

## COMPARATIVE STUDY OF THE PHYTOCHEMICAL AND ANTIBACTERIAL PROPERTIES OF TWO DIFFERENT VARIETIES OF *Dioscorea bulbifera* TUBERS

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### ABSTRACT

The phytochemical composition and the antibacterial potential of two different varieties (edible and wild types) of *Dioscorea bulbifera* tubers were determined using standard microbiological and physico-chemical methods. Extraction of bioactive compounds of the samples was done using aqueous, ethanol and methanol solvents. The antibacterial potentials of the extracts were tested against clinical bacterial isolates of *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Escherichia coli*, *Staphylococcus aureus*, *Shigella dysenteriae* and *Streptococcus pyogenes*. The percentage yield from aqueous, ethanol and methanol extracts for the wild variety of *D. bulbifera* were 14.2%, 7.1% and 5.7% respectively while that from the edible variety were 13.8%, 6.4% and 5.2% respectively. Four 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.

**Key words:** *Dioscorea bulbifera*, extracting solvents, phytochemicals, clinical isolates.

{ **Citation:** Okwu G. I., Enweani, I. B., Amengialue O. O., Obiazi H. A. K. Comparative study of the phytochemical and antibacterial properties of two different varieties of *Dioscorea bulbifera* tubers. American Journal of Research Communication, 2016, 4(7): 46-59} [www.usa-journals.com](http://www.usa-journals.com), ISSN: 2325-4076.

## INTRODUCTION

The emergence and re-emergence of diseases has left researchers with no option than to focus spot-light on the discovery of bioactive metabolites from medicinal plants to compliment synthetic orthodox drugs in the fight against diseases caused by infectious agents. The use of medicinal plants in the treatment of diseases has been in practice since ancient times in different parts of the world. Plants have the major advantage of still being the most effective and cheaper alternative sources of drugs (Pretorious and Watt, 2001). The local use of natural plants as primary health remedies, due to their pharmacological properties is quite common in Asia, Latin America and Africa (Bibitha *et al.*, 2002).

Most medicinal plants are known to produce certain bio-active molecules which are responsible for their antimicrobial properties (Sonibare *et al.*, 2009; Kuete, 2010). The substances that can inhibit pathogens and have little toxicity to host cells are considered candidates for developing new antimicrobial drugs. On the other hand, indiscriminate use of commercial antimicrobial drugs in the treatment of infectious diseases has resulted in multiple drug resistance to many human pathogenic microbes (Brody, 2012). This situation has necessitated a more radical approach in the search for new antimicrobial substances from various sources which could be used as novel antimicrobial chemotherapeutic agents (Banter and Grein, 1994; Borris, 1996;

Sonibare and Abegunde, 2012). In line with this, a recent survey of a community in parts of South Western Nigeria identified *Dioscorea* species as veritable indigenous species used by traditional medical practitioners in managing various disease conditions (Sonibare and Abegunde, 2012).

Yam as it is generally called is the common name of some tuber crops species in the genus *Dioscorea* under the family Dioscoreaceae (Engler and Prantl, 1888). They are widely distributed throughout the tropics with only a few species in the temperate regions of the world (Coursey, 1967; FAO, 1996). Apart from being used as food, yams are also known for their medicinal characteristic properties due to their complex phytochemical profile. Some of the major secondary metabolites or phytochemicals present in plants include protease inhibitors, lactins, alkaloids, non-protein amino acids, cynogen, glycosides, saponins, steroids, tannin, terpenes, phenolic compounds and flavonoids (Kuethe, 2010). Phytochemicals have complex and unique structure, and their production is often enhanced by both biotic and abiotic stresses.

*Dioscorea bulbifera* is such a peculiar yam species of the genus *Dioscorea* following the two different types of it that exist (edible and wild type). *Dioscorea bulbifera* has been used widely in the Chinese system of medicine to treat diseases of the lungs, kidneys, spleen, diarrhea, improving digestion and metabolism. It has also shown anti-tumour, anti-fungal and anti-inflammatory properties (Crabbe, 1979). Mbiantcha *et al.*, (2010), studied the analgesic and anti-inflammatory properties of extract from *Dioscorea bulbifera* in mice and rats. In Asia, it is used to treat disorder and traditionally to lower glycemic index, providing a more sustained form of energy and better protection against obesity and diabetes mellitus, and also to treat hypercholesterolemia (Brand-Miller *et al.*, 2003). This study is to evaluate and compare the phytochemical and antimicrobial characteristic of both edible and wild type of *Dioscorea bulbifera* with a view of exploiting their medicinal benefits.

## MATERIALS AND METHODS

**Sample collection and extract preparation:** Freshly harvested tubers of both edible and wild type of *Dioscorea bulbifera* were collected from Ewu market, in Esan Central Local Government Area of Edo State, Nigeria. The tubers were washed, peeled, cut into thin slices, sundried and thereafter oven dried at 55<sup>0</sup>C for 2hrs. The sliced dried tubers were finally pounded into fine powder using sterile mortar and pestle. The extraction of bioactive compound of samples was done using three different extracting solvents (distilled water, ethanol and methanol). Ten grams of powdered sample was dissolved into 100ml of each of the extracting solvents and allowed to soak for 48hrs. The solutions were later filtered using whatman filter paper No 1, and the filtrate was evaporated to dryness using a water bath at 60<sup>0</sup>C. The extracts were weighed and preserved in the refrigerator for further use.

**Test Bacterial Isolates:** A total of six clinical bacterial isolates including *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus pyogenes* and *Shigella dysenteriae* were used in this study. The organisms were collected from University of Benin Teaching Hospital (UBTH) laboratory, Ugbowo in Egor Local Government Area of Edo State, Nigeria. The isolates were sub-cultured and thereafter maintained on nutrient agar slants.

**Determination of Saponin:** The saponin content of each sample was determined using the standard method as described by AOAC (1990). The samples (2g in triplicate) were defatted by using soxhlet apparatus. They were later connected to a flat bottom flask containing 200ml of acetone. Continuous extraction was carried out using a heating mantle. The sample was subsequently fixed to another flat bottom flask containing 200ml of methanol. Heat was applied until the methanol was completely dried up. The total saponin content was calculated as a percentage of the dried material.

**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 each 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 precipitate the alkaloid,

thereafter the residue was filtered with whatman filter paper No 1, and oven-dried at 60<sup>0</sup>C for 30 minutes. Finally the residue was weighed and the percentage alkaloid content calculated.

**Determination of Flavonoid:** Flavonoid content of the sample was determined using the method described by Boham and Kocipari (1994). Ten (10) grams of the powdered sample was extracted with 100ml of 80% methanol and continuously stirred at room temperature (29.0± 2<sup>0</sup>C) for 2 – 4 hrs. The solution was filtered through whatman filter paper and the filtrate was transferred into a weighed crucible, thereafter, evaporated to dryness in a water bath and weighed. The flavonoid content was expressed as a percentage of the sample.

**Determination of Tannin:** The amount of total tannin in the samples was determined using the method described by AOAC (1990) with slight modifications. The samples (1g in triplicate) was dissolved in 80ml of distilled water and boiled for 30mins. The solution was cooled, transferred into a 100ml volumetric flask and made up to mark with distilled water. The solution was filtered with whatman filter paper No 1. Folin Denis reagent and saturated sodium carbonate solution were prepared in accordance with the standard for tannin content analysis. Also standard solution of tannic acid was freshly prepared and aliquots (0ml, 0.5ml, 1ml, 1.5ml, 2ml, 2.5ml) were dispensed into 25ml volumetric flasks. 1.25ml of Folin-Denis reagent and 2.5ml of sodium carbonate solution were added to each flask. Each mixture was made up to the volume of the flask with distilled water. The colour was measured after 30mins using spectrophotometer at 760nm. To (5ml) of the filtrate in the volumetric flask was added 1.25ml of Folin-Denis reagent and 2.5ml of Sodium carbonate solution. The colour was measured after 30mins using spectrophotometer at 760nm. The amount of Tannin content was measured by extrapolation.

**Determination of steroids:** The gravimetric method described by Harborne (1973) was used in the determination of the steroid content of the samples. One gram of the sample was weighed into conical flask and 10ml of chloroform was added. The solution was stirred continuously at room temperature. Thereafter, the mixture was made up to 100ml with chloroform and filtered with whatman filter paper No 1. Three ml of the filtrate was pipetted into a test tube and 2ml of Liberman-Burchard reagent was added and thoroughly mixed. The absorbance of the mixture was read using spectrophotometer at 640nm. Standard cholesterol solution ranging from 0 - 2.5mg/ml were prepared and also treated with 2ml each of liberman-Burchard reagent and their

absorbance were read using spectrophotometer at 640nm. Finally, the amount of steroid present was measured by extrapolation.

**Determination of antimicrobial activity:** Antibacterial activity of the extracts of *Dioscorea bulbifera* (wild and edible types) was determined by the agar well diffusion method as described by Navarro *et al.*, (1966) and Oyetayo *et al.*, (2009) with slight modifications. Briefly, the concentration of the target bacterial cell suspensions was adjusted to about  $10^6 - 10^7$  cfu/ml. The bacteria were seeded on nutrient agar plates using the spread plate method. Small wells (6mm in diameter) were made on the agar plates using a sterile cork borer. One hundred microliters of the extracts of each type was loaded into the different wells. All the preloaded plates with respective extracts and test organisms were incubated at  $37^{\circ}\text{C}$  for 24hrs. After incubation, the zones of inhibition were measured in millimeters and their means recorded.

**Determination of Minimum Inhibitory Concentration (MIC):** The MIC is defined as the lowest concentration of the compound that inhibits the growth of microorganisms. The MIC values were studied for the bacterial strains that were sensitive to the extracts in the agar well diffusion method. The broth dilution method as described by Barley and Elvyn, (1970) was employed in the determination of the minimum inhibitory concentration (MIC) against the test organisms. To each 5ml of the various extracts that has been serially diluted to various concentrations (50mg/ml, 25mg/ml, 12.5mg/ml and 6.25mg/ml) was added 5ml of nutrient broth in a test tube.

Thereafter, 50 $\mu$ l of the test organism suspension was added to each serially diluted test tube and then incubated at  $37^{\circ}\text{C}$  for 24hrs. The first test tube in the above series with no sign of visible growth was reported as the minimum inhibitory concentration.

## RESULTS

The percentage yield of aqueous, ethanol and methanol extracts of *Dioscorea bulbifera* tuber is shown in Table 1. The result showed that aqueous extract had the highest percentage yield of

14.2% and 13.8%, followed by ethanol extract of 7.1% and 6.4% while methanol had the lowest of 5.7% and 5.2% for wild and edible types respectively.

Table 2 showed the results of the phytochemical screening of *D. bulbifera*. Both the wild and edible types were evaluated for their active components namely: alkaloids, saponins, flavonoids, steroids and tannins. There were slight differences observed in the contents among the two varieties. Of the five phytochemicals screened for, saponin had the highest percentage composition which ranged from 6.8 - 11.50 % followed by alkaloids which ranged from 2.20-2.60% while steroid had the least range of 0.01% and 0.03% for wild and edible types respectively.

Table 3 summarized the antibacterial activities of the various extracts. The aqueous extract had no antimicrobial activity on all test isolates. Both ethanol and methanol extracts of the wild type showed antimicrobial activity against four out of the six test organisms viz; *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus*, and *Streptococcus pyogenes*. However, methanol extract of the wild type indicated higher zones of inhibition on the test organisms than the ethanol extract. Highest zone of inhibition (24.5mm) was observed with methanol extract on *Staph. aureus* followed by *Escherichia coli* ( 18.5mm) while the least (3.5mm) was recorded with ethanol extract on *Streptococcus pyogenes* and *Pseudomonas aeruginosa* . Extract from the edible type were active against two (2) out of the six (6) test organisms (*Escherichia coli* and *Staphylococcus aureus*) with slight differences in their zones of inhibitions.

The result obtained from the minimum inhibitory concentration (MIC) assay is shown in Table 4. The MIC values were studied for the bacterial species that were sensitive to the extracts on the agar well diffusion method. It was observed that the MIC values of ethanol and methanol extracts from both wild and edible types ranged between 25.0 – 50.0mg/ml. Among the selected organisms studied, extracts from the wild type inhibited the growth of the entire organisms better than the edible type. *Escherichia coli* and *Staphylococcus aureus* showed higher sensitivity to the metabolic compounds of the wild type than the other test organisms.

**Table 1: Percentage Yield of *Dioscorea bulbifera* Tuber extracts**

Extracting solvent	Yield (%)	
	(Wild type)	(Edible type)
Aqueous	14.2	13.8
Methanol	5.7	5.2
Ethanol	7.1	6.4

**Table 2: Percentage Active ingrediets of *Dioscorea bulbifera* Tuber extracts**

Parameters	Percentage Active ingredients	
	Wild type	Edible type
Alkaloids	2.60	2.20
Saponin	11.50	6.80
Flavonoid	0.06	0.40
Steroid	0.01	0.03
Tannin	0.24	0.27



**Table 3: Antimicrobial activity of tuber extracts of *Dioscorea bulbifera* on test organisms**

Test Organisms	Diameter of zone of inhibition (mm)					
	Aqueous	Wild type		Aqueous	Edible type	
		Ethanol	Methanol		Ethanol	Methanol
<i>Pseudomonas aeruginosa</i>	-	3.5	10.5	-	-	-
<i>Bacillus subtilis</i>	-	-	-	-	-	-
<i>Escherichia coli</i>	-	15.5	18.5	-	15.0	16.5
<i>Staphylococcus aureus</i>	-	21.5	24.5	-	22.5	24.5
<i>Shigella dysenteriae</i>	-	-	-	-	-	-
<i>Streptococcus pyogenes</i>	-	3.5	7.5	-	-	-

**Key:** - : negative, 1.0 - 3.0: resistant, 3.5 – 24.5: susceptible

**Table 4: Minimum inhibitory concentration (MIC) of *Dioscorea bulbifera* tuber extracts on test organisms**

Test Organisms	MIC (mg/ml)					
	Aqueous	Wild type		Aqueous	Edible type	
		Ethanol	Methanol		Ethanol	Methanol
<i>Pseudomonas aeruginosa</i>	++	50.0	50.0	++	++	++
<i>Escherichia coli</i>	++	25.0	25.0	++	50.0	50.0
<i>Staphylococcus aureus</i>	++	25.0	25.0	++	50.0	25.0
<i>Streptococcus pyogenes</i>	++	50.0	50.0	++	++	++

**Key:** ++ is high growth; 20 – 50 is highly susceptible.

## DISCUSSION AND CONCLUSION

The results of this investigation revealed the phytochemical composition of both wild and edible types of *Dioscorea bulbifera* and their potential antibacterial activities.

The percentage yield of *D. bulbifera* tuber extracts as presented in Table 1 revealed that aqueous extract had the highest percentage yield followed by ethanol, then methanol in both wild and edible types. This variation among the different extracting solvents could be attributed to the polarity of the solvents used (Siddiq *et al.*, 2005). The highest percentage yield recorded with the aqueous solvents indicated that the extract contained more polar compounds that could dissolve more readily in water.

The results obtained from the phytochemical screening of *D. bulbifera* (Table 2) revealed saponin as the highest active ingredients assayed followed by alkaloid while flavonoid, steroid and tannin were in trace amounts. These phytochemicals are believed to confer antimicrobial property to plants (Geissman, 1963; Lewis and Elvin-Lewis, 1995). Similar observation was made by Okwu and Ndu (2006) in their study on evaluation of phytonutrients, mineral and vitamin contents of some varieties of yam. Alkaloid has been reported by Oliver-Bever (1989) to contain dihydrodioscorine (a compound that causes paralysis of the central nervous system in animal), however, it has been exploited as drug due to its analgesic effect.

The test organisms were resistant to the aqueous extracts of both wild and edible types of *D. bulbifera* as shown in Table 3. The non-antimicrobial activity observed with the aqueous extract could be attributed to the fact that most of the bioactive ingredients of plants are localized deep within plant tissues and water may not have penetrated deep enough to leach them out. Secondly, most bioactive compounds are not soluble in water. This observation is in close agreement with Siddiq *et al.*, (2005) which indicated that 100% acetone and deionized water extract of *Moringa oleifera* leaves had the lowest anti-oxidant activity which was due to the low phenolic content of the extract. High polarity solvents such as water is not suitable in extracting bioactive components of plants when compared with solvents with intermediate polarity such as alcohols; alcohols maximizes bio-availability (Siddiq *et al.*, (2005).

Ethanol and methanol extracts of *D. bulbifera* tuber were shown to exhibit various degrees of antibacterial effects against Gram positive and Gram negative the test organisms. *Pseudomonas*

*aeruginosa*, *Escherichia coli*, *Staphylococcus aureus* and *Streptococcus pyogenes* were susceptible to both ethanol and methanol extracts of the wild types. This observation is an indication that the antibacterial principle in *D. bulbifera* tuber extract has broad spectrum activity and is consistent with the findings of Uaboi-Egbenni, (2003) on the inhibitory effect of crude extract of *Dennettia tripetala* (pepper fruit) on selected bacterial pathogens. Methanol extracts demonstrated the highest zones of inhibition suggesting that it was the best extracting solvent amongst the solvents used in this study. Similar research by Butnairiu and Coradini (2012) on evaluation of biologically active compounds from *Calendula officinalis* flowers using spectrophotometer confirmed that methanol extract gave the highest anti-oxidant activity which correlated to the polyphenolic content of the sample.

The MIC values (Table 4) revealed that extract of the wild type inhibited the growth of the organisms better than that of the edible type. However, *Escherichia coli* and *Staphylococcus aureus* presented high susceptibility to metabolic compounds of *D. bulbifera*. *Escherichia coli* and *Staphylococcus aureus* are recognized as important food-borne pathogens and the potential of their inhibition by *D. bulbifera* tuber extracts may generate more interest.

There has been renewed interest in traditional medicine and increasing demand on drugs from plant sources. Green plant medicine is safe and dependable than costly synthetic drugs which are now losing their potency due to resistant factors and abuse, many of which have adverse side effect (Agbafor *et al.*, 2011). Information obtained could lead to synthesis of potent antimicrobial agent, which may be able to stand the test of time.

Conclusively, the wild variety demonstrated a better and more promising source of antimicrobial effect than the edible types. This potential should be appreciated and hence its use in modern medicine. However, more studies should be carried out on the bioactive compounds and then mechanisms of action prior to its application as therapeutic agents.

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