**Drug Discovery**

# Free radicals scavenging potency of ethanolic fruit extract of Selim pepper (*Xylopia aethiopica*)

**Peter Folorunsho Ayodele[1*], Adio Jamiu Akamo[2], Lukman Bolaji Bello[1], Samuel Tobi Farohunbi[1], Adekemi Titilayo Adesulu-Dahunsi[1], Oluseyi Adeboye Akinloye[2]**

**To Cite:**
Ayodele PF, Akamo AJ, Bello LB, Farohunbi ST, Adesulu-Dahunsi AT, Akinloye OA. Free Radicals scavenging potency of ethanolic fruit extract of Selim pepper (*Xylopia aethiopica*). *Drug Discovery* 2024; 18: e9dd1974
doi: https://doi.org/10.54905/disssi.v18i41.e9dd1974

**Author Affiliation:**
[1]Department of Biological Sciences, Faculty of Computing and Applied Sciences, Thomas Adewumi University, Oko, Kwara State, Nigeria
[2]Department of Biochemistry, College of Biosciences, Federal University of Agriculture, Abeokuta, Ogun State, Nigeria

**[*]Corresponding Author**
Department of Biological Sciences, Faculty of Computing and Applied Sciences, Thomas Adewumi University, Oko, Kwara State, Nigeria
Email: ayodele.peter@pg.funaab.edu.ng / peter.ayodele@tau.edu.ng
ORCID: 0000-0001-6659-9800

**Peer-Review History**
Received: 15 December 2023
Reviewed & Revised: 19/December/2023 to 15/February/2024
Accepted: 19 February 2024
Published: 23 February 2024

**Peer-Review Model**
External peer-review was done through double-blind method.

Drug Discovery
pISSN 2278–540X; eISSN 2278–5396



## ABSTRACT

*Introduction:* Free radicals (FRs) are unstable molecular species with unpaired electrons, capable of causing damage to cells and the human body, leading to illness and aging. The ability to scavenge the free radicals possesses a property referred to as anti-oxidant property. This study aimed to investigate the ability of Selim pepper (*Xylopia aethiopica*) fruit ethanolic extract (XAFEE) to scavenge some free radicals. *Materials and Methods:* A powdered sample of dried *Xylopia aethiopica* fruit was soaked in 95% ethanol (1: 10 w/v) for 48 hours on a shaker to ensure maximum mixture and extraction. Filtration of the extract with clean Whatmann No.1 paper followed. The filtrate was placed in a water bath to allow evaporation of the solvent, and the consequent concentration followed using a rotary evaporator. XAFEE-GC-MS and the *in-vitro* analyses against standard (vitamin C) on 2, 2-azinobis-3-ethylbenzothiazoline-6-sulphonic acid radical (ABTS•), 2, 2-diphenyl-1-picryl hydroxyl radical (DPPH•), nitric oxide (NO), hydroxyl radical (OH•), and hydrogen peroxide ($H_2O_2$) were carried out. *Results:* XAFEE-GC-MS analysis confirmed the presence of thirteen (13) bioactive compounds. The extract showed higher remarkable inhibitions of ABTS•, DPPH•, NO, OH•, and $H_2O_2$ than the standard vitamin C. *Conclusion:* The bioactive compounds of Selim pepper (*Xylopia aethiopica*) fruit and its subsequent free radicals-scavenging activities suggest, it is an ideal natural anti-oxidant agent and, thus can be explored in feed systems, animal feed formulation, and drug preparation.

**Keywords:** Chimba; Eeru; Ethiopian pepper; Free radicals; GC-MS analysis of *Xylopia aethiopica* Selim pepper; Uda

## 1. INTRODUCTION

Investigating the therapeutic potency of plants in recent scientific developments is of great importance (WHO, 2020). Plants are rich in organic molecules such as vitamins, minerals, and other biologically important molecules needed for healthy life. So far, only a few of these plant species have been utilized for medicinal purposes, and fewer have been investigated to validate their therapeutic uses and safety (Salmerón-Manzano *et al*., 2020). The name 'Xylopia' is coined from the Greek word '*xylon pikron*', which means 'a bitter wood' in English. The name *aethiopica* refers to its origin, 'Ethiopia'. The standard English names for *Xylopia* '*aethiopica*' are Selim pepper, Negro pepper, and Ethiopian pepper. In Nigeria, *Xylopia aethiopica* is known by the Yorubas, Igbos, and Hausa as '*eeru*', '*uda*', and '*chimba*', respectively (Akinloye *et al*., 2019).

Some reports showed the plant has played vital roles in indigenous medicine. Almost all parts of *X. aethiopica* are medicinally useful, but the fruits are most commonly used for therapeutic purposes (Ayodele *et al*., 2021; Oloyede and Aduramigba-Modupe, 2011). Extracts of the fruits in some regions are domesticated for treating bronchitis, inflammation, rheumatism, cough, dysentery, malaria, amenorrhea, and hyperlipidemia (Ayodele *et al*., 2021; Wood *et al*., 2012). The seeds are crushed and applied topically on the forehead to relieve headaches and neuralgia. It can also be used as a cuisine, decoction, concoction, or chewed and swallowed to relieve aches and pains (Igwe *et al*., 2003). The abilities of various extracts of *X. aethiopica* at different concentrations for managing sickle cell disease have also been established. Several studies have reported that *X. aethiopica* fruit possesses anti-inflammatory, antimicrobial, antifungal, antitubular, antitumor, and antimutagenic properties (Fetse *et al*., 2016; Wood *et al*., 2012). Thus, this study investigated the bioactive compounds of the ethanol extract of *X. aethiopica* fruit and its *in-vitro* scavenging abilities on some free radicals.

## 2. MATERIALS AND METHODS

**Collection and identification of the fruit**

The dried fruit of the plant (*X. aethiopica*) was procured from an herb-selling shop in the East Bank of Ogun state, Nigeria. The plant was botanically identified and authenticated in the Department of Botany, Federal University of Agriculture, Abeokuta, Ogun State, Nigeria. A voucher specimen with reference 'FUNAAB-0061' was deposited at the herbarium for reference purposes.

**Extraction of plant material**

The fruit was washed with clean tap water and air-dried, and then it was crushed into powder using a dry and clean mortar. The powdered sample was soaked in 95% ethanol for 48 hours on a shaker to ensure maximum extraction. The extract was then filtered using clean Whatmann No.1 filter paper. The filtrate concentration was achieved using a rotary evaporator.

**Determination of *in-vitro* anti-oxidant (free radicals scavenging) activities of XAFEE**

***{2, 2 – azinobis-(3-ethylbenzothiazoline-6-sulphonic acid)} (ABTS•) assay***

This assay is based on the principle that when 2, 2''-azinobis-(3- ethylbenzothiazoline-6-sulphonic acid) (ABTS) is incubated with a peroxidase, such as metmyoglobin and $H_2O_2$, a relatively stable radical cation, ABTS•, is formed. The formation of ABTS• on interaction with ferryl myoglobin produces a relatively stable blue-green color, measurable at 600 nm. The color-producing suppressive activities of the anti-oxidants in samples are proportional to the concentration of the samples (Miller *et al*., 1993).

***{1, 1 diphenyl-2-picry hydrazyl} (DPPH•) assay***

The method is based on reducing a methanolic solution of colored free radical DPPH by a free radical scavenger. The procedure involves measuring the decrease in DPPH absorbance at its absorption maxima of 516 nm, which depends on the concentration of free radical scavenger added to the DPPH reagent solution. A solution of 3.0 ml of 100 mM DPPH solution in methanol was gently added to 0.5 ml of plant extract (at various concentrations) in methanol. After 10 minutes, the absorbance was compared with a control of methanol. The concentration required to inhibit or change the absorbance by 50% ($IC_{50}$) was calculated as an absorbance change of 0.4. This 0.4 change was determined from a standard $IC_{50}$ generated from the scavenging action of vitamin C at 29 mM following the method of Ursini *et al*. (1994). The percentage of inhibition was calculated using the formula:

Inhibition (%) = ($A_0 – A_1 / A_0$) × 100. Where $A_0$ is the absorbance of the control, and $A_1$ is the absorbance of the test.

***Nitric oxide radical (NO) scavenging assay***

The nitric oxide produced from sodium nitroprusside in an aqueous solution at physiological pH reacts with oxygen to produce nitrite ions using the Griess reaction reagent described by Green *et al*. (1982). Consequently, 3.0 ml of 10 mM sodium nitroprusside in phosphate buffer (10 mM, pH 7.4) was added to 2.0 ml of extract (tested sample), methanol (blank), and vitamin C (standard) in different concentrations (20 -100 μg/ml). The resulting solutions were then incubated at $25^0$C for 60 mins.

***Hydroxyl radical (HO•) scavenging assay***

The HO• scavenging ability of XAFEE was measured by the method of Kunchandy and Rao (1990). The percentage of hydroxyl radical (HO•) scavenging activity of XAFEE was calculated as follows:

% Scavenged (HO•) = ($A_0$ – $A_1$ / $A_0$) × 100. Where $A_0$ is the absorbance of the control, and $A_1$ is the absorbance of the test. Vitamin C was used as standard.

***Hydrogen peroxide radical ($H_2O_2$) scavenging assay***

The ability of plant extracts to scavenge hydrogen peroxide was determined according to the method of Ruch *et al*. (1989). The percentage of hydrogen peroxide scavenging activity of XAFEE was calculated as follows:

% Scavenged ($H_2O_2$) = ($A_0$ – $A_1$ / $A_0$) × 100. Where $A_0$ is the absorbance of the control, and $A_1$ is the absorbance of the test. Vitamin C was used as standard.

**Chromatographic analysis of X. aethiopica fruit ethanol extract**

Gas chromatography-mass spectrometry of XAFEE was performed at the Federal University of Technology, in the capital of Ondo state, with an Agilent 19091S-933HP-1MS instrument, with capillary column $35^0$C: 30 m x 250 μm x 0.25 μm. Helium gas (99.99%) was used as the mobile phase at a flow rate of 1.0 ml/min, 1 μL of the XAFEE with a split ratio of 10:1 sample size was injected using the splitless injection technique, and a fused capillary silica column (30 X 0.25m). The GC analysis came to completion in 18.33 mins. Compounds were identified with Wiley-9 combined with the NIST-11 mass spectral database.

**Data analysis**

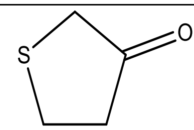
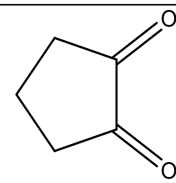
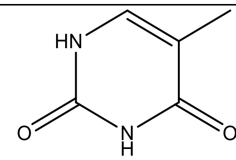
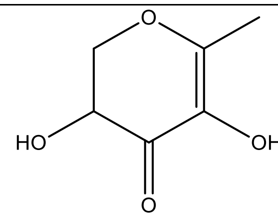
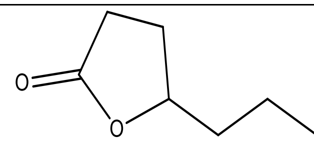
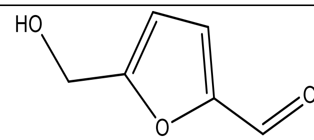
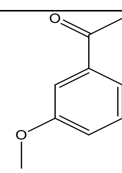
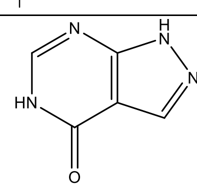
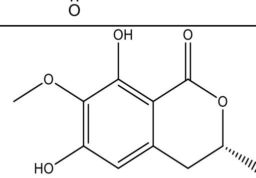
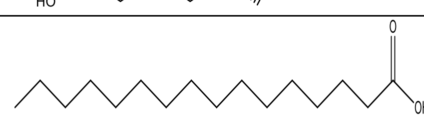
Graph prism (V: 20), using one-way analysis of variance together with post hoc Duncan's multiple range tests was used as a statistical package to analyze data. A statistically significant association between variables was said to exist if the p-value $< 0.05$.

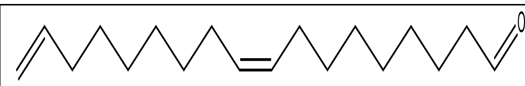
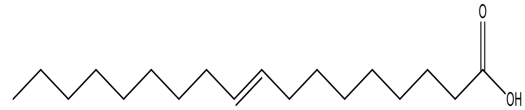
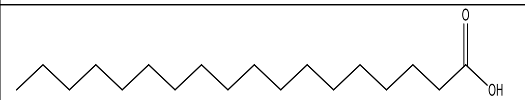
## 3. RESULTS AND DISCUSSION

According to Aliyu *et al*. (2022); Fetse *et al*. (2016), studies on the *X. aethiopica* plant have dwelt much on the taxonomy, ethnobotany, and pharmacology, but a handful of information on the bioactive compounds present in the *X. aethiopica* fruit ethanol extract is generally lacking. Thus, this study provides information on the bioactive composition and the *in-vitro* antioxidant potentials of the ethanol extract of *X. aethiopica* fruit.

Table 1 and Figure 1 show the bioactive compounds and the chromatogram of XAFEE, generated by GC-MS as the detector received signals from the detected compounds in XAFEE. From our study, there were thirteen major bioactive compounds present in the XAFEE, with allopurinol having the most significant area percentage of 35.53%; retention time (RT) of 8.49 mins, followed by 9-octadecenoic acid with an area percentage of 18.28%; RT of 13.58 mins, and 9,17-octadecadienal with the smallest area percentage of -0.18%; RT of 13.43 mins. The XAFEE showed a significant ($p < 0.05$) increase in inhibition concentration on ABTS•, DPPH•, NO, OH•, and $H_2O_2$ compared to vitamin C. The results are in line with the studies of Moukette *et al*. (2015); Adefegha and Oboh (2012). Figures 2, 3, 4, 5, and 6 show the *in-vitro* antioxidant activities of XAFEE, which were examined by exploring the scavenging activities of the stable 2,2′-azinobis(3-ethylbenzthiazoline)-6-sulfonic acid (ABTS), 1, 1- diphenyl-2-picrylhydrazyl (DPPH), nitric oxide (NO), hydroxyl (OH) and hydrogen peroxide ($H_2O_2$) radicals with a standard (vitamin C).

**Table 1** Bioactive compounds of *X. aethiopica* fruit ethanol extract assessed by GC-MS

| Peak | RT (min.) | Area % | Bioactive compounds | Structures |
|---|---|---|---|---|
| 1 | 2.64 | 2.81 | Dihydro-3-(2H)-thiophenone |  |
| 2 | 3.07 | 5.13 | 1,2-Cyclopentanedione |  |
| 3 | 3.07 | 5.13 | Thymine |  |
| 4 | 4.78 | 2.50 | 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-Pyran-4-one |  |
| 5 | 4.78 | 2.50 | 2(3H)-Furanone, dihydro-5-propyl- |  |
| 6 | 5.60 | 6.05 | 5-Hydroxymethylfurfural |  |
| 7 | 6.842 | 4.93 | 3-Methoxyacetophenone |  |
| 8 | 8.49 | 35.53 | Allopurinol |  |
| 9 | 12.58 | 1.38 | 3,4-dihydro-6,8-dihydroxy-7-methoxy-3-methyl-(R1H-2-Benzopyran-1-one |  |
| 10 | 12.90 | 8.56 | n-Hexadecanoic acid |  |

| | | | | |
|---|---|---|---|---|
| 11 | 13.43 | -0.18 | 9,17-Octadecadienal |  |
| 12 | 13.58 | 18.28 | 9-Octadecenoic acid |  |
| 13 | 13.66 | 5.59 | Octadecanoic acid |  |

Quantitative values in 2 decimal places; RT (Retention time)

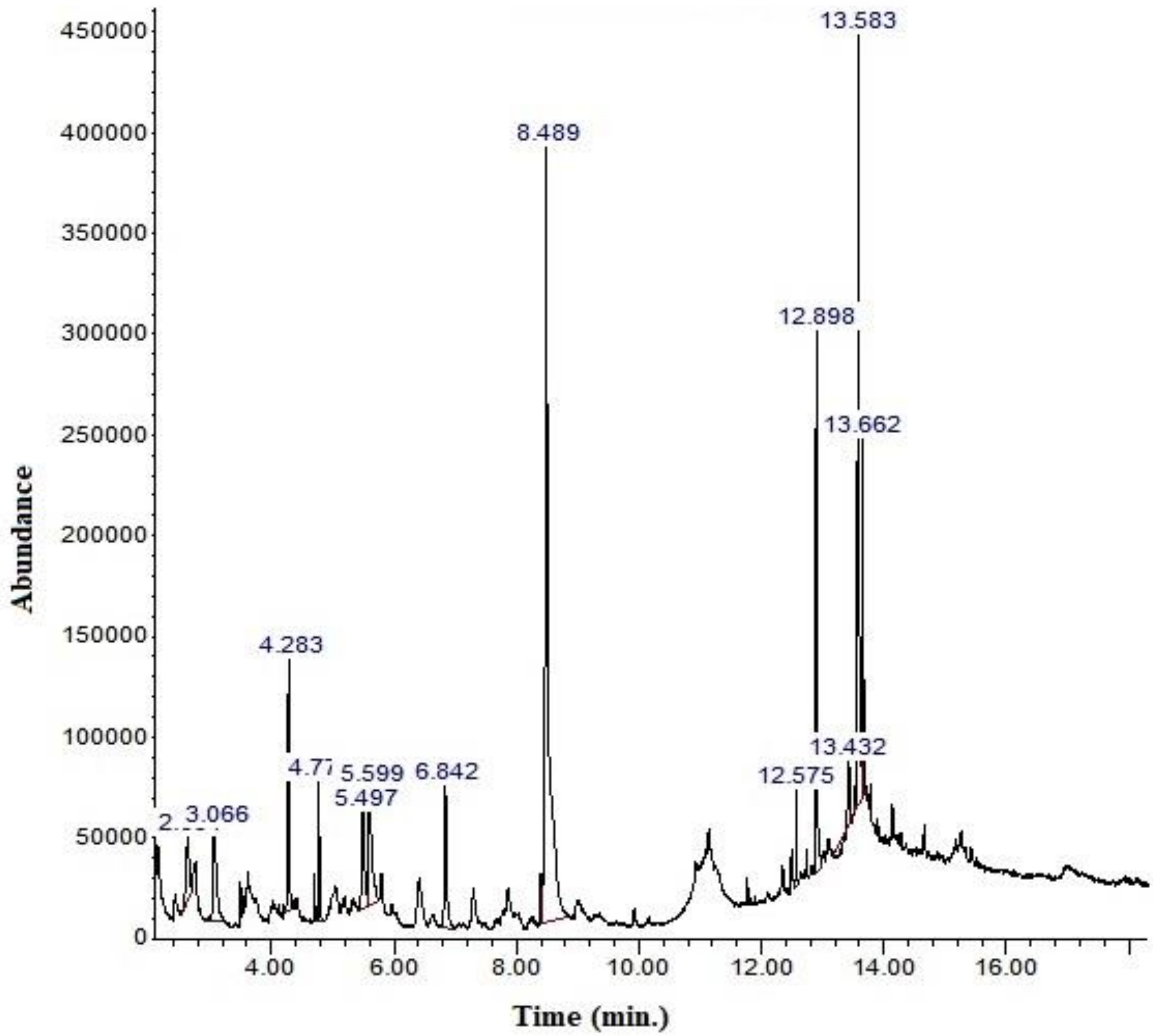



**Figure 1** GC-MS chromatogram of *X. aethiopica* fruit ethanol extract

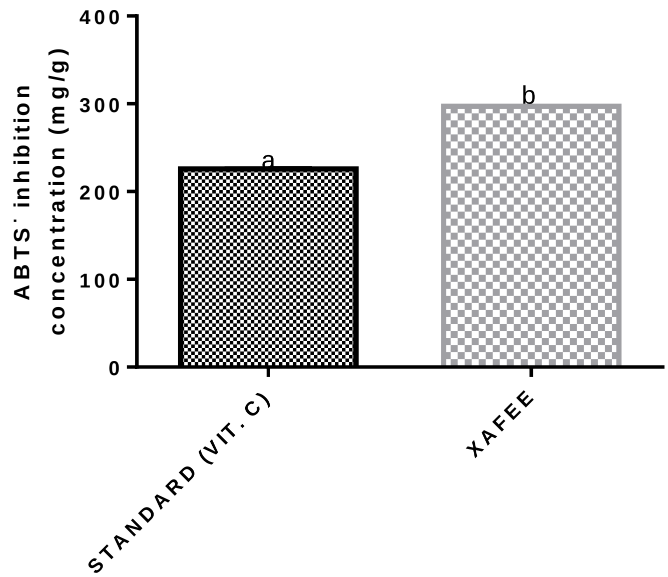



**Figure 2** Ability of *X. aethiopica* fruit extract to scavenge ABTS•

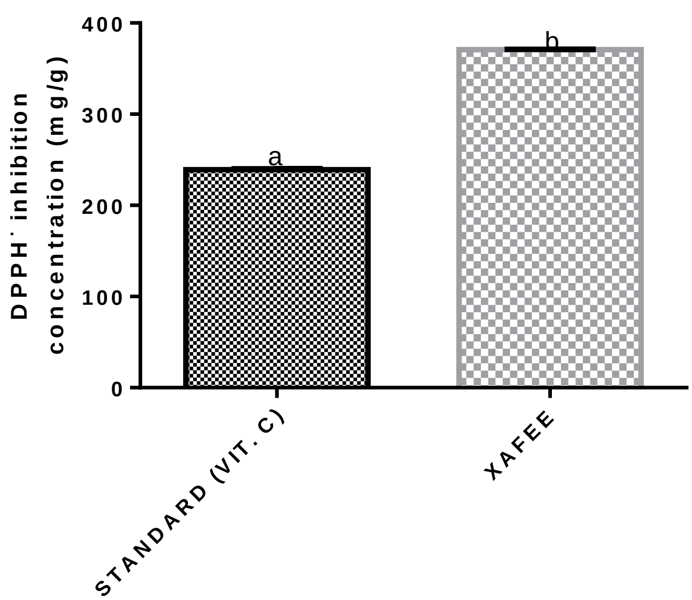



**Figure 3** Ability of *X. aethiopica* fruit extract to scavenge DPPH•

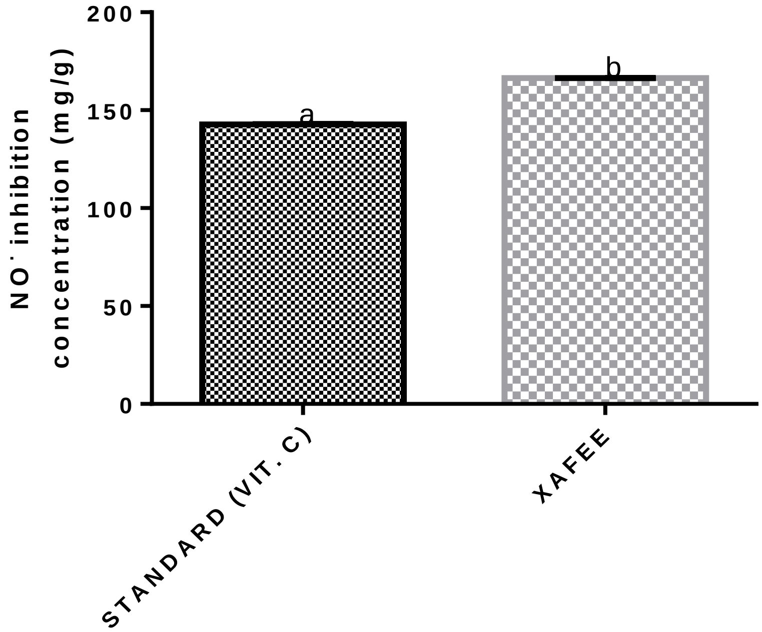



**Figure 4** Ability of *X. aethiopica* fruit extract to scavenge NO•

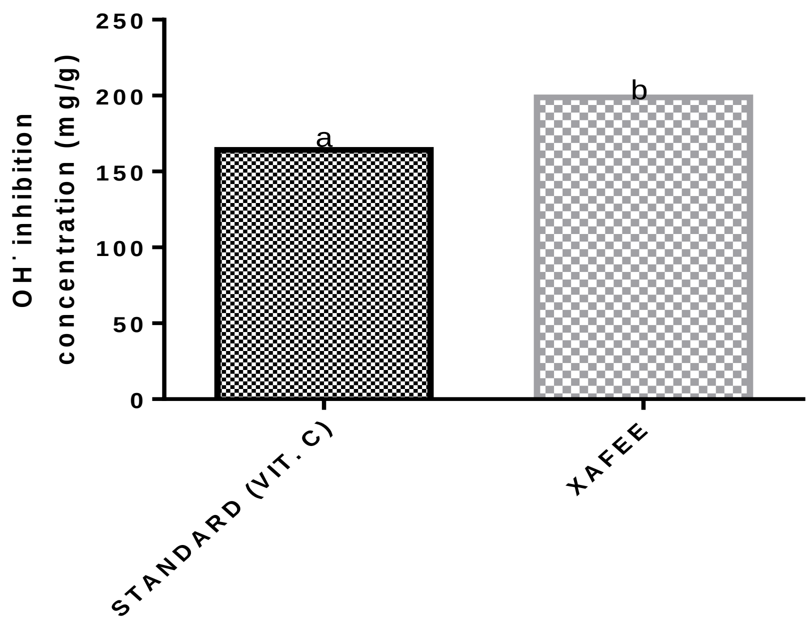



**Figure 5** Ability of *X. aethiopica* fruit extract to scavenge OH•

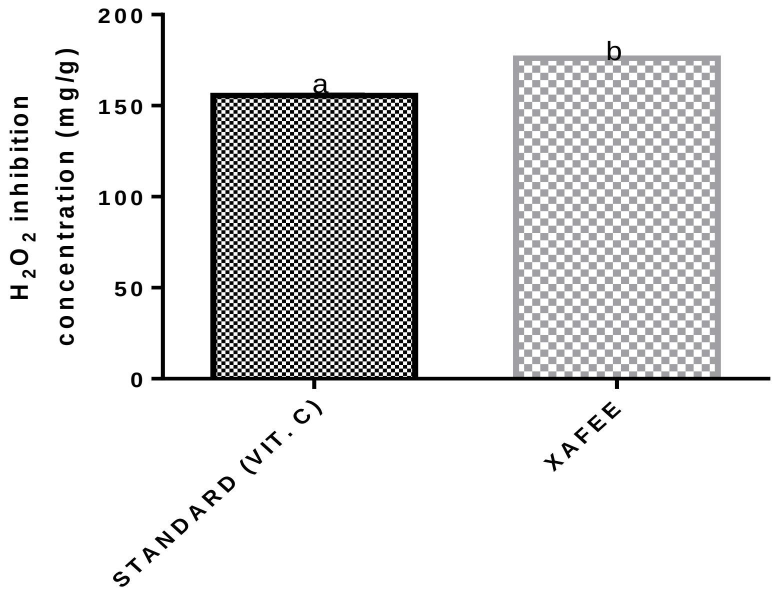



**Figure 6** Ability of *X. aethiopica* fruit extract to scavenge $H_2O_2$ radical

The XAFEE showed a significant increase ($p < 0.05$) in inhibition concentration ($IC_{50}$) compared to the vitamin C. Anti-oxidants may prevent and, or improve different diseased states by neutralizing free radicals or their actions. The *in-vitro* anti-oxidant activities of XAFEE against ABTS•, DPPH•, OH•, NO, and $H_2O_2$ radicals observed in this study could be attributed to its ability to trap FRs by donating hydrogen atoms or electrons. This agrees with the report of Kaviarasan *et al*. (2007), who observed that *X. aethiopica* extracts displayed a higher percentage inhibition on some of these FRs. It also correlates quite very well with the study reported by Moukette *et al*. (2015), who observed that vitamin C showed a significant decrease ($p < 0.05$) in $IC_{50}$ on nitric NO, OH•, DPPH•, and ABTS• as compared to *X. aethiopica* extracts.

This extract showed suitable free radical scavenging activity, indicating some of the bioactive compounds in XAFEE serve as good electron donors, and possess the ability to terminate radical chain reactions by converting free radicals and reactive oxygen species to more stable products. These findings are perfectly related to the reports of Aliyu *et al*. (2022); Moukette *et al*. (2015); Adefegha and Oboh (2012). Nevertheless, some of the bioactive compounds revealed in the XAFEE have demonstrated anti-inflammatory, modulation of cell membrane structure, and regulation of blood uric level, as reported in the studies of Ayodele *et al*. (2021), Fetse *et al*. (2016); Wood *et al*. (2012), and this might be undoubtedly responsible for the ability of XAFEE to scavenge ABTS•, DPPH•, OH•, NO, and $H_2O_2$ radicals (Figure 2-6), showing a higher percentage inhibition on the FRs compared with the standard vitamin C.

## 4. CONCLUSION

Selim pepper (*X. aethiopica*) contains bioactive compounds with good anti-oxidants and protective potentials against oxidative damage caused by FRs. The free radicals-scavenging activities of the *Xylopia aethiopica* fruit suggest, it is an ideal anti-oxidant agent. Thus, it can be explored as a natural source of antioxidants in food systems, animal feed preparation, and drug formulations.

**Acknowledgments**

The authors appreciate all the institutional members for their kind support.

**Authors contributions**

Peter Florunsho Ayodele: Conceived the idea, collected the data, performed the experiment, and drafted the manuscript.

Adio Jamiu Akamo: Supervised the research work.

Lukman Bolaji Bello, Samuel Tobi Farohunbi, Adekemi Titilayo Adesulu-Dahunsi: Reviewed and edited the manuscript.

Oluseyi Adeboye Akinloye: Supervised the research work

**Ethical approval**

The dried fruit of the Selim pepper (*X. aethiopica*) was procured from an herb-selling shop in the East Bank of Ogun state, Nigeria. The ethical guidelines for plants and plant materials were followed in the study for sample collection and identification (Ethical approval code: FUNAAB-0061).

**Informed consent**

Not applicable

**Conflicts of interests**

The authors declare that there are no conflicts of interests.

**Funding**

The study has not received any external funding.

**Data and materials availability**

All data associated with this study are present in the paper.

## REFERENCES

1. Adefegha S, Oboh G. Effect of diets supplemented with Ethiopian pepper [*Xylopia aethiopica* (Dun.) A. Rich (*Annonaceae*)] and Ashanti pepper [*Piper guineense Schumach.* Thonn (Piperaceae)] on some biochemical parameters in normal rats. Asian Pac J Trop Biomed 2012; 2(2):S558–S566. doi: 10.1016/S2221-1691(12)60274-3
2. Akinloye OA, Ayodele PF, Ayobami OA. Median Lethality Dose of Xylopia aethiopica Fruit Ethanol Extract. J Anal Tech Res 2019; 1:032-035.
3. Aliyu M, Akande KE, Egwim EC. Free radicals scavenging activity of selim pod (*Xylopia aethiopica*). Proceedings of the 2nd International Conference of Agriculture and Agricultural Technolgy (ICAAT) 2022; 298-302.
4. Ayodele PF, Akinloye DI, Akamo AJ, Agboola DA, Akinloye OA. Effects of *Xylopia aethiopica* fruit ethanol extract on cadmium-induced inflammatory changes and dyslipidemic rats. Asian J Res Biochem 2021; 9(4):9-16a.
5. Fetse JP, William K, Reimmel KA. Ethnopharmacological importance of *Xylopia aethiopica* (DUNAL) A. RICH (Annonacea) - A Review. Br J Pharm Res 2016; 11(1):1-21. doi: 10.9734/BJPR/2016/24746
6. Green LC, Wagner DA, Glogowski J, Skipper PL, Wishnok JS, Tannenbaum SR. Analysis of nitrate, nitrite, and [15N] nitrate in biological fluids. Anal Biochem 1982; 126(1):131-8. doi: 10.1016/0003-2697(82)90118-x
7. Igwe SA, Afonne JC, Ghasi SI. Ocular dynamics of systemic aqueous extracts of *Xylopia aethiopica* (African guinea pepper) seeds on visually active volunteers. J Ethnopharmacol 2003; 86(2-3):139-42. doi: 10.1016/s0378-8741(02)00371-9
8. Kaviarasan S, Naik GH, Gangabhagirathi R, Anuradha CV, Priyadarsini KI. *In-vitro* studies on antiradical and antioxidant activities of fenugreek (*Trigonella foenum graecum*) seeds. Food Chem 2007; 103(1):31-37. doi: 10.1016/j.foodchem.2006.05.064
9. Kunchandy E, Rao MNA. Oxygen radical scavenging activity of curcumin. Int J Pharm 1990; 58:237–240. doi: 10.1016/0378-5173(90)90201-E
10. Miller NJ, Rice EC, Davies MJ, Gopinathan V, Milner A. A novel method for measuring antioxidant capacity and its application to monitoring the antioxidant status in premature neonates. Clin Sci (Lond) 1993; 84(4):407-12. doi: 10.1042/cs0840407
11. Moukette BM, Pieme CA, Nya-Biapa PC, Ngogang JY. *In-vitro* antioxidant and anti-lipoperoxidation activities of bark

extracts of *Xylopia aethiopica* against ion mediated toxicity on liver homogenates. J Complement Integr Med 2015; 12(3):195-204. doi: 10.1515/jcim-2015-0002

12. Oloyede AM, Aduramigba-Modupe AO. Antimicrobial activities of crude ethanolic extract of *Xylopia aethiopica*. Int J Curr Res 2011; 3(9):005-007.
13. Ruch RJ, Cheng SJ, Klaunig JE. Prevention of cytotoxicity and inhibition of intercellular communication by antioxidant catechins isolated from Chinese green tea. Carcinogenesis 1989; 10(6):1003-8. doi: 10.1093/carcin/10.6.1003
14. Salmerón-Manzano E, Garrido-Cardenas JA, Manzano-Agugliaro F. Worldwide research trends on medicinal plants. Int J Environ Res Public Health 2020; 17(10):3376. doi: 10.3390/ijerph17103376
15. Ursini F, Maiorino M, Morazzoni P, Roveri A, Pifferi G. A novel antioxidant flavonoid (IdB 1031) affecting molecular mechanisms of cellular activation. Free Radic Biol Med 1994; 16(5):547-53. doi: 10.1016/0891-5849(94)90054-x
16. Wood E, Ameyaw EO, Boakye-Gyasi E, Abotsi WK. Analgesic effects of an ethanol extract of the fruits of *Xylopia aethiopica* (Dunal) A. Rich (Annonacea) and the major constituents, xylopic acid murine models. J Pharm Bioallied Sci 2012; 4(4):291-301. doi: 10.4103/0975-7406.103251
17. World Health Organization (WHO). General guidelines for methodologies on research and evaluation of traditional medicine 2020.