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ANTIMICROBIAL AND PHITOCHEMICAL SCREENING OF TUBERS OF *Dioscorea dumetorum* SOURCED WITHIN BENUE STATE

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Abstract

The emergence and re-emergence of diseases have 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 aim of this research is to investigate the phytochemical and antimicrobial activity of D. dumetorum. Alkaloids, saponins, flavonoids, steroids and tannins were determined. The MIC values were studied for the bacterial strains that were sensitive to the extracts in the agar well diffusion method. The result showed that aqueous extract had the highest percentage yield of 12.1% 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. The aqueous extract had no antimicrobial activity on all test isolates. Both ethanol and methanol extracts showed antimicrobial activity against four test organisms viz; Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus, and Streptococcus pyogenes. However, methanol extract indicated higher zones of inhibition on the test organisms than the ethanol extract. Highest zone of inhibition (20.0mm) was observed with methanol extract on Staphylococcus aureus followed by Escherichia coli (16.0mm) while the least (6.0mm) was recorded with ethanol extract on Streptococcus pyogenes and Pseudomonas aeruginosa. The tuber demonstrated a better and more promising source of antimicrobial effect. This potential should be appreciated and hence its use in modern medicine.

Key word: Bioactive, Antimicrobial, Phitochemical, tuber

Introduction

Dioscorea dumetorum (Family: Dioscoreaceae) as the common English name (Bitter yam) suggests has a bitter taste and occurs wild throughout Africa, predominantly in the tropics. It has trifoliate (three branched) compound leaves which differentiate it between other yams having single heart-shaped leaves, and a slender stem that twines anticlockwisely. The stem is covered with hairs and spikes. The tuber is coarse and juicy and is usually produced in clusters. *D. dumetorum* tubers like tubers of other wild plants are widely used in times of food shortage. The fresh tubers of *D. dumetorum* are said to cause poisoning. Most plant diseases are caused by pathogenic microorganisms' infections and pests' infestation (Okwu, *et al*., 2016). 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 (Okwu, *et al*., 2016). 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).

Yam is a generic name for *Dioscorea* species that grow in all continents. There are over 600 species known, few of these species have been domesticated, while majority of the species of *Dioscorea* grow in the wild (Eleazu *et* al, 2013; Abdussalam *et* al, 2016; Kumar *et al*, 2017. They are one of the staple tubers consumed in many tropical countries and they are widely cultivated in West Africa (Sonibare & Abegunde, 2012; Kumar *et* al, 2017; Ifediba *et al*, 2017). The tubers of the common yellow yam *Dioscorea dumetorum* (Kunth) Pax is known in Nigeria as food, which however must be carefully prepared (Okwu, *et al*., 2016). In Benue State it is boiled over night and hawked around with pepper as a meal during the day.

The tribes that utilize it has names that differentiate it from the edible variety.

Among the Igede people it is called *Ari rika* (which implies do not eat) or *Ijunyalegwu* (meaning yam for the spirits), Tiv identifies it as *Anumbeikyo* (which means forest yam), Idoma, it is called *Achu* (meaning bush yam). These wild yams are annual, semi-perennials or perennials (Kumar *et al,* 2017), depending on the habitat. They are climbers and climb by twining. The difference between this wild *Dioscorea dumetorum* and the other variety is the thorny vines with hard spikes that are piercing.

The tubers are in clusters and are more fibrous with rough thin skin (bark) compared to the other varieties. Its composition has not been widely studied except for the folkloric uses as antimicrobial agents among the local tribes (Ifediba *et al*, 2017). The Igede people of North-central Nigeria have been using its aqueous extracts as biocontrol against pests and fungal infection in stored food materials (Okwu, *et al*., 2016).

The efficacy of the aqueous extract of *D. dumetorum* in reducing blood glucose, lipids and ketones in diabetic animals has been reported by Nimenibo-Uadia (2003).

Aim of the Study

The aim of this research is to investigate the phytochemical and antimicrobial activity of *D. dumetorum.*

Objectives of the Study

The objectives of this study include:

(i) To extract phytochemical components of *Dioscorea dumetorum.*
(ii) To investigate the antimicrobial activity of the phytochemical constituents of this yam against some plant pathogens.

MATERIALS AND METHODS

Sample collection and extract preparation: Freshly harvested tubers of both edible and wild type of *Dioscorea dumetorum* were collected from Ugbokolo market, in Okpokwu Local Government Area of Benue State, Nigeria. The tubers were washed, peeled, cut into thin slices, sundried and thereafter oven dried at 55^0C 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 60^0C. 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, Escherichia coli, Staphylococcus aureus,* and *Streptococcus pyogenes* were used in this study. The organisms were collected from Benue Stae University Teaching Hospital (BSUTH) laboratory, Makurdi Benue 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 Okwu, *et al*., (2016). 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 Okwu, *et al*., (2016) 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 600C 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 Okwu, *et al*., (2016). Ten (10) grams of the powdered sample was extracted with 100ml of 80% methanol and continuously stirred at room temperature (29.0± 20C) 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 Okwu, *et al*., (2016) 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 Okwu, *et al*., (2016) 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

Determination of antimicrobial activity: Antibacterial activity of the extracts of *Discorea dumetorum* was determined by the agar well diffusion method as described by Okwu, *et al*., (2016) and Oyetayo *et al*., (2009) with slight modifications. Briefly, the concentration of the target bacterial cell suspensions was adjusted to about 106 – 107cfu/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 370C 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 Okwu, *et al*., (2016) 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, 50µl of the test organism suspension was added to each serially diluted test tube and then incubated at 370C for 24hrs. The first test tube in the above series with no sign of visible growth was reported as the minimum inhibitory concentration.

Results

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

Extracting Solvent	Yield (%)
Aqueous	12.1
Methanol	3.9
Ethanol	5.6

Table 1 shows the percentage yield of aqueous, ethanol and methanol extracts of *Dioscorea dumetorum* tuber. The result showed that aqueous extract had the highest percentage yield of 12.1% 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: Percentage Active ingredients of *Dioscorea dumetorum* Tuber extracts

Parameter	Percentage Active Ingredients
Alkaloids	2.30
Saponin	9.30
Flavonoid	0.04
Steroid	0.02
Tannin	0.19

Table 2 showed the results of the phytochemical screening of *D. dumetorum*. 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 of 9.3% followed by alkaloids with 2.3% while steroid had 0.02% and 0.09% for tannin.

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

		Diameter of zone of inhibition (mm)	
Test Organisms	**Aqueous**	**Ethanol**	**Methanol**
Pseudomonas aeruginosa	-	4.0	9.0
Escherichia coli	-	13.5	16.0
Staphylococcus aureus	-	19.0	20.0
Streptococcus pyogenes	-	5.0	6.0

Key: - : negative

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 showed antimicrobial activity against four test organisms viz; *Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus,* and *Streptococcus pyogenes.* However, methanol extract indicated higher zones of inhibition on the test organisms than the ethanol extract. Highest zone of inhibition (20.0mm) was observed with methanol extract on *Staphylococcus aureus* followed by *Escherichia coli* (16.0mm) while the least (6.0mm) was recorded with ethanol extract on *Streptococcus pyogenes* and *Pseudomonas aeruginosa* .Ethanol extract were more active against two (2) out of the four (4) test organisms (*Escherichia coli* and *Staphylococcus aureus*) with slight differences in their zones of inhibitions.

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

		MIC (mg/ml)	
Test Organisms	**Aqueous**	**Ethanol**	**Methanol**
Pseudomonas aeruginosa	15.0	17.0	14.0
Escherichia coli	18.0	24.0	28.0
Staphylococcus aureus	19.0	27.0	21.0
Streptococcus pyogenes	14.0	16.0	15.0

Table 4 shows the result obtained from the minimum inhibitory concentration (MIC). 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 ranged between 14.0 – 28.0mg/ml. Among the selected organisms studied, extracts inhibited the growth of the entire organisms. *Escherichia coli* and *Staphylococcus aureus* showed higher sensitivity compared to other test organisms.

Discussion and Conclusion

The study showed that the phytochemical composition of *Diosorea dumetorum* have potential antibacterial activities.

The percentage yield of *D. dumetorum* tuber extracts revealed that aqueous extract had the highest percentage yield followed by ethanol, then methanol (Table 1). 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. dumetorum* revealed saponin as the highest active ingredients assayed followed by alkaloid while flavonoid, steroid and tannin were in small amounts (Table 2). These phytochemicals are believed to confer antimicrobial property to plants (Okwu, *et al*., 2016). 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 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 (Oyetayo *et al*., 2009).

Table 3 showed that the organisms were resistant to the aqueous extracts of *D. dumetorum*.. 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 (Siddiq*et al*., 2005). 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. dumetorum* 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. dumetorum* 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.

Escherichia coli and *Staphylococcus aureus* presented high susceptibility to metabolic compounds of *D. dumetorum* (Table 4). *Escherichia coli* and *Staphylococcus aureus* are

recognized as important food-borne pathogens and the potential of their inhibition by *D. dumetorum* 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).

Conclusively, the tuber demonstrated a better and more promising source of antimicrobial effect. 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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