Global Scientific and Academic Research Journal of Multidisciplinary Studies
ISSN: 2583-4088 (Online)
Frequency: Monthly
Published By GSAR Publishers
Journal Homepage Link- https://gsarpublishers.com/journals-gsarjms-home/

Antioxidant Potential of Methanol Extract of *Piper guineense* leaf and *Xylopia aethiopica* seeds

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Article History

Received: 05/07/2025
Accepted: 18/07/2025
Published: 21/07/2025

Vol – 4 Issue –7

PP: - 100-105

Abstract

This study was carried out to evaluate antioxidant analyses of crude extract from Piper guineense leaf and Xylopia aethiopica seed. Antioxidant potentials of the plant extracts such as nitric oxide, FRAP, Hydroxyl Radical Scavenging Activity, and DPPH were conducted using established protocols. Results revealed that there is high percentage scavenging activity sample gallic acid when compared with sample P. guineense and X. aethiopica. there was an increase in the percentage scavenging activity of the gallic acid than the extracts. These plants can contribute important nutrients to diets. Its diverse phytochemicals might be responsible for its therapeutic functions. The results suggest that Piper guineense leaf and Xylopia aethiopica seed have potential antioxidant properties which could be exploited in medicine and food industry.

Keywords: *antioxidant, seed, Piper guineense leaf, Xylopia aethiopica seed.*

Introduction

Piper guineense leaf and *Xylopia aethiopica* seeds are two plant materials commonly used in traditional medicine and as spices in cooking, particularly in South Eastern Nigeria and other parts of Africa [1]. Nursing mothers in these regions often consume soups prepared with these plants, believing they offer nutritional and health benefits. However, there is scanty scientific evidence to support these claims, and issues have been raised about potential toxicity effects on both mothers and their babies. Medicinal plants are widely known to show diverse positive effects in animals, and humans. Most spices are used for the management or treatment of some diseased conditions in herbal medicine [2]. Some of which include *Xylopia aethiopica* (*X. aethiopica* and *Piper guineense* (*P. guineense*).

P. guineense (Family: *Piperaceae*) a tropical plant of West African origin (Anyanwu and Nwosu, 2014) is popularly known as "African black pepper." In Nigeria, it is commonly called "Uziza" in Igbo and "Iyere" in Yoruba. Its seeds and leaves are used as spices in preparation of certain types of foods such as the popular "hot soup" or "pepper soup" usually consumed by nursing mothers after childbirth to aid uterine contraction and consequently, placenta expulsion or the expulsion of some remains in the woman's womb [3].

In traditional medicine, the leaves have been associated with management of problems of infertility in women and for treating respiratory ailments [4]. Its parts are used in herbal medicine for treatment of rheumatic pains. Researchers have reported that the seeds and leaves possess antiparasitic and antimicrobial activities [5]. Some people use the seeds as an aphrodisiac. [6]. [7] reported the effect of aqueous extract of the seeds on antioxidant enzymes, liver marker enzymes and indices of haematology in albino rats.

X. aethiopica (Family: Annonaceae) popularly called "African pepper" is reported to grow in forest zones and often along rivers and in arid areas [8]. The colour of the matured fruit usually changes from green to brownish-black after drying [9]. In Nigeria, it is commonly called "Uda" in Igbo, "Erunje" in Yoruba and "Kimba" in Hausa (Sara et al, 2015). The fruits are also used in preparation of hotsoup usually given to nursing mothers after childbirth [10].

This study will contribute significantly to the understanding of the nutritional and pharmacological properties of *Piper guineense* leaf and *Xylopia aethiopica* seeds. The results will provide valuable information on the potential health benefits and risks associated with the consumption of these plants, particularly among nursing mothers and their babies. This knowledge will inform the development of safe and effective traditional medicine practices, as well as guidelines for the use of these plants in cooking.

Materials and Methods

Plant collection and Processing;

Fresh leaf of *Piper guineenses* (Uziza) was collected from a native compound at Ekwe in Isu L.G.A OF Imo State while

*Corresponding Author: **Ubah, C.E**

the *seeds* of *Xylopia aethiopiaca* (Uda) was purchased from Relief market, Owerri and the plants and was identified by a taxonomist. Freshly collected samples was air-dried at room temperature, ground into powder, weighed and stored for extraction

Antioxidant Assay

Ferric Reducing Antioxidant Property (FRAP)

The principle of the assay is the quantification of ferric degradation product, by its condensation with the extract. The reducing property of the extracts will be determined as described by [11].

0.25 ml of the extracts will be mixed with 0.25 ml of 200 mMSodium phosphate buffer pH 6.6 and 0.25 ml of 1% Potassium ferrocyanide. The mixture will be incubated at 50^{o}C for 20 min, thereafter 0.25 ml of 10% trichloroacetic acid will be added and centrifuge at 2000 rpm for 10 min, 1 ml of the supernatant will be mixed with 1 ml of distilled water and 0.2 ml of ferric chloride and the absorbance was measured at 700 nm.

DPPH SPECTROPHOTOMETRIC ASSAY

The scavenging ability of the natural antioxidants of the leaves towards the stable free radical DPPH was measured by the method of [12]. The leaf samples (20µl) were added to 0.5ml of 0.1mM methanolic solution of DPPH (2.2-diphenyl-2-picryl hydrazyl hydrate) and 0.48ml of methanol. The mixture was allowed to react at room temperature for 30 minutes. Methanol served as the blank and DPPH in methanol, without the leaf samples, served as the positive control while butylated hydroxytoluene (BHT) served as reference. After 30 minutes of incubation, the discolouration of the purple colour was measured at 518nm in a spectrophotometer (Genesys 10-S, USA). The radical scavenging activity was calculated as follows:

$$\text{Scavenging activity \%} = 100 - \frac{\text{A518 (sample) - A518 (blank)}}{\text{A518 (blank)}} \times 100$$

MEASUREMENT OF NITRIC OXIDE SCAVENGING ACTIVITY

The extent of inhibition of nitric oxide radical generation in vitro was followed as per the method reported by Pulido *et al.*, (2000).

PRINCIPLE

Sodium nitroprusside in aqueous solution, at physiological pH, spontaneously generates nitric oxide, which interacts with oxygen to produce nitrite ions that are estimated spectrophotometrically at 546nm.

PROCEDURE

The reaction was initiated by adding 2.0ml of sodium nitroprusside, 0.5ml of PBS, 0.5ml of leaf samples (50mg) and incubated at 25^{o}C for 30 minutes. Griess reagent (0.5ml) was added and incubated for another 30 minutes. Control tubes were prepared without the samples. The absorbance was read at 546nm against the reagent blank, in a spectrophotometer (Genesys 10-S, USA).

Measurement Of Hydroxyl Radical Scavenging Activity

The extent of hydroxyl radical scavenging from Fenton reaction was quantified using 2'-deoxyribose oxidative degradation as described by Elizabeth and Rao (1990).

PRINCIPLE

The principle of the assay is the quantification of 2'-deoxyribose degradation product, malondialdehyde, by its condensation with thiobarbituric acid.

PROCEDURE

The reaction mixture contained 0.1ml of deoxyribose, 0.1ml of FeCl3, 0.1ml of EDTA, 0.1ml of H2O2, 0.1ml of ascorbate, 0.1ml of KH_2PO_4-KOH buffer and 20^{o}l of sample samples in a final volume of 1.0ml. The mixture was incubated at 37^{o}C for 1 hour. At the end of the incubation period, 1.0 ml of TBA was added and heated at 95^{o}C for 20 minutes to develop the colour. After cooling, the TBARS formation was measured spectrophotometrically (Genesys 10-S, USA) at 532nm against an appropriate blank. The hydroxyl radical scavenging activity was determined by comparing the -absorbance of the control with that of the samples. The per cent TBARS production for positive control (H_2O_2) was fixed at 100% and the relative per cent TBARS was calculated for the sample treated groups.

ESTIMATION OF TOTAL PHENOLS

The number of total phenols in the sample tissues was estimated by the method proposed by [13].

PRINCIPLE

Phenols react with phosphomolybdic acid in Folin-Ciocalteau reagent to produce a blue-coloured complex in alkaline medium, which can be estimated spectrophotometrically at 650nm.

PROCEDURE

The sample (0.5g) was homogenized in 10X volume of 80% ethanol. The homogenate was centrifuged at 10,000rpm for 20 minutes. The sample ion was repeated with 80% ethanol. The supernatants were pooled and evaporated to dryness. The residue was then dissolved in a known volume of distilled water. Different aliquots were pipette out and the volume in each tube was made up to 3.0ml with distilled water. Folin-Ciocalteau reagent (0.5ml) was added and the tubes were placed in a boiling water bath for exactly one minute. The tubes were cooled and the absorbance was read at 650nm in a spectrophotometer (Genesys 10-S, USA) against a reagent blank. Standard catechol solutions (0.2-1ml) corresponding to 2.0-10µg concentrations were also treated as above. The concentration of phenols is expressed as mg/g tissue.

ESTIMATION OF FLAVONOIDS

The method proposed by Cameron *et al.* (1943) was used to sample and estimate flavonoids.

PRINCIPLE

Flavonoids react with vanillin to produce a coloured product, which can be measured spectrophotometrically.

EXTRACTION OF FLAVONOIDS

*Corresponding Author: **Ubah, C.E**

The samples (0.5g) were first sampled with methanol: water mixture (2:1) and secondly with the same mixture in the ratio 1:1. The samples were shaken well and they were allowed to stand overnight. The supernatants were pooled and the volume was measured. This supernatant was concentrated and then used for the assay.

PROCEDURE

A known volume of the sample was pipetted out and evaporated to dryness. Vanillin reagent (4.0ml) was added and the tubes were heated in a boiling water bath for 15 minutes. Varying concentrations of the standard were also treated in the same manner.

The optical density was read in a spectrophotometer (Genesys 10-S, USA) at 340nm. A standard curve was constructed and the concentration of flavonoids in each sample was calculated. The values of flavonoids were expressed as mg/g sample.

RESULTS

ANTIOXIDANT ASSAY

Results of Nitric Oxide Scavenging activities

The graph below shows that there is high percentage scavenging activity sample gallic acid when compared with sample *P. guineense* and *X. aethiopica*. As the concentration increases, the graph shows that there is increase in the percentage scavenging activity of the gallic acid than the extracts. There was a decrease in percentage inhibition at point 20 in sample *P. guineense.*

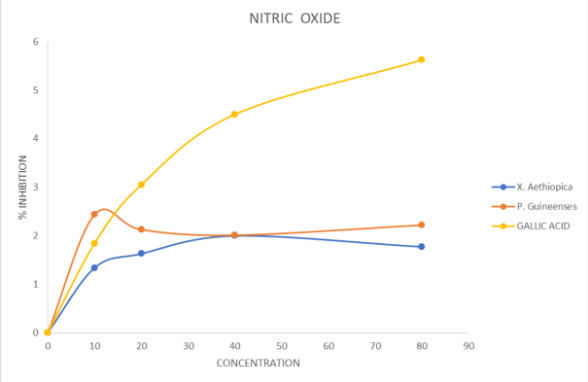


FIG 4.1 NITRIC OXIDE SCAVENGING ACTIVITY

RESULTS OF FRAP SCAVENGING ACTIVITY

The graph below shows that there is high percentage scavenging activity of gallic acid when compared with the Sample *X. aethiopica* and *P. guineense*. As the concentration increases, the graph shows that there is increase in the percentage scavenging activity of the gallic acid than the extracts.

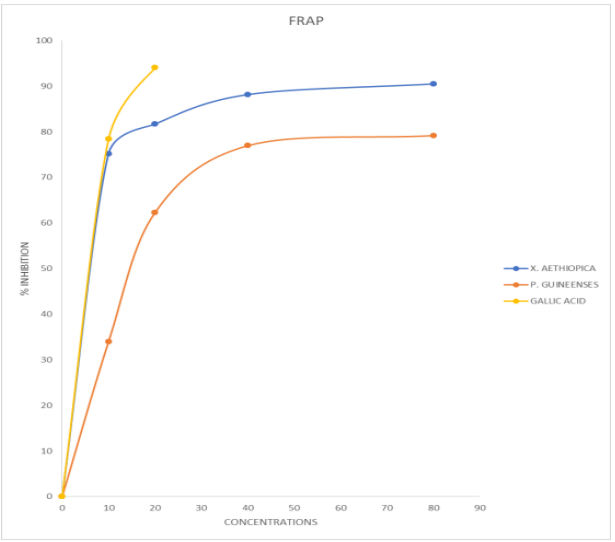
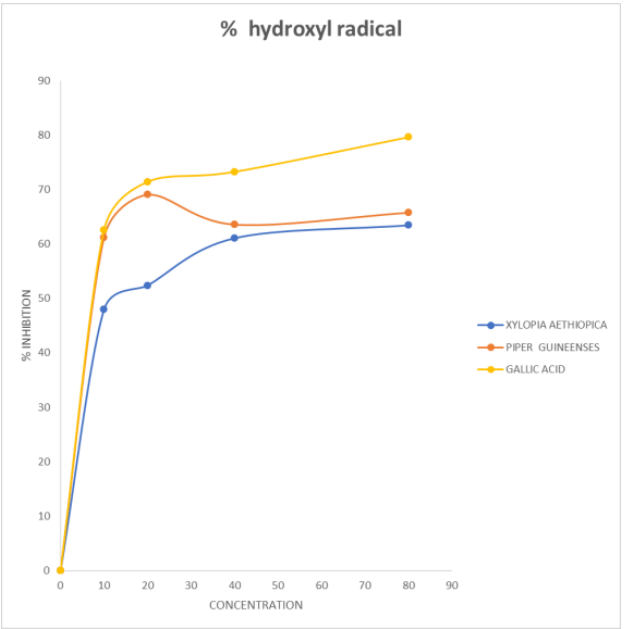


FIG 4.1.2 FRAP SCAVENGING ACTIVITY

Results of hydroxyl radical scavenging activity

The graph below shows that there is high percentage scavenging activity of gallic acid when compared with the Sample *P. guineenses* and *X. aethiopica*. As the concentration increases, the graph shows that there is increase in the percentage scavenging activity of the gallic acid than the extracts.

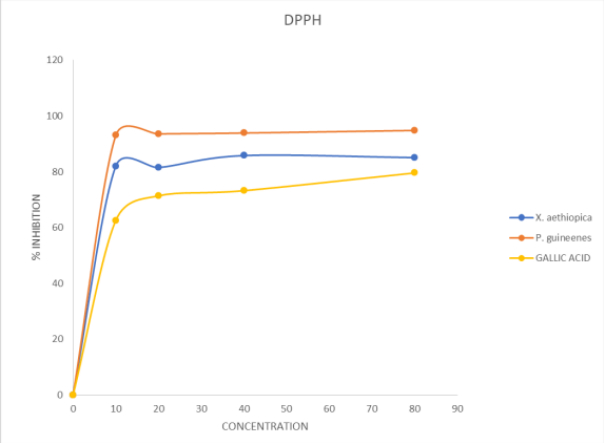


% HYDROXYL RADICAL SCAVENGING ACTIVITY

RESULTS OF DPPH SCAVENGING ACTIVITY

The graph below shows that there is high percentage scavenging activity of Sample *X. aethiopica* and *P. guineense* when compared with the Sample gallic acid. As the concentration increases, the graph shows that there is increase in the percentage scavenging activity of the extract than the standard.

*Corresponding Author: **Ubah, C.E**



% DPPH SCAVENGING ACTIVITY

Table 4.1 shows the mean, standard deviation and the significant difference of the sample compared with the gallic acid, the table shows that there is statistical difference at ($P<0.05$).

a=significant difference

b=Not significant different

TABLE 4.1 MEAN COMPOSITION OF THE SAMPLES

CONCENTRATIONS	NITRIC OXIDE	FRAP	HYDROXYL RADICAL	DPPH
10 *X. aethiopica*	1.78±0.12a	75.19±1.65a	47.94±1.04a	82.04±0.05a
20 *X. aethiopica*	2.17±0.17a	81.66±0.82a	52.37±0.84a	81.52±0.20a
40 *X. aethiopica*	2.66±0.30a	88.72±0.61a	61.04±0.67a	8a5.81±0.11a
80 *X. aethiopica*	2.35±0.14a	90.48±0.36a	63.42±0.57a	85.09±0.11a
10 *P. guineenses*	2.43±0.08a	33.95±0.45a	61.17±0.65a	93.05±0.14a
20 *P. guineenses*	2.12±0.09a	62.25±0.23a	69.03±0.60a	93.57±0.05a
40 *P. guineenses*	2.00±0.04a	76.93±1.22a	63.54±0.57a	93.89±0.00a
80 *P. guineenses*	2.20±0.06a	79.14±0.43a	65.73±0.67a	94.82±0.00a
10GALLIC ACID NO	1.84±0.14a	78.37±1.23a	62.56±0.99a	78.37±1.23a
20 gallic acid NO	3.05±0.12a	94.00±0.36a	71.39±0.64a	94.00±0.36a
40GALLIC ACID NO	4.50±0.12a	4.50±0.12a	73.23±0.71a	4.50±0.12a
80GALLIC ACID NO	5.62±0.13a	5.62±0.13a	79.59±0.18a	5.62±0.13a

TOTAL FLAVANOID

The concentration of total flavonoids present in the samples are shown in fig. 4.1.5, from the graph, *X. aethiopica* has the highest % inhibition and there was a decrease in the scavenging activity at concentration 20 and 40 in the extract percentage inhibition. *P. guineense* has the lowest scavenging activity

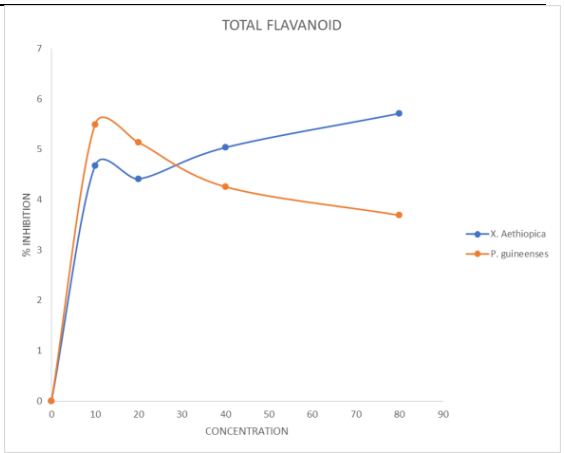


FIG. 4.1.5 TOTAL FLAVONOID

RESULTS OF TOTAL PHENOLIC COMPOUND

The concentration of total phenol present in the samples are shown in fig. 4.1.6, from the graph *X. aethiopica* has the highest % inhibition and there was a decrease in the

*Corresponding Author: **Ubah, C.E**

scavenging activity at concentration 20 and 40 in the extract. *P. guineense* has the lowest scavenging activity.

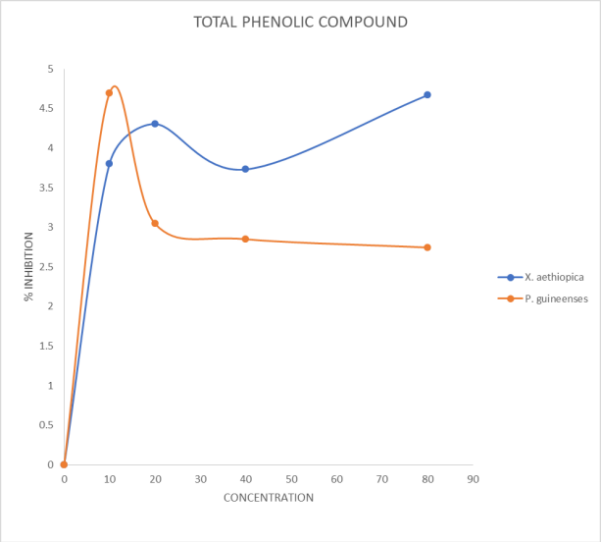


FIG 4.1.6 TOTAL PHENOLIC COMPOUND

RESULTS OF ANTIHAEMOLYTIC ASSAY

The percentage inhibition of the samples compared with the standard Aspirin are shown in fig. 4.1.7, The graph below shows that there is high percentage inhibition of the standard when compared with the Sample *X. aethiopica* and *P. guineense.* As the concentration increases, the result shows that there is increase in the percentage inhibition of the standard than the extracts.

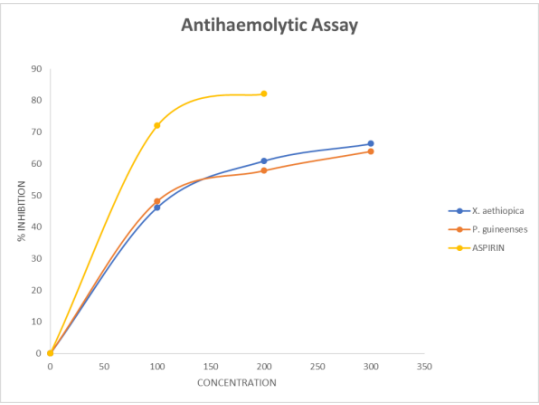


FIG 4.1.7 ANTIHAEMOLYTIC ASSAY

Discussion

The plants exhibit potent antioxidant properties due to their high content of vitamins A, C, E, and various phenolic compounds. Antioxidants are crucial for neutralizing free radicals and reducing oxidative stress in the body [14]. This activity helps in preventing cellular damage, which is a major factor in the development of chronic diseases such as cancer, cardiovascular diseases, and neurodegenerative disorders. A study by [15] demonstrated the high antioxidant activity of *Piper guineense* and *Xylopia aethiopica* attributing this effect to their significant content of phenolic compounds and flavonoids. The digestive health benefits of Uziza are well-documented in traditional medicine. The leaves and seeds of Uziza stimulate the secretion of digestive enzymes and gastric juices, enhancing the digestive process. This can help in preventing digestive disorders such as indigestion, bloating, and constipation. [16] noted that *Piper guineense* is commonly used in West African medicine to improve digestion and treat gastrointestinal disorders, citing its ability to stimulate digestive enzyme production. [17] studied the antioxidant activity of *P.guineense.* The result showed that the leaves of this plant exhibited free radical scavenging effects. This could be attributed to the presence of phenolic compounds in the plant which is a major group of compounds that act as primary antioxidants or free radical scavengers. In another study, the seed extracts of P.guineense was found to rapidly scavenge nitric oxide in vitro at different intervals [18]

Conclusion

The results of the present study on antioxidant analysis of *Piper guineense* and *Xylopia aethiopica* showed that the plant extract contained some phytoconstituents which are pharmacologically important. This plant part could represent potential source of lead molecules with pharmacological activities for the development of new novel pharmaceutical products for treatment of malaria and other diseases. Also, the presence of compounds with biological activities justifies the traditional use of the plants for the treatment of malaria and other diseases. This study revealed that the plants contain varied and considerable amounts of secondary metabolites

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