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Phytochemical Constituents and *in vitro* Antioxidant Capacity of Methanolic Leaf Extract of *Oxytenanthera abyssinica* (A. Rich Murno)

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Authors' contributions

This work was carried out in collaboration between all authors. Author IBO conceptualized and designed the work, interpretation of results, laboratory analysis and drafting of the original manuscripts and final approval of the version. Author EM involved in the project design, involved in result interpretation and laboratory analysis, critical revision of draft article for suitability and intellectual content and final approval of the version. Author HJB involved in statistical analysis and critical revision of the manuscript. All authors read and approved the final manuscript.

Research Article

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ABSTRACT

Aims: The antioxidant and phytochemical properties of leaf extracts of the Nigerian *Oxytenanthera abyssinica* were evaluated at different concentrations using ascorbic acid standard. This preliminary study investigates the potentially bioactive components of the plant which may renew research into its medicinal value.
Study Design: Cold extraction of the leaf parts followed by evaluation of phytochemicals and antioxidant capacity using chromatographic and spectrophotometric methods**.**
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Methodology: The antioxidant properties were determined using three assay models: the 2,2-diphenyl-1-picrylhydrazyl (DPPH), ferric reducing antioxidant power (FRAP) colorimetric test and a modified version of ***in vitro*** antioxidant TBARS after cold extraction maceration. The activity was determined at different concentrations (10µg/ml,50µg/ml,100µg/ml,200µg/ml and 400µg/ml) of the extract and expressed as % inhibition. phytochemicals were determined by standard detection and spectrophotometric methods.
Results: The yield of the methanolic leaf extract of *Oxytenanthera abyssinica* was 3.92% w/w dry matter. Steroids (steroid glycoside), alkaloids, saponins, tannins, cardiac glycosides, flavonoids, phlobatanins, anthroquinone and terpenes were detected while cyanogenic glycosides were absent. The quantitative analysis yielded (in mg/100g): flavonoids (1.51±0.23), alkaloids (1.40±0.02), polyphenols (1.31±0.32), tannins (0.071±0.40) and saponins (1.2±0.10). *Oxytenanthera abyssinica*'s DPPH reduction was highest at 400µg/ml (82.10 ± 0.01%) with IC_{50} of 56.2µg/ml. The ferric reducing power of the extract at 400µg/ml was 61±1.52% (FRAP: 0.61) and the inhibition of lipid peroxidation measured as TBARS was 81.0 ±1.11%.
Conclusion: There is an indication that *Oxytenanthera abyssinica* contains important phytochemicals and an antioxidant capacity comparable with standard antioxidant compounds that may be linked to its beneficial effects on health.

Keywords: Oxytenanthera abyssinica; antioxidants; phytochemicals; flavonoids; medicinal plant; Nigeria.

1. INTRODUCTION

Phytochemicals may be described as non-nutritive plant chemicals that have protective or disease preventive properties. They are regarded as non essential nutrients [1]. Normally, they are naturally occurring bioactive molecules produced by plants for protection from the elements of the earth and the sun's harmful rays. These amazing phyto-compounds provide food resources for the human cells. Consumption of plant foods containing these compounds have been scientifically validated to help slowdown the aging process and reduce the risk factors of many diseases including cancer, heart disease, stroke, high blood pressure, cataracts, osteoporosis, diabetes and urinary tract infections [2-4].

It has been noted that one of the mechanisms of action of phytochemicals is associated to their antioxidative activity. Oxidation, a chemical reaction that transfers electrons from a substance to an oxidizing agent, produces free radicals [5] and initiates chain reactions that may damage cells [6,7]. Antioxidants terminate these chain reactions by removing free radical intermediates, inhibit other oxidation reactions, or enhance the endogenous antioxidant defences of the organism [8]. They act by being oxidized themselves and are often reducing agents such as thiols, ascorbic acid and/or phenols [9]. These antioxidants may be natural (ascorbic acid and tocopherols) as well as synthetic molecules as propyl gallate [PG], tertiary butylhdroquinone [TBHQ], butylated hydroxyanisole [BHA] and butylated hydroxytoluene [BHT] thus they can be synthesize in the body or may be obtained from the diet [10,11].

It is known that many herbs, especially from tropical and subtropical climates, have antioxidant activity linked to their ethnomedicinal value. *Oxytenanthera abyssinca* is a tropical drought resistant plant (bamboo) that grows in open grassland, lowlands and highlands, though mostly on hills or along intermittent watercourses [12,13]. They are

distributed mainly in the tropics and also occur naturally in subtropical and temperate zones of all continents, except Europe. The species is found at latitudes 46º N to 47º S and from sea level to 4000m elevation [14]. It is often found on very poor soils in Sub-Saharian Africa [15,16] and can survive fire in its natural habitat. Evidence indicates that each plant flowers once in a life time, it dies and does not regrow spontaneously in the same region [17-20]. In Nigeria, this herb is known ethnically by the following names: Nnyanyanga (Efik), Kewal (Fulfulde), Kawu (Gwari), Goora (Hausa), Achala Oyibo or Otosi (Igbo), Gamare (Kanuri), Eman (Loke), Takarwa (Ngizim), Apako (Yoruba) and Shuwa (Arabic).

In Sub-Sahara African countries, the bamboo has various uses such as roofing and furniture construction, shelterbelts and windbreaks (whole plant), fuel for cooking and heating (charcoal), split stems for basket weaving, fresh/dried leaves for fodder and sap for wine making [21-23]. Also the stems are widely used for fencing and fish-traps, stakes, drainages, trellises, tool handles, paper making, household implements, arrow shafts [24] and the seeds and young shoots as famine food [25]. The bamboo therefore is highly valuable to the local population. Current trend in parts of Africa shows that the herb is used as a poverty eradication crop [26] especially in Sub-Saharan African countries as contrasted by its high medicinal use in the Chinese population. Some African countries now fully protect the species where harvesting is carefully controlled and reintroduction programmes have been effectively established. In ethnomedicine, the rhizome is used in the treatment of dysentery and the leaves are marketed for treating diabetes, colics and rheumatism. *Oxytenanthera abyssinica* has wide applications in various countries, for instance in Ethiopia the root is applied in the treatment of skin diseases while in Senegal leaf decoctions are taken to treat polyuria, oedema and albuminuria [27].

Generally, the economic potential of this herb in Sub-Saharan Africa has masked its ethnomedicinal value. Hence, current research efforts tend towards *Oxytenanthera abyssinica*'s potential as a poverty alleviation plant. Therefore, we investigated for the first time the antioxidant and phytochemical properties of the Nigerian *Oxytenanthera abyssinica* as a preliminary investigation in determining its potentially bioactive compounds.

2. MATERIALS AND METHODS

2.1 Collection and Identification of Plant Materials

Leaves of O*xytenanthera abyssinca* were plucked from the forest of Michael Okpara University of Agriculture, Umudike, Nigeria (Latitude $05^0\ 29^1$ N to $05^0\ 42^1$, Longitude $07^0\ 24^1$ E to $07^0\ 33^1$). The matured leaves were identified and confirmed by experts of the Department of Forestry, College of Natural Resources and Environmental Management, Michael Okpara, University of Agriculture Umudike, Nigeria. A voucher specimen with the number Ibeh 2010-59 was deposited in the University herbarium for future reference.

2.2 Sample Preparation

The leaves of *Oxytenanthera abyssinca* were first washed with distilled water to remove debris and dust particles, air-dried at room temperature and pulverized into a uniform material using a Thomas-Willey mini-milling machine(model 4, 3375-e25). Plant extraction was done by cold maceration of 300g in 80% methanol with intermittent shaking at 2h interval for 48h. The extract was then filtered (Whatman filter paper no.1) and evaporated to

dryness at 40ºc. The obtained crude extract was packed in airtight plastic containers and stored at 4^0C until analysis.

The percentage yield of the extract was calculated using the formula:

$$\% \text{ Yield} = \frac{\text{weight of the extract}}{\text{weight of plant material}} \times \frac{100}{1}$$

2.3 Phytochemical Determination

For the phytochemical detection of major constituents of O*xytenanthera abyssinca* we used thin-layer chromatography (TLC) on silica gel 60 F_{254} with layer thickness 0.25 mm(Merck, Darmstadt, Germany) after dissolving the extract (2mg) in 2ml in methanol. The plates were developed, then left to dry for about 10 min before they were viewed under UV fluorescence light at 254 and 366nm. Spraying was done with the required detection reagent to determine the compounds present. For flavonoids, TLC was developed in n-butanol/acetic acid/water (4:1:5), then spots were visualized with 1% $Alcl_3$ solution in methanol under UV light (366nm) (Ce 3041 Buck Scientific, UK). Alkaloids, saponins, tannins, anthraquinones, flavonoids, terpenoids, steroids and cardiac glycosides were all identified based on the methods of Harbone [28,29] and Trease and Evans [30]. Quantitative determination was carried out as previously described by Trease and Evans [31], Sofowara [32] and Harborne [33].

2.4 Antioxidant Assay

2.4.1 Inhibition of Lipid Peroxidation

A modified version of the thiobarbituric acid reactive substances (TBARS) assay was used to determine the level of lipid peroxides formed using egg yolk homogenate as lipid-rich media [34]. Egg homogenate (0.5 ml, 10% v/v) was added to 0.1 ml of extract (1mg/ml) and the volume made up to 1 ml with distilled water. Then 0.05 ml of $FeS0_4$ was added and the mixture incubated for 30 min. Acetic acid (1.5 ml) and thiobarbituric acid (1.5 ml) in SDS were sequentially added. The resulting mixture was vortexed and heated at 95℃ for 60 min. After cooling 5 ml of butan-1-ol was added and the mixture centrifuged at 3000 rpm for 10 min (Ultra-8 digital CR Scientific, Koningsweg, Netherlands). The absorbance of the organic upper layer was measured at 532 nm and converted to percentage inhibition using the formula:

Inhibition of Lipid Peroxidation (%) = (1 – E/C) X 100

Where C = absorbance of fully oxidized control and E = absorbance in the presence of extract

2.4.2 Ferric Reducing Antioxidant Power (FRAP) Assay

The reductive potential of O*xytenanthera abyssinca* was determined according to the method of Benzie and Strain, [35] based on the chemical reduction of Fe^{3+} to Fe^{2+}. At low pH, we measured the reduction of ferric tri(2-pyridyl)-1,3,5-triazine (Fe III TPTZ) complex to ferrous form by monitoring the change in absorption at 593nm. The calculation was done by:

FRAP value of sample (µM)=

$$\frac{(\Delta \text{ in absorbance of sample from 0−4 min}) \times \text{FRAP value of standard (1000µm)}}{(\Delta \text{ in absorbance of standard from 0 to 4 min})}$$

2.4.3 Determination of DPPH Radical Scavenging Activity

Rapid thin layer chromatography (TLC) screening for antioxidant activity was carried out by spotting a concentrated methanolic solution of the extract on silica gel plates. The plates were developed in methanol: ethyl acetate (2:1) then air-dried and sprayed with 0.2% w/v DPPH spray in methanol. The presence of yellow spots was detected. Radical scavenging activity of extracts was performed according to the DPPH spectrophotometric method of Mensor *et al*. [36] using vitamin C (Emzor Pharmaceutical Industries, Nigeria) as a reference antioxidant. Methanol (1.0 ml) plus extract solution (2.5 ml) was used as blank while 1 ml of 0.3 mm DPPH plus methanol (2.5 ml) was used as a negative control. The free radical scavenging properties of the extracts against 2,2-diphenyl-1-picryl hydrazyl (DPPH) radical were measured at 518 nm, as an index of their antioxidant activity. The concentrations of the extracts and vitamin C used were 10, 50, 100, 200 and 400 $\mu g ml^{-1}$ and the assay was carried out in triplicates for each concentration. IC_{50} values (the concentration of extracts required to scavenge 50% of DPPH free radicals) were also obtained. The absorbance (abs) of the resulting mixture measured at 518nm was converted to percentage antioxidant activity (AA %) and thus calculated by the equation:

AA% = [100 – ((ABS sample —ABSblank) × 100)] /ABS control

2.5 Statistical Analysis

The statistical analysis was done using one way analysis of variance (ANOVA), using spss® version 17. The differences between the means were tested using posthoc LSD. A *p*-value of $p<0.05$ was considered to be statistically significant. We presented results as mean ± standard deviation. Assays were done in triplicates.

3. RESULTS AND DISCUSSION

3.1 Extraction Yield

The yield of the methanolic leaf extract of *Oxytenanthera abyssinica* was 3.92% w/w dry matter.

3.2 Phytochemical Analysis

Preliminary **phytochemical screening** of *Oxyteneanthera abyssinica* shows the presence of steroids (steroid glycosides), alkaloids, saponins, tannins, cardiac glycosides, flavonoids, phlobatanins, anthraquinones, and terpenes, while cyanogenic glycosides were absent (Table 1). The quantitative analysis yielded high levels flavonoids, alkaloids, polyphenols, and moderate levels of tannins and saponins (Table 2).

Table 1. Phytochemical Screening of Leaf Extracts of *Oxytenanthera abyssinica*

Plant Metabolite	Extract Content
Cyanogenic glycosides	-
Cardiac glycosides	++
Steroid glycoside	++
Saponins	+
Tannins	++
Alkaloids	++
Phlobatanins	+
Terpenoids	++
Flavonoids	++
Anthroquinones	+

+ = Trace, +++ = Abundant, - = Absent

Table 2. Phytochemical Composition of the Leave Extracts of *Oxytenanthera abyssinica* (mg/100g dry weight)

Plant metabolite	Composition
Polyphenols	1.31±0.32
Saponins	1.2±0.10
Tannins	0.07±0.40
Alkaloids	1.40±0.02
Flavonoids	1.51±0.23

Results are mean of triplicate determinations on a dry weight basis ± standard deviation

3.3 Antioxidant Activity

The percentage inhibition of DPPH by *Oxytenanthera abyssinica* and vitamin C, FRAP and the inhibition of lipid peroxidation measured as TBARS of the extract showed a concentration-dependent antioxidant activity resulting from reduction of DPPH (Table 3), FRAP and inhibition of TBARS (Fig. 1) radicals to non-radical forms. The leaf extract had a comparable reduction capacity in all the concentrations measured when compared with the scavenging activity of ascorbic acid. Ic_{50} values for *Oxytenanthera abyssinica* and ascorbic acid were 56.2 and 55.10 µg/ml, respectively (Table 3). The FRAP of the extract at 400µg/ml was 61±1.52% (FRAP: 0.61) and that of the inhibition of lipid peroxidation (measured as TBARS) was 81.3±1.11% (Fig. 1).

Table 3. Antioxidant activity Measured As % Reduction of DPPH

	Antioxidant activity (% Inhibition)	
Concentration (µg/ml)	***Oxytenanthera abyssinica***	**Ascorbic acid**
10	72.60 ± 0.01	73.64 ± 1.82
50	73.85 ± 0.01	74.10 ± 0.09
100	72.83 ± 0.01	74.62 ± 3.46
200	79.97 ± 0.01	77.16 ± 2.11
400	82.10 ± 0.01	87.00 ± 0.11
	56.2‡	55.1‡

**Indicates significant difference at p<0.05; ‡indicates IC_{50} value measured at µg/mL*

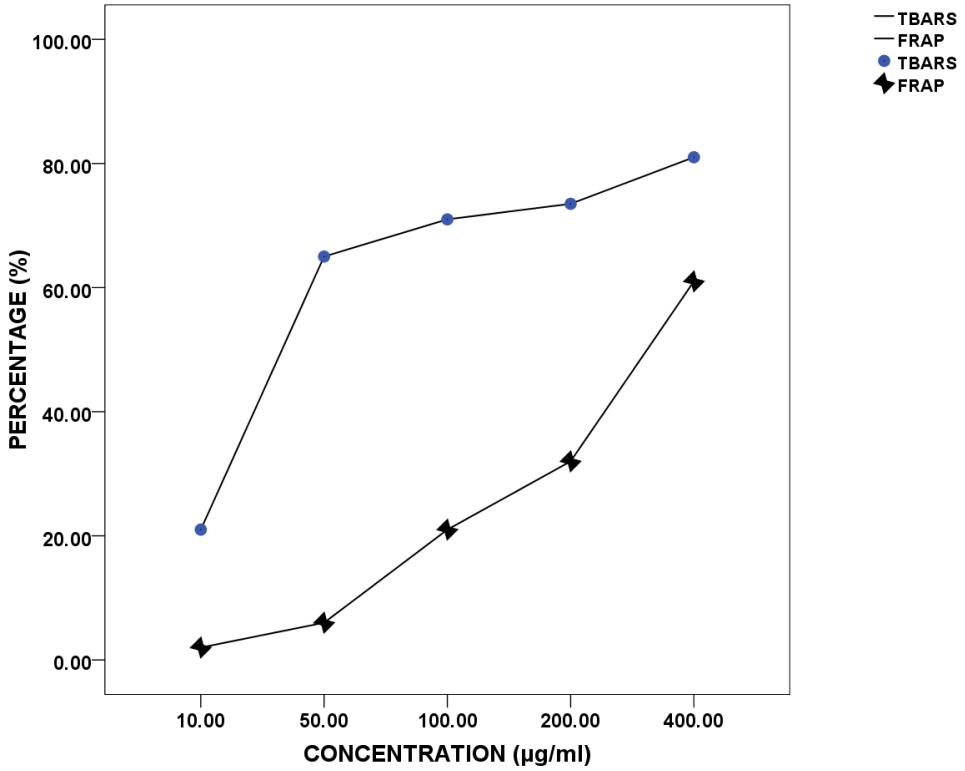

Fig. 1. Ferric reducing potential (FRAP) and inhibition of lipid peroxidation (TBARS) by *Oxytenanthera abyssinica*

4. DISCUSSION

For most regions in Africa *Oxytenanthera abyssinica* currently has major attraction as a natural product amenable to sustainable management. It is grown throughout tropical Africa outside the humid forest zone. The plant has strong stems used as various construction materials, wine making, charcoal for fuel energy, and leaves are used for fodder [37]. Despite its wide rural significance, *Oxytenanthera abyssinica* remained neglected in the forestry and agricultural sectors until recently, when the international network for bamboo and rattan (INBAR) included this species among 38 priority bamboos of economic importance [38]. The economic benefits of the plant have masked its traditional ethno-medicinal exploits in the treatment of various ailments.

Scientific investigations on the constituents of this plant with relevance to its ethno-medicinal value are scanty and not well documented in Africa. Our TLC assay revealed the presence of various phytochemicals in the plant (Tables 1 and 2). These phytochemicals may be related with its ethno-medicinal use in the treatment of various diseases. Our results are comparable with other medicinal plants such as nettle (*Urtica dioica* l.)[39] which, has antioxidant and antimicrobial effects [40]. The therapeutic effects of this medicinal plant is evidently seen in the treatment of prostate hypertrophy, diabetes mellitus, rheumatic disease, hypertension, osteoarthritis, diarrhea, rheumatoid arthritis, inflammation, skin disease and urinary tract infection [41]. Generally, alkaloids are known to have antimicrobial,

antifungal and anti-inflammatory effects [1] and also act as anti-hypertensive agents [42](Sofowora, 1993). The folkloric use of *Oxytenanthera abyssinica* in the treatment of skin diseases, colics, diabetes, rheumatism, poluria, oedema and albuminuria may owe its efficacy to the presence and concentration of the phytochemicals contained in the plant. These phyto-compounds have been linked to specific actions against infections and diseases [42]. The absence of cyanogenic glycosides support its low toxicity and considerable wide application as fodder and famine food source.

At high experimental concentrations (non-existent *in vivo*), the antioxidant capabilities of flavonoids *in vitro* may be stronger than those of vitamins C and E, though depending on concentrations tested [43]. Phenolics are a major group of chemical species that act as primary antioxidants [44]. They ultimately have high redox potentials which allow them to act as reducing agents, hydrogen donors and/or singlet oxygen quenchers [45]. This attribute is possibly due to the delocalization of electrons over the phenolics and stabilization by the resonance effect of the aromatic nucleus that prevents the continuation of the free radical chain reaction [46]. Phenolics contents were high in the methanolic leaf extracts of the plant. These are potent water soluble antioxidants which prevent oxidative cell damage suggesting their possible implication in the protection of various diseases. Our results indicated a high antioxidant capacity (DPPH [Table 3], TBARS [Fig 1] and FRAP) when compared with ascorbic acid. DPPH is a fast, reliable, reproducible and widely used to measure the *in vitro* general antioxidant activity of pure compounds as well as plant extracts [47]. The decrease in absorbance by the DPPH radical with concomitant increase in the concentration of the extract (Table 3) may have resulted in the rapid discolouration of DPPH, suggesting that the antioxidant activity of the methanolic extract of *oxytenanthera abyssinica* is due to its proton donating ability. The data also showed that the antioxidant activity is concentration dependent, having maximal effect at 400µg/ml. The extract may have a comparable antioxidant capacity with that of ascorbic acid, requiring 56.2 µg/ml (IC_{50} value) to reach 50% inhibition of DPPH radical activity, though the value is higher than ascorbic acid (55.1µg/ml). A lower DPPH radical-scavenging activity is, however, associated with a higher IC_{50} value. It has been shown that the reduction mechanism of DPPH correlates with presence of hydroxyl groups on the antioxidant molecules [48] which may suggest that the antioxidant activity of *Oxytenanthera abyssinica* is probably due to the presence of substances with an available hydroxyl group such as flavonoids or condensed tannins. All extracts tested (100-400 µg ml^{-1}) revealed good scavenging activity for DPPH, FRAP and an appreciable inhibition of TBARS in a dose dependent manner. Meir et al.[49] noted that the reducing power of compounds could serve as indicator of potential antioxidant properties. In the FRAP assay, it is likely that the presence of antioxidants in the extract reduced Fe^{3+} complex to the ferrous form with a reduction of about 61%. This suggests that the leave extract acts as electron donor and could neutralize free radicals.

Antioxidants are widely consumed in the diet, naturally and as food additives and also consciously taken as therapeutic agents in the form of supplements such as herbs. Antioxidants from natural resources such as extracts of green tea, rosemary, oregano, liquorice and bamboo leaves are also widely used in the market. The numerous physiological actions of phenolic compounds may be linked to their ability to act as strong antioxidants and free radical scavengers. As such, the ethno-medicinal use of *Oxytenanthera abyssinica* could be attributed to the complex mixture of phytochemicals present. Furthermore, the potential effects of antioxidants in controlling degenerative diseases with marked oxidative damage have been reported [50-52]. The high antioxidant capacity and inhibition of lipid peroxidative activity of the plant may suggest its potential in the prevention of various oxidative related diseases. Therefore, leave extracts of

Oxytenanthera abyssinica could be exploited as sources of bioactive metabolites for nutritional, medicinal and commercial purposes as Antwi-boasiako et al. [25] recently reported.

5. CONCLUSION

Oxytenanthera abyssinica has demonstrated to possess significant antioxidant activity in the model used and showed a wide variety of phytochemicals. The plant contains putatively bioactive compounds that may be responsible for its ethno-medicinal use, thus should be explored further. Furthermore, detailed studies on the isolation and characterization of the plant phenols as well as *in vivo* assays to describe novel biological antioxidants is needed.

CONSENT

Not applicable.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and Michael Okpara University, Umudike, Nigeria.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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