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# *In vitro* antimicrobial and antioxidant analysis of *Dioscorea dumetorum* (Kunth) Pax and *Dioscorea hirtiflora* (Linn.) and their bioactive metabolites from Nigeria

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## ABSTRACT

*Objectives:* To analyze total phenols, flavonoids, antioxidant potential and antimicrobial activity of tuber extracts of two *Dioscorea* species.

*Methodology and results:* 1, 1-diphenyl-2-picrylhydrazyl (DPPH) assay and Folin-Ciocalteu reagent were used to determine total antioxidant activity and total phenolic content of tuber extracts of *D. dumetorum* (edible and wild species) and *D. hirtiflora*. Total Flavonoids were determined by repeatedly extracting 1 g of powdered tuber sample with 10ml of 80% aqueous methanol at room temperature for 2-4 hours. Anti-microbial activity was determined by Agar diffusion and pour plate methods. Quantitative analysis of bioactive components revealed that total phenols are the major bioactive constituents found in the extracts expressed as mg of GAE per gram, which ranged from 22.83 ± 0.33mg/g to 64.33 ± 2.55mg/g. Mean concentrations of flavonoids ranged from 7.53± 0.07 mg/g to 25.60 ± 0.39 mg/g. Anti-oxidant efficiency was higher in *D. hirtiflora* and non-edible *D. dumetorum* than edible *D. dumetorum* when compared to standard antioxidants like Ascorbic acid. Antimicrobial activity profile showed direct influence of concentrations of bioactive components.

*Conclusion and application of findings:* The results of our study confirmed the folkloric uses of the studied plants and provided evidence that tuber extracts of *D. dumetorum* and *D. hirtiflora* might indeed be potential sources of natural antioxidant and antimicrobial agents.

**Key words***: Dioscorea*, antimicrobial activity, antioxidant activity, bioactive compounds.

## INTRODUCTION

Medicinal plants have been shown by many studies as sources of diverse nutrients and non-nutrient molecules. Many of the medicinal plants display antioxidant and antimicrobial properties which can protect the human body against both cellular oxidation reactions and pathogens. Thus it is important to characterize different types of medicinal plants for their antioxidant and antimicrobial potential (Mothana & Lindequist, 2005; Bajpai et al., 2005; Wojdylo et al., 2007). Medicinal plants are known to produce certain bioactive molecules which are responsible for their antimicrobial properties (Rios & Recio, 2005; Kuete et al., 2008; 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 microorganisms. 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 (Navarro et al., 1996). In line with this, our 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 diseased conditions.

*Dioscorea* which is commonly known as yam and a large genus in the family Diosoreaceae is one of the staples in many tropical countries. Yam is widely grown in many West African countries (Wait, 1963; Coursey, 1970). Some yams are used as medicines in oriental countries to prevent diarrhea and diabetes (HSU et al., 1984; Yen, 1992). Studies have shown that yam extracts can reduce blood sugar (Undie & Akubue, 1986; Hikino et al., 1986; Araghiniknam et al., 1996), inhibit microbial activity (Hu et al., 1996a & b; Kelmanson et al, 2000) and show antioxidative activity (Farombi et al., 2000; Chan et al., 2004). Traditionally, *D. dumetorum* and *D. hirtiflora* have been widely used. *D. dumetorum* has been used in the treatment of schistosomiasis, jaundice and malaria, as a topical anesthetic, sometimes used externally as a rubefacient and also applied to suppurating abscesses (Adjanohoun et al., 1972). *D. hirtiflora* has been used in treating abscesses and scabies (Haerdi et al., 1964). In the interview conducted during the course of this research in Laniba village of Akinyele local government area of Oyo-State, Nigeria, Villagers were found to use these plants in the treatment of boils, jaundice, dysmenorrheal, and gonorrhea. The objectives of this study therefore were: 1) to determine the antioxidant capacity of 2 species of *Dioscorea*; 2) to screen for antimicrobial and antioxidant activities of the two species; and 3) to test for the correlation between bioactive metabolites and antioxidant capacity.

## MATERIAL AND METHODS

**Plant collection and extract preparation:** The plant materials (tubers) of *Dioscorea dumetorum* and *D. hirtiflora* were collected between September – October, 2010 from Laniba village of Akinyele local government and Bode market in Oyo-State, Nigeria. Plant authentication was done by Mr. O.S. Shasanya, of Forest Herbarium Ibadan (FHI) where voucher specimens were deposited in under FHI NO 108942 and FHI NO-108911 for *D. dumetorum* and *D. hirtiflora* respectively. The plant materials were scrapped with a knife to remove the attached dirt, peeled, cut into pieces and gently sun-dried for quick removal of water. It was then oven-dried at temperature of 40 $^0$C until they were properly dried and then milled. Five hundred (500) g each of the powdered samples were macerated with 95 % methanol for 72 hours. The filtrate was evaporated to dryness using a rotary evaporator. The extracts were weighed and preserved for further use in the refrigerator.

**Biological materials:** A total of 8 organisms were used in this study. The microorganisms maintained on Nutrient and sabouraud dextrose Agar (Int'l Diagnostic Group Plc., Topley house UK) were supplied by Pharmaceutical Microbiology Laboratory, Faculty of pharmacy, University of Ibadan, Nigeria.

**Determination of total phenolics:** The amount of total phenolics in the extracts was determined using Folin-Ciocalteu procedure (Singleton et al., 1999) with slight modifications. Samples (2.5ml in triplicates) were introduced into test- tubes; 0.5ml of Folin-Ciocalteu's reagent was added. The content in the tubes were mixed and allowed to stand for 30 minute. Absorption was measured at 765nm. The total phenolic content was expressed as Gallic acid equivalent in mg/g dry material.

**Determination of flavonoid contents :** Flavonoid determination was done using the methods of Bohm and Kocipai (Boham & Kocipai, 1994). One (1) g of the powdered samples was extracted repeatedly with 10ml of 80% aqueous methanol at room temperature for 2-4 hours. The whole solution was filtered through Whatman paper No. 1. The filtrate was later transferred into a Petri dish and evaporated into dryness over a water bath and weighed to a constant weight. The flavonoid content was expressed as milligram per gram.

**DPPH radical scavenging activity:** Radical scavenging activity of plant extracts was measured by slightly modified method of Miliauskas et al. (2004). To 2.5ml of the extract was added 3ml of methanol and 0.5ml of 1,1-diphenylpicrylhydrazyl (DPPH). The

mixture was shaken and left to stand at room temperature for 30 minutes. The absorbance of the resulting solution was read spectrophotometrically at 517nm. Ascorbic acid was used as standard antioxidant while a blank of methanol (Analytical BDH) was ran with each assay. All determinations were carried out in triplicates. The same procedure was repeated using control sample (DPPH without extracts). The inhibition of DPPH was calculated as a percentage according to the following equation;

$$\% \text{ inhibition} = \frac{\text{Absorbance of control - Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

**Antimicrobial activity:** Antimicrobial activity of methanolic extracts of *Dioscorea dumetorum* and *D. hirtiflora* was determined by the agar well diffusion method (for bacteria) and pour plate method (for fungi) (Oyetayo et al., 2009) with slight modifications. Briefly, the methanol extracts were dissolved in absolute methanol to final concentrations of 200 mg/ml, 100 mg/ml, 50 mg/ml, 25 mg/ml, 12.5 mg/ml, and 6.25 mg/ml, using serial dilution. Small wells (8mm in diameter) were made in the agar plates by a sterile cork borer. One hundred (100) microliters of the extract of each plant was loaded into the different wells. Concentration of target microbial cells suspensions was adjusted to about $10^6$-$10^7$ CFULML. Bacteria were cultured on Nutrient Agar; and fungi on Sabouraud Dextrose Agar. Gentamycin (10 ug/ml) for bacteria, Tioconazole (0.5 mg/ml) for fungi were used as positive control and methanol was used as a negative control for test organisms. All the preloaded plates with respective extracts and test organisms were incubated at 37 $^0$C for 24 hours for bacteria and at 26 $^0$C for 48 hours for fungi. After the incubation period, the zone of inhibition was measured in millimeters. All the tests were carried out in triplicate and their means recorded.

**Minimum Inhibitory Concentration (MIC) Assay:** The MIC was defined as the lowest concentration of the compounds to inhibit the growth of microorganisms. The MIC values were studied for the microbial strains, being sensitive to the extracts in the agar well diffusion method. The crude methanolic extracts were partitioned into n-hexane, ethyl acetate and aqueous fractions and the fractions were tested on the organisms. Ethyl acetate fraction was found more effective than the other two. The MICs of the ethyl acetate fractions of the three samples were thus determined against three bacteria and a fungus (the more sensitive organisms): *Bacillus subtilis, Salmonella typhi, Proteus mirabilis and Candida albicans* respectively, MIC was determined by Agar dilution Assay method (Forbes et al., 1998). In the dish dilution assay, the agar and the extracts were poured together into the Petri-dish at different concentrations of extracts (0.78 mg/ml, 1.56 mg/ml, 3.13 mg/ml, 6.25 mg/ml, 12.5 mg/ml, 25 mg/ml, and 50 mg/ml) and left on the bench for about two hours to set. Different organisms were then inoculated on the different plates with different concentrations. The bacteria plates were incubated at 37 $^0$C for 24 hours while that of fungal plates was incubated at 26 $^0$C for 24-48 hours. The first plate in the above series with no sign of visible growth was reported as the minimum inhibitory concentration.

**Statistical analysis:** Experimental results were the mean ±S.D of three measurements. Student t-test and Pearson correlation coefficients were from Excel programme (SPSS Series). Differences were considered significant at $p < 0.05$.

## RESULTS AND DISCUSSION

In the present study, two *Dioscorea* species were evaluated for their total phenol and flavonoid. Methanolic extracts were screened for the DPPH scavenging assay and antimicrobial efficacy. The two *Dioscorea* species showed significant and satisfactory results but *D. hirtiflora* had the highest activity in all assay carried out. The total phenolic and flavonoid contents of the plants are shown in Table 1.

**Table 1:** Total phenol and Flavonoid contents of the plant extracts

| Samples | Total phenols (mgGEA/g) | Total flavonoid (mg/g) |
|---|---|---|
| *D. dumetorum* (edible) | 22.83±0.17$^a$ | 7.53±0.03$^a$ |
| *D. dumetorum* (non-edible) | 28.67±0.33$^{ab}$ | 25.60±0.20$^c$ |
| *D. hirtiflora* | 64.33±0.67$^c$ | 13.33±0.33$^b$ |

Data are represented as means±SEM (n=3)

Different letters in the same column represent significantly different values ($p$<0.05) as separated by independent paired student t-test

The differences in the contents among plants used were statistically significant ($p < 0.05$). Total phenols are the major bioactive components found in the extracts of the plants expressed as mg of GAE per gram which ranged from 22.83 ± 0.33 mg GAE/g to 64.33 ± 2.55 mg GAE/g. Average concentration of flavonoid ranged from 7.53 ± 0.07 mg/g to 25.60 ± 0.39 mg/g. The total phenolic compound amount was quite high in *Dioscorea hirtiflora* methanol extracts (64.33 ± 2.55 mgGAE/g). In contrast to this, the total flavonoid concentration was 13.33 ± 0.65mg/g. DPPH radical scavenging activity of extracts compared well with that of Ascorbic acid (shown in Table 2). Among the studied extracts, radical scavenging activity was found significant in *D. hirtiflora* (38.27 ± 0.45) followed by non-edible *D. dumetorum.*

**Table 2:** Free radical scavenging effect % inhibition on DPPH of plant extracts

| Samples | % Inhibition |
|---|---|
| *D. dumetorum* (edible) | 16.97±0.23[a] |
| *D. dumetorum* (non-edible) | 26.87±0.13[c] |
| *D. hirtiflora* | 38.27±0.23[d] |
| Ascorbic acid | 88.50±0.00[b] |

Data are represented as means±SEM (n=3)

Different letters in the same column represent significantly different values ($p<0.05$) as separated by independent paired student t-test

Positive DPPH test suggest that extract of all the samples were scavengers of free radicals. In summary, using the DPPH radical scavenging method the antioxidant activity of the plant extracts were in order *D. hirtiflora* > non-edible *D.dumetorum* > edible *D.dumetorum.* This result was also consistent with the order of total phenol content of each extract. Several studies have reported the relationship between phenolic content and antioxidant activity. Velioglu , (Velioglu et al., 1998) and Odukoya ., *(2005*) reported a strong relationship between total phenolic content and antioxidant activity in selected fruits, vegetables and grain products. Sodifiya . (2006) and Yang . (2002) also reported correlation between total phenolic content and antioxidants activity in some Nigerian medicinal plant extracts while Kahkonen ., (1999) found no correlation between antioxidant activity and phenolic content of some plant extracts. As shown in Figure 1, our findings showed a good correlation between total phenolic content and free radical scavenging activity.

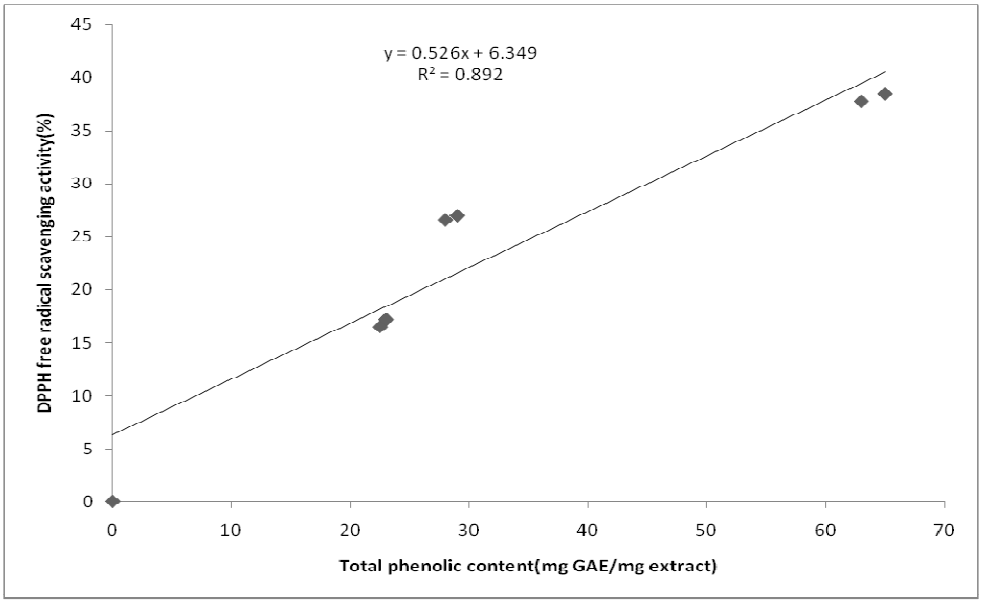


**Figure 1:** Linear correlation between total phenolic content and DPPH free radical scavenging activity of the two *Dioscorea* species

**Antimicrobial assay:** The antimicrobial activity of tuber extracts of *D. dumetorum* (edibles species), *D. dumetorum* (non-edibles species) and *D. hirtiflora* was assayed *in vitro* by agar well diffusion method against eight organisms (5 bacteria and fungi). Table 3 summarizes the microbial growth inhibition of both the tuber extracts of the screened plant species. Plant samples were shown to exhibit various degrees of antimicrobial effects against the tested microorganisms. The highest *in vitro* anti-microbial activity was by *D. hirtiflora* followed by non-edible *D. dumetorum and* then edible *D. dumetorum* against *Staphylococcus aureus* and *Candida albicans.* Non-edible *D. dumetorum* demonstrated the highest *in-vitro* antibacterial activity against *Proteus mirabilis.* In all, the methanol extracts from *D. hirtiflora* showed antimicrobial activity against all the 8 tested organisms; non-edible *D dumetorum* tuber extract inhibited 5 out of 8 organisms and edible *D dumetorum* extract inhibited 4 out of 8 organisms.

**Table 3:** Result of antimicrobial activity of crude methanolic extract of *D. dumetorum* and *D. hirtiflora* Zone of inhibition (Mean± standard error)

| Plants samples | Conc.(mg/mL) | S.a | E.c | B.s | P.m | S.t | C.a | A.n | P.c |
|---|---|---|---|---|---|---|---|---|---|
| | 200 | 20±1.3 | 0 | 0 | 16±1.2 | 0 | 20±1.3 | 14±1.1 | 0 |
| | 100 | 18±1.2 | 0 | 0 | 0 | 0 | 16±1.1 | 14±1.0 | 0 |
| Edible | 50 | 0 | 0 | 0 | 0 | 0 | 14±1.0 | 12±1.2 | 0 |
| *D. dumetorum* | 25 | 0 | 0 | 0 | 0 | 0 | 12±1.2 | 10 | 0 |
| | 12.5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 6.25 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Non-edible | 200 | 20±1.3 | 0 | 22±1.4 | 22±1.4 | 16±1.2 | 14±1.1 | 0 | 0 |
| *D. dumetorum* | 100 | 18±1.3 | 0 | 20±1.2 | 20±1.3 | 14±1.1 | 12±1.0 | 0 | 0 |
| | 50 | 0 | 0 | 16±1.1 | 0 | 12±1.0 | 10±1.1 | 0 | 0 |
| | 25 | 0 | 0 | 12±1.0 | 0 | 0 | 0 | 0 | 0 |
| | 12.5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 6.25 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *D.hirtiflora* | 200 | 20±1.3 | 22±1.4 | 26±1.3 | 20±1.4 | 22±1.1 | 24±1.3 | 20±1.3 | 18±1.2 |
| | 100 | 18±1.3 | 20±1.1 | 20±1.3 | 18±1.2 | 18±1.2 | 18±1.3 | 18±1.2 | 16±1.0 |
| | 50 | 16±1.1 | 18±1.1 | 18±1.2 | 16±1.1 | 16±1.3 | 16±1.2 | 16±1.3 | 12±1.0 |
| | 25 | 14±1.0 | 16±1.0 | 16±1.2 | 10 | 14±1.1 | 14±1.0 | 14±1.2 | 10±0.8 |
| | 12.5 | 12±1.0 | 16±1.0 | 14±1.4 | 0 | 12±1.0 | 12±1.3 | 12±1.3 | 0 |
| | 6.25 | 10 | 14±1.1 | 10 | 0 | 0 | 10 | 10 | 0 |
| | -ve | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | +ve | 38 | 36 | 38 | 38 | 36 | 22 | 26 | 24 |

**Key:** S.a - *Staphylococcus aureus,* E.c - *Escherichia coli, B.s - Bacillus subtilis,* P.m - *Proteus mirabilis,* S.t - *Salmonella typhi,* C.a - *Candida albicans,* A.n - *Aspergillus niger,* P.c - *Penicillum chrysogenum*
-ve (Negative control) -Methanol., +ve (Positive control) -Gentamycin 10ug/ml (Bacteria), -Tioconazole 0.5mg/ml (Fungi). Values represents mean ± S.E, n=3
Figures are mean Diameter of zone of inhibition in mm, 0 = no inhibition, Diameter of cork borer= 8mm

It is noteworthy that only *D. hirtiflora* inhibited the growth of *Escherichia coli* and *Penicillin chrysogenum. Escherichia coli* is a Gram-negative, non-sporing *bacillus* that is associated with acute gastroenteritis in infants and certainly in older children and adults. *E. coli* may also be the causative organism in appendicular abscess, peritonitis, cholecystitis and wound infections (Hugo, 1994). *Candida albicans* on the other hand (a fungus) is yeast like fungus present is the mouth, intestine and vagina. It is responsible for infections in

these sites where there is impairment of the defense mechanisms (Rogers, 1990). *Candida* is tolerant of acid and not sensitive to any of the antibacterial antibiotic except polyene antibiotics. Thus, the activity of the methanolic extracts of *D. hirtiflora* against it is remarkable and comparable with positive control. In general, the extracts of both plants are more effective against Gram-positive than Gram-negative organisms. This tendency may be due to several possible reasons such as permeability barrier provided by presence of cell-wall with multilayer structure in Gram-negative bacteria or the membrane accumulate in mechanisms or presence of enzymes in periplasmic space which are able to breakdown foreign molecules induced from outside (Holetz et al., 2002; Abu-Shanab et al., 2004; Parekh & Chanda,2007). This observation therefore supports the fact that in general, the Gram-negative bacteria are more resistant than the Gram-positive bacteria (Grierson & Afolayan, 1999; Afolayan, 2003). The MIC values (Table 4) indicate that among the selected micro-organisms studied, extract of *D. hirtiflora* inhibited the growth of the entire organism better than *D. dumetorum* (edible) and *D. dumeforum* (non-edible). *Bacillus subtilis* presented high sensitivity to metabolic compounds of *D. hirtiflora, B subtilis* is widely recognized as important food-borne pathogens and the potential of its inhibition presented by *D. hirtiflora* may receive more attention (Getha et al., 2009).

**Table 4:** Minimum Inhibitory Concentration (MIC) (mg/ml) of ethyl acetate fractions of *D. dumetorum* and *D. hirtiflora* on selected microorganisms

| Plant samples | *Bacillus subtilis* | *Salmonella typhi* | *Proteus mirabilis* | *Candida albicans* |
|---|---|---|---|---|
| *D.dumetorum (*edible) (mg/ml) | 50 | 50 | 50 | 6.25 |
| *D.dumetorum* (non-edible) (mg/ml) | 25 | 50 | 50 | 50 |
| *D. hirtiflora* (mg/ml) | 1.56 | 6.25 | 3.125 | 1.56 |

Key: Sample A = Edible *D. dumetorum*
Sample B= Non-edible *D dumetorum*
Sample C= *D. hirtflora*

## CONCLUSION

From the present investigation, it can be concluded that the methanolic extracts of the plants (*Dioscorea dumetorum (edible and non-edible) and D. hirtiflora)* showed biopharmaceutical potentiality, where antimicrobial efficacy is directly influenced by the phenolic contents and DPPH scavenging activity had good correlations with phenolic contents of the plant. However, whether such extracts will act as effective therapeutic agents remain to be investigated the identification of the bioactive compound and study of mechanisms of actions is necessary prior to application. The results obtained might then be considered sufficient for further studies on the isolation and identification of the active principles and to evaluate the possible synergism among extract components for their antioxidant and anti-microbial activity.

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