.90261502191424	10	2.1373404479479	17.862659552052
5.2927503362298	20	12.935964991126	27.064035008874
98.181679844856	40	33.718039928356	46.281960071644
1257.0066928864	50	43.826479641744	56.173520358256
0.20778672769666	5	-2.5467462256648	12.546746225665
0.20778672769666	5	-2.5467462256648	12.546746225665
2.3981139063835	15	7.4007247419135	22.599275258087
10.785954538733	25	18.444594441895	31.555405558105

Nitric Oxide Radical scavenging ability of Tannic acid

"Evaluation"

"X"	"Fn"	"95 Pred -"	"95 Pred +"
4.5115026645362	10	4.2917029148888	15.708297085111
11.635278537869	20	14.308486223688	25.691513776312
36.214569211006	40	34.414824356999	45.585175643001
58.374147489667	50	44.415537225848	55.584462774152
95.235762745142	60	54.381201177766	65.618798822234
166.28694832325	70	64.387440616505	75.612559383495
349.92604255676	80	74.497791740874	85.502208259126
1.8065113574266	5	-0.77051511065259	10.770515110653
1.8065113574266	5	-0.77051511065259	10.770515110653
7.7685665339231	15	9.2987367598529	20.701263240147
16.22088579461	25	19.331456465517	30.668543534483

Reducing Power of *D. bulbifera*

"Evaluation"

"X"	"Fn"	"95 Pred -"	"95 Pred +"
480.27310371399	0.5	0.41592379384727	0.58407620615273
78.861723542213	0.1	0.019468347483099	0.1805316525169
166.8650662899	0.2	0.11632792754498	0.28367207245502
262.08399057388	0.3	0.21551392393422	0.38448607606578
365.93041181564	0.4	0.31639623000053	0.48360376999947

Reducing Power of Ascorbic Acid

"Evaluation"

"X" "Fn" "95 Pred -" "95 Pred +"

4.7012668475509	0.1	0.00065021302951792	0.19934978697048
11.561032682657	0.2	0.10456082113879	0.29543917886121
18.03012534976	0.3	0.20666379044864	0.39333620955136
24.179761558771	0.4	0.30746841113409	0.49253158886591
30.066681206226	0.5	0.40742424819485	0.59257575180515

Hydroxyl Radical Scavenging ability of *D. bulbifera*

"Evaluation"

"X"	"Fn"	"95 Pred -"	"95 Pred +"
13.09871584177	10	4.1822294520794	15.817770547921
43.354049324989	20	14.583635658329	25.416364341671
239.40512418747	40	34.764517970066	45.235482029934
633.60266208649	50	44.857993363258	55.142006636742
4.5906043797731	5	-0.75023721889126	10.750237218891
4.5906043797731	5	-0.75023721889126	10.750237218891
25.62402933836	15	9.3404523609802	20.65954763902
68.375566005707	25	19.778552455685	30.221447544315
3368.936650455	60	54.191477785419	65.808522214581

Hydroxyl Radical Scavenging ability of Tannic acid

"Evaluation"

"X"	"Fn"	"95 Pred -"	"95 Pred +"
33.982682228088	10	2.2038033459325	17.796196654068
43.509282171726	20	12.181716595347	27.818283404653
61.647609472275	40	32.795372214526	47.204627785474
74.559977054596	50	42.816110418452	57.183889581548
99.672806859016	60	52.975827044002	67.024172955998
27.418701350689	5	-2.5660871208804	12.56608712088
27.418701350689	5	-2.5660871208804	12.56608712088
39.03154194355	15	7.1164818956534	22.883518104347
47.789949476719	25	17.344223776706	32.655776223294

DPPH Radical Scavenging ability of *D. bulbifera*

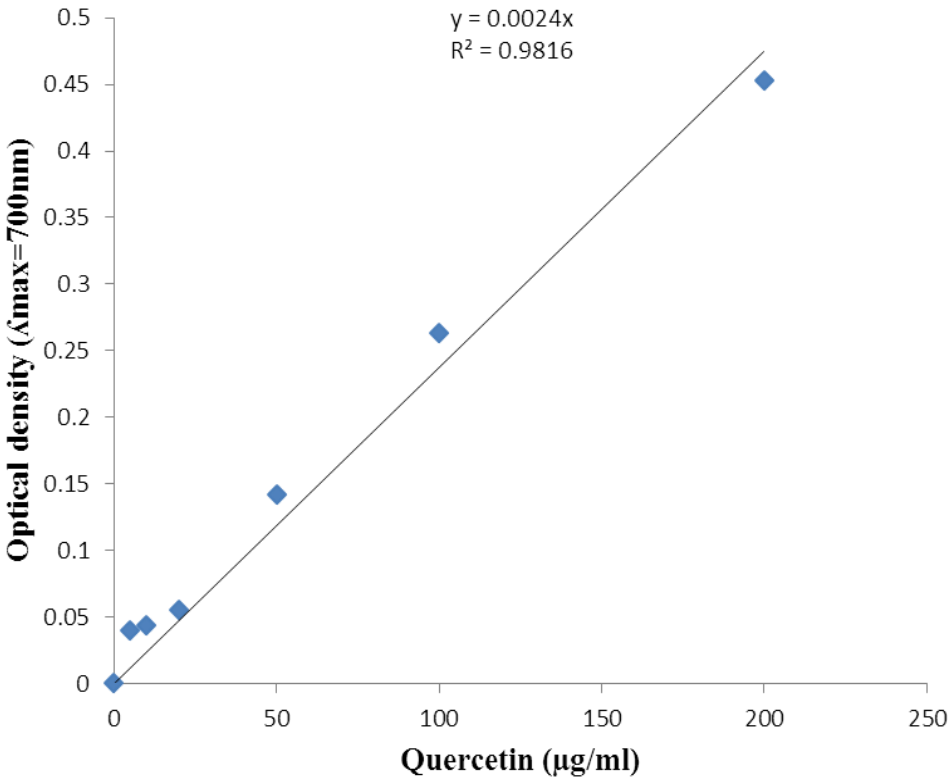
"Evaluation"

"X"	"Fn"	"95 Pred -"	"95 Pred +"
35.646806359291	10	4.5361812181457	15.463818781854
222.43077874184	20	14.71056954887	25.28943045113
1294.1547679901	40	34.676837235277	45.323162764723
2285.7789099216	50	43.836833974032	56.163166025968
3655.1043465734	60	49.892221242539	70.107778757461
5462.5382497907	70	52.423162649794	87.576837350206
7774.0404307842	80	51.761324157993	108.23867584201
4.4618277996778	5	-0.56420938673606	10.564209386736
4.4618277996778	5	-0.56420938673606	10.564209386736
105.64487695694	15	9.6708124389836	20.329187561016
393.04055452347	25	19.669322679486	30.330677320514
14207.682058215	100	41.157891647808	158.84210835219

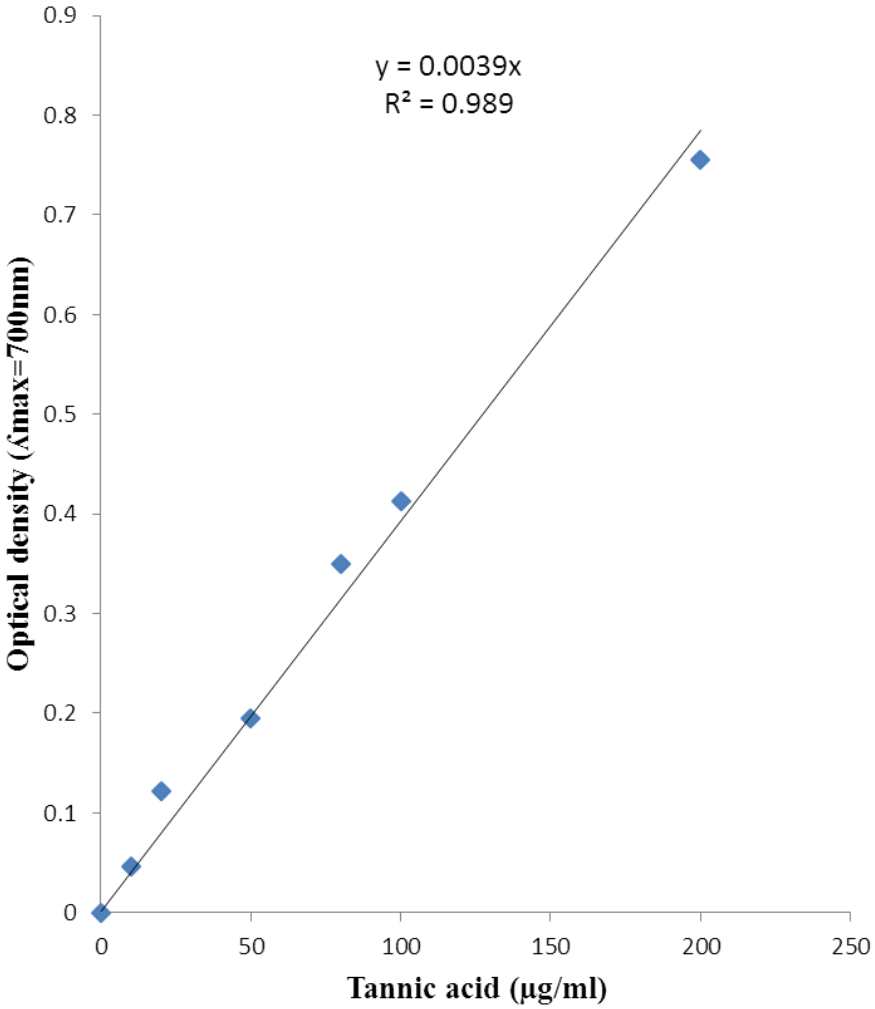
DPPH Radical Scavenging ability of Tannic Acid

"Evaluation"

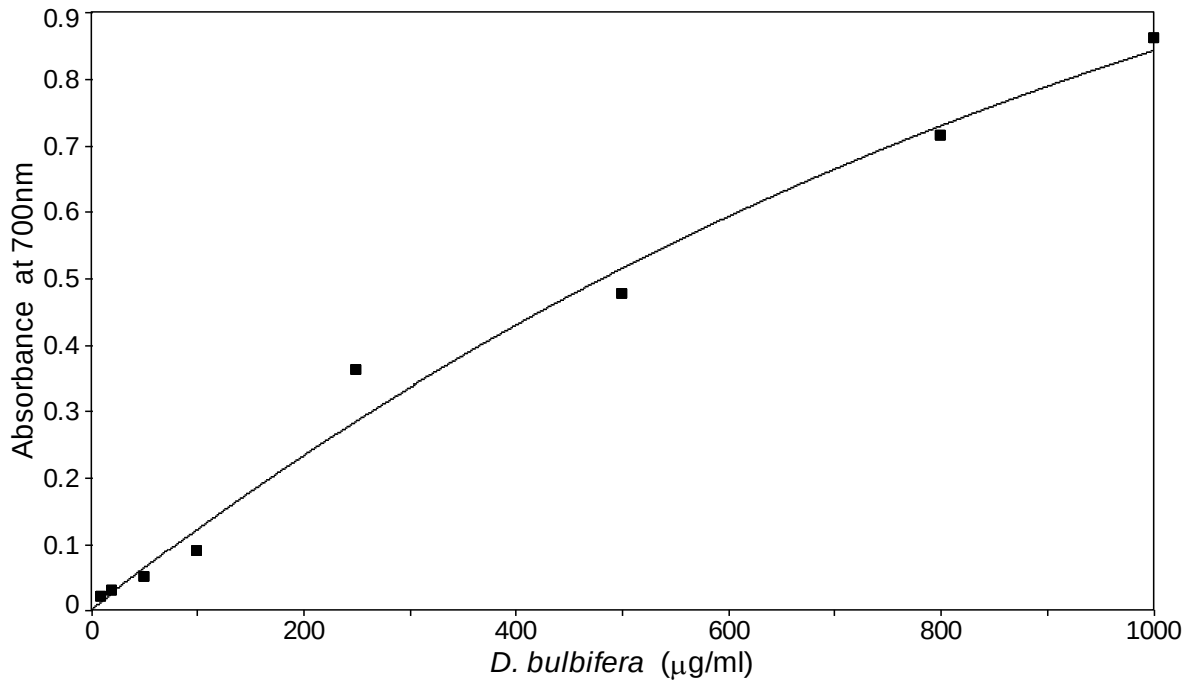
"X"	"Fn"	"95 Pred -"	"95 Pred +"
14.973573610187	10	1.2365423492959	18.763457650704
81.62963449955	20	11.407785166657	28.592214833343
395.50564289093	40	31.466769267777	48.533230732223
651.01415157318	50	41.390789714259	58.609210285741
977.28357315063	60	51.411662676264	68.588337323736
1377.9993534088	70	61.445478739853	78.554521260147
1856.8521404266	80	71.060460109376	88.939539890624
1.9535195082426	5	-3.874663847772	13.874663847772
1.9535195082426	5	-3.874663847772	13.874663847772
41.268194913864	15	6.313283261738	23.686716738262
136.69190406799	25	16.483204821421	33.516795178579
3063.9733076096	100	86.33302751507	113.66697248493



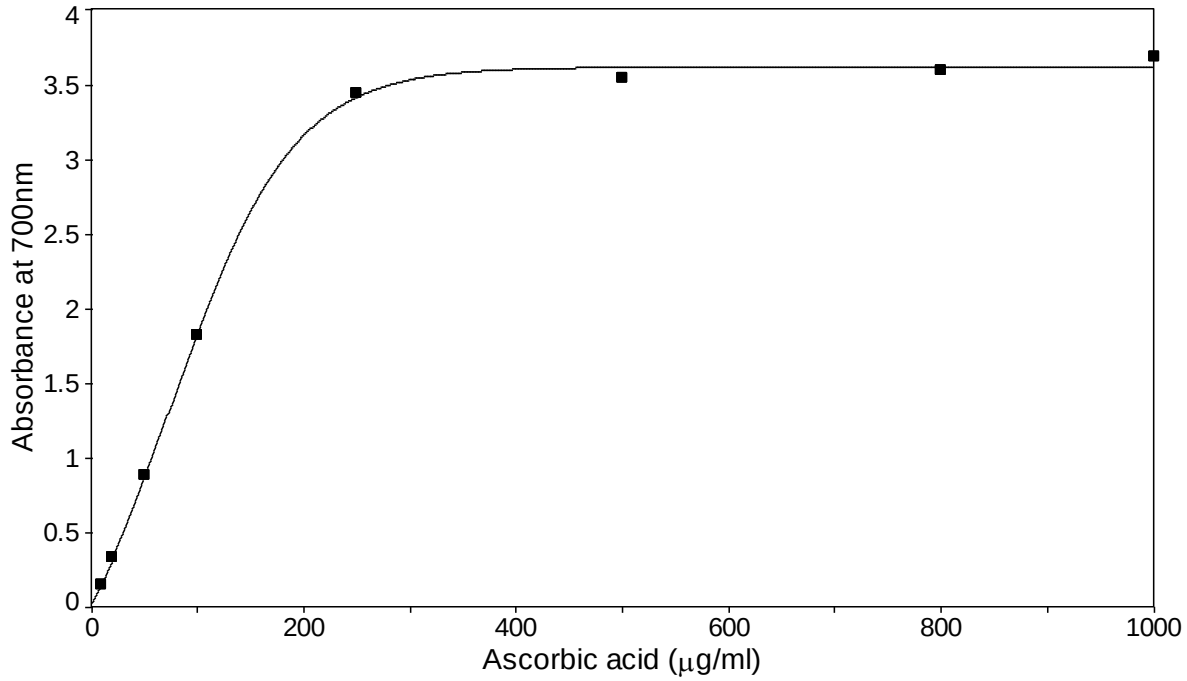
Calibration curve for calculation of flavonoid content



Calibration curve for Tannic acid



Reducing power properties of *D. bulbifera* extract



Reducing power properties of *Ascorbic acid*

T-TEST GROUPS = Reducing Power Of *D. bulbifera* Extract And Ascorbic acid (1 2)

/MISSING=ANALYSIS

/VARIABLES=ReducingPower

/CRITERIA=CI(.95).

T-Test

[DataSet0]

Group Statistics

	ReducingPowerOfDbulbiferaExtractAndAscorbicacid	N	Mean	Std. Deviation	Std. Error Mean
ReducingPower	D. bulbifera Extract	3	460.6333	33.82339	19.52795
	Ascorbic acid	3	30.0700	2.16709	1.25117

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
ReducingPower	Equal variances assumed	9.196	.039	22.003	4	.000	430.56333	19.56799	376.23389	484.89277
	Equal variances not assumed			22.003	2.016	.002	430.56333	19.56799	347.02269	514.10397

T-TEST GROUPS = Threshold Inhibitory Concentration Of Nitric Oxide (1 2)

/MISSING=ANALYSIS

/VARIABLES=IC5 IC10 IC20 IC50 IC80

/CRITERIA=CI(.95).

T-Test

[DataSet0]

Group Statistics

	ThresholdInhibitoryConcentrationOfNitricOxide	N	Mean	Std. Deviation	Std. Error Mean
IC5	D. bulbifera	3	.2133	.03055	.01764
	Tannic acid	3	1.8133	.11504	.06642
IC10	D. bulbifera	3	.9167	.07638	.04410
	Tannic acid	3	4.5133	.09018	.05207
IC20	D. bulbifera	3	5.2933	.16010	.09244
	Tannic acid	3	11.6433	.38812	.22408
IC50	D. bulbifera	3	1257.0000	128.55738	74.22264
	Tannic acid	3	58.3667	8.09674	4.67466
IC80	D. bulbifera	0[a]	.	.	.
	Tannic acid	0[a]	.	.	.

a. t cannot be computed because at least one of the groups is empty.

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
IC5	Equal variances assumed	2.128	.218	-23.283	4	.000	-1.60000	.06872	-1.79079	-1.40921
	Equal variances not assumed			-23.283	2.281	.001	-1.60000	.06872	-1.86339	-1.33661
IC10	Equal variances assumed	.038	.855	-52.713	4	.000	-3.59667	.06823	-3.78611	-3.40723
	Equal variances not assumed			-52.713	3.894	.000	-3.59667	.06823	-3.78815	-3.40518
IC20	Equal variances assumed	1.869	.243	-26.197	4	.000	-6.35000	.24240	-7.02300	-5.67700
	Equal variances not assumed			-26.197	2.662	.000	-6.35000	.24240	-7.17999	-5.52001
IC50	Equal variances assumed	8.299	.045	16.117	4	.000	1198.63333	74.36970	992.14994	1405.11673
	Equal variances not assumed			16.117	2.016	.004	1198.63333	74.36970	881.04776	1516.21890

ONEWAY Relative Percentage Inhibition BY Effect Of *D. bulbifera* Extract On HbSS Polymerization

/STATISTICS DESCRIPTIVES HOMOGENEITY

/MISSING ANALYSIS

/POSTHOC=TUKEY DUNCAN ALPHA (0.05).

Oneway

[DataSet0]

Descriptives

Relative Percentage Inhibition

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
0	3	.0000	.00000	.00000	.0000	.0000	.00	.00
20	3	3.9600	.27404	.15822	3.2792	4.6408	3.75	4.27
50	3	-1.9833	.11504	.06642	-2.2691	-1.6976	-2.10	-1.87
100	3	-9.8867	.53257	.30748	-11.2096	-8.5637	-10.36	-9.31
200	3	1.9767	.11240	.06489	1.6975	2.2559	1.88	2.10
500	3	15.8367	.74380	.42943	13.9890	17.6844	15.24	16.67
Total	18	1.6506	7.93000	1.86912	-2.2929	5.5941	-10.36	16.67

Test of Homogeneity of Variances

Relative Percentage Inhibition

Levene Statistic	df1	df2	Sig.
4.819	5	12	.012

ANOVA

Relative Percentage Inhibition

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	1067.168	5	213.434	1365.489	.000
Within Groups	1.876	12	.156		
Total	1069.043	17			

Post Hoc Tests

Multiple Comparisons

Dependent Variable: Relative Percentage Inhibition

	(I) Effect OfD.bulbiferaExtractOnHbSSPolymerization	(J) EffectOfD.bulbiferaExtractOnHbSSPolymerization	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Tukey HSD	0	20	-3.96000*	.32281	.000	-5.0443	-2.8757
		50	1.98333*	.32281	.001	.8991	3.0676
		100	9.88667*	.32281	.000	8.8024	10.9709
		200	-1.97667*	.32281	.001	-3.0609	-.8924
		500	-15.83667*	.32281	.000	-16.9209	-14.7524
	20	0	3.96000*	.32281	.000	2.8757	5.0443
		50	5.94333*	.32281	.000	4.8591	7.0276
		100	13.84667*	.32281	.000	12.7624	14.9309
		200	1.98333*	.32281	.001	.8991	3.0676
		500	-11.87667*	.32281	.000	-12.9609	-10.7924
	50	0	-1.98333*	.32281	.001	-3.0676	-.8991

	20	-5.94333*	.32281	.000	-7.0276	-4.8591
	100	7.90333*	.32281	.000	6.8191	8.9876
	200	-3.96000*	.32281	.000	-5.0443	-2.8757
	500	-17.82000*	.32281	.000	-18.9043	-16.7357
	0	-9.88667*	.32281	.000	-10.9709	-8.8024
	20	-13.84667*	.32281	.000	-14.9309	-12.7624
100	50	-7.90333*	.32281	.000	-8.9876	-6.8191
	200	-11.86333*	.32281	.000	-12.9476	-10.7791
	500	-25.72333*	.32281	.000	-26.8076	-24.6391
	0	1.97667*	.32281	.001	.8924	3.0609
	20	-1.98333*	.32281	.001	-3.0676	-.8991
200	50	3.96000*	.32281	.000	2.8757	5.0443
	100	11.86333*	.32281	.000	10.7791	12.9476
	500	-13.86000*	.32281	.000	-14.9443	-12.7757

500	0	15.83667*	.32281	.000	14.7524	16.9209
	20	11.87667*	.32281	.000	10.7924	12.9609
	50	17.82000*	.32281	.000	16.7357	18.9043
	100	25.72333*	.32281	.000	24.6391	26.8076
	200	13.86000*	.32281	.000	12.7757	14.9443

*. The mean difference is significant at the 0.05 level.

Homogeneous Subsets

Relative Percentage Inhibition

	EffectOfD.bulbifera ExtractOnHbSPolymerization	N	Subset for alpha = 0.05					
			1	2	3	4	5	6
Tukey HSD[a]	100	3	-9.8867					
	50	3		-1.9833				
	0	3			.0000			
	200	3				1.9767		
	20	3					3.9600	
	500	3						15.8367
	Sig.		1.000	1.000	1.000	1.000	1.000	1.000
Duncan[a]	100	3	-9.8867					
	50	3		-1.9833				
	0	3			.0000			
	200	3				1.9767		
	20	3					3.9600	
	500	3						15.8367
	Sig.		1.000	1.000	1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

ONEWAY Relative Percentage Inhibition Of HbSS Polymerization BY Effect Of HBA On HbSS Polymerization

/STATISTICS DESCRIPTIVES HOMOGENEITY

/MISSING ANALYSIS

/POSTHOC=TUKEY DUNCAN ALPHA(0.05).

Oneway

[DataSet0]

Descriptives

Relative Percentage Inhibition Of HbSS Polymerization

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
0	3	.0000	.00000	.00000	.0000	.0000	.00	.00
20	3	30.6900	1.53039	.88357	26.8883	34.4917	29.14	32.20
50	3	-25.7400	1.29290	.74646	-28.9518	-22.5282	-26.74	-24.28
100	3	11.8800	.65483	.37807	10.2533	13.5067	11.32	12.60
200	3	-42.5567	2.13388	1.23199	-47.8575	-37.2558	-44.14	-40.13
500	3	88.1300	3.91547	2.26060	78.4034	97.8566	84.18	92.01
Total	18	10.4006	43.43497	10.23772	-11.1991	32.0003	-44.14	92.01

Test of Homogeneity of Variances

Relative Percentage Inhibition Of HbSS Polymerization

Levene Statistic	df1	df2	Sig.
2.297	5	12	.110

ANOVA

Relative Percentage Inhibition Of HbSS Polymerization

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	32023.487	5	6404.697	1579.662	.000
Within Groups	48.654	12	4.054		
Total	32072.140	17			

Post Hoc Tests

Multiple Comparisons

Dependent Variable: Relative Percentage Inhibition Of HbSS Polymerization

	(I) Effect Of HBA On HbSS Polymerization	(J) Effect Of HBA On HBSS Polymerization	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Tukey HSD	0	20	-30.69000*	1.64407	.000	-36.2123	-25.1677
		50	25.74000*	1.64407	.000	20.2177	31.2623

	100	-11.88000*	1.64407	.000	-17.4023	-6.3577
	200	42.55667*	1.64407	.000	37.0343	48.0790
	500	-88.13000*	1.64407	.000	-93.6523	-82.6077
20	0	30.69000*	1.64407	.000	25.1677	36.2123
	50	56.43000*	1.64407	.000	50.9077	61.9523
	100	18.81000*	1.64407	.000	13.2877	24.3323
	200	73.24667*	1.64407	.000	67.7243	78.7690
	500	-57.44000*	1.64407	.000	-62.9623	-51.9177
	0	-25.74000*	1.64407	.000	-31.2623	-20.2177
	20	-56.43000*	1.64407	.000	-61.9523	-50.9077
50	100	-37.62000*	1.64407	.000	-43.1423	-32.0977
	200	16.81667*	1.64407	.000	11.2943	22.3390
	500	-113.87000*	1.64407	.000	-119.3923	-108.3477

	0	11.88000*	1.64407	.000	6.3577	17.4023
	20	-18.81000*	1.64407	.000	-24.3323	-13.2877
100	50	37.62000*	1.64407	.000	32.0977	43.1423
	200	54.43667*	1.64407	.000	48.9143	59.9590
	500	-76.25000*	1.64407	.000	-81.7723	-70.7277
	0	-42.55667*	1.64407	.000	-48.0790	-37.0343
	20	-73.24667*	1.64407	.000	-78.7690	-67.7243
200	50	-16.81667*	1.64407	.000	-22.3390	-11.2943
	100	-54.43667*	1.64407	.000	-59.9590	-48.9143
	500	-130.68667*	1.64407	.000	-136.2090	-125.1643
	0	88.13000*	1.64407	.000	82.6077	93.6523
500	20	57.44000*	1.64407	.000	51.9177	62.9623
	50	113.87000*	1.64407	.000	108.3477	119.3923

	100	76.25000*	1.64407	.000	70.7277	81.7723
	200	130.68667*	1.64407	.000	125.1643	136.2090

*. The mean difference is significant at the 0.05 level.

Homogeneous Subsets

Relative Percentage Inhibition Of HbSS Polymerization

	Effect Of HBA on HbSS Polymerization	N	Subset for alpha = 0.05					
			1	2	3	4	5	6
Tukey HSD[a]	200	3	-42.5567					
	50	3		-25.7400				
	0	3			.0000			
	100	3				11.8800		
	20	3					30.6900	
	500	3						88.1300
	Sig.		1.000	1.000	1.000	1.000	1.000	1.000
Duncan[a]	200	3	-42.5567					
	50	3		-25.7400				
	0	3			.0000			
	100	3				11.8800		
	20	3					30.6900	
	500	3						88.1300
	Sig.		1.000	1.000	1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.