Trop J Nat Prod Res, February 2024; 8(2):6451-6456 **ISSN 2616-0684 (Print)**
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

In Vitro Antioxidant and Antidiabetic Potentials of the Seed, Bark and Whole Pod of Okra (*Abelmoschus esculentus* (L.) Moench): A Comparative Study

Olorunfemi R. Molehin[1,*], Oluwakemi V. Adeleke[2], Stephen A. Adefegha[3], Adeyemi O. Adeola[4], Adeniyi S. Ohunayo[5]

[1]*Department of Biochemistry, Faculty of Science, Ekiti State University, Ado-Ekiti, P.M.B. 5363 Ado-Ekiti, 360001, Nigeria.*
[2]*Biochemistry Unit, Department of Science Laboratory Technology, Faculty of Science, Ekiti State University, Ado-Ekiti, P.M.B. 5363 Ado-Ekiti, 360001, Nigeria*
[3]*Functional and Nutraceutical Unit, Department of Biochemistry, Federal University of Technology Akure, P.M.B. 704 Akure, 340001, Nigeria*
[4]*School of Nursing, Jos, Plateau State College of Nursing Sciences, Vom, P.M.B. 07, Vom, Nigeria*
[5]*Biotechnology Unit, Department of Science Laboratory Technology, Faculty of Science, Ekiti State University, Ado-Ekiti, P.M.B. 5363 Ado-Ekiti, 360001, Nigeria*

ARTICLE INFO

Article history:
Received 10 November 2023
Revised 27 January 2024
Accepted 02 February 2024
Published online 01 March 2024

ABSTRACT

Okra is an indigenous vegetable consumed for its sliminess and nutritional benefits. The aim of this study was to assess and compare the *in vitro* antioxidant, and antidiabetic activities of the seed, bark and whole pod of okra. The total phenolic and total flavonoid contents of the different parts were evaluated according to standard methods. The antioxidant capacity was assessed using the 1,1-diphenyl-2-picrylhdrazyl (DPPH), hydroxyl (OH), and 2,2-azino-bis (3-ethylbenzthiazoline-6-sulphoric acid) (ABTS) radical scavenging assays, ferric reducing antioxidant power (FRAP) assay, and ferrous ion-induced lipid peroxidation assay using standard procedures. The *in vitro* antidiabetic activity was evaluated using the α-amylase, and α-glucosidase inhibitory assays. The whole okra pod exhibited a significantly higher total phenolic content (5.0 mg GAE/g) and enhanced radical scavenging activity compared to both the seed and bark of the pod ($p < 0.05$). Although, the seed had a higher content of total flavonoid (2.23 mg QE/g) than the bark and whole pod, the bark and whole pod of okra showed a higher ferric reducing antioxidant power than the seed. Similarly, the whole pod showed a higher lipid peroxidation inhibition, and higher α-glucosidase inhibitory activity than the bark and seed of the pod. In order to enjoy all the nutritional and pharmacological benefits associated with okra consumption, it is recommended that no part of the pod should be considered a waste.

Keywords: Anti-hyperglycemic, Antioxidant, Enzyme inhibition, Nutraceuticals

Introduction

Consumption of certain foods from plants especially those in the vegetable and fruit category produce certain therapeutic effect and their intake serve a lot of health benefits.[1] These food sources are classified as functional foods because they confer health-promoting benefits beyond the basic diets to the consumers.[2] Okra (*Abelmoschus esculentus* L.) forms part of the regularly consumed indigenous vegetables in Nigeria, especially in the southwestern part. These vegetables are highly valued for their nutritional, antioxidant, and pharmacological benefits and the bioactive compounds they possess.[3-5] Okra is consumed in the tropical and temperate regions of the world, including the African continent, Asia, Southern Europe, and the North American continent; this contributes to an estimated trade value of more than $5 billion.[6,7] It is a seeded pod with all parts of the pod edible and indigenously prepared in various homes in Nigeria as soup and used in diabetes management.

The seeds serve as a viable alternative to coffee upon roasting and grinding,[6] while also proving highly valuable for producing cottonseed oil.[8] The seed exhibits a notable abundance of phenolic compounds, positioning it as a prospective source of antioxidants and nutraceuticals.[9,10] The oligomeric catechins and other flavonoid derivatives confer antioxidant potentials on okra and are thus essential for healthy living, disease management, and prevention.[11]

In the past, okra was consumed for its delicious and appetizing taste when prepared as soup but in recent years, a huge focus has been placed on it therapeutic potentials. Different parts of this vegetable can serve enormous benefits as functional food, providing more than nutrients but therapeutic value. Owing to the bioactive compounds they possess and their associated bioactivities, they have found potential applications in the treatment and management of diseases.[12] They have been shown in several studies to possess pharmacological activities such as antidiabetic,[13,14] antioxidant,[15] anticancer,[16] and immunomodulatory activities.[17] The presence of these broad-spectrum activities in functional food initiates a shift towards the industrialization of natural products especially in the pharmaceutical industry. The antioxidant potential and nutritional benefits of the pod of okra have been elucidated and properly documented in some literatures.[18-20] It becomes imperative to check if these benefits ensued from a synergistic effect between the components within the pod. The present study sought to undertake a comparative study of the in vitro antioxidant, and antidiabetic activities of whole okra pod, its bark, and seeds with a focus on radical scavenging, ferric reducing antioxidant power, lipid peroxidation, and possible enzyme (α-amylase, and α-glucosidase) inhibitory activities.

*Corresponding author. E mail: olorunfemi.molehin@eksu.edu.ng
Tel: +2348034621267

Citation: Molehin OR, Adeleke OV, Adefegha SA, Adeola AO, Ohunayo AS. *In Vitro* Antioxidant and Antidiabetic Potentials of the Seed, Bark and Whole Pod of Okra (*Abelmoschus esculentus* (L.) Moench): A Comparative Stud. Trop J Nat Prod Res. 2024; 8(2):6450-6455. http://www.doi.org/10.26538/tjnpr/v8i2.38

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria.

Materials and Methods

Collection of plant materials

Fresh okra (*Abelmoschus esculentus* (L.) Moench) pods were purchased in July, 2023 from the well-known King's market located at Ado Ekiti, Ekiti State, Nigeria. The okra pods were authenticated at the herbarium of the Ekiti State University, Nigeria and a voucher number UHAE 2023078 was issued. The okra pods were cleaned, after which they were separated into bark and seeds components, resulting in three distinct samples (whole pod, bark, and seeds). These samples were air-dried while shielded from direct sunlight and subsequently ground into fine powder.

Preparation of extracts

Ten grams (10 g) each of powdered pod, bark, and seed samples were macerated separately in one hundred milliliters (100 mL) of distilled water and shaken in an orbital shaker for 24 h. The mixture was filtered using Whatman grade 1 filter paper, and then centrifuged at 805 × g for 10 min. A translucent supernatant solution was obtained. The supernatant was freeze-dried and preserved at 4°C until subsequent utilization. Prior to subsequent use for the experiments, the samples were reconstituted in water.[21]

In vitro evaluation of antioxidant activity

Assessment of total phenolic content

The total phenolic content was quantified using the method described by Singleton *et al.*[22] Gallic acid was used as the standard, and used to prepare the calibration curve. Total phenolic content was estimated from the equation of the standard curve and was expressed as milligram gallic acid equivalent per gram of the extract (mg GAE/g extract).

Assessment of total flavonoid content

The total flavonoid content was determined using the method described by Meda *et al.*[23] Quercetin was used as the reference standard for the preparation of the calibration curve, and the total flavonoid content was expressed as milligram quercetin equivalent per gram extract (mg QE/g extract). The determination of non-flavonoid polyphenols was done by subtracting the total flavonoid content from the total phenolic content.

DPPH free radical scavenging assay

The assessment of the scavenging potential of the extracts against the 1,1-diphenyl-2-picrylhdrazyl (DPPH) radical was done according to the method previously described by Gyamfi *et al.*[24] The results were presented as percentage radical scavenging activity relative to the control.

ABTS radical scavenging assay

The procedure described by Re *et al.*[25] was used to assess the scavenging activity of the extracts against the 2,2-azino-bis (3-ethylbenzthiazoline-6-sulphoric acid) (ABTS) radical. Trolox was used as the comparative reference standard, and the ABTS radical scavenging activity was expressed as trolox equivalent antioxidant power.

Ferric reducing antioxidant power (FRAP) assay

The FRAP assay which is based on the ability of the extracts to reduce ferric chloride ($FeCl_3$) solution was done following the method described by Pulido *et al.*[26] The absorbance measurements were done spectrophotometrically at 700 nm against a reagent blank, while employing ascorbic acid as the reference standard.

Fenton reaction (OH radical scavenging ability) assay

The capacity of the extracts to impede Fe^{2+}/H_2O_2-induced degradation of deoxyribose was evaluated according to the procedure outlined by Halliwell and Gutteridge.[27] The measurement of absorbance was performed at 532 nm, followed by the calculation of the percentage (%) hydroxyl (OH) radical scavenging activity.

Lipid Peroxidation Inhibition Assay

Five male albino rats of the Wistar strain weighing between 200 and 250 grams were sacrificed by cervical dislocation. The pancreas were quickly harvested, cooled on ice and weighed. Subsequent to its preparation in chilled saline solution (0.9% NaCl), the tissue underwent homogenization utilizing a Teflon glass homogenizer with approximately 10 up-and-down strokes at a speed of about 1200 revolutions per minute. After centrifugation at 4000 × g for 10 min, the resulting supernatant was collected for lipid peroxidation assessment, while the pellet was discarded. The lipid peroxidation assay was conducted following the modified procedure of Ohkawa *et al.*[28] Thiobarbituric acid reactive species (TBARS) production was quantified at 532 nm. This allowed for the calculation of the percentage of lipid peroxidation inhibition.

Assessment of in vitro antidiabetic activity

α-Amylase Inhibition assay

The ability of the extracts to inhibit α-amylase was evaluated using the dinitrosalicylic acid technique according to Worthington Biochemical Corporation's instructions.[29]

α-Glucosidase Inhibition assay

Five male albino rats (Wistar strain) weighing between 200 – 250 g were humanely euthanized via cervical dislocation. The intestine was swiftly harvested, chilled and their weights determined. The tissue was homogenized in 0.1M phosphate buffer (pH 7.4) and then centrifuged at 10,000 × g for 10 min. The resulting supernatant was used for the α-glucosidase inhibition assay. The measurement of absorbance was done at 400 nm,[30] and the results were expressed as a percentage inhibition of *α*-glucosidase.

Statistical analysis

Data were presented as the mean ± standard deviation. The program GraphPad Prism 6.0 was used to analyze the data. IC_{50} values were determined from non-linear regression analysis.

Results and Discussion

Total phenolic and flavonoid contents

The total phenolic and total flavonoid contents of the aqueous extracts of the bark, seed, and whole pod of okra is shown in Table 1. The whole pod extract demonstrated the highest total phenolic content (5.00 mg GAE/g) compared to the other extracts, while the seed extract exhibited the highest total flavonoid content (2.23 mgQE/g), compared to the other extracts ($p < 0.05$). Due to their broad spectrum of pharmacological actions and purported positive effects, phenolic compounds have a wide variety of functions and are the most extensively studied secondary metabolites in plants.[31] They show versatile health benefits such that they are used as antioxidants, antimicrobials, anti-hypoglycemic, anti-inflammatory agents, and immune system promoters, making them an interesting candidate for nutraceutical applications.[32] Their high content in medicinal foods has been associated with protecting the human system against the damaging effects of free radicals as well as in the mitigation of diseases arising from oxidative stress. Flavonoids are responsible for the antioxidant, anti-diabetic, and ant-inflammatory effects of okra and are considered as one of its most significant components.

Antioxidant activity

The 2,2-azinobis (3-ethylbenzothiazoline-6-sulfonate (ABTS) radical scavenging effect of the okra whole pod, bark, and seed components is shown in Figure 1. Although no notable differences in the ABTS radical scavenging capability were observed among the various samples, the seed exhibited the lowest ferric-reducing antioxidant power (Table 1). The outcomes of the 2,2-diphenyl-1-picrylhydrazyl (DPPH) and hydroxyl (OH) radical scavenging activity of the whole, bark, and seed components of the okra pod are presented in Figure 2. The extracts displayed substantial scavenging activity against DPPH and OH radicals, with the whole pod demonstrating the highest DPPH radical scavenging ability, and the bark exhibiting a superior OH radical scavenging ability, as evident from their IC_{50} values (Table 2).

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Medicinal plants exhibit antioxidative properties through multiple mechanisms, such as scavenging reactive oxygen species, metal chelation linked to the production of free radicals, boosting antioxidant defense, and hydrogen or electrons donation for molecular stability.[33] Ademiluyi and Oboh[34] have suggested a correlation between a plant's radical scavenging capacity and its phenolic content. The observed radical scavenging activity in the okra (*Abelmoschus esculentus* L) pods is likely attributable to the presence of polyphenols, flavonoids, and other phenolic compounds.

The existence of hydrogen-donating groups enables the mitigation of highly reactive radicals through reduction, leading to the formation of non-radical entities and subsequently contributing to the reduction in radical concentrations.[35]

Figure 3 shows the Fe^{2+}-induced lipid peroxidation inhibitory activity of the aqueous extracts of the whole, bark, and seed of the okra pod. Fe^{2+} is capable of inducing reactive oxygen species generation and initiating lipid peroxidation chain reaction.[36] The pathogenesis of diabetes mellitus revolves around the loss of pancreatic ß-cell function from oxidative stress. Protecting these cells against oxidative stress is an approach to prevent or reduce the occurrence of diabetes mellitus. As a pro-oxidant, Fe^{2+} was used to induce lipid peroxidation in rat pancreas. The extracts exhibited a dose-dependent response, resulting in a significant ($p < 0.05$) decrease in the concentration of malondialdehyde (MDA).

Table 1: Total phenolic content (TPC), total flavonoid content (TFC), and ferric reducing antioxidant power of the whole, bark, and seed components okra (*Abelmoschus esculentus* L.) pod

	Whole		Bark	Seed	
TPC (mg GAE/g)	5.00 ± 0.59[a]	±	4.29 ± 0.10[a]	2.96 ± 0.22[b]	±
TFC (mg QE/g)	0.48 ± 0.07[b]	±	0.21 ± 0.00[c]	2.23 ± 0.12[a]	±
FRAP	3.84 ± 0.29[a]	±	4.28 ± 0.21[a]	2.19 ± 0.26[b]	±

The values presented indicate the means ± standard deviation of triplicate measurements.
Values with the same superscript letter within rows exhibit no significant differences.

Table 2: DPPH and OH radical scavenging activities of whole, bark and seed of okra (*Abelmoschus esculentus* L.) pod

Activity	IC_{50} (µg/mL)		
	Whole	Bark	Seed
DPPH RSA	0.96 ± 0.00[a]	1.16 ± 0.01	1.39 ± 0.01
OH RSA	0.07 ± 0.00[b]	0.05 ± 0.00[a]	0.08 ± 0.00[b]

The values presented indicate the means ± standard deviation of triplicate measurements.
Values with the same superscript letter within rows exhibit no significant differences.

Notably, the aqueous extract of the whole okra pod exhibited superior inhibition of lipid peroxidation in comparison to both the bark and the seed extracts. The aqueous extracts of the whole, bark and seed of okra pod inhibited lipid peroxidation in the pancreas by complexing with the Fe^{2+} molecule and scavenging hydroxyl radicals,[37-39] thus preventing lipid peroxidation measured by its end-product, malondialdehyde.

In vitro antidiabetic activity

The *in vitro antidiabetic activity* of the extracts as measured by their α-amylase and α-glucosidase inhibitory activities are shown in Figure 4. These enzymes (α-amylase and α-glucosidase) are pivotal in the process of starch breakdown into simpler sugar molecules for absorption from the alimentary tract. Delays in the digestion and absorption of carbohydrates are crucial in preventing the post-prandial rise in blood sugar levels and the related complications in people with diabetes mellitus, especially type 2 diabetes. Inhibiting the relevant enzymes (α-amylase and α-glucosidase) can effectively slow down the digestion process and impede the absorption of sugars, thereby contributing to the management of blood glucose levels[40] and the pathological rise in the level of blood glucose.

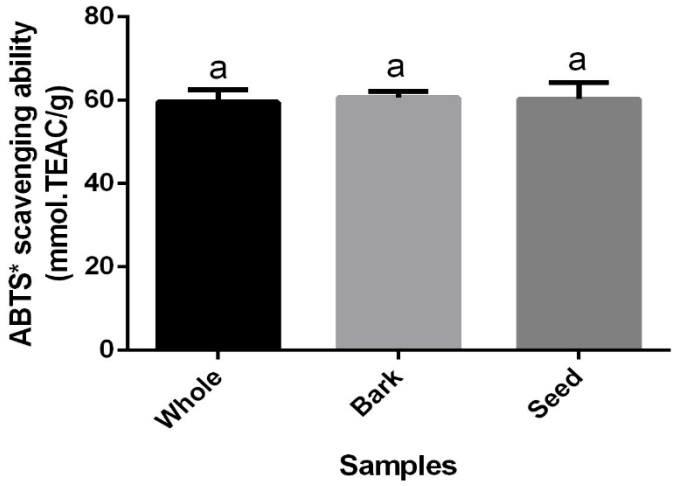

Figure 1: ABTS radical scavenging activity of whole, bark and seed of okra (*Abelmoschus esculentus* L) pod.
Bars represent mean ± standard deviation of triplicate measurements (n = 3).
Bars with the same letter show no significant difference ($p > 0.05$).

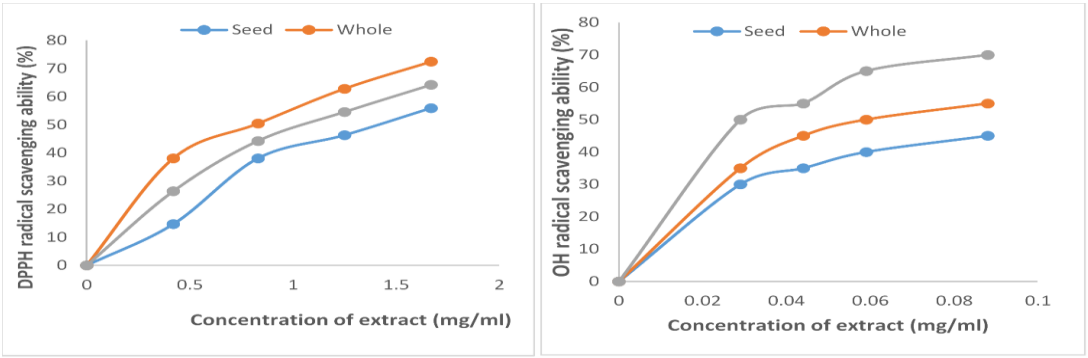

Figure 2: DPPH and OH radical scavenging activity of whole, bark and seed of okra (*Abelmoschus esculentus* L) pod.
The lines represent mean ± standard deviation of triplicate measurements (n = 3).

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

The extracts showed a significant ($p < 0.05$) dose-dependent inhibitory effect on both α-amylase and α-glucosidase, in line with other recorded medicinal plants renowned for their natural suppression of these enzymes. The IC_{50} values of the extracts as presented in Table 3 revealed that the aqueous extract of the bark of okra pod (IC_{50} = 0.04 mg/mL) had the highest inhibitory effect on α-amylase than the aqueous extracts of the seed and the whole pod with IC_{50} values of 0.07 mg/mL, and 0.11 mg/mL, respectively. However, for the α-glucosidase inhibitory activity, the whole pod extract (IC_{50} = 0.04 mg/mL) showed a more potent α-glucosidase inhibitory effect than extract of the seed (IC_{50} = 0.07 mg/mL) as well as the bark (IC_{50} = 0.09 mg/mL). The inhibition of carbohydrate metabolizing enzyme by different parts of okra pod correlates with the investigation conducted by Abbas *et al*,[41] which demonstrated the antihyperglycemic effect of dehydrated okra fruits on alloxan-induced diabetic rats. These effects were attributed to oxidative stress reduction and improved antioxidant capacity.[41]

Conclusion

The consumption of the whole pod of okra provides the consumer with all of its nutritional and pharmacological benefits. All part of the pod contains appreciable contents of total phenol and flavonoid with good radical scavenging activity. Furthermore, the inhibition of carbohydrate metabolizing enzymes (α-amylase and α-glucosidase) by okra justifies the recommendation of okra as part of the regularly consumed vegetable by diabetic patients. No part of the okra pod should be considered distinct from the other, as the whole pod, bark, and seed play different important roles in maintaining health.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

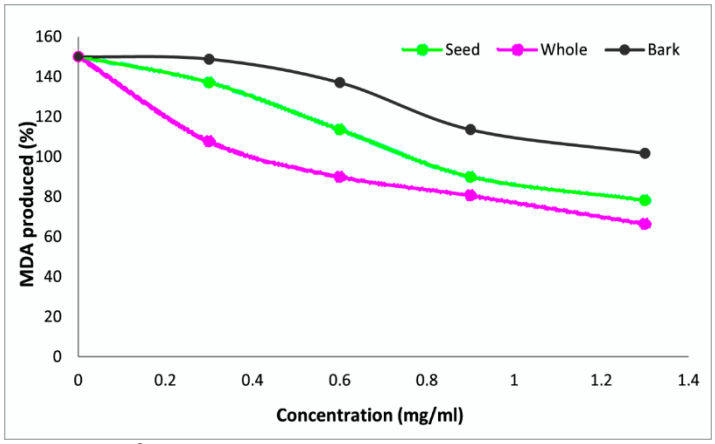

Figure 3: Fe^{2+}-induced lipid peroxidation inhibitory activity of whole, bark and seed of okra (*Abelmoschus esculentus* L) pod. The lines represent mean ± standard deviation of triplicate measurements (n = 3).

Table 3: The *α*-amylase, *α*-glucosidase and Fe^{2+} induced lipid peroxidation inhibitory activities of whole, bark and seed of okra (*Abelmoschus esculentus* L.) pod

Activity	IC_{50} (µg/mL)		
	Whole	Bark	Seed
α- amylase	0.11 ± 0.00^a	0.04 ± 0.00^b	0.07 ± 0.00
α- glucosidase	0.04 ± 0.00^b	0.09 ± 0.00^a	0.07 ± 0.00
LPO (Fe^{2+})	1.44 ± 0.01^a	2.57 ± 0.02^a	1.69 ± 0.02

The values presented indicate the means ± standard deviation of triplicate measurements.
Values with the same superscript letter within rows exhibit no significant differences.

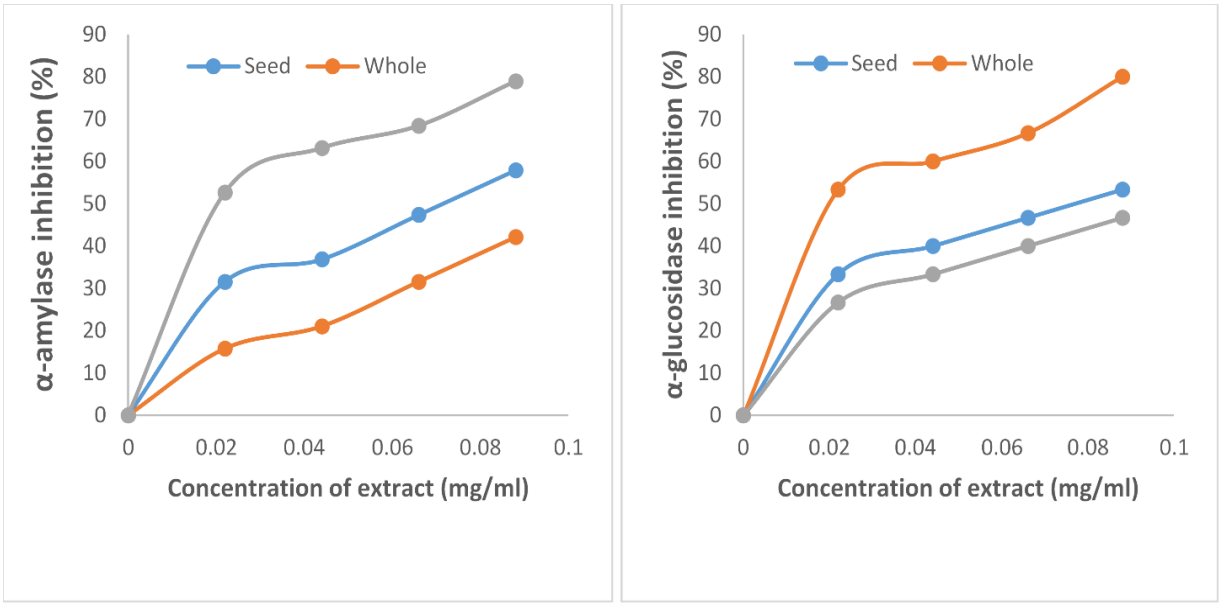

Figure 4: α-amylase and α-glucosidase inhibitory activity of whole, bark and seed of okra (*Abelmoschus esculentus* L) pod. The lines represent mean ± standard deviation of triplicate measurements (n = 3).

Acknowledgments

No external funding was received by the authors for thus study.

References

1. Rana A, Samtiya M, Dhewa T, Mishra V, Aluko RE. Health benefits of polyphenols: A concise review. J Food Biochem. 2022; 46(10):e14264.
2. Olaiya CO, Soetan KO, Esan AM. The role of nutraceuticals, functional foods and value-added food products in the prevention and treatment of chronic diseases. Afr J Food Sci. 2016; 10(10):185-193.
3. Lawal OM, Idowu-Adebayo F, Enujugha VN. Nutritional assessment of Nigerian ethnic vegetable soups (Marugbo, Tete and Ila). J Nutr Food Lipid Sci. 2018; 1:32-39.
4. Fagbemi TN and Oluwajuyitan TD. Food safety and value addition: a panacea to food security and wealth creation in emerging countries of sub-Sahara Africa (SSA)– Nigeria in focus, in: Strategies and Tactics of Sustainable Agriculture in the Tropics, College Press & Publishers Ibadan, Oyo State, Nigeria, 2020; 61-72 p.
5. Ogie-Odia EA, Mensah JK, Ehilen OE, Eseigbe DA. Nutrient, mineral and phytochemical properties of selected underutilized Amaranthus vegetable species in Ekpoma, Edo state, Nigeria. Niger J Biotechnol. 2022; 38(1):1-12.
6. Gong X, Huang X, Yang T, Wen J, Zhou W, Li J. Effect of drying methods on physicochemical properties and antioxidant activities of okra pods. J Food Proc Preserv. 2019; 43(12):e14277.
7. Davies R. Some physical properties of okra fruits and seeds. Int J Res Stud Sci Engineer Technol. 2020; 8(1):23-29.
8. Rathod V and Kavya DO. Breeding of Okra for Resistance to Yellow Vein Mosaic Virus. Int J Plant Soil Sci. 2023; 35(20):954-965.
9. Dantas TL, Alonso Buriti FC, Florentino ER. Okra (*Abelmoschus esculentus* L.) as a potential functional food source of mucilage and bioactive compounds with technological applications and health benefits. Plants. 2021; 10(8):1683.
10. Petropoulos S, Fernandes Â, Barros L, Ferreira I. Chemical composition, nutritional value and antioxidant properties of Mediterranean okra genotypes in relation to harvest stage. Food Chem. 2018; 242: 466-474.
11. Devi N. Evaluation for Heterosis in Okra (*Abelmoschus esculentus* L.) Moench). Int J Pure Appl Biosci. 2017; 5(6):590-593.
12. Islam MT. Phytochemical information and pharmacological activities of Okra (*Abelmoschus esculentus*): A literature-based review. Phytother Res. 2019; 33:72-80.
13. Zhang T, Xiang J, Zheng G, Yan R, Min X. Preliminary characterization and anti-hyperglycemic activity of a pectic polysaccharide from okra (*Abelmoschus esculentus* (L.) Moench.). J Funct Foods 2018; 41:19-24.
14. Shen D-D, Li X, Qin Y-L, Li M-T, Han Q-H, Zhou J, Lin S, Zhao L, Zhang Q, Qin W, Wu DT. Physicochemical properties, phenolic profiles, antioxidant capacities, and inhibitory effects on digestive enzymes of okra (*Abelmoschus esculentus*) fruit at different maturation stages. J Food Sci Technol. 2019; 56(3):1275-1286.
15. Olawuyi IF and Lee WY. Structural characterization, functional properties and antioxidant activities of polysaccharide extract obtained from okra leaves (*Abelmoschus esculentus*). Food Chem. 2021; 354:129437.
16. Khan S, Rafi Z, Baker A, Shoaib A, Alkhathami AG, Asiri M, Alshahrani MY, Ahmad I, Alraey Y, Hakamy A, Saeed M. Phytochemical screening, nutritional value, anti-diabetic, anti-cancer, and anti-bacterial assessment of aqueous extract from *Abelmoschus esculentus* pods. Proc. 2022; 10(2):183.
17. Hayaza S, Darmanto W, Wahyuningsih SP, Susilo RJ, Husen SA, Winarni D, Doong RA. Immunomodulatory activity of G Levels, and cell apoptosis in DEN-induced mice. Res J Pharm Technol. 2022; 15(2):546-550.
18. Khan S, Rafi Z, Baker A, Shoaib A, Alkhathami AG, Asiri M, et al. Phytochemical screening, nutritional value, anti-diabetic, anti-cancer, and anti-bacterial assessment of aqueous extract from *Abelmoschus esculentus* Pods. *Proc*. 2022; 10(2):183.
19. Panighel G, Ferrarese I, Lupo MG, Sut S, Dall'Acqua S, Ferri N. Investigating the in vitro mode of action of okra (*Abelmoschus esculentus*) as hypocholesterolemic, anti-inflammatory, and antioxidant food. *Food Chemistry:* Mol Sci. 2022; 5:100126.
20. Wahyuningsih SPA, Savira NII, Anggraini DW, Winarni D, Suhargo L, Kusuma BWA, Nindyasari F, Setianingsih N, Mwendolwa AA. Antioxidant and nephroprotective effects of okra pods extract (*Abelmoschus esculentus* L.) against lead acetate-induced toxicity in mice. Scientifica (Cairo). 2020; 2020:4237205.
21. Oboh G, Akinyemi JA, Ademiluyi AO, Adefegha SA. Inhibitory effect of aqueous extract of two varieties of Ginger on Key Enzymes linked with Type-2 Diabetes. J Food Nutr Res. 2010; 49(1):14-20.
22. Singleton VL, Orthofor R, Lamuela-Raventos RM. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin–Ciocaltau reagent. Meth Enzymol. 1999; 299:152-178.
23. Meda A, Lamien CE, Romito M, Millogo J, Nacoulma OG. Determination of the total phenolic, flavonoid and proline contents in Burkina Fasso honey, as well as their radical scavenging activity. Food Chem. 2005; 91(3):571-577.
24. Gyamfi MA, Yonamine M, Aniya Y. Free-radical scavenging action of medicinal herbs from Ghana *Thonningia sanguinea* on experimentally-induced liver injuries. Gen Pharmacol. 1999; 32(6): 661-667.
25. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Rad Biol Med. 1999; 26(9-10):1231-1237.
26. Pulido R, Bravo L, Saura-Calixto F. Antioxidant activity of dietary polyphenols as determined by a modified ferric reducing/antioxidant power assay. J Agri Food Chem 2000; 48(8):3396-3402.
27. Halliwell B and Gutteridge JMC. Formation of a thiobarbituric-acid-reactive substance from deoxyribose in the presence of iron salts: the role of superoxide and hydroxyl radicals. FEBS Lett. 1981; 128(2):347 - 352.
28. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem. 1979; 95(2):351 - 358.
29. Worthington V. Alpha amylase, In: Worthington K. and Worthington V (Edition), Worthington enzyme manual, Worthington Biochemical Corporation, Freehold, New Jersey 1993; 36 – 41 p.
30. Apostolidis E, Kwon YI, Shetty K. Inhibitory potential of herb, fruit, and fungal-enriched cheese against key enzymes linked to type 2 diabetes and hypertension. Innov Food Sci Emerg Technol. 2007; 8:46 - 54.
31. Tsimogiannis D and Oreopoulou V. Classification of phenolic compounds in plants. In: Polyphenols in plants, Academic Press. 2019; 1(01):263-284 p.
32. Meng XH, Liu C, Fan R, Zhu LF, Yang SX, Zhu HT, et al. Antioxidative flavan-3-ol dimers from the leaves of *Camellia fangchengensis*. J Agric Food Chem. 2018; 66:247 - 254.
33. Michalak M. Plant-derived antioxidants: Significance in skin health and the ageing process. Int J Mol Sci. 2022; 23(2):585.
34. Ademiluyi AO and Oboh G. Antioxidant properties of methanolic extracts of mistletoes (*Viscum album*) from

cocoa and cashew trees in Nigeria. Afr J Biotechnol. 2008; 7(17):3138 - 3142.
35. Gangwar M, Gautam MK, Sharma AK, Tripathi YB, Goel RK, Nath G. Antioxidant capacity and radical scavenging effect of polyphenol rich *Mallotus philippenensis* fruit extract on human erythrocytes: An *in vitro* study. Sci World J. 2014; 2014:279451.
36. Afolabi OB, Oloyede OI, Agunbiade SO. Inhibitory potentials of phenolic-rich extracts from *Bridelia ferruginea* on two key carbohydrate-metabolizing enzymes and Fe2+-induced pancreatic oxidative stress. J Integr Med. 2018; 16(3):192-198.
37. Adefegha SA, Oboh G, Ejakpovi II, Oyeleye SI. Antioxidant and antidiabetic effects of gallic and protocatechuic acids: A structure-function perspective. Comp Clin Pathol. 2015; 24(6):1579-1585.
38. Ademosun AO and Oboh G. Anticholinesterase and antioxidative properties of water-extractable phytochemicals from some citrus peels. J Basic Clin Physiol Pharmacol. 2014; 25(2):99 - 204.
39. Adetuyi FO, Karigidi KO, Akintimehin ES, Adeyemo ON. Antioxidant properties of *Ageratum conyzoides* L. Asteraceae leaves. Bangladesh J Sci Ind Res. 2018; 53(4):265 - 276.
40. Gong L, Feng D, Wang T, Ren Y, Liu Y, Wang J. Inhibitors of α-amylase and α-glucosidase: Potential linkage for whole cereal foods on prevention of hyperglycemia. Food Sci Nutr. 2020; 8(12):6320-6337.
41. Abbas AY, Muhammad I, AbdulRahman MB, Bilbis LS. Antioxidant effect of ex-maradi okra fruit variety (*Abelmuscus esculentus*) on alloxan-induced diabetic rats. Trop J Nat Prod Res. 2020; 4(3):105-112.