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Antibacterial activities and phytochemical properties of extracts of *Dioscorea bulbifera* Linn (Air Potatoe) tubers and peels against some pathogenic bacteria

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ABSTRACT

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 proximate profile of this tuber included moisture content, ash, crude protein, fat, fibre and carbohydrate with varying percentages. 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.

Keywords: Antibacterial activity, Pathogens, Bulbils, Peels and *Dioscorea bulbifera*.

INTRODUCTION

The global resurgence of medicinal plants as remedy in recent years, occasioned by the emergence of multiple antibiotic resistances^[1], coupled with questions concerning the safety of synthetic compounds, has however, not left Africa behind. African continent is endowed with the richest biodiversity in the world, with an avalanche of many food plants used as herbs, health foods and for therapeutic purposes. Over 5,000 different species of plant substances have been recognized to occur in Africa continent, and many of them have been found to be useful in traditional medicine for prophylaxis and cure of diseases^[2]. Several species of yam plants (genus *Dioscorea*, family Dioscoreaceae) produce edible tubers, bulbils or rhizomes that also have medicinal and pharmacological properties, which are of considerable economic importance^[2]. They serve as major source of carbohydrate staple throughout the tropics, particularly western and eastern part of Nigeria where the most widely cultivated species include *Dioscorea rotundata* (white yam), *D. alata* (water yam), *D. cayenensis* (yellow or guinea yam), *D. dumatorium* (cluster or bitter yam), *D. esculenta* (Chinese yam), and *D. bulbifera* (aerial yam)^[3]. *Dioscorea bulbifera* is widely distributed in Asia and Africa in the wild state with a vigorously twining herbaceous vine, with small or absent underground tubers^[4], long petioled leaves, with a rare, fragrant, male and female flowers arising from leaf axils on separate plants^[5].

The therapeutic potentials of *Dioscorea bulbifera* has been documented in many parts of the world include; the treatment of sore throat, gastric cancer and carcinoma of rectum, and goiter in China^[6, 7]; treatment of tumor and leprosy in Bangladesh^[8]. Furthermore, various extracts of this plant have been reported to be usable as; anorexiant^[9], antitumor^[10], antihyperlipidemic^[11], antioxidant^[12], plasmid curing agent^[13], antihyperglycemic^[14], analgesic and anti-inflammatory^[15]. The potential of this tuber as antimicrobial agent against noble pathogens is however scarcely evaluated. Hence, the present study was designed to validate the antibacterial properties of the ethanolic and aqueous extract of the peel and bulbils of *Dioscorea bulbifera* Linn.

MATERIALS AND METHODS

Plant collection and authentication

Tubers of *Dioscorea bulbifera* Linn (Aerial yam) were obtained from the experimental garden of the Department of Microbiology at the back of Microbiology Laboratory, Obaekere, Federal University of Technology, Akure (FUTA), Ondo-State, Nigeria. Authentication of the plant tubers was done in The Department of Crop, Soil and Pest, FUTA. Several tubers of *D. bulbifera* were harvested, packed in clean sterile manila papers, labelled with a voucher specimen and transported to the Laboratory of Department of Microbiology, FUTA, Ondo-State, Nigeria; for analysis.

Preparation of plant materials

The tuber were separated into bulbils and peels, washed and sanitized in a 6% sodium hypochlorite solution (50 ppm), (Reckitt Benckiser, Nig. Ltd) [16]. Plant materials were thinly sliced (approximately 25mm) and dried in an oven-drier (HME Global Laboratory Oven Model No. DHG-9101-1SA, England) set at 60°C for 48hrs. The plants' parts were separately grounded using a 12-speed blender (Excella,) for 5mins and stored in an air-tight container at 4°C in the Laboratory Refrigerator, until used.

Proximate analysis of plant materials

The proximate composition; including moisture content, crude protein, fat, ash, fiber and carbohydrate, of the dried powdered bulbils and peel of *D. bulbifera* were separately estimated using respective standard techniques described by [17].

Preparation of plant extracts

One hundred grams (100 g) of dried powdered bulbils and peels of *Dioscorea bulbifera* were macerated separately in a 3L flask using distilled water and ethanol as solvent, making a ratio of 1 to 15 of the plant materials and solvents; described by [18]. Each mixture was placed for extraction in an orbital shaker (Stuart Orbital incubator, S1500) for 24hrs at a speed of about 100rpm at room temperature (25°C) and filtered through a 90mm diameter filter paper (Whatman No. 1, Whatman® Schleicher and Schuel) [19]. Extracts were collected and concentrated under reduced pressure using rotary evaporator (SearchTech Instruments, RE52-1) at 40°C for 10mins and reconstituted with 50% dimethylsulphoxide (DMSO) to make a stock extracts, which were stored at 4°C until used.

Phytochemical analysis

Standard phytochemical screenings described by [20] were separately carried out on the crude extracts of the bulbils and peels of *D. bulbifera* to detect the presence and absence of alkaloids, saponins, tannins, coumarin, flavonoids and other bioactive compounds. Each of the detected bioactive compounds was quantify using different techniques described by [21].

Antibacterial properties of *D. bulbifera*

Test microorganisms

Ten (10) strains of bacteria including five (5) Gram-negative isolates; *Escherichia coli*; *Proteus vulgaris*; *Klebsiella pneumonia*; *Serratia liquefaciens*; and *Pseudomonas aeruginosa*, and five (5) strains of Gram-positive; *Staphylococcus aureus*; *Bacillus cereus*; *Micrococcus leteus*; *Streptococcus pneumonia*; and *Citrobacter freundii*; were subjected to susceptibility test in the course of this research. All test organisms were obtained from the stock culture of The Microbiology Laboratory of Microbiology Department, FUTA, Ondo-State, Nigeria.

Standardization of microbial culture

Suspension of each of the test organisms was prepared and standardized according to The National Committee for Clinical Laboratory Standards [22], by adjusting its density to equal the turbidity of a barium sulphate (BaSO₄) (0.5 McFarland turbidity standards) which corresponds to approximately 1.0x10⁸ cfu/ml.

Antibacterial assay

Agar well diffusion method described by [23] was adopted to assay for the antibacterial properties of the plant extract against the array of bacteria selected. Aliquot of 100 µL of the logarithmic phase of each bacterial suspension (optical density equivalent to 10⁷-10⁸ cfu/ml) was seeded on a sterile molten Mueller-Hinton agar (Biotech, India) with a sterile swab. Using a sterilized 6mm diameter cork borer, wells were bored at equidistance on the agar, into which 100 µl of increasing concentrations of reconstituted extract was added. A pre-incubation diffusion of the extracts into the seeded medium was allowed for 1 hr. Bacteria plates were incubated at 37°C in an incubator for 18-24 hrs after which diameters of zones of inhibition (mm) were measured and expressed in millimetres (ml) [24].

Determination of Minimum inhibitory concentration (MIC)

Tube dilution method used by [25] was used to determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the extracts against test organisms with slight modification; in that, the inhibition of bacteria were visualized by observing colour reaction with *p*-iodonitrotetrazolium violet (Bio Medicals, Inc.), in place of traditional turbidity measurement to eliminate difficulties of observing turbidity for each 96 wells.

Statistical analysis

Data obtained were subjected to analysis of variance (ANOVA) using the Statistical Program for the Social Sciences (SPSS) version 20.0 with 95% confidence level and 5% significance level.

RESULTS

Proximate composition of *Dioscorea bulbifera* Linn

Analysis of the proximate components of *D. bulbifera* showed that it contains all important contents in varying concentration as illustrated in Fig 1. The proximate composition of the bulbils of the tuber ranged as follow; moisture content (4.58 – 4.61%); ash (4.46 – 4.52%); crude protein (8.29 – 8.44%); fat (1.96 – 1.99%); fibre (2.22 – 2.28%) and carbohydrate (78.31 -78.34%); while those of the peel of the tuber ranged as follow; moisture content (3.68 – 3.74%); ash (9.00 – 9.11%); crude protein (8.47 – 8.50%); fat (2.98 – 3.02%); fibre (22.67 – 22.58%) and carbohydrate (53.05 – 53.20%).

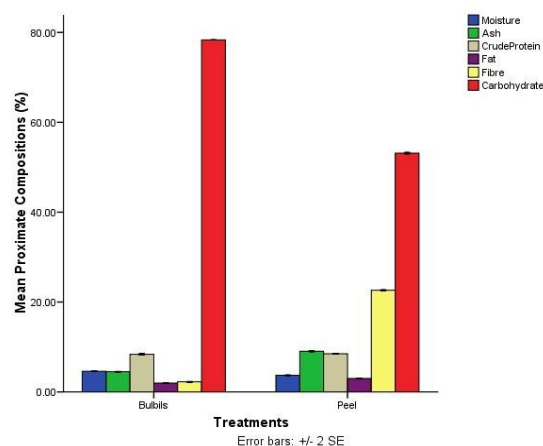


Figure 1: Proximate composition of the bulbils and peel of *Dioscorea bulbifera*

Phytochemical properties

The phytochemical screening for the presence of bioactive components in the tuber of *D. bulbifera* samples are presented in table 1. The analysis indicated the presence of saponin, tannin, flavonoids, terpenoid, phlobatannin and cardiac glycosides, while steroids, alkaloids and anthraquinones were absent in the samples. The peels of the tubers were recorded to contain higher amount of these bioactive compounds in the following order; saponin with the average of 32.28 mg/g, followed by terpenoids with 22.90 mg/g, cardiac glycosides with 15.90 mg/g, flavonoid with 9.17 mg/g, tannin followed with 4.79 mg/g and phlobatannin with 1.87 mg/g was the least among the

bioactive ingredients detected in the peel of *D. bulbifera*. The average concentrations of the active compounds in the bulbils were as follow; saponin (21.37 mg/g), terpenoid (20.40 mg/g), cardiac glycosides (12.37 mg/g), flavonoid (6.33 mg/g), tannin (4.25 mg/g) and phlobatannin (1.56 mg/g).

Table 1: Phytochemical composition of *Dioscorea bulbifera*

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

Key: ND- Not Detected

Antibacterial assay of *Dioscorea bulbifera*

Antibacterial effect of ethanolic extract

The results of antibacterial activities of ethanolic extracts of the bulbils, peels and whole tuber of *D. bulbifera* against ten (10) clinical pathogens, are depicted in table 2. Different concentrations of the ethanolic extracts of each part of the tuber exhibited varying antibacterial actions in a dose dependant manner against the species of bacteria tested. From the result shown in table 2, the ethanolic extract of the peel of *D. bulbifera* demonstrated considerable inhibitory effect against most of the test pathogens with inhibition zones above the stipulated standard (15 mm) for the reference antibiotic (Nitrofurantoin) that was attained against *P. aeruginosa*; *S. pneumonia* with 16 mm and *S. aureus* with exactly 15 mm, at 250 µg/ml. 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*. No inhibition was however recorded against *Staphylococcus aureus* at 1000 µg/ml of the ethanolic extract of the peel of the tuber.

The antibacterial activity of the ethanolic extract of the bulbils of *Dioscorea bulbifera* was not profound in this present study compared to that of the peel. The inhibition zones recorded at different concentrations against majority of the test organisms was below the standard for the reference antibiotic. *Klebsiella pneumonia* was susceptible to this extract at 250, 500 and 1000 µg/ml with inhibition zones of 18, 18 and 22 mm respectively, while *Escherichia coli* and *Staphylococcus aureus* were susceptible to the effect of the ethanolic extract of the bulbils of *D. bulbifera* only at 1000 µg/ml with 15 mm and 17 mm respectively. Except for against *Micrococcus luteus* that showed 15 mm at 1000 µg/ml and *Streptococcus pneumonia* with 16 mm at 500 µg/ml and 18 mm at 1000 µg/ml, the inhibition zones recorded against all the test isolates were below the standard stipulated for the reference antibiotic.

Antibacterial effect of aqueous extract

The antibacterial effects of the aqueous extracts of the bulbils, peel and whole tuber of *D. bulbifera* evaluated against ten pathogens are represented in Table 3. Only *Proteus vulgaris* and *Pseudomonas aeruginosa* were susceptible to the antibacterial effect of aqueous extract of the whole tuber of *D. bulbifera* at 1000 µg/ml showing inhibition zones of 16 mm and 17 mm against the isolates respectively.

Minimum inhibitory concentrations (MIC) of aqueous and ethanolic extract of *D. bulbifera*

The minimum inhibitory concentrations of ethanolic and aqueous extract of each part (bulbils, peel and whole tuber) of *D. bulbifera* are shown in Table 4. The aqueous extracts of the bulbils and peels of the tuber were not evaluated for MIC, as they did not show positive results in the antibacterial assay. In line with the inhibitory effect of these extract as recorded in Tables 3 and 4, the whole tuber of *D. bulbifera* extracted with water showed inhibitory effects against *Serratia liquefaciens*; *Micrococcus luteus*; *Streptococcus pneumonia* at 250 µg/ml and *Escherichia coli*; *Proteus vulgaris* at 500 µg/ml. The ethanolic extract of the peels exhibited the highest inhibitory action, having the least MIC of 125 µg/ml as against *Escherichia coli*, *Klebsiella pneumonia*, *Serratia liquefaciens*, *Bacillus cereus* and *Citrobacter freundii*. Ethanolic extract of the bulbils showed least MIC of 125 µg/ml against *Pseudomonas aeruginosa*, *Proteus vulgaris* and *Citrobacter freundii* while, that of whole tuber had the least MIC of 125 µg/ml against *Proteus vulgaris* and *Staphylococcus aureus* only.

Minimum bactericidal concentration (MBC) of aqueous and ethanolic extract of *D. bulbifera*

Table 5 showed that the minimum concentration of ethanolic and aqueous extract of the bulbils, peel and whole tuber of *D. bulbifera* required to totally eradicate the growth of tested pathogens was 250 µg/ml. The ethanolic extract of the peel actually demonstrated more potency as bactericidal agent with a lower MBC of 250 µg/ml against *Serratia liquefaciens* and *Citrobacter freundii*; although the ethanolic extract of the bulbils also showed MBC of 250 µg/ml against *Proteus vulgaris*. Meanwhile, one or more bacterial isolates showed resistance to the bactericidal effect of ethanolic and aqueous extract of the whole tuber and the ethanolic extract of the bulbil, but the ethanolic extract of the peel of *Dioscorea bulbifera* confer bactericidal effect on all the test isolates at varying concentration ranging between 250 µg/ml and 1000 µg/ml.

DISCUSSION

Recently in the field of nutrition, there has been a tremendous interest in the assessment of nutrients, vitamins and mineral composition of staple foods such as yam, because they are considered to be economically, socially and culturally important in many tropic and subtropic regions of the world [26]. It is traditionally known that yam tuber of which *D. bulbifera* is not an exception, have potential ability to provide one of the cheapest sources of dietary energy in the form of carbohydrate [27]. Hence the high value of carbohydrate recorded for the bulbil of the tuber in this study is closely related to the report of [28], which proven the edibility of the bulbil of this staple food; and as a potential source of energy to the human body. The slight above average values obtained for the carbohydrate inherent in the peel of *D. bulbifera* in this study is significantly supportive of the cultural attitude of eating this tuber. The peel is often taken for waste, although could serve other benefits such as animal feeds or sometimes reprocessed to make another form of diet.

The moisture content which reflects the total quality of solid matter in this yam sample (*D. bulbifera*), was higher in the bulbils with average of 4.60% compared to the peel with average of 3.71%, which is in accordance with the report that the peels of *D. bulbifera* should have lower moisture content [29]. Low percentages obtained for moisture content for the bulbils in this study was in contrary to the report in previous literatures; [30] recorded a range between 64.13 and 68.60%; and [31] recorded a range between 61.55 and 71.09. This could be due to level of maturity, in accordance with [28] who reported that the moisture content of yam tubers decreased with maturity which continued after harvest and resulted in about 20% loss in weight on storage. Following the report of [32], that related moisture content of food to a property that affects the quality and shelf life of food and food products, including bacterial growth and texture. This consequently proves that the tuber in this study is suitable for prolonged storage, permissive rate of spoilage and industrial food product processing.

Table 2: Antibacterial activities of ethanolic extract of *Dioscorea bulbifera*

| Test organisms | Zone of inhibition (mm) by Concentration of extract (µg/mL) | | | | | | | | | | | | | | |
|------------------------|---|--------------------------|-------------------------|--------------------------|--------------------------|-------------------------|--------------------------|--------------------------|-------------------------|--------------------------|-------------------------|-------------------------|-------------------------|-------------------------|------------------------|
| | Bulbils | | | | | Peel | | | | | Whole tuber | | | | |
| | 1000 | 500 | 250 | 125 | 62.5 | 1000 | 500 | 250 | 125 | 62.5 | 1000 | 500 | 250 | 125 | 62.5 |
| <i>E. coli</i> | 14.66±0.57 ^h | 13.00±0.00 ^g | 11.06±0.57 ^c | 10.66±0.57 ^d | 9.66±0.57 ^c | 17.66±0.57 ⁱ | 17.00±0.00 ⁱ | 13.66±0.57 ^g | 12.00±0.00 ⁱ | 11.00±0.00 ^g | 13.66±0.57 ^g | 11.00±0.00 ^g | 8.66±0.57 ^b | 0.00±0.00 ^a | 0.00±0.00 ^a |
| <i>K. pneumonia</i> | 22.00±0.00 ^g | 17.66±0.57 ^f | 18.00±0.00 ^f | 12.00±0.00 ^c | 10.33±0.57 ^b | 21.66±0.57 ^g | 17.00±0.00 ^e | 12.66±0.57 ^c | 10.00±0.00 ^b | 10.33±0.57 ^b | 12.00±0.00 ^c | 10.00±0.00 ^b | 10.33±0.57 ^b | 0.00±0.00 ^a | 0.00±0.00 ^a |
| <i>P. aeruginosa</i> | 10.66±0.57 ^g | 9.00±0.00 ^b | 14.00±0.00 ^g | 12.33±0.57 ^e | 11.00±0.00 ^d | 20.00±0.00 ^h | 13.66±0.57 ^g | 12.00±0.00 ^e | 11.00±0.00 ^d | 9.66±0.57 ^c | 13.00±0.00 ^f | 10.66±0.57 ^d | 9.00±0.00 ^b | 8.66±0.57 ^b | 0.00±0.00 ^a |
| <i>P. vulgaris</i> | 10.00±0.00 ^d | 9.00±0.00 ^c | 8.66±0.57 ^c | 9.00±0.00 ^c | 0.00±0.00 ^a | 17.33±0.57 ^h | 14.00±0.00 ^g | 11.66±0.57 ^f | 10.00±0.00 ^d | 9.00±0.00 ^c | 11.00±0.00 ^e | 9.00±0.00 ^c | 0.00±0.00 ^a | 8.00±0.00 ^b | 0.00±0.00 ^a |
| <i>S. liquefaciens</i> | 17.00±0.00 ⁱ | 12.33±0.57 ^f | 10.33±0.57 ^d | 10.00±0.00 ^{cd} | 12.00±0.00 ^{ef} | 0.00±0.00 ^a | 17.00±0.00 ⁱ | 15.00±0.00 ^h | 13.00±0.00 ^g | 12.00±0.00 ^{ef} | 13.00±0.00 ^g | 11.66±0.57 ^e | 9.66±0.57 ^c | 9.00±0.00 ^b | 0.00±0.00 ^a |
| <i>S. aureus</i> | 12.66±0.57 ^e | 11.00±0.00 ^c | 10.66±0.57 ^c | 10.00±0.00 ^b | 9.00±0.00 ^a | 19.00±0.00 ^h | 13.66±0.57 ^f | 12.00±0.00 ^d | 11.00±0.00 ^c | 10.33±0.57 ^{bc} | 15.00±0.00 ^g | 12.66±0.57 ^e | 11.00±0.00 ^c | 10.66±0.57 ^c | 9.00±0.00 ^a |
| <i>M. luteus</i> | 14.00±0.00 ^{ef} | 13.66±0.57 ^{de} | 11.66±0.57 ^c | 11.00±0.00 ^c | 10.00±0.00 ^b | 14.66±0.57 ^f | 14.00±0.00 ^{ef} | 13.66±1.15 ^{de} | 11.00±0.00 ^c | 9.66±0.57 ^{ab} | 11.00±0.00 ^c | 9.66±0.57 ^{ab} | 11.00±0.00 ^c | 13.00±0.00 ^d | 9.00±0.00 ^a |
| <i>B. cereus</i> | 0.00±0.00 ^a | 10.00±0.00 ^c | 12.33±0.57 ^c | 14.00±0.00 ^g | 13.00±0.00 ^f | 22.00±0.00 ⁱ | 17.66±0.57 ⁱ | 16.00±0.00 ^h | 11.00±0.00 ^d | 8.66±0.57 ^b | 17.66±0.57 ⁱ | 16.00±0.00 ^h | 10.66±0.57 ^d | 10.00±0.00 ^c | 9.00±0.00 ^b |
| <i>S. pneumonia</i> | 12.00±0.00 ^f | 10.00±0.00 ^d | 8.66±0.57 ^c | 9.00±0.00 ^c | 8.00±0.00 ^b | 19.00±0.00 ^h | 12.66±0.57 ^g | 11.00±0.00 ^e | 9.66±0.57 ^d | 10.00±0.00 ^d | 11.00±0.00 ^e | 9.00±0.00 ^c | 0.00±0.00 ^a | 0.00±0.00 ^a | 0.00±0.00 ^a |
| <i>C. freundii</i> | 0.00±0.00 ^a | 14.00±0.00 ^f | 14.00±0.00 ^f | 8.66±0.57 ^c | 8.66±0.57 ^c | 17.00±0.00 ^h | 16.00±0.00 ^g | 16.33±0.57 ^g | 12.00±0.00 ^e | 9.00±0.00 ^c | 10.00±0.00 ^d | 9.00±0.00 ^e | 8.00±0.00 ^c | 0.00±0.00 ^a | 0.00±0.00 ^a |

Table 3: Antibacterial activities of aqueous extract of *Dioscorea bulbifera*

| Test organisms | Zone of inhibition (mm) by Concentration of extract (µg/mL) | | | | | | | | | | | | | | |
|------------------------|---|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-------------|-----------|-----------|-----------|-----------|
| | Bulbils | | | | | Peel | | | | | Whole tuber | | | | |
| | 1000 | 500 | 250 | 125 | 62.5 | 1000 | 500 | 250 | 125 | 62.5 | 1000 | 500 | 250 | 125 | 62.5 |
| <i>E. coli</i> | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 |
| <i>K. pneumonia</i> | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 |
| <i>P. aeruginosa</i> | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 |
| <i>P. vulgaris</i> | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 |
| <i>S. liquefaciens</i> | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 |
| <i>S. aureus</i> | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 |
| <i>M. luteus</i> | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 |
| <i>B. cereus</i> | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 |
| <i>S. pneumonia</i> | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 |
| <i>C. freundii</i> | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 |

Key: mm- Millimeter
 µg/mL- Micro gram per millilitre
 Values with similar superscript(s) along column are not significantly different from each one.

Table 4: Minimum inhibitory concentration (MIC) of ethanolic and aqueous extract of *Dioscorea bulbifera*

| Test Organisms | Minimum inhibitory concentration (µg/mL) | | | |
|--------------------------------|--|------|-------------|-----------------|
| | Ethanolic extract | | | Aqueous extract |
| | Bulbil | Peel | Whole tuber | Whole tuber |
| <i>Escherichia coli</i> | 250 | 125 | 250 | 500 |
| <i>Klebsiella pneumonia</i> | 250 | 125 | 500 | NI |
| <i>Pseudomonas aeruginosa</i> | 125 | 250 | 250 | NI |
| <i>Proteus vulgaris</i> | 125 | 250 | 125 | 500 |
| <i>Serratia liquefaciens</i> | 250 | 125 | 500 | 250 |
| <i>Staphylococcus aureus</i> | 250 | 250 | 125 | NI |
| <i>Micrococcus luteus</i> | 500 | 250 | 250 | 250 |
| <i>Bacillus cereus</i> | 250 | 125 | 500 | NI |
| <i>Streptococcus pneumonia</i> | 250 | 250 | 250 | 250 |
| <i>Citrobacter freundii</i> | 125 | 125 | 250 | NI |

Key: NI- No Inhibition

Table 5: Minimum bactericidal concentration (MBC) of ethanolic and aqueous extract of *Dioscorea bulbifera*

| Test Organisms | Minimum bactericidal concentration (µg/mL) | | | |
|--------------------------------|--|------|-------------|-----------------|
| | Ethanolic extract | | | Aqueous extract |
| | Bulbil | Peel | Whole tuber | Whole tuber |
| <i>Escherichia coli</i> | 500 | 500 | 1000 | 1000 |
| <i>Klebsiella pneumonia</i> | 500 | 500 | 1000 | NE |
| <i>Pseudomonas aeruginosa</i> | 500 | 500 | 1000 | NE |
| <i>Proteus vulgaris</i> | 250 | 500 | 500 | 1000 |
| <i>Serratia liquefaciens</i> | 1000 | 250 | NE | 1000 |
| <i>Staphylococcus aureus</i> | 1000 | 500 | 500 | NE |
| <i>Micrococcus luteus</i> | 1000 | 500 | 500 | NE |
| <i>Bacillus cereus</i> | 500 | 1000 | NE | NE |
| <i>Streptococcus pneumonia</i> | NE | 500 | 500 | 1000 |
| <i>Citrobacter freundii</i> | 1000 | 250 | NE | NE |

Keys: NE - No Effect

The ash content which reveals the substantial amount of minerals and trace elements in the sampled yam in this study had range of percentages that are closely similar to the values obtained in study by [33] who recorded values of 3.37% - 4.27%. According to [34], the actual value of mineral element in the studied sample, revealed by the ash content, depends wholly on the chemical composition of soil, cultural practices, time of planting and the amount of water available to the tuber plant.

Although there are few reports on the protein content of *Dioscorea bulbifera* in previous literature, the crude protein content of sampled tuber in this study showed a significant higher range of value than those documented in available reports [28]. The higher values obtained for the peels over the bulbils of the tuber in this study agreed with the report of [29] who indicated that the peels of yam tubers contained more nitrogen than the bulbils; and that of [34] which reported that the peel contained higher levels of protein than the tissue of *D. bulbifera* on both wet and dry basis. The range of values recorded for the crude fibre content of *D. bulbifera* peels and bulbils in this study correspond with the report of [34] that recorded the fibre in the tissue/bulbil of this same tuber on wet and dry weight basis to be 1.69% and 2.89 respectively while the peels recorded 3.75% and 6.36% of crude fibre respectively.

Available literatures pointed at the fact that yam tubers generally contained low and similar levels of crude fats which do not exceed 2%; and the distribution of fats is such that the peels contains higher levels of fats than the bulbil [28]. This supports the findings in this study, showing higher values of fat in the peel of the tuber. The result recorded for the proximate composition of *D. bulbifera* in this present study is however in support of the report available in previous literatures, affirming the fact that the tuber peels of *D. bulbifera* are richer in ash, fat, protein and crude fibre than the bulbil of the tubers [29]. Although the availability of the nutrients in the peel may be limited by the high fibre content, because it may affects digestibility of the nutrients [34]. More so, yams according to [34] and [31] are generally low in fat and protein, but high in moisture content and carbohydrate.

The presence of these phenolic compounds in plants has however been reported to possess considerable antimicrobial properties, which is attributed to their redox properties [35]. The result of the phytochemical screening of the extract of *D. bulbifera* revealed that the whole tuber contain saponins, tannins, flavonoids, terpenoids and cardiac glycosides. Each of these bioactive agents has two or more health promoting effects, and this may be responsible for their antibacterial potentials. [36] reported that plant extracts containing bioactive agents with antimicrobial properties have been found useful in treating bacterial and fungal infections.

Saponins were detected in the tuber of *D. bulbifera* in this present study with the highest concentration among other bioactive compounds. This is consistent with the findings of [37]; and implies that the tuber possesses some functions. According to [38], presence of saponin in *D. bulbifera* as observed in this study, suggest that the tuber may have hypocholesterolemic effect, in that, saponins reduces the uptake of certain nutrient including glucose and cholesterol at the gut through intralumeral physicochemical interactions. This action tends to lessen the metabolic burden that would have been placed on the liver- hypocholesterolemic effect. Saponins have also been reported to possess the properties of precipitating and coagulating red blood cells [39]. Therefore, in medicine, *D. bulbifera* can be applied as antibleeding agent to arrest lost of blood in case of injuries. Vitamins and some vital minerals such as zinc and iron are reported to form insoluble complexes with saponin [40]. This also may have contributed to the antimicrobial activity of *D. bulbifera* against some pathogenic microorganism that requires these vitamin and mineral for their metabolic processes.

Glycosides recorded very high concentration after saponins, in the studied tubers of *D. bulbifera* in the form of cardiac glycosides. Glycosides, as reported in available literature, are the product of sugar condensation with the host of different varieties of organic hydroxyl or thiol compounds, of which the hemiacetal moiety of the carbohydrate plays an ignoble role in the condensation [41]. Therefore, the high value of this bioactive compound could be attributed to high percentage of carbohydrate earlier reported, which will serve as noble source of the sugar for condensation that will consequently yield the

compound. These compounds have been reported, although to be chemically unrelated but possess common properties of intense bitter taste^[41]. The bitters act on gustatory nerves, which results in increase flow of saliva and gastric juices^[41].

The review of^[41] also reported that the tannic acid present in bitter principle of these cardiac glycosides either serves as an antiprotozoal, or to reduce thyroxine metabolisms. Terpenoid also called terpene are among the most widespread and chemically diverse groups of bioactive compounds in plants^[41]. A number of terpenoid as a bioactive compound has been isolated and have been shown to elicit antibacterial and antiprotozoal properties. Therefore, the considerable amounts of terpenoid in the tuber of *D. bulbifera* indicated that the tuber elicit medicinal benefits.

Presence of tannin in the extract of *D. bulbifera* is of health benefit, following the report of^[42] that advocated that the consumption of tannin containing beverages especially green teas and red wine, can prevent or cure a variety of illness.^[43] also stated that many physiological activities such as stimulation of phygocytic cells and wide range of anti-infective actions in plants have been attributed to tannins because of its molecular actions of forming complexes with proteins^[44]. Tannins were able to inhibit the growth of insect and disrupt the digestive activities in ruminant animals^[43]. The mode of antimicrobial activities confer on plants by tannins, which include their ability to inactivate microbial adhesions, enzymes and cell envelope transport proteins^[41], are all indicative of the antimicrobial efficacy of the extracts of *D. bulbifera* against the test microorganisms in this study.

Although, alkaloids which are one of the most efficient therapeutic phytochemicals in plants were not detected in the sampled tuber of *D. bulbifera*, but the presence of flavonoids is of alternative advantage due to their anti-inflammatory and antimicrobial properties reported by^[39]. Flavonoids are important class of polyphenols, structurally made of more than one benzene ring, which are found in plants^[45]. It has long been reported by^[46] that alkaloids and flavonoids are responsible for the antimicrobial activities in higher plants. Therefore, the considerable amount of flavonoids detected in the sampled tuber indicated the pharmacological properties embedded in the tuber of *D. bulbifera*.

An antimicrobial agent is any compound that kills or inhibits the growth of microorganisms such as bacteria, thus is called antibacterial agent^[47]. Continuous and increasing need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action against new and re-emerging infectious diseases has long been eminent. This is due to the increased failure of chemotherapeutics and antibiotic-resistance exhibited by pathogenic microbial infectious agents^[48]. The varied antibacterial activity recorded for peel, bulbils and whole tuber of this yam could be attributed to different metabolites constituted by this plant parts, following the fact that these phytochemicals are routinely described as the major antibacterial factors found in plant^[49]. From the result obtained in this work, the ethanolic extract of the peel of *D. bulbifera* demonstrated better inhibitory efficacy against most (about 80%) of the etiologically significant bacteria tested including *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Escherichia coli*, *Streptococcus pneumonia*, *Micrococcus luteus*, *Proteus vulgaris* and *Staphylococcus aureus*, for the fact that the peels of this tubers could be more enriched with several bioactive chemical.

Furthermore, the minimum inhibitory concentration obtained for the ethanolic extract of this tuber in the present study contradict the general believe that plant extracts are more effective against gram-positive than gram-negative bacteria^[50], as both gram-positive and gram-negative isolates tested against the extract were inhibited at concentrations ranging between 125 µg/ml and 500 µg/ml. Following the report of^[1], the significant activity displayed by this tuber also reinforce the hypothesis that *D. bulbifera* could be explored as potential antimicrobial drug. Moreover,^[1] also emphasized that active compounds from *D. bulbifera* are substrates of multi-drug resistant (MDR) bacteria efflux pumps, suggesting a possible use as an inhibitor in the fight against these strains.

According to the report of^[51], a critical study on the minimum bactericidal concentration of the extract of *D. bulbifera* also deduced

that the killing effect of the ethanolic extract of the tuber is inevitable of all the sensitive organisms, since none of their MBCs was up to fourfold of their corresponding MICs. Although, the mechanisms of antibacterial activity of the tuber (*D. bulbifera*) is not evaluated in this present study; although membrane disruption and formation of complex with bacterial cell wall are suspected mechanisms because of the terpenoids and flavonoids content of the tuber^[52].

CONCLUSION

The present study affirmed the fact that plant extracts especially *D. bulbifera*, in this regard; represent a novel source for bioactive compounds employable as new antibacterial agents. Considering the medical importance of the studied microorganisms, the efficacy of ethanolic extract of the peel of *D. bulbifera* as a chemotherapeutic was also established. It is however, deducible that the aqueous extract of *Dioscorea bulbifera* is not effective as antibacterial agent. More so, it is conclusive that *D. bulbifera* is a rich source of relevant bioactive agents that do not only enhances the antibacterial properties of the tubers but also ascertain its health promoting qualities. It is therefore recommended that pharmaceutical industries should exploit the broad antibacterial potentials embedded in the extract of the peel of *D. bulbifera* to produce novels antibiotics of broad spectrum to circumvent the ever emerging trend of multiple drug resistant pathogenic microorganisms.

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