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Antioxidant Activity and Phenolic Content of *Okoubaka aubrevillei* (Anunuebe) Fruit *Pychnobotrya nitida* (Osu) Leaf Extracts

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Abstract: The antioxidant activity and the total phenolic content of *Okoubaka aubrevillei* (Anunuebe) and *Pychnobotrya nitida* (Osu) plant extracts were investigated in this study to justify their ethno medicinal importance. The antioxidant activity of crude extracts of both plants was investigated using the scavenging effect on DPPH (2, 2-Diphenyl-1-Picrylhydrazyl Radical) assay, while the total phenolic content was determined by the Folin-Ciocalteu reagent method. When compared to standard drug vitamin C at various concentrations, both extracts exhibited potent antioxidant activity that increased with increasing concentration of extracts. The high positive linear correlation between the antioxidant capacity and the total phenolic content suggests that the antioxidant capacity of both plants can be largely attributed to their phenolic compounds. Based on these findings, *O. aubrevillei* fruit and *P. nitida* leaf could be potential natural antioxidant sources for preventing free radical-induced diseases.

Key words: Medicinal plants, *Okoubaka aubrevillei*, *Pychnobotrya nitida*, phenolic content, antioxidant capacity.

1. Introduction

During normal cellular metabolism in the human body, free radicals and other reactive oxygen species are constantly formed in the mitochondria and microsome organelles as natural by-products [1, 2], due to partial reduction of molecular oxygen [3]. They are also produced externally from other sources such as food, drugs, cigarette smoke, and other pollution from the environment [4, 5], such as radiation and toxic chemicals. Excessive production of reactive oxygen species can cause oxidative damage to a variety of biomolecules, including lipids, proteins, DNA (Deoxyribonucleic Acid), and cell membranes. This may also influence the onset of diseases associated with metabolic dysfunction [2]. To maintain a cellular balance between reactive oxygen species production and clearance, eukaryotic cells have developed several anti-oxidative defense mechanisms, including enzymes and antioxidants [3]. Although most of the free radicals are scavenged by endogenous enzymes such as catalase, superoxide dismutase, and peroxidase-glutathione system [6], their actions may not be sufficient to remove all the free radicals [7]. As a result, much emphasis has been placed on the use of antioxidants, particularly natural antioxidants, to inhibit and protect damage caused by free radicals and reactive oxygen species [8].

Antioxidants, also known as free radical scavengers [9], are substances that help the body's defense mechanism against diseases caused by the free radical attack [10] and are thus capable of preventing or suppressing damage caused by free radicals and reactive oxygen species when they are present.

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Antioxidant sources can be both natural and synthetic; however, due to their safety, natural antioxidants such as plant-based antioxidants are now preferred over synthetic antioxidants [8]. Plants provide the majority of natural antioxidants in the form of phenolic compounds such as flavonoids, phenolic acids, and tocopherols [11]. Phenolic compounds are one of the most abundant and widespread groups of plant metabolites [12], and several studies have described the antioxidant properties of medicinal plants high in phenolic compounds [13]. The roles of natural antioxidants, particularly phenolic compounds that can delay oxidative degeneration in lipids, are rapidly gaining attention, as evidenced by the growing interest in plant materials high in phenols [7]. There is, therefore, a need to search for, develop, and use more effective antioxidants derived from plants.

In Nigeria, many indigenous plants have medicinal values as they are used for the treatment of various illnesses in line with their traditional uses in their various localities [14]. *Okoubaka aubrevillei* bark is widely used as a medicine in the treatment of skin problems [15], to counteract poisoning, cure tachycardia, and is taken as a vapor bath or as nose drops to cure edema. It is also used to disperse hematomas when used in a compress [16]. While several studies have been carried out on different parts of *O. aubrevillei*, including the bark and leaves, there is a dearth of information on the phenolic content and antioxidant activities of this plant.

Pychnobotrya nitida is a medicinal plant that belongs to the family *Apocynaceae* and is locally known as Osu in Igbo land. It is a perennial shrub of about 50 cm high and it grows as a wild plant. Although most of the farmers that grow this plant use it mainly for marking boundaries in their farmlands, the plant has also been found to be of some medicinal use as it is being used by the locals for the treatment of various ailments. There is no known published work done on this plant yet to ascertain the phytocompounds responsible for its medicinal potentials.

Plant antioxidants such as polyphenols and vitamins have been linked to protection against various disorders in the literature, and potential sources of antioxidant compounds have been sought in a variety of plant materials [17]. The potentials of using *Okoubaka aubrevillei* and *Pychnobotrya nitida* plants as sources of plant-derived antioxidant compounds are still largely unexplored despite their folklore acclaimed medicinal potentials in the treatment of various ailments. Hence, this study was designed to investigate the phenolic contents and antioxidant potential of *O. aubrevillei* and *P. nitida*to justify their ethno medicinal importance.

2. Material and Methods

2.1 Collection of Plant Materials

The leaves of *Pychnobotrya nitida* were collected from Awgu in Enugu State, while the fruits of *Okoubaka aubrevillei* were obtained from Eke Awka market in Anambra State, both in Nigeria. The samples were identified by an expert at the Tree Crops and Tropical Ecological Centre at No.7 Dona Drive Independence Layout Enugu Nigeria.

2.2 Preparation of Plant Materials

Thoroughly washed mature leaves of *P. nitida* and fruits of *O. aubrevillei* were shade dried and then powdered using an electric blender. A measure of 50 g of the powder was filled in the thimble and extracted successively with ethanol using a Soxhlet extractor as described by Gopalasatheeskumar [18]. All the extracts were concentrated using a rotary flask evaporator and preserved at 5 ℃ in airtight bottles until further use.

2.3 DPPH (2, 2-Diphenyl-1-Picrylhydrazyl Radical) Scavenging Assay

The antioxidant activity of crude extracts of *O. aubrevillei* and *P. nitida* was investigated using the scavenging effect on DPPH method [19]. The DPPH antioxidant assay is based on the principle that in the

presence of free radical scavengers, DPPH can decolorize. The color changes from purple to yellow as the DPPH radical's molar absorptivity at 517 nm decreases from 9,660 to 1,640 when the odd electron of the DPPH radical is paired with hydrogen from a free radical scavenging antioxidant to form the reduced DPPH-H. The odd electron in the DPPH radical is responsible for the 517 nm absorbance as well as the visible deep purple color [20].

The measurement was as described by Umaru et al. [21], with slight modification. Briefly, the sample was prepared by diluting 5mg of crude extract into 5 mL of methanol, producing a concentration of 1,000 µg/mL. The stock solution was sonicated to ensure the homogeneity of the sample. Eight other concentrations were prepared at 7.81 µg/mL, 15.62 µg/mL, 31.25 µg/mL, 62.5 µg/mL, 125 µg/mL, 250 µg/mL, 500 µg/mL and 1,000µg/mL, diluted from the 1,000µg/mL stock solution. Analysis was done in triplicate. The solution was mixed vigorously and left to stand at room temperature for 30 min in the dark after which its absorbance was measured using an ultraviolet spectrophotometer. Methanol was used as blank (only methanol) and negative control (1mL methanol mixed with 3 mL DPPH), while ascorbic acid (vitamin C) was used as the standard. The concentration of the sample required to inhibit 50% of the DPPH free radical was calculated as IC_{50}, and the value was determined using the log dose inhibition curve which was performed by using PRISM version 3.02 software, based on the calculated values of the DPPH scavenging activity (%) of the sample. DPPH scavenging activity (%) was calculated with the formula:

$$\text{DPPH scavenging activity}(\%) = \frac{A_0 - A_1}{A_0} \times 100$$

where: A_0 = Absorbance of the control; A_1 = Absorbance in presence of the sample.

2.4 Determination of Total Phenolic Content

The total phenolic content of the plants was determined by the Folin-Ciocalteu reagent method. An aliquot of 0.2 mL of 10% Folin-Ciocalteu reagent and 1 mL of 7.5% solution of Na_2CO_3 was added to 0.4 mL of the crude extracts (1 mg/mL). To the resulting mixture, 0.8 mL of distilled water was added and was incubated for 30 min at 40 ℃. The absorbance of the sample was measured at 760 nm. Gallic acid was used as standard (1 mg/mL). All the tests were performed in triplicates. The results were determined from the gallic acid calibration curve (mgGAE/g). A calibration curve was prepared with 1,000 µg/mL, 500 µg/mL, 250 µg/mL, 125 µg/mL, 62.5 µg/mL, 31.25 µg/mL, 15.62 µg/mL, 7.81 µg/mL, 3.91 µg/mL and 1.95 µg/mL concentrations [13].

2.5 Statistical Analysis of Data

Data were expressed as means ±SD (Standard Deviations) of three replicate determinations and then analyzed with *R* [22], using the following packages; plyr, car, emmeans, polynom, ggplot2, ggtext. ANOVA (One-Way Analysis of Variance) and Tukey's post hoc test were used to determine the differences among the means. The Pearson correlation analysis was performed between antioxidant activity and total phenolic content. Significant difference was determined at 5% probability level ($p < 0.05$).

3. Results and Discussion

3.1 DPPH Scavenging Activity of Plant Extracts Compared with Standard Ascorbic Acid

The DPPH assay was chosen because of its relative accuracy and reliability for assessing antioxidant capacity in plant extracts [23]. Antioxidants in the extracts of both *O. aubrevillei* fruit and *P. nitida* leaf extracts reacted with DPPH, reducing it to DPPH-H. The degree of discoloration indicated the extracts' scavenging potential in terms of hydrogen donating ability. The results showed that both plant extracts had good scavenging ability and are therefore primary antioxidants that can limit free radical damage by reacting with free radicals in the body (Fig. 1). It also

showed that the percent inhibition of DPPH radicals for *O. aubrevillei* fruit and *P. nitida* leaf extracts increased gradually with an increase in concentration, thus showing a dose-dependent inhibition of DPPH activity. This observation suggests that the antioxidant activities of the plant extracts increased with an increase in concentration. This is consistent with previous studies where the antioxidant activity of plant extracts increased as the concentration increased [7, 24]. The concentration of plant extract required to inhibit 50% of DPPH free radicals (IC_{50}) was 719.30 µg/mL and 1,030.73 µg/mL for *O. aubrevillei* fruit and *P. nitida* leaf extracts, respectively, while that of ascorbic acid (standard) was 26.05 µg/mL (Fig. 2). Comparatively, this suggests that the *O. aubrevillei* fruit extract has a better antioxidant capacity than the *P. nitida* leaf extract. Contrary to the observed higher antioxidant activity of the fruit extract in the present study, previous studies have shown that leaf extracts have better antioxidant activity than fruit extracts [25, 26].

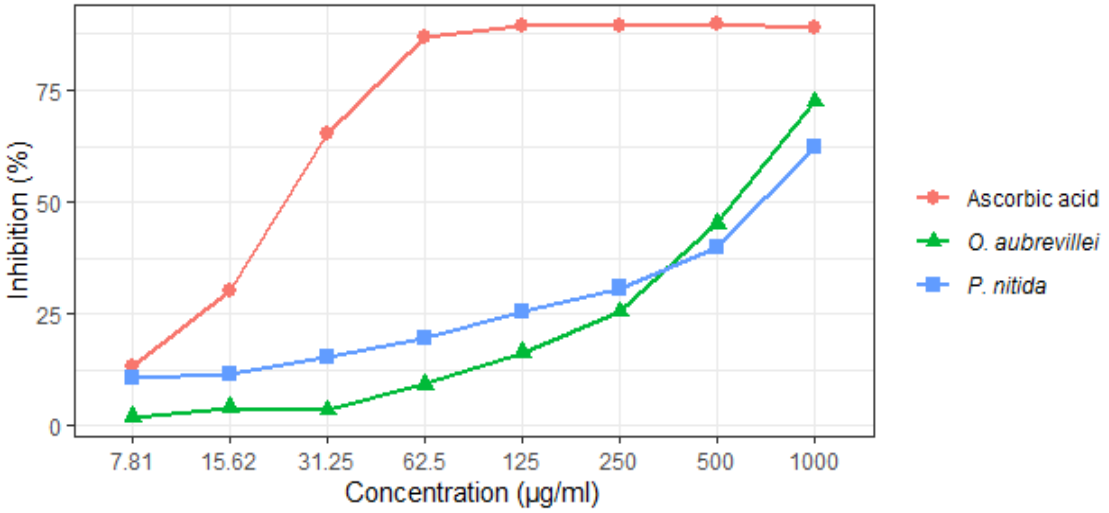

Fig. 1 Dose inhibition curve of *O. aubrevillei* fruit and *P. nitida* leaf extracts compared to ascorbic acid (standard).

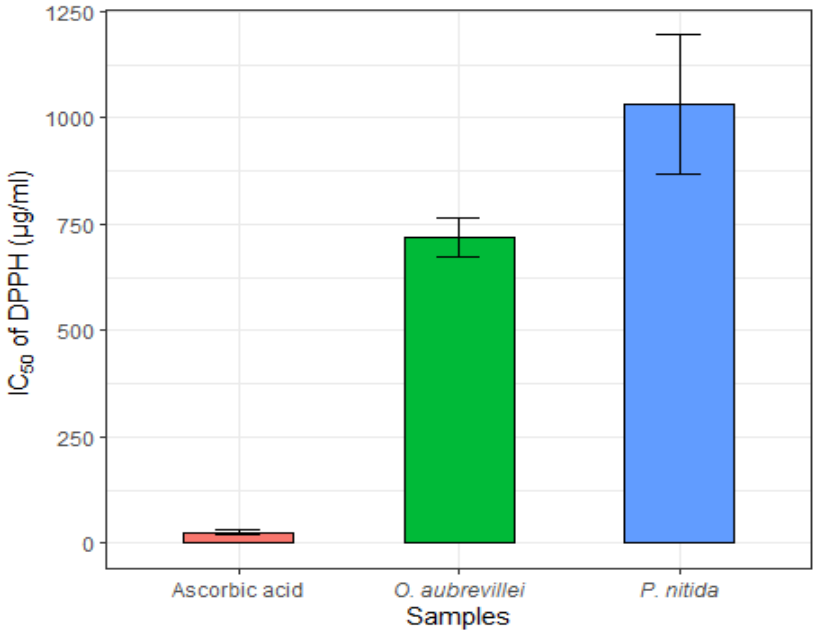

Fig. 2 Comparison of the IC_{50} of the *O. aubrevillei* fruit and *P. nitida* leaf extracts with ascorbic acid. Values are mean ±SD for three determinations.

This disparity may be due to the fact that, while the present study involved different plants, the other studies compared the leaves and fruits from the same plant species. It, therefore, suggests that, although fruit phenolic compounds are thought to be potent antioxidants capable of mediating the scavenging of reactive oxygen species and other radical species [27], they may not be more potent than those of the leaves from same plant species.

3.2 Total Phenolic Content

Phenolic compounds are one of the major constituents in most plants that are efficient free radical scavengers [28] exhibiting antioxidant activity by inactivating lipid free radicals, or by preventing the decomposition of hydroperoxides into free radicals [7, 29]. Table 1 shows that both plants had high phenolic content which may have contributed to the plants' antioxidant property and curative ability in adsorbing and neutralizing free radicals, considering the fact that significant linear correlations (R^2 and R) were observed between the DPPH antioxidant activity and the total phenolic content in *O. aubrevillei* (R^2 = 0.9991 and R = 0.9982) and *P. nitida* (R^2 = 0.9335 and R = 0.9661) (Fig. 3). This is consistent with previous studies that found a linear correlation between the total phenolic contents

Table 1 Antioxidant activity by DPPH method and total phenolic content of the plant extracts (mean ± SD).

Plant extract	Plant part	DPPH antioxidant activity (%)	Total phenolic content (mg GAE/g)
Okoubaka aubrevillei	Fruits	0.42 ± 0.01	93.13 ± 40.26
Pychnobotrya nitida	Leaves	0.37 ± 0.01	53.81 ± 5.62

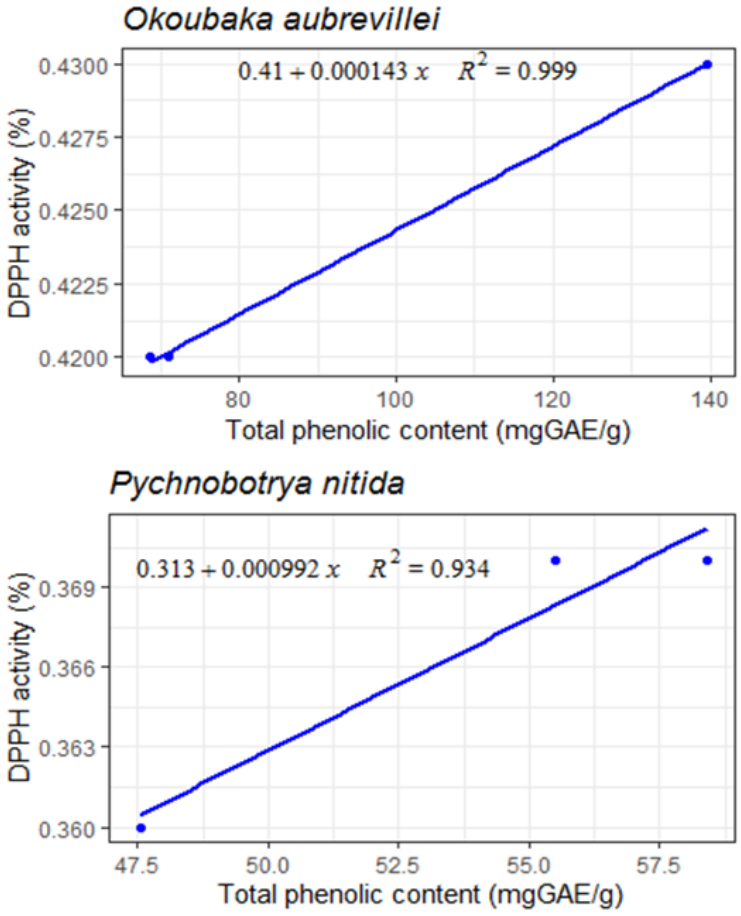

Fig. 3 Linear regression between DPPH antioxidant activity and total phenolic content for both plants.

and antioxidant activity of several plant species [30, 31] thus corroborating the findings that phenolic compounds are a major antioxidant constituent in fruits and vegetables, and their antioxidant activity and total phenolic content have a direct association

4. Conclusion

The antioxidant capacity and total phenolic content of *O. aubrevillei* fruits and *P. nitida* leaves were determined. The strong positive linear correlations between antioxidant capacity determined by DPPH assays and total phenolic content suggest that both plants' antioxidant activities are primarily attributed to their phenol compounds. The results indicate that *O. aubrevillei* fruit was more potent since it exhibited the highest scavenging activity. Although the results of this study may support the traditional use of the plants as sources of antioxidants, the use of these plants as natural antioxidants will necessitate additional research to isolate and identify their phenolic compounds, as well as in vivo studies to better understand the functionality of the plant materials to possibly standardize them as natural antioxidants. Since these are local and available plants, they may provide cheaper, more compatible and efficacious alternatives to synthetic drugs. This is expected to positively impact both public health and the nation's economy.

Conflict of Interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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