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FUNCTIONAL FOODS & NUTRACEUTICALS ASSOCIATION OF NIGERIA

Functional Food and Nutraceuticals Association of Nigeria (FFNAN)

6TH PHARMA-FOOD CONGRESS
IBADAN 2024

THEME:

Functional Foods and Phytomedicines: An emerging nexus for Healthcare Management in Humans and Animal Husbandry

VENUE:

Assembly Hall, North Campus, The Polytechnic Ibadan, Nigeria

DATE:

Monday 11th - Thursday 14th, November 2024

BOOK OF PROCEEDINGS

Edited by: Ganiyu Oboh

FFNAN

FUNCTIONAL FOODS & NUTRACEUTICALS ASSOCIATION OF NIGERIA

JOSEPH AYO BABALOLA UNIVERSITY
IKEJI - ARAKEJI
FOR KNOWLEDGE & GODLY SERVICE

7th JABU 2025 Pharma-Food Congress

Theme:

THE FUTURE OF WELLNESS: Functional Foods, Nutraceuticals and Phytomedicines as Key Drivers

Sub-themes

- The role of functional foods and phytomedicines in combatting emerging health challenges
- The impact of artificial intelligence in food product and nutraceuticals development
- Promoting novel functional feeds & therapeutic strategies for animal care
- Influence of market forces on the development of functional food products
- Regulation, characterization and standardization of Functional Foods and Phytomedicines

JOSEPH AYO BABALOLA UNIVERSITY, IKEJI-ARAKEJI, NIGERIA

Sunday, September 28 – Wednesday, October 1, 2025

Registration:
Members: #30,000
Non members - #35,000
Students - #15,000

Account Details - 2150416113
(UBA) Functional Food Group

Registration: Early birds (registration before 30th June, 2025) will be qualified for free accommodation during the conference.
Abstract Submission: ffnanigeria@yahoo.com

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Editor's Note

Health care management is not just crucial to human health but also to livestock management. This is more so as we depend largely on these animal resources for food. Hence, it may be safe to say that healthy livestock management can greatly promote healthy humans. Considering the pivotal role of functional foods and phytomedicine in promoting health and wellness, this conference, therefore, aims to foster the role of functional foods and phytomedicine in promoting healthy living in animals and, consequently, humans. In order to achieve these objectives, a convergence of academics from different relevant disciplines, industrialists, entrepreneurs, and policymakers is crucial to work together to build a cross-sectoral partnership to encourage and implement functional foods and phytomedicines for sustainable management of both human and animal health, in line with the global best practices.

The 6th Pharma-Food Congress, organized by the Functional Foods and Nutraceuticals Association of Nigeria (FFNAN), was held at The Polytechnic, Ibadan, from November 11th to 14th, 2024. Themed *"Functional Foods and Phytomedicines: An Emerging Nexus for Healthcare Management in Humans and Animal Husbandry,"* the congress brought together experts, researchers, and students to explore the intersection of nutrition, health, and phytomedicine for sustainable management of both human and animal health.

This four-day event was a convergence of ideas across academia, industry, and public health, all centered on the growing relevance of functional foods and phytomedicines in human and animal healthcare. Activities included pre-conference workshops, keynote lectures, plenary sessions, technical research presentations, and roundtable discussions. It featured workshops led by scholars from the Federal University of Technology, Akure (FUTA), covering topics like the valorization of citrus and cassava by-products and modeling the effects of elevated CO_2 on crop nutrition, highlighting innovation, sustainability, and indigenous resource utilization. The keynote lecture by Prof. Lateef O. Sanni (NSPRI) was a major highlight of the conference alongside other insightful plenary sessions and conference paper presentations by participants. A standout moment was the roundtable discussion on male infertility linked to herbal aphrodisiacs, fruits, and herbal malaria treatments, which was an engaging and relevant dialogue. Some of the full-length articles of papers presented at the conference have now been thoroughly revised and uniquely selected for this book of proceedings.

Prof. Ganiyu Oboh*fnas,faas*
Convener, Pharma-Food Congress

The Therapeutic Potential of *Talinum triangulare* and *Spondias mombin* in the Management of Neurodegenerative Disorders

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Abstract
Neurodegenerative disorders (NDDs) such as Alzheimer's disease (AD), and Parkinson's disease (PD) are characterized by gradual neuronal loss resulting in severe cognitive and motor impairments. Due to the progressive nature of NDDs and the available medications not being curative, the use of alternative medications is on the rise. The role of medicinal plants in NDDs has been beneficial because of their multi-receptor target property which has been attributed to the presence of several bioactive phytochemicals. Talinum triangulare and Spondias mombin are traditionally used in Nigeria for the prevention and management of various diseases because of their availability and nutritional potentials. This study aimed to review the therapeutics potential of the medicinal plants Talinum triangulare and Spondias mombin on NDDs in order to provide scientific validation for their ethnobotanical uses. Hence, this article aims to review the therapeutics potential of two medicinal plants Talinum triangulare and Spondias mombin on NDDs.

Keywords Talinum triangulare; Spondias mombin; Neurodegenerative disorders

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1. Introduction

Neurodegenerative diseases are characterized by the gradual loss of nervous system structure and functionality (Kakkar *et al.*, 2024). The primary target of these disorders is neurons, the basic unit of the nervous system, which includes the brain and spinal cord. These diseases include Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD), Amyotrophic lateral sclerosis (ALS), and Multiple sclerosis (MS) (Konishi *et al.*, 2017). The pathophysiological mechanisms underlying NDDs are multi-factorial, however, they share common features such as protein misfolding, oxidative stress, mitochondrial dysfunction, cholinesterase malfunction and neuroinflammation. The symptoms associated with NDDs are dependent on the central nervous system (CNS) tissue affected, however, decline of neuronal functioning, motor skills, and cognitive ability are common in all (Wareham *et al.*, 2022). There is currently no cure for neurodegenerative disorders, however, there are both pharmacological and the non-pharmacological interventions which aimed at improving the quality of life of patients as well as helping patients to undertake daily activities.

NDDs affect millions of people worldwide and according to recent data by WHO in 2022, up to 30% of all people over the age of 85 suffer from Alzheimer's disease, and 5% of people over the age of 65 suffer from Parkinson's disease (Shusharina *et al.*, 2023). In 2019 alone, neurological disorders were globally responsible for nearly 10 million deaths and 349 disability-adjusted life-years (DALYs) lost (Zhang *et al.*, 2023). Global, regional, and national data has shown that the burden of all neurological diseases, or persons who died due to neurological diseases has increased significantly in all countries worldwide (Wang *et al.*, 2023).

The progressive and multi-factorial pathophysiologic nature of NDDs coupled with severe side effects of conventional medications have necessitated the continuous search for newer and alternative medicines. The use of medicinal plants as neuroprotective agents is gaining traction

because of their multitude of phytochemicals which exhibit various bioactivities such as alleviation in inflammatory responses, antioxidative properties, and cholinesterase inhibiting properties (Luthra and Roy, 2022). Several medicinal plants such as *Talinum triangulare, Spondias mombin, Carica papaya, Jatropha carcus* are used independently or in combination with other herbs for the management of neurodegenerative diseases among Yoruba people of western Nigeria, Nigeria (Adetuyi *et al.*, 2023, Sonibare and Ayoola, 2015). Despite the huge popularity and usage of traditional medicine, it's still been looked down or not accepted by the society especially among health care professionals. This is mainly due to insufficient scientific evidence to justify their ethnomedicinal uses and assess their safety profile. Currently, investigations are geared towards confirming the ethnobotanical uses of medicinal plants with the aim of discovering new target drugs capable of preventing and delaying the progression of NDDs (Sonibare and Ayoola, 2015). Therefore, this article intends to summarize research studies done to justify the ethnobotanical-neuroprotective effect of *Talinum triangulare* and *Spondias mombin* on various neurodegenerative diseases.

2. Methods

The key words "neurodegenerative diseases", "medicinal plants", "pharmacological activity" *Talinum triangulare* and neurodegenerative diseases'', "*Spondias mombin*'' and neurodegenerative diseases" were searched using various search engines such as Google scholar, Pubmed, Web of Science. Relevant scientific articles regarding *Talinum triangulare*, *Spondias mombin*, neurodegenerative diseases were pooled from different scientific databases. Pharmacological activity and the plant parts used in managing neurodegenerative diseases were summarized.

3. Hallmarks of Neurodegenerative diseases

Neurodegenerative diseases (NDDs) are heterogeneous group of neurological diseases characterized by progressive loss of neurons in the central nervous system (CNS) or peripheral nervous system (PNS) (Willson *et al*., 2023). The structural and functional collapse of the nervous system and the associated neuronal loss impairs nerve to nerve communication leading to impaired memory, cognition, behaviour, and sensory, and / or motor function. Age is the single most contributing risk factor to the development of all NDDs, although, recent findings revealed that a combination of an individual's genetic makeup and environmental factors can equally contribute to increasing the risk for NDDs (Liu *et al.*, 2022)

NDDs are of significant health burden worldwide with various mechanisms proposed to explain the neurodegeneration underlying the diseases. Such mechanisms include pathological protein aggregation, synaptic and neuronal network dysfunction, aberrant proteostasis, cytoskeletal abnormalities, altered energy metabolism, DNA and RNA defects, inflammation, and neuronal cell death (Willson *et al*., 2023).

4. Medicinal Plants and Neurodegenerative Diseases

The use of medicinal plants in mitigating against various neurodegenerative diseases is as old as man. Current therapies are mainly symptomatic in management with no cure yet. Medicinal plants are important source of lead compounds in the drug discovery process with several scientific studies reporting the beneficial effect of phytocompounds on the brain and/or neuronal function. Vegetables are well known for their nutritional benefits, with some of them possessing potent antioxidant, acetylcholinesterase inhibitory, and neuromodulatory activities, which are crucial in delaying the onset of NDDs. These vegetables act as functional foods with their regular consumption part of non-pharmacological measures in management of NDDS.

Talinum triangulare, belongs to the family Talinaceae (Figure 1A). It is commonly called Waterleaf, Talinum, Ceylon spinach, Philippine spinach, Florida spinach, Potherb Fameflower, Lagos bologi etc., and locally in Nigeria, as Efo Gbure (Yoruba), Mgbolodi (Igbo), Alenruwa (Hausa), and Ebe-dondon (Edo). It is endemic in tropics such as Nigeria, where it's popularly consumed as vegetable soup (Osaretin and Ebuehi, 2017). The folkloric neuroprotective of *T. triangulare* could be attributed to its high protein content which are important in production of brain neurotransmitters. It is used industrially to manufacture natural polymers due to its richness in mucilage and pectin (Akin-Ajani *et al*., 2022). The leaves, are consumed for managing gastrointestinal disorders, indigestion and treatment of edema in several regions of West Africa (Barman *et al*.,

2023). The indigenous people of Javan islands of Indonesia, also make use of *T. triangulare* to boost their immune system and stamina. Various compounds like quercetin, kaempferol, apigenin, lycopene, ergosterol, cinchonine, caffeine and colchicine are reported. These compounds are shown to have a wide range of bioactivity. Other health benefits of *T. triangulare* include management of anemia (Adeyemi *et al*., 2018), to combat neurologic disorders (Ofusori *et al*., 2008), as hepatoprotective agent (Liang *et al*., 2011), amongst others.

Spondias mombin, "Yellow mombin", known for its edible fruit belongs to the family Anacardiaceae (Figure 1B). It is commonly known as Yellow Mombin, Hog plum, Amra or Cajazeira and in Nigeria, it is locally known as Iyeye or Yeye (Yoruba), Ngulungwu (Igbo) and Isada (Hausa). The fruit pulp possesses high fibre content, ascorbic acid, and carotenoid content which is responsible for its bright yellow colour. A 100 g portion of yellow mombin fruit pulp can provide more than 37% of the Recommended Daily Intake (RDI) of vitamin A, 5.8% of the RDI for magnesium (for adults), 4.6% of the phosphorus RDI and 8.2% of potassium RDI and 4% of the RDI for iron for men (Tiburski *et al*., 2011)

It has also demonstrated antioxidant capacity (phenolic compounds), in form of free radical scavenging, and modulatory effects against the onset, development or degenerative pathophysiology of chronic diseases (Ogunro *et al*., 2023). The young leaves of *S. mombin* are eaten as vegetable and the shoots can be eaten raw or boiled. In folk medicine, the bark and flowers are used in treatment of digestive tract ailments, gonorrhea, malarial fever, rheumatism, heamorrhages with the leaves used as abortifacient (Nworu *et al*., 2007). The leaves of *S.mombin* have been reported to contain antiviral ellagitannins and caffeoyl esters, as well as antibacterial and molluscicidal phenolic acids. Its anti-inflammatory and antihelminthic properties have also been investigated and confirmed in its methanolic leaf extract (Nworu *et al*., 2011)

5. Therapeutic potential of *Talinum triangulare* and *Spondias mombin* on Neurodegenerative diseases

5.1 *Talinum triangulare* and Spondias mombin on learning and memory

According to a study conducted by Eru *et al*., (2022), Alzheimer's type cognitive dysfunction was intraperitoneally induced in rats with scopolamine hydrobromide for seven days and the ameliorative effect of aqueous extract of *T. triangulare* was observed. The study revealed there was learning impairment induced by scopolamine, indicated by general elevation in escape latency using the Morris water maze test compared to the negative control. However, rats treated with *T. triangulare* at 1750 and 875 mg/kg body weight leaf showed reduced escape latency time thereby indicating the reversal of the learning deficits induced by scopolamine. Also, aqueous extract of *T. triangulare* significantly improved the memory output of treated rats when subjected to memory assay according to a study conducted by Osaretin and Ajagun-Ogunleye (2017). In the experiment, the animals had a longer time in the illuminated compartment than the dark after being exposed to electric shock in the dark compartment during the training test.

According to Elufioye and Oyelude (2015), *Spondias mombin* was investigated for its memory enhancing abilities. In the study, the memory enhancing activities of the extract was evaluated in scopolamine induced amnesic mice which was administered interperitoneally and Morris water maze test was carried out at various doses (0.2 mL of 4 mg/kg, 6mg/kg and 8mg/kg) by determining the escape latency. The study showed that the mice showed characteristics that indicated impairment of spatial memory which was reversed after administration of the ethyl acetate extract. The activity was observed to be dose dependent through days 1 to 3 thus inferring the ability of *Spondias mombin* to enhance memory and cognition due to the activity of its constituents.

A

B

Figure 1 Macroscopic outlook of the A) Talinum triangulare and B) Spondias mombin respectively.

5.2 Effect of *Talinum triangulare* on the cerebrum

The effect of orally administered aqueous extract of T. *triangulare on* the cerebrum of mice was investigated by Ofusori *et al*., (2008), the results revealed that mice fed with 20, 30, and 40 mg/kg body weight of the plant extract had distinct neuronal appearance, with no stroma vacouations when compared with the control (vehicle-fed). This indicated no brain cell death or damage. Also, the lipid peroxidation index marker, Maloniadeldehyde (MDA), was significantly reduced in the brain of the treated mice compared to control with an increment in the activity of the antioxidant enzyme, catalase. This result indicates that T. *triangulare* can protect the cerebrum from insults especially oxidative stress-induced damage.

5.3 Effect of *S. mombin* on the hippocampus

According to the study by Elufioye and Oyelude (2011), the hippocampus and forebrain was extracted and histopathological assessment was carried out. The study revealed a reduction in the density of the brain cells of the control group whereas there was an increase in the density and number of cells in mice injected with the ethyl acetate extract of *Spondias mombin* at the different doses which could be responsible for the cognitive enhancing activity of the plant extracts

Conclusion

There are recent rapid development of various surgical, pharmacological, and non-pharmacological interventions for managing neurodegenerative diseases, however, most patients with neurological disorders are clinically diagnosed only when nerve damage is very severe, losing the optimal treatment time. Overall, the prognosis of patients is still poor, and most drugs have adverse side effects. Therefore, the regular consumption of medicinal plants with anti-neurodegenerative activity can help in preventing and/or ameliorate neurodegeneration. These plants can also be produced as herbal medicinal products for standardization, acceptable presentation and improved patient compliance and adherence; all aiding therapeutic outcome.

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Declaration of Conflict of Interest

None

Author's contribution

Tolulope O. Ajala – Conceptualization, Review and Editing, Hamidat A. Alaka – Writing, Review and Editing, Oluwatobiloba A. Shanu - Writing, Review and Editing.

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Functional Beverages from Tropical Fruits for the Management of Hypertension: A Narrative Review

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Abstract

Hypertension, a prevalent cardiovascular condition, poses a significant global health challenge. Dietary interventions have gained attention as potential strategies for its control, with functional beverages emerging as a promising avenue. This review examines the impact of functional beverages on blood pressure regulation. Google Scholar, PubMed, and ScienceDirect were searched for publications on developing functional beverages from tropical plants. The market for functional beverages is anticipated to reach $208.13 billion, growing at a 7.5% compound annual growth rate by 2024. Analysis of studies investigating the effects of five functional beverages, including green tea, roselle, guava, beetroot, and dairy alternatives on hypertension was reported. The bioactive compounds and underlying mechanisms responsible for the observed blood pressure-modulating effects are elucidated, encompassing vasodilation, nitric oxide bioavailability enhancement, and renin-angiotensin-aldosterone system modulation. While emphasizing the need for robust clinical trials and standardized methodologies to validate their efficacy this review guides future research directions and underscores the potential of functional beverages for promoting cardiovascular health and managing hypertension.

Keywords: Hypertension, functional beverage, green tea, guava tea, beetroot.

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1.0 Introduction

1.1 Brief overview of hypertension as a global health concern.

Hypertension is the consistent rise in blood pressure measured over time. It is considered as a blood pressure reading greater than 130 mmHg for the systolic and 80 mmHg for the diastolic measurements respectively (Armstrong, 2018). It accounts for about 16.5% of yearly deaths worldwide, with estimated predictions of annual deaths of about 23.5 million by the year 2030 (World Health Organisation, 2021). Cardiovascular diseases account for about 18 million annual deaths, and hypertension is a critical risk factor in the pathogenesis and progression of chronic illnesses such as heart failure, atherosclerosis, stroke, coronary artery disease, and peripheral disease. Furthermore, it is a foremost cause of kidney damage/failure, dementia, and even blindness and it has been reported that 45 and 51% of deaths from heart attack and stroke, respectively are due to hypertension (Gakidou et al., 2017; Zhou, et al., 2017; World Health Organisation, 2019). Also, obesity, physical inactivity, alcoholism, and unhealthy diet are risk factors for the incidence of hypertension (Getiye et al., 2016; Armstrong, 2018). Meresa et al. (2017) reported that over 25% of the world's adult population is hypertensive, and it is projected that by the year 2025, about 75% of the world's hypertensive adult population will be from developing countries including Sub-Saharan Africa. The

World Health Organisation (WHO) has reported that Africa has the highest hypertension prevalence (27%) compared to the Americas (18%) (WHO, 2019). Although there are numerous antihypertensive drugs, hypertension is still poorly managed, thus raising serious concerns to the scientific community and the continuous research in the area of alternative therapies.

1.2 Functional beverages as a potential vehicle for hypertension management.

A functional beverage is “any non-alcoholic drink that provides additional health benefits due to the inclusion of any bioactive component from a plant, animal, marine or microorganism source” (Gayathry & John, 2021). They are beverages that provide beyond basic hydration due to additional health benefits. They are formulated with specific ingredients or additives that are believed to impact the body and mind positively. The concept of "functional foods" is not at all novel as it was originally mentioned in the old Indian Vedic literature and is also a fundamental component of traditional Chinese medicine. The goal of formulating functional foods follows the Eastern viewpoint that "Food and medicine have a similar ancestor. The contemporary focus on creating foods with added health advantages first appeared in Japan in the 1980s for food products enhanced with ingredients that had beneficial physiological effects. In the article "Japan Explores the boundary between food and medicine" published in the year 1993 issue of Nature, the term "functional food" first appeared (Cong et al., 2020). So, it can be inferred that functional beverages intersect with conventional beverages and orthodox medicine (pharmaceuticals). According to Shahidi & Ambigaipalan (2015), the rise in demand for functional foods and beverages with therapeutic applications is mostly attributable to the new century's trend of easy access to knowledge, which gives people the means to look for a high quality of life. Widely used as beverages, infusions of some vegetable species' leaves, flowers, fruits, and seeds have significant effects on human health due to their phytochemical constituents, which promote an improvement in oxidative balance. This practice also goes hand in hand with maintaining hydric status (Valduga et al., 2019).

1.3 Importance of lifestyle interventions and complementary strategies.

The prevalence of noncommunicable diseases, especially hypertension, has increased significantly (especially in the last two decades), despite several scientific advances in the form of novel drugs and cutting-edge diagnostic methods. As a result of this pattern, major organizations such as the American Heart Association, the National Institutes of Health, and the National Heart, Lung, and Blood Institute have begun to develop an integrative strategy for combating this rising pandemic. Notwithstanding this, medication and diagnostic procedures continue to be the cornerstones of patient care; however, it is crucial to pay attention to lifestyle factors, including food, exercise, and stress management (Challa et al., 2021). Beverages and dietary interventions are critical vehicles used to supply essential nutrients (both macro and micronutrients) needed by the body for optimum function and survival. Some tropical plants have been reported to reduce blood pressure and have been exploited to produce functional beverages for hypertension management. The emergence of functional foods and beverages as adjuncts to conventional therapies has spurred interest in harnessing the medicinal properties of tropical plants. These plants have been an integral part of traditional medicine systems, and their bioactive compounds hold potential for managing hypertension. This review discusses different functional beverages that have been developed from tropical plants that hold promise for hypertension management, highlighting their mechanisms of action, clinical efficacy, and potential benefits.

2.0 Methods and search strategy

Publications available between 2013 and 2023 were used for this review. Public databases including Google Scholar, PubMed, and ScienceDirect, were checked for publications on developing functional beverages from tropical plants. Keywords including “hypertension”, “functional beverages”, “functional drinks”,

"tropical plants", and "functional foods + tropical plants", were used for the search. The search was limited to articles published in the last ten years due to the enormous volume of literature available in functional foods and beverage research over the years.

3.0 Results and Discussion

3.1.1 Bioactive compounds and their mechanisms of antihypertensive action

Bioactive compounds of plants are naturally produced as secondary metabolites. They are non-nutritive or extra-nutritional components present in foods (Martirosyan & Miller, 2018). They are compounds other than primary metabolites, which do not contribute to the energy needs of plants but are believed to be helpful to plants for survival from predators. These secondary metabolites of plants have been reported to exert both pharmacological and toxicological effects in humans and animals (Martirosyan & Miller, 2018). Functional beverages with antihypertensive activity usually contain bioactive compounds such as polyphenols, flavonoids, peptides, and minerals. These compounds exert various mechanisms of action, including vasodilation, reduction of oxidative stress, inhibition of angiotensin-converting enzyme (ACE), and modulation of nitric oxide production.

Flavonoids are mainly known for their antioxidant benefits to both plants and animals (humans). Quercetin is the most ubiquitous flavonoid studied for various biological activities. It exerts its antihypertensive effect through the decrease in expression of nuclear factor kappa-B (NF-kB) (Patel et al., 2018), vasodilation of endothelial tissues, inhibition of platelet-derived growth factor-induced cell migration/proliferation in the vascular smooth muscle cell and attenuation of angiotensin II-induced vascular smooth muscle cell abnormal growth (Hügel et al., 2016). Furthermore, Kaempferol has been reported to reduce blood pressure indicators such as systolic blood pressure (SBP), diastolic blood pressure (DBP), and pulse pressure (Ikewuchi et al., 2014) through the inhibition of angiotensin-converting enzyme (ACE) (Irondi et al., 2016) and enhanced endothelial function through increased nitric oxide (NO) production.

Phenolic acids including ferulic acid enhance endothelial function via improved relaxation of the endothelium and antioxidant status (Fukuda et al., 2015). Some terpenoids especially triterpenes and tetraterpenes have been reported to ameliorate hypertensive complications through mechanisms such as inhibition of ACE and nicotinamide adenine dinucleotide phosphate (NAD(P)H) oxidases, enhancement of NO production and endothelial function (Shafei e al., 2017; Teng & Chen, 2017; Li et al., 2017).

Some alkaloids including neoliensinine and lobeline, have also been investigated for antihypertensive effects. Neoliensinine and other alkaloids in lotus seed exert their antihypertensive effect via relaxation of the vascular smooth muscle (Yang et al., 2018), while lobeline counteracted the vasoconstrictive effect of endothelin-I and inhibiting the proliferation of vascular smooth muscle (Ren-Ren et al., 2015).

An increased intake of minerals such as potassium, magnesium, and calcium by dietary means has been shown in some studies to reduce blood pressure in patients with hypertension **(**Wabo et al., 2022). Calcium, in combination with other ions, such as sodium, potassium, and magnesium, provides an ionic balance to the vascular membrane, and vasodilatation, resulting in reduced blood pressure (Kant et al., 2020). A consistent body of evidence indicates that high potassium levels are associated with lower blood pressure (Filippini et al., 2020). A potassium-rich diet, as well as an elevation in serum potassium levels, even within the physiologic range, promote endothelium-dependent vasodilation by hyperpolarizing the endothelial cell via sodium pump stimulation and potassium channel opening (Staruschenko, 2018). Natriuresis, modulation of baroreceptor sensitivity, reduced vasoconstrictive sensitivity to norepinephrine and angiotensin II, increased serum and urinary kallikrein, increased sodium/potassium ATPase activity, alteration in DNA synthesis, and proliferation in vascular

smooth muscle and sympathetic nervous system cells are also proposed mechanisms by which potassium can influence blood pressure (Kant et al., 2020).

3.1.2 Antihypertensive Mechanisms of some Bioactive Compounds

Some enzymes, particularly those of the renin-angiotensin system (RAS), such as angiotensin-converting enzyme (ACE), arginase, nitric oxide synthase, cholinesterase, and those involved in the oxidative stress pathway, have been linked to hypertension pathogenesis (Obode et al. 2020). Because the RAS is important in the regulation of blood pressure and volume homeostasis, proper regulation of the RAS pathway is an important method for the treatment of hypertension (Azmir et al., 2013). The RAS pathway begins in the kidneys, where angiotensinogen is converted to angiotensin I by renin. When activated by ACE, angiotensin I is inactive and can be transformed into its vasoconstrictive form, angiotensin II. Angiotensin II is a critical regulator that is required for sodium and potassium balance as well as modulating cellular growth and remodeling (Obode et al., 2020).

Plant bioactive compounds have been reported to modulate some pathways in the pathophysiology of hypertension including increased hydrogen sulphide (H_2S) production, inhibition of angiotensin II and ACE expression, and mitigation of inflammation. For instance, the organosulphur compounds of garlic have been scientifically proven to enhance the production of H_2S and inhibition of ACE in animal models of hypertension (Piragine et al., 2020).

3.2 Functional beverages from tropical plants

Tropical plants thrive in tropical regions, which are typically characterized by warm temperatures, high humidity, and abundant rainfall. These plants have adapted to the unique conditions of the tropics and often display lush foliage, vibrant colours, and exotic shapes. The tropics are home to an incredibly diverse range of plant life, each with its unique characteristics and adaptations.

There has been an increase in the demand and production of functional beverages and this has been attributed to the recognition of the critical correlation between health and diet. Furthermore, the decline in health owing to hectic work schedules and lifestyles, excessive consumption of convenience foods, and insufficient physical activities have been identified as important factors driving the functional foods market (Gupta et al., 2023). The study by Kumar et al. (2022) revealed that the market for functional beverages is anticipated to reach $208.13 billion, growing at a 7.5% compound annual growth rate by 2024. The specific health benefits offered by functional beverages vary depending on the composition of the various raw materials and ingredients used for the formulation.

Functional beverages could be classified into energy drinks, sports drinks, protein shakes, antioxidant drinks, digestive health drinks, immune boosters, weight management drinks, functional teas, herbal infusions, and cognitive enhancement drinks. The classification of functional beverages is not mutually exclusive, and some beverages may fall into multiple categories. However, it is essential to note that the classification may evolve as new research and ingredients emerge, driving innovation in the functional beverage industry.

3.3 Antihypertensive functional beverages

3.3.1 Green Tea-Based Beverages: Green tea possesses a myriad of bioactive compounds with different pharmacological activities. Polyphenolic compounds account for 40% of the dry weight of green tea leaves (Tallei et al., 2021). The main classes of polyphenols found in green tea include phenolic acids, flavonoids, and lignans. The flavonoids in green tea such as flavanols (flavan-3-ols), flavones, flavanones, and anthocyanidins account for about 30% of the dry weight of the leaves (Cavusoglu et al., 2022). Flavan-3-ols such as catechins (epicatechin (EC), epigallocatechin (EGC), epicatechin gallate (ECG), epigallocatechin gallate (EGCG), catechin, and gallocatechin) are the main bioactive compounds that contribute to the biological activities of green tea beverage.

Antihypertensive Effect of Green Tea: There have been inconsistent results on the effects of green tea on blood pressure. A meta-analysis of randomized control trials from twenty-five studies showed that the acute intake of green tea did not affect blood pressure (Liu et al., 2014). However, most studies have reported a decrease in diastolic and systolic blood pressure in hypertensive patients consistently consuming green tea. Another meta-analysis of twenty-four clinical trials reported that green tea consumption significantly reduced systolic blood pressure (SBP) levels by −1.17 mmHg and diastolic blood pressure DBP by −1.24 mmHg (Xu et al., 2020). Yildirim et al (2023) also reported that green tea supplementation reduced systolic blood pressure by 2.99 mmHg and diastolic pressure by 0.95 mmHg. In human population research, a variety of factors could influence the effect of tea drinking on blood pressure. For example, the duration of tea consumption affects blood pressure. A meta-analysis of randomized controlled trials found that green tea reduces SBP and DBP in hypertension patients by -3.53 and -0.99 mmHg, respectively. This reduction was more significant with longer tea use (> 3 months) (Madhavi-Roshan et al., 2020). Significant reductions in both systolic and diastolic blood pressure were observed in a cross-sectional observational study involving 75 healthy participants and 75 Type-2 diabetic patients following a 90-day green tea intake regimen (Shah et al., 2017). In another study of four weeks, 20 obese pre-hypertensive women took 500 mg green tea extract capsules three times a day, which significantly lowered their systolic blood pressure. This crossover, double-blind, randomized, placebo-controlled clinical trial revealed no change in diastolic blood pressure or other measures (Nogueira et al., 2017). In a double-blind, placebo-controlled study, 56 obese, hypertensive participants who took one green tea extract capsule daily for three months saw a significant drop in both SBP and DBP (Bogdanski et al., 2012). Furthermore, in pre-diabetic patients, supplementing with 3 cups of green tea per day for 14 weeks resulted in the suppression of mean arterial pressure in both men and women (Toolsee et al., 2017).

The synergistic effect of the phytochemical constituents and route of administration may affect the antihypertensive effect of green tea. For instance, the caffeine content of tea could result in a short-term rise in blood pressure. The state of blood pressure is another factor to be considered in the antihypertensive effect of green tea. A study reported that green tea achieved better blood pressure control in hypertensive patients compared to normotensive patients and those already well-controlled by antihypertensive therapy (Teramoto et al., 2022). Nonetheless, *in vitro* and *in vivo* studies have shown that green tea and its secondary metabolites may trigger smooth muscle relaxation, reduce vascular inflammation, inhibit renin, and endothelin-1 activity, improve nitric oxide activity, and reduce vascular oxidative stress (Yan et al., 2020).

Green tea exhibits antihypertensive effects via different biochemical mechanisms. The beverage contains various phytochemical compounds that act on vascular tone and balance vasoconstricting substances such as angiotensin II (Ang-II), prostaglandins, endothelin-1 (ET-1), and vasodilating substances, such as prostacyclin and endothelium-derived hyperpolarizing factors (Chaudhary et al., 2023). Green tea also boosts ventricular function and exerts positive effects by activating nitric oxide (NO) production from endothelium in PI3-kinase-dependent pathways (Lorenz et al., 2017). Green tea also decreases oxidative stress and controls free radical production by blocking pro-oxidant enzymes and stimulating antioxidant enzymes. Green tea catechins block the oxidative stress-causing nuclear factor kappa B, redox-sensitive transcription factors, and activator protein-1. Green tea catechins also have anti-inflammatory properties by reducing inflammatory markers like cytokines, adhesion molecules, and nuclear factor kappa B (Bagheri et al., 2020).

3.3.2 Hibiscus-Based Beverages: *Hibiscus sabdariffa* is a tropical plant also known as rozelle, sorrel, or red sorrel. It belongs to the Malvaceae family. Due to the abundant availability of the plant, several studies have been conducted to validate its folkloric claims as

a medicinal plant. The anticancer, antibacterial, antioxidant, nephron- and hepatoprotective, diuretic, anti-cholesterol, anti-diabetic, and antihypertensive activities of this herb have been reported in several studies (Jalalyazdi et al., 2019). These biological activities are attributed to the bioactive compounds present in the *H. sabdariffa* calyx. Flavonoids are the most abundant bioactive compounds in the flower of *H. sabdariffa*. The plant is a rich source of anthocyanin which confers antioxidant effects on various biological systems. The presence of cyanidin-3-glucoside, delphinidin-3-glucoside, cyanidin-3-sambubioside, and delphinidin-3-sambubioside has been reported in extracts of *H. sabdariffa*.

The ACE-inhibitory activity, vascular smooth muscle relaxation activity, cyclic guanosine monophosphate (cGMP) pathway, and phosphatidylinositol-3-kinase/protein kinase B pathway activation (which stimulates NO synthase enzyme) have all been associated withwith the antihypertensive effect of *H. sabdariffa* (Amin et al., 2020). Furthermore, its antioxidant activity is associated with anti-atherosclerotic properties including reduction of low-density lipoprotein (LDL) oxidation, and other steps of formation of atherosclerotic plaque.

3.3.3 Guava-based beverages

Guava (*Psidium guajava*) is a tropical fruit that is low in calories and rich in nutrients such as vitamin C, folate, copper, potassium, and fiber. The plant (including its leaves and fruit) is considered "magical" because of its array of nutrients and the medicinal uses of its parts. It is one of the most important sources of medicine and has been used traditionally as a medicinal plant globally for several ailments (Daswani *et al*., 2017). Guava trees produce fragrant fruits, but their leaves are also used to create a functional beverage. Guava leaf tea is made by steeping guava leaves in hot water. A comprehensive review carried out by Morais-Braga *et al*. (2016) on the toxicity studies with various parts and extracts of guava showed the plant is safe for use. Guava leaf extracts contain chemicals with a variety of biological properties, including antioxidant, antihypertensive, hypoglycemic, and anticancer properties.

Mechanism of antihypertensive action of *Psidium guajava*: Incorporating guava-based beverages into one's diet may offer several potential benefits for hypertension management. For example, guava juice or smoothies can provide a convenient and tasty way to increase one's intake of potassium, which is an important nutrient for blood pressure regulation. Additionally, guava-based beverages may offer a natural alternative to traditional hypertension medications; or work with traditional hypertension medications (Díaz-de-Cerio *et al*., 2017). Several studies have investigated the potential of guava in reducing blood pressure and improving cardiovascular health. In a study carried out by de Assis Braga et al. (2022), *Psidium guajava* leaves exhibited a hypotensive effect after 4 weeks of treatment to weaned male Wistar rats which had been placed prior on a high-salt diet for 16 weeks. In another study, the oral administration of the aqueous extract of different varieties of guava leaf reduced systolic blood pressure and diastolic blood pressure in cyclosporine-induced hypertensive rats. There was no significant difference in the activity of the aqueous extracts of the three varieties when compared, (Babatola and Oboh, 2021). Therefore, guava-based beverages may offer a natural and effective therapeutic approach to the management of hypertension.

3.3.4 Beetroot (*Beta vulgaris*) and Nitrate-Rich Beverages: Most common drugs used for the treatment of hypertension including calcium channel blockers, statins, angiotensin-I converting enzyme inhibitors, and beta-adrenergic receptor blockers mostly contribute to the increase in the bioavailability of nitric oxide (NO). The reduction of NO has been linked with cardiovascular problems, especially hypertension. This molecule is important in the reduction of blood pressure due to its crucial role in the cardiovascular system. NO contributes to vascular tone modulation and microcirculatory blood flow. It also plays a major role in upholding vascular integrity via its antioxidative, anti-inflammatory, and anti-aggregatory activities (Calstrom et al., 2018).

Hence, the production of NO via the reduction of inorganic nitrates and nitrites formed endogenously is critical to the treatment and management of hypertension.

Studies have shown that dietary intervention with fruits and vegetables rich in nitrates may reduce blood pressure due to the increase in bioavailability of NO which is supplied by these foods (Borgi et al., 2016; Jakubcik et al., 2021). The blood pressure-reducing effect of most fruits and vegetables has been linked to the actions of vitamins, antioxidants, and phenolic compounds present. However, there are indications that antioxidants and vitamins show little or no effect on reduction of blood pressure. High levels of nitrate (NO_3) have been identified as a potent antihypertensive agent due to its blood pressure-reducing effect. Hence there has been a great interest in the exploration of the antihypertensive effect of fruits and vegetables with high levels of inorganic NO_3. Beetroot contains high levels of inorganic NO_3 and NO_2 which can be reduced to NO, a bioactive molecule capable of inducing vasodilation in endothelial tissues. Dietary nitrate can be reduced to NO_2 by oral bacteria from NO_3 reductase. The NO_2 released in the stomach reacts with low pH to form NO which easily enters into the bloodstream. This NO is capable of inducing vasodilation in the blood vessels. Also, NO_2 in the stomach can be absorbed into the duodenum which may contribute to the production of NO. Endogenously, NO can also be formed via the arginine and O_2 pathways in reactions catalyzed by NO synthase. The increase in NO contributes to muscle relaxation and vasodilation in the endothelium via different cellular mechanisms, thereby leading to the reduction of blood pressure (Jakubcik et al., 2021).

Recent studies have shown that NO_3 supplied by beetroot juice is capable of reducing blood pressure compared to NO_3 in a salt form. There are indications that NO_3 supplied through beetroot juice can interact with other minerals, vitamins, and phenolic compounds which may induce intense pressure and vascular responses. The study of Hobbs et al. (2012) revealed that beetroot showed a significant hypotensive effect at a low dose. Also, Jajja et al. (2014) also reported that beetroot juice concentrates also reduced daily systolic pressure in older overweight subjects after three weeks. The study of Jakubcik et al. (2021) showed that beetroot juice rich in nitrate did not exhibit any effect on systolic and diastolic blood pressure in healthy adults. However, the same study revealed that beetroot juice significantly reduced mean arterial pressure. Furthermore, a systematic review conducted by Ocampo et al. (2018) revealed that beetroot juice supplementation reduced diastolic and systolic blood pressure. Results from the systematic review showed that beetroot juice supplementation may be an effective adjuvant therapy.

3.3.5 Dairy Alternative Beverages: Scientific evidence has shown an inverse relationship between the intake of milk or milk products (dairy) and blood pressure. (McGrane et al., 2011). Dairy milk and dairy product intake are now promoted in public health plans around the world and are seen as essential in human nutrition (Walther et al., 2022). On the one hand, there is a trend toward replacing fresh milk with more heavily processed dairy products such as sweetened drinks or fermented milk such as yogurt, sour milk, or cheese (Walther et al., 2022). Moreover, talks on sustainability and carbon footprint have resulted in criticisms of the environmental implications of animal products, promoting a transition toward a plant-based diet in the general community, rather than only hardcore vegan customers (Espinosa-Marrónet et al., 2022). The prevalence of lactose intolerance is becoming better well-known, which often leads to reduced conventional dairy consumption despite the availability of lactose-free dairy products and the substitution of dairy milk with plant-based drinks (Aydar et al., 2020). People who are allergic to milk protein may benefit from plant-based protein sources. Studies have also corroborated that dairy consumption could positively impact blood pressure (Thorning et al., 2016).

Antihypertensive activity of bioactive compounds of dairy alternatives: Bioactive peptides generated from milk proteins are food components that retain numerous biological

features and have therapeutic effects, in addition to their nutritional value (Marcone et al., 2016).

Angiotensin-converting enzyme is a multifunctional enzyme that plays a central role in regulating endogenous pathways that regulate blood pressure, such as the renin-angiotensin and bradykinin systems (Guang et al., 2012). Milk proteins contain several ACE inhibitory peptides (Marcone et al., 2016). Several ACE inhibitory peptides decreased blood pressure in a dose-dependent manner after intravenous or oral administration in spontaneously hypertensive rats and hypertension humans (Marcone et al., 2016). Interestingly, there was little influence on normotensive patients' blood pressure, indicating that hypotension is an improbable adverse effect. As a result, ACE inhibitory peptides may be utilized to treat moderate hypertension or as a complement to standard medication. Furthermore, *in vivo* studies revealed that ACE activity was lower in the aortas of hypertensive rats after oral administration of fermented milk compared to a control group, indicating that these peptides were absorbed without further cleavage by digestive enzymes, reached the abdominal aorta, and exerted antihypertensive activity (Marcone et al., 2016). Antihypertensive peptides may also affect blood pressure via methods other than ACE inhibition. Endogenous vasodilators, such as prostaglandins and nitric oxide, are released into the bloodstream (Marcone et al., 2016). Because of their high antioxidant activity and fatty acid content, plant-based milk substitutes have been shown to reduce the risk of cardiovascular disease, atherosclerosis, cancer, and diabetes (Sethi et al., 2016; Aydar et al., 2020). Emerging studies on the role of the microbiome may shed light on the ability of plant-based diets to modify inflammatory indicators and support cardiovascular health in the long term (Ramsing et al., 2023; Tomovaet al., 2019).

4.0 Conclusion:

Functional beverages have emerged as a novel avenue for addressing hypertension, offering a holistic approach to high blood pressure management. By harnessing the potential of bioactive compounds from various sources, these beverages contribute to the diverse toolkit available for individuals seeking to mitigate the risks associated with hypertension. It's important to note that functional beverages should not replace medical advice or prescribed treatments, but they can complement a holistic approach to managing hypertension. Several experimental investigations have explored the antihypertensive activity of tropical plants however, few of these studies have been translated into useful products such as functional beverages. Further work is encouraged in developing functional beverages to produce standardized and reproducible products while maintaining their sensory appeal and efficacy. Also, there is a need for robust clinical trials and standardized methodologies to validate their efficacy. This review thus guides future research directions and underscores the potential of functional beverages as a modality for promoting cardiovascular health and managing hypertension.

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African Star Apple Supplemented Diet Prevents Potassium Bromate-induced Oxidative Stress in Rat's Brain

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Abstract
African Star Apple (ASA), *Chrysophyllum albidum* is a seasonal fruit with enormous economic potential and natural antioxidants. Its peel is a rich source of fibre, minerals and phytochemicals required to maintain cell viability and protection of neural cells from inflammation and oxidative stress associated with aging and brain diseases. Twenty-five male Wister rats were grouped into five and treated for 7 days as follows: Group 1(basal diet), Group 2 (10mg/kg $KBrO_3$), Group 3 (10mg/kg $KBrO_3$ + 2000 mg/kg ascorbic acid + basal diet), Group 4 (10mg/kg $KBrO_3$ + supplemented diet) and Group 5 (Supplemented diet). Animals were euthanized, whole brain harvested, homogenized and used for biochemical analysis. Administration of $KBrO_3$ increased malondialdehyde (MDA) level and acetylcholinesterase (AChE) activity significantly ($p<0.05$) while decreasing nitric oxide (NO) level, superoxide dismutase (SOD) and catalase activities in the brain however, groups whose diets were supplemented with ASA peels were able to cause a reversal to these parameters. This implies that ASA supplemented diets could be utilized for the maintenance and treatment of neurological and brain disorders occurring as a result of oxidative stress.

Key words: Oxidative stress, potassium bromate, antioxidants, supplemented diet, African Star Apple.

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Introduction

The African Star Apple, also known as, *Chrysophyllum albidum* belongs to the family *Sapotaceae*. It is a seasonal fruit with great economic (Ibrahim *et al*., 2017) and industrial use (Adzinyo *et al.,* 2015), that grows in diverse ecozones in Nigeria, Uganda and Niger Republic (Agbaje *et al*., 2020). In Nigeria, it is also known as "agbalumo" in the South-West and "Udara" in the South-East. *C. albidum* been a rich source of natural antioxidants have been established to promote health by acting against oxidative stress-related diseases such as diabetes, cancer and coronary heart diseases (Arueya and Ugwu, 2017). The fruit pulp has been reported to contain significant amount of ascorbic acid, vitamins, iron, fat, food flavours, carbohydrates and mineral elements. The fruit's peel is a rich source of fibre and mineral while the seed shell pericarp has been reported to be a good source of carbohydrates and minerals (Ibrahim *et al*., 2017).The high pectin content in *C. albidum* is suggestive of its vast medicinal benefit as well as its detoxifying action and effectiveness in diarrheal therapy however, there is a significant gap in its neuroprotective property in disease conditions related to oxidative stress and neuro-inflammation in experimental animals (Ajayi *et al*., 2020). Phytochemicals are required to maintain cell viability and protection of neural cells from inflammation and oxidative stress associated with aging and brain diseases (Cerbo *et al*., 2020).

Increased hippocampal neurogenesis by diet has been linked repeatedly to improved cognition performance, brain plasticity and mental health (Aryal *et al*., 2019). Also, there has been expanding evidence showing strong connection between poor dieting, cellular aging and cognitive impairment failure (Betts *et al*., 2016).

Potassium bromate ($KBrO_3$) is a well-known flour improver that act as a maturing agent (Mode *et al*., 2023). It is a food additive that gives strength and elasticity to dough and has

been in use for over 90 years (Oloyede and Sunmonu, 2009). $KBrO_3$ has also been found useful in the production of beer, cheese, fish paste products; and also, in pharmaceutical and cosmetic industries (Ahmad and Mahmood, 2016). The International Agency for Research on Cancer (IARC) has classified potassium bromate as a possible human carcinogen (group 2B) and its application in food processing has been banned in several countries including United Kingdom in 1990, Nigeria in 1993 and Canada in 1994 (Oloyede and Sunmonu, 2009). The biotransformation of $KBrO_3$ leads to the generation of free radicals which causes oxidative damage to essential cellular macromolecules, leading to nephrotoxicity and multiple organ toxicity in experimental animals (Hassan *et al*., 2019).

Materials and Methods

Sample Collection and Preparation

African Star Apple was purchased from a local market in Isara, Remo-North Local Government, Ogun State, Nigeria. It was identified and authenticated at the herbarium, University of Ibadan, Nigeria with voucher number ULH-23224. The peel was air dried, grounded and stored in an air-tight container for diet formulation.

Diet Formulation

Diet was formulated according to the method of Akinyemi, 2014 with a brief modification as follows: basal diet (BD) contained 13.68 g of casein, 10 g of corn oil, 3 g of vitamin premix, 2 g of sucrose and 71.32 g of corn starch while the supplemented diet (SD) contained 13.68 g of casein, 10 g of corn oil, 3 g of vitamin premix, 2 g of sucrose, 51.32 g of corn starch and 20 g of ASA peel.

Experimental Animals

Twenty-five (25) male Wister rats with an average weight of 110-200 g were obtained from the Animal House of Olabisi Onabanjo University teaching Hospital (OOUTH) and allowed to acclimatize for seven (7) days under standard conditions of 12 hours light/dark cycle with access to water and food *ad libitum*. Thereafter they were grouped into five as follows:

Group 1 (rats were given distilled water + BD); Group 2 (rats were given 10 mg/kg $KBrO_3$ + BD); Group 3 (rats were given 10 mg/kg $KBrO_3$ + 2000 mg/kg ascorbic acid + BD); Group 4 (rats were given 10 mg/kg $KBrO_3$ + SD); Group 5 (SD).

After seven days, the animals were decapacitated by cervical dislocation under light ether, whole brain was harvested, rinsed in normal saline, weighed and a 10% homogenate was prepared. The homogenate was centrifuged at 3000 rpm for 15 minutes and stored in the refrigerator for further analysis.

Biochemical Analysis

Lipid peroxidation was detected by measuring the MDA formed according to the method described by Varshney and Kale, (1990). Catalase activity was determined according to the method described by Caliborne, (1984). SOD was determined according to the method described by Misra and Fridovich, (1972). NO level was determined according to the method described by Green *et al*., 1982. AChE was determined according to the method of Ellman *et al*., 1961. All data are expressed as mean ± standard error of mean (SEM). Statistical analysis was carried out using Standard Package for Social Sciences (SPSS) software version 20, IBM software, USA. Statistical significance was determined by one-way analysis of variance (ANOVA), followed by Duncan's test. $p < 0.05$ represented a significant difference in all experimental values.

Results

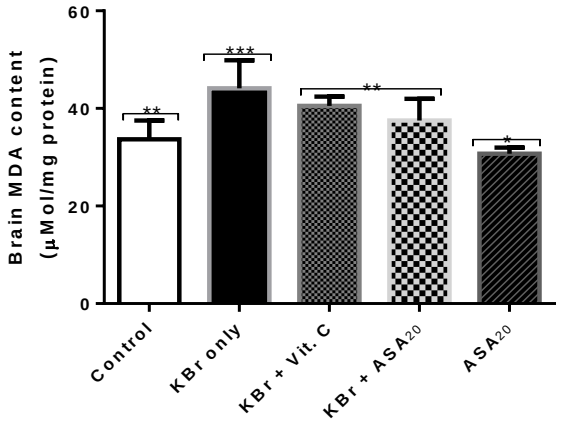

***Figure 1**: Effect of African Star Apple peel supplemented diet on brain malondialdehye content of rats exposed to potassium bromate-induced oxidative stress. Results are expressed as mean ± SEM (n=10). Bars with different asterisks are significantly different (p<0.05).*

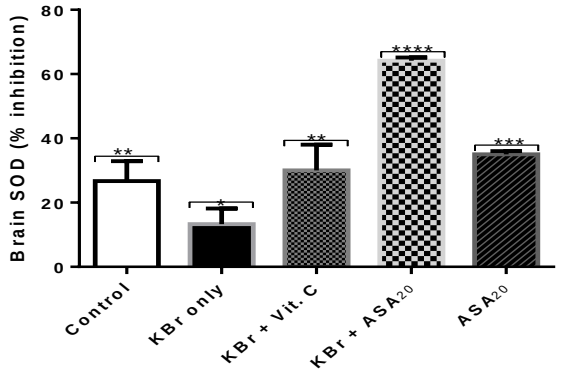

***Figure 2**: Effect of African Star Apple peel supplemented diet on brain Superoxide Dismutase of rats exposed to potassium bromate-induced oxidative stress. Results are expressed as mean ± SEM (n=10). Bars with different asterisks are significantly different (p<0.05).*

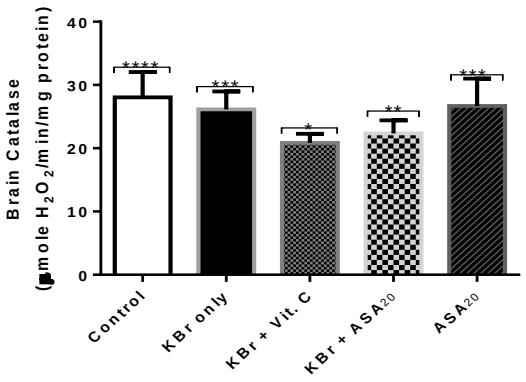

***Figure 3**: Effect of African Star Apple peel supplemented diet on brain Catalase of rats exposed to potassium bromate-induced oxidative stress. Results are expressed as mean ± SEM (n=10). Bars with different asterisks are significantly different (p<0.05).*

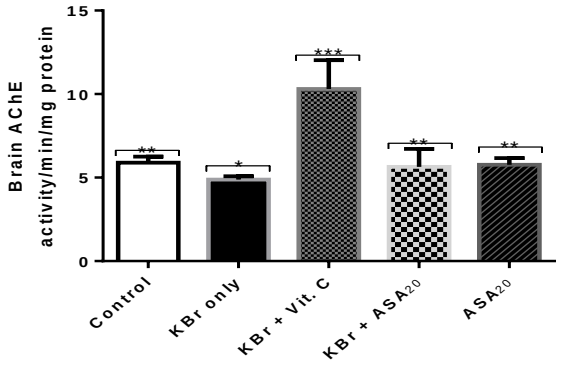

***Figure 4:** Effect of African Star Apple peel supplemented diet on brain Acetylcholinesterase of rats exposed to potassium bromate-induced oxidative stress. Results are expressed as mean ± SEM (n=10). Bars with different asterisks are significantly different (p<0.05).*

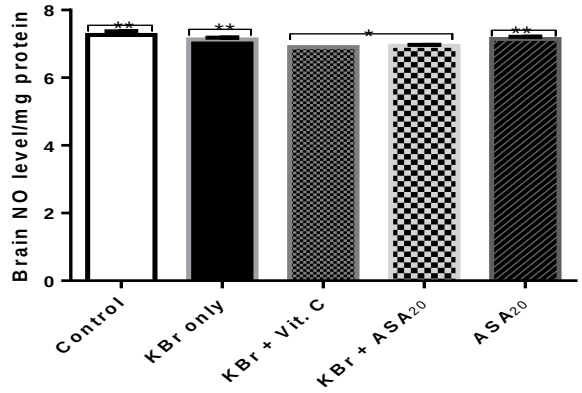

***Figure 5**: Effect of African Star Apple peel supplemented diet on brain Nitric oxide level of rats exposed to potassium bromate-induced oxidative stress. Results are expressed as mean ± SEM (n=10). Bars with different asterisks are significantly different (p<0.05*

Discussion

Oxidative stress arises from the overproduction of free radicals due to an imbalance in antioxidant production by cells. Potassium bromate is a major food additive and water disinfection by-product is able to induce oxidative stress in living cells (Ajarem *et al*., 2016). Natural products especially from plant sources have the ability to reduce oxidative stress due to their antioxidant capability (Agnihotri *et al.,* 2020). MDA, a secondary product of lipid peroxidation, is a marker of oxidative tissue damage (Gulcin and Beydemir, 2013). Elevated level of MDA is an indicator of oxidative damage and causes functional degradation due to increased oxidative stress (Reckziegel *et al*., 2016; Gebaly *et al*., 2012). In the present study, administration of $KBrO_3$ increased MDA level significantly ($p<0.05$) but treatment with ASA supplemented diet led to its reduction as shown in Figure 3.1. This is in line with the work of Josiah *et al*., 2012 who reported a similar increase in brain MDA level of rats administered with $KBrO_3$ but decreased upon treatment with ethanolic extract of *Vernonia amygdalina.* In another study by Agu *et al.,* (2023) the same trend was observed as $KBrO_3$ increased MDA level in *Rattus norvegicus* brain and was reversed when *Allium cepa* was administered. The antioxidant enzymes, SOD and Catalase were reduced significantly ($p<0.05$) upon administration of $KBrO_3$ as shown in Figures 3.2 and 3.3 respectively but administration of ASA supplemented diet led to an increase in SOD inhibition while the catalase activity reduced. The observed increase in SOD is in line with the discovery of Agu *et al.,* 2023.

The AChE activity increased significantly ($p<0.05$) upon administration of $KBrO_3$ and decreased in the group treated with ASA supplemented diet as show in figure 3.4. This agrees with the findings of Saad *et al*., (2017) who discovered that $KBrO_3$ caused an increase in AChE activity while a co-treatment with vanillin reversed that effect. ASA supplemented diet appears to have a good memory booster effect by decreasing AChE activity in rats compared with those exposed to $KBrO_3$ only. This could be as a result of bioactive substances such as flavonoids, polyphenols etc present in ASA peel as they have been shown to neutralize free radicals by crossing the blood-brain barrier to protect the brain and nervous system. Increased AChE activity leads to an increased acetylcholine breakdown and ultimately to a cholinergic deficit which is often a characteristic feature of memory disorders, disorientation etc (Shihana *et al*., 2019).

NO is a proinflammatory mediator which induces inflammation when overproduced (Smallwood *et al*., 2018). NO level in this study remained on the same level in animals administered with $KBrO_3$ only when compared with control animals but treatment with ASA supplemented diet caused a reduction in NO level as shown in Figure 3.5. This implies that the supplemented diet was able to reduce inflammation when administered to $KBrO_3$ exposed rats.

Conclusion

The ability of ASA supplemented diet to reduce MDA content, catalase activity, NO level and AChE activity while increasing the activity of SOD in the brain indicates that it could be a potential diet used for the maintenance and treatment of neurological disorders. However, extensive research on the molecular and cellular interventions of ASA on specific neurological disorders should be investigated.

Acknowledgement

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Declaration of conflict of interest

No conflict of interest is associated with this research work.

Author's contribution

A.O. Osifeso: Conceptualization, Methodology, Supervision and Writing of manuscript. T.M. Sefiu: Investigation. A.F. Kareem: Supervision. O.E. Olajide: Supervision.

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Nutritional Composition of Oven-Dried Eggs Produced by Laying Bird-Fed Sweet Orange Peel-Based Diet

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Abstract
A total of ninety-six point-of-lay Isa-Brown birds were randomly distributed to four experimental diets: control diet (0.00% dried *Citrus sinensis* peel (DCSP)), diet B (2.50% DCSP), diet C (5.00% DCSP), and diet D (7.50% DCSP). The birds were fed 100 grams of feed for three weeks, which was increased to 110g for the remaining experimental period, which lasted for four months. Eggs were collected thrice daily and in the second month of egg production, six eggs collected across the treatment groups were subjected to proximate analysis. The crude protein, ash, crude fiber, and nitrogen-free extract of the egg yolk powder were significantly influenced ($P<0.05$) by dietary DCSP except for the moisture content and metabolizable energy. All parameters observed were affected ($P<0.05$) by dietary DCSP except for the nitrogen-free extract and metabolizable energy in the albumen powder. Likewise in the whole egg powder, the crude protein, ash, crude fat, and nitrogen-free extract were significantly influenced ($P<0.05$) by dietary DCSP except for the moisture and metabolizable energy content. The crude fat and ash content of the different egg components decreased with the inclusion of DCSP into the diet of the birds while the moisture content of the egg components was in safe range to prevent spoilage over a long period in humid conditions. Conclusively, DCSP can be utilized in the diet of laying birds up to 7.50% for the enrichment of laying hen's egg.

Keywords: Citrus peel, egg, proximate composition, laying bird

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INTRODUCTION

Eggs are a good source of high-quality proteins, vitamins, and other bioactive nutrients such as lecithins and carotenoids (Andersen, 2015). They are recommended as part of a healthy diet in the 2015–2020 US dietary guidelines. Still, the intake of eggs and egg yolk, especially by the adult group, has declined due to the high amount of cholesterol, which is a dietary risk factor for cardiovascular health (Zhuang *et al.*, 2021). Eggs, like any individual food, are usually consumed as part of an overall diet, which can influence total cholesterol and saturated fat intake thereby predisposing adults to cardiovascular-related diseases. Cardiovascular disease is one of the major causes of death globally (WHO, 2022). Hence, dietary cholesterol has been limited to below 300 mg/day with specific recommendations to restrict egg consumption, which is high in cholesterol, to a maximum of three eggs per week (Carter *et al.*, 2023) as eggs are high in cholesterol but low in saturated fat, with an average large whole egg (50 g) containing 244 mg of cholesterol but only 1.2 g of saturated fat (FSANZ, 2014).

Nutrient profiling is essential for ranking food according to their nutritional composition for reasons related to preventing disease and promoting health. The proximate composition of food provides information for product development, quality control (QC), or regulatory purposes. However, the bird species, diet, or environment where the laying bird is being raised could influence the proximate composition of eggs. Sweet orange peel contains bioactive compounds with varying bioactive compounds that positively lower cholesterol

content (Abbasi *et al.,* 2015). Also, the drying form of processing removes water and allows for better storage of egg solids and their transportation. Therefore, eggs can be dried and processed into egg powder during the period of peak production, stored, and released to the market when production falls or during scarcity provided the nutrient quality of the egg powder is unaltered. The acceptability of this dry egg form depends on its quality. Therefore, this study aims to determine the effects of oven drying on the nutritional properties of whole eggs, egg yolk, and egg albumen.

MATERIALS AND METHOD

Fresh *Citrus sinensis* peels were gathered from local vendors at different markets in Akure, Southwestern Nigeria, and sundried until they were crispy and ground with the aid of a hammer mill. Ninety-six Isa-Brown point-of-lay birds procured from reliable sources were randomly assigned to four different diet groups (A, B, C, and D) comprising 0.0, 2.50, 5.0 and 7.50% dried *Citrus sinensis* peel (DCSP) on arrival in a complete randomized design. The diets were formulated according to (NRC's, 1994) nutritional recommendations with DCSP utilized as a substitute for wheat offal. The diet groups were replicated 3 times comprising 8 birds per replicate (24 birds/treatment group). The birds were provided with 100g of feed in the first 3 weeks of the experiment after which it was increased to 110g till the end of the three weeks of the experiment daily. Pre-lay diet was introduced initially to the birds as shown in Table 1.0 but at 10% hen-day production, the experimental diet as shown in Table 2.0 was fed to the birds. Fresh clean water was provided for the birds copiously and recommended medication was provided when due. Eggs were collected three times daily (morning, afternoon, and night). At the end of the 2nd month of egg production, 6 eggs were collected from each treatment group. The eggs were carefully deshelled and homogenized. The samples were oven-dried at 40^{o}C using (UNISCOPE Oven, SM 9052 Laboratory Incubator, Surgifriend Medicals, England) and allowed to cool. The egg flakes were scooped, milled, and then sieved. The nutritional quality of the egg and its components were assessed using their proximate compositions as a guide. The AOAC (2005) methods were used in determining the moisture content, while the ash content was determined by the furnace method. The crude protein content was determined using the Kjeldahl method. The fat content was determined using ether extraction by the reflux soxhlet method, while the carbohydrate was calculated by difference and the food energy values (FEV) estimated by the method described by (Osuagwu, 2008). All data obtained were subjected to compare means using the Statistical Package for Social Sciences (SPSS,2017) version 21.0.

Table 2: Gross composition (g/100g) of the experimental diets for pullet at the laying phase (23–40 weeks of age)

Ingredients (%)	**Composite Sweet Orange Peel (CSOP) levels (%)**			
	0.00	**2.50**	**5.00**	**7.50**
Maize	54.00	54.00	54.00	54.00
DCSP	0.00	2.50	5.00	7.50
Wheat Offal	8.00	5.50	3.00	0.50
Groundnut Cake	9.00	9.00	9.00	9.00
Soybean Meal	18.00	18.00	18.00	18.00
Limestone	9.70	9.70	9.70	9.70
Di-Calcium Phosphate	0.50	0.50	0.50	0.50
Premix	0.25	0.25	0.25	0.25
Methionine	0.15	0.15	0.15	0.15
Lysine	0.15	0.15	0.15	0.15
Salt	0.25	0.25	0.25	0.25
Total	**100**	**100**	**100**	**100**
Calculated Nutrient:				
Crude Protein (%)	17.17	17.02	16.88	16.73

Metabolizable Energy (kcal/kg)	2646.20	2689.85	2733.50	2777.15
Crude Fibre (%)	4.19	4.19	4.19	4.19
Calcium (%)	3.61	3.61	3.61	3.60
Phosphorus (%)	0.41	0.41	0.40	0.39
Lysine (%)	0.83	0.81	0.78	0.76
Methionine (%)	0.45	0.44	0.44	0.43

RESULTS

Table 3 shows the proximate composition of the yolk, albumen, and whole egg powder. DCSP significantly influenced (P<0.05) the dry matter, crude protein, ash, crude fat, and nitrogen-free extract (NFE) contents of the yolk powder and whole egg powder except for the moisture content and metabolizable energy. The moisture content range recorded in the different egg components was 9.94±0.18% to 13.63±0.17%. In the albumen powder, the NFE and metabolizable energy were unaffected by the dietary inclusion of *Citrus* peel into the feed of laying birds. Significant differences (P<0.05) were also observed in the crude protein, ash, crude fat, and Nitrogen-free extract of the whole egg powder. An increase was noticed in the crude protein contents of the yolk powder when compared with the control group. The values varied from 30.27±0.00% in eggs collected from the control group to 33.45±0.38% respectively in eggs of birds fed 7.50% CSOP. In the yolk powder, the ash content of birds gradually declined from 4.98±0.13% in eggs collected from the control group to 2.61±0.05% in eggs of birds fed diet D (7.50%). Although the ash content of the albumen powder did not follow a regular pattern, a decreasing trend was noticed in the crude fat content of the albumen powder of eggs from the control diet (5.34±0.17) to 1.72±0.21% in the albumen powder of eggs collected from 7.50% DCSP dietary group.

Table 3: Proximate Composition of egg yolk, albumin, and whole egg from Isa-Brown layers fed varying levels of dietary DCSP

	Parameters (%)	Diet A	Diet B	Diet C	Diet D	P-Value
Yolk	Moisture content	10.50±0.47	10.33±0.21	10.43±0.19	11.06±0.39	0.20
	Crude protein	30.27 ± 0.00^{b}	32.51 ± 0.40^{ab}	31.06 ± 0.44^{ab}	33.45 ± 0.38^{a}	0.00
	Ash content	4.98 ± 0.13^{a}	4.92 ± 0.30^{a}	4.30 ± 0.24^{b}	2.61 ± 0.05^{c}	0.01
	Crude fat	41.34 ± 0.44^{a}	41.89 ± 0.11^{a}	38.91 ± 0.18^{b}	37.75 ± 0.46^{c}	0.00
	NFE	23.41 ± 0.10^{ab}	20.68 ± 0.01^{b}	25.73 ± 0.25^{a}	26.19 ± 0.42^{a}	0.01
	Metabolizable Energy	6.25±0.95	6.55±0.72	6.31±0.50	6.62±1.09	0.21
Albumen	Moisture content	13.32 ± 0.17^{a}	12.43 ± 0.07^{a}	9.94 ± 0.18^{b}	13.63 ± 0.17^{a}	0.04
	Crude protein (%)	34.93 ± 0.00^{b}	34.79 ± 0.00^{b}	33.85 ± 0.37^{b}	35.77 ± 0.41^{a}	0.02
	Ash content (%)	5.32 ± 0.24^{b}	6.23 ± 0.24^{a}	3.75 ± 0.02^{c}	5.08 ± 0.19^{b}	0.00
	Crude fat (%)	5.34 ± 0.17^{a}	3.64 ± 0.07^{b}	3.72 ± 0.09^{b}	1.72 ± 0.21^{c}	0.00

	NFE (%)	54.41±2.55	55.34±1.52	58.68±2.11	57.43±0.59	0.64
	ME (MJ/kg)	6.10±0.34	6.03±0.04	5.95±0.70	6.14±0.52	0.20
Whole Egg	Moisture content	11.37±0.01	12.46±0.17	12.94±0.04	12.39±0.52	0.10
	Crude protein	29.86±0.40^{a}	30.05±0.00^{a}	26.05±0.00^{b}	25.85±0.27^{b}	0.00
	Ash content	3.85±0.08^{c}	5.32±0.08^{b}	7.26±0.17^{a}	3.55±0.08^{c}	0.00
	Crude fat	27.07±0.29^{a}	25.92±0.00^{b}	23.42±0.21^{b}	14.87±0.00^{c}	0.01
	NFE	39.22±1.83^{c}	38.71±2.15^{c}	43.27±2.28^{b}	55.73±3.78^{a}	0.04
	ME (MJ/Kg)	5.90±0.75	5.89±1.25	5.28±0.37	5.13±0.54	0.59

a, b, c = Means with different superscripts on the same row are significantly (P<0.05) different
Values are means ± standard error; Diet A: 0.00% Citrus peel, Diet B: 2.50% Citrus peel, Diet C:5.00% Citrus peel, Diet D: 7.50% Citrus peel, NFE – Nitrogen-free extract, ME- metabolizable energy, ME = 10 x (3.5 x % crude protein) + 8.5 x %crude fat + 3.5 x %Nitrogen-free extract;1 Kcal = 0.004184MJ

DISCUSSION

Usually, the moisture contents of food above 15% encourage microbial activities which speed up spoilage of food (Hassan *et al.*, 2008). Although the moisture content of the egg parts (albumen, yolk, and whole egg) collected across the treatment groups was higher than the values reported by (Adegbenro, 2020) for whole egg, albumen, and yolk powder, the values obtained were still within the safe range. This indicated that there is the possibility of storing egg powder for an extended period in a humid environment as reported by (Oladeji *et al.*, 2019). The values varied from 30.27±0.00% to 33.45±0.38% respectively which differ greatly from the findings of (Rehault-Gobert *et al.*, 2019) who stated that the concentration of protein is, on average 12.5g per 100g of whole raw fresh egg while egg yolk with its vitelline membrane and egg white contains 15.9g protein and 10.90g protein per 100g respectively. However, it was lower than the values reported by (Ndife *et al.*, 2010) and (Adegbenro, 2020) for whole egg and albumen (45.21% and 62.04% respectively) but was in close range with the values reported for egg yolk powder (26.20% - 32.21%). The crude fat of the whole eggs progressively declined as the level of DCSP incorporation into the diet increased while the reverse was observed in the NFE content of the whole egg powder. This could be attributed to the presence of hesperidin, a bioflavonoid that helps to eliminate or reduce fat.

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DECLARATION OF CONFLICT OF INTEREST

The authors declare no competing interest

AUTHOR'S CONTRIBUTION

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Haematology and antioxidant profile of two breeds of meat-type chickens fed dietary inclusion of Composite sweet orange peel

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Abstract

Haematological and antioxidant profiling are very important physiological testimonials that reveal the welfare, health and general thriftiness of livestock to any dietary intervention directed towards their production and management. This experiment was thus conducted to investigate the response of two meat-type chickens- the Arbor Acre and Cobb 500 breeds to varied dietary inclusion of composite sweet orange peels (CSOP). Four treatment diets namely the Control (0.00%), 2.50%, 5.00% and 7.50% CSOP designated as T1, T2, T3 and T4 respectively were formulated and used in an-eight-week study on 192 birds, randomly allocated to the four dietary treatments and replicated three times in a 4 x 2 factorial experiment using a Completely Randomised Design. Haematological and antioxidant parameters were determined from blood sampled on the birds at the end of the experiment. Results showed significant ($p<0.05$) treatment effect on haemogram parameters like erythrocyte sedimentation rate (ESR), packed cell volume (PCV), red blood corpuscles (RBC), haemoglobin (Hb) concentration, mean corpuscular volume (MCV) and mean corpuscular haemoglobin (MCH). The ESR, MCV and MCH decreased with increase in dietary CSOP while PCV, RBC and Hb showed an upward trend. There was neither treatment, breed nor their interactive effect on the antioxidant parameters that were investigated but an upward trend with increase in dietary CSOP was observed. The decreased ($p<0.05$) ESR with corresponding increase in PCV, RBC and Hb confirmed the blood-boosting potential of Dietary CSOP. Conclusively, the utilisation of CSOP in the production of these breeds of broiler chickens would not pose any deleterious threat to their health and wellbeing as far as haematology and antioxidant parameters are concerned.

Keywords: Anti-oxidants, broilers, composite sweet orange peels, haematology.

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Introduction

Haematological parameters could monitor an animal's health condition, including their response to environmental and nutritional stress (Iyaode *et al.*, 2020). Several studies have reported mixed findings on the influence of dietary sweet orange peels on RBC indices. Amaga *et al.* (2019) observed no significant differences in RBC count, packed cell volume (PCV), or hemoglobin (Hb) concentration in broilers fed diets containing varying durations of water-soaked sweet orange peels (SOP), suggesting no detrimental effects on oxygen transport capacity. Similarly, Dinu *et al.* (2007) found no alterations in these parameters when sweet orange peels replaced 5% of the control diet. Conversely, Oluremi *et al.* (2020) reported a slight decrease in RBC count and Hb concentration in birds fed increasing sweet orange peel levels (up to 10%), though PCV remained unaffected. These discrepancies might be attributed to variations in sweet orange peels processing methods, dietary inclusion levels, and broiler breed differences. Orayaga *et al.* (2016) also reported a value range 1.76 $x10^6$/ml - 3.76 $x10^6$/ml of RBC but however reported that no significant ($P> 0.05$) was observed among the treatment means. Broiler finisher chickens in the sweet orange fruit peel meal (SOFPM) based diet groups had haematology variables satisfactorily comparable to the haematology variables of chickens in the maize-based diet group.

Citrus peels contain a lot of phyto-chemicals like hesperidins naringenin and hysteritine (Alireza *et al.,* 2018) which are potent anti-oxidants and when ingested are capable of potentiating the natural anti-oxidant enzymes like catalase, super-oxide dismutase (SOD) and glutathione in the body. Faiz *et al.* (2017), reported that catalase and SOD activity increased non-significantly as the amount of citrus waste in increased, while lipid peroxidase, glutathione peroxidase, and glutathione activities decreased up to 5% in citrus waste-fed broiler groups. However, Vitamin E and C activity, as well as glutathione activities and serum vitamin C levels, were found to be highest in birds fed citrus waste-based diets supplemented with enzymes (Abbasi *et al.,* 2015). According to Abd El Latif *et al.* (2023), antioxidant levels in broiler blood, such as SOD, catalase, and glutathione peroxidase increased as the amount of citrus waste in the feed increased. The study suggested that, the antioxidant action of orange peel meal could be attributed to the flavones present in them. One of the most significant flavanones to be extracted from orange peel, hesperidins, has demonstrated diuretic and antioxidant effects (Tirkey *et al.,* 2005). This experiment thus aimed to profile the haematological and anti-oxidant responses of Arbor Acre and Cobb 500 breeds of broilers fed varied levels of CSOP.

Materials and Methods

This experiment was carried out at the Teaching and Research Farm of The Federal University of Technology, Akure, Ondo State, Nigeria. The study location lies between latitude 7.49^{o} North of the Equator and 5.820^{o} East of the Greenwich Meridian in the humid tropical rainforest region. The climatic condition of Akure follows the pattern of southwest Nigeria and it has an average annual rainfall of about 1300mm and 1650mm and annual daily temperature ranging between 21^{0}C and 38^{o}C. Fresh sweet orange (*Citrus sinensis*) peels were collected from FUTA community, Ilara-Mokin, Iju, and Akure, Ondo State, Nigeria. The peels were subjected to sun-drying to reduce the moisture content to about 12% and were tagged as sun-dried composite sweet orange peels (CSOP) and stored in air-tight storage bags at room temperature for subsequent analyses.

A total of 200-day-old broiler chickens comprising one hundred (100) Arbor Acre and one hundred (100) Cobb500 breeds were purchased from a reliable hatchery out of which 192 birds were randomly allocated to four (4) dietary treatments with each replicated three times in a 4 x 2 factorial experiment using a Completely Randomized Design. Four on-farm feeds were formulated to contain 0% (control), 2.5%, 5.0%, and 7.5% inclusion level of Composite Sweet Orange Peel (CSOP). Each of the treatment had 48 birds comprising of 24 Arbor Acre and 24 cobb500 breeds with each treatment replicated three times to have 8 birds/replicate for Arbor Acre and Cobb 500 breeds respectively. The birds were raised on deep litter, standard management practices were observed, that is. vaccination and medication. Feed and drinking water were administered *ad libitum* throughout the experimental period of eight (8) weeks. The gross composition of the starter and finisher diets are presented in Tables 1 and 2.

Table 1: Gross Composition (g/100g) of Broiler Starter Diets (0– 4 weeks of age)

Ingredients (%)	**Composite Sweet Orange Peel (CSOP) levels (%)**			
	0.00	**2.50**	**5.00**	**7.50**
Maize	50.00	50.00	50.00	50.00
CSOP	0.00	2.50	5.00	7.50
Wheat Offal	7.50	5.00	2.50	0.00
Groundnut Cake	8.45	8.45	8.45	8.45
Soybean Meal	27.90	27.90	27.90	27.90
Fishmeal	1.30	1.30	1.30	1.30
Premix	0.15	0.15	0.15	0.15
Limestone	1.30	1.30	1.30	1.30
Bone meal	0.70	0.70	0.70	0.70

Methionine	0.10	0.10	0.10	0.10
Lysine	0.10	0.10	0.10	0.10
Salt	0.10	0.10	0.10	0.10
Vegetable oil	2.70	2.70	2.70	2.70
Total	**100.00**	**100.00**	**100.00**	**100.00**
Calculated Nutrient:				
Crude Protein (%)	20.94	20.74	20.55	20.36
Metabolizable Energy (kcal/kg)	3000.43	3019.94	3039.37	3058.84
Crude Fibre (%)	4.10	4.20	4.31	4.41
Calcium (%)	0.80	0.80	0.80	0.79
Phosphorus (%)	0.46	0.46	0.45	0.44
Lysine (%)	1.21	1.19	1.17	1.15
Methionine (%)	0.41	0.40	0.40	0.39

CSOP = Composite Sweet Orange Peel

Table 2: Gross Composition (g/100g) of Broiler finisher Diets (5-8 weeks of age)

Ingredients (%)	**Composite Sweet Orange Peel (CSOP) levels (%)**			
	0.00	**2.50**	**5.00**	**7.50**
Maize	51.40	51.40	51.40	51.40
CSOP	0.00	2.50	5.00	7.50
Wheat Offal	7.50	5.00	2.50	0.00
Groundnut Cake	18.15	18.15	18.15	18.15
Soybean Meal	15.00	15.00	15.00	15.00
Premix	0.15	0.15	0.15	0.15
Limestone	1.55	1.55	1.55	1.55
Bone meal	0.90	0.90	0.90	0.90
Methionine	0.10	0.10	0.10	0.10
Lysine	0.10	0.10	0.10	0.10
Salt	0.15	0.15	0.15	0.15
Vegetable oil	5.00	5.00	5.00	5.00
Total	**100.00**	**100.00**	**100.00**	**100.00**
Calculated Nutrient:				
Crude Protein (%)	18.80	18.60	18.41	18.21
Metabolizable Energy (kcal/kg)	3132.01	3151.48	3170.95	3190.42
Crude Fibre (%)	3.44	3.55	3.66	3.76
Calcium (%)	0.82	0.82	0.82	0.82
Phosphorus (%)	0.45	0.44	0.43	0.43
Lysine (%)	0.98	0.96	0.94	0.91
Methionine (%)	0.37	0.37	0.36	0.35

CSOP = Composite Sweet Orange Peel

At the end of 8 weeks, blood samples (2mls) from two birds from each replicate was collected after slaughtering into a sterile EDTA bottle and another set of blood sample were collected into a plain bottle without an anti-coagulant to prepare the serum for biochemical analyses. The haematological assay was carried out at the Department of Animal Production and Health Laboratory while the serum assay was carried out at the Department of Biochemistry Laboratory of The Federal University of Technology, Akure. Serum Antioxidant enzymes like catalase, super-oxide dismutase and glutathione were assayed through standard methods. All data collected were subjected to analysis of variance (ANOVA) with the aid of Statistical Package for Social Sciences (SPSS). Where significant difference was observed, the means were compared using Duncan's Multiple Range Test.

Results and Discussion

Table 3 shows the effect of diet, breed, and their interactions on haematological profile of two breeds of Meat-type chickens. Lymphocytes, Heterophil, Monocyte, Basophils and Eosinophils were not significantly (P> 0.05) different. Dietary effects on Erythrocyte Sedimentation Rate (ESR), PCV, RBC and haemoglobin were significantly (P< 0.05) different. Observed result showed that ESR was highest for birds on diet A (0.00% CSOP) inclusion level while birds on diet B (2.50% CSOP) showed the lowest observed value. Breed and interaction effects did not significantly (P> 0.05) influence the haematological profile of the birds.

The observed range of 2.50 to 4.17mm/hr in this study is within the range of 0.80 to 4.34mm/hr reported by Mitruka and Rawnsley (1977). The higher ESR observed in birds on diet A (control) when compared to those on sweet orange-based diets implied that the birds on diet A (control) are likely to have a higher rate of inflammation in the body than those on composite sweet orange-based diets (Pfafferotti *et al.* 1999). Hence, the utilization of the CSOP-based diets would help to prevent tissue inflammation in the prime cuts of the meat-type chicken, thus, production of a more wholesome carcass of the best quality. The higher PCV, RBC, and haemoglobin in all the CSOP diets relative to the control further supports the claims in the literature (Amaga *et al.*, 2019); Majekodunmi *et al.*,2022) of the blood-boosting potentials of dietary sweet orange peels.

Table 3: Haematological Profile of Two Breeds of Meat-type Chickens Fed Dietary Inclusion of Composite Sweet Orange Peel (CSOP)

Diet	Breed	ESR (mm/hr)	PCV (%)	RBC (10^6mm^{-3})	Hb (g/dl)	MCV (fL)	MCH (pg)	MCHC (%)	LYMP (%)	HET (%)	MON (%)	EOS (%)	BAS (%)
A		4.17^a	25.00^b	2.07^b	8.28^b	123.40^a	40.90^a	33.13	61.00	22.67	11.50	3.50	1.33
B		2.50^b	29.17^a	2.68^a	9.70^a	108.30^b	36.00^b	33.26	58.33	22.67	11.17	3.50	1.33
C		2.83^b	28.50^a	2.69^a	9.45^a	108.80^b	36.10^b	33.16	59.17	24.83	11.17	3.50	1.33
D		2.67^b	28.67^a	2.80^a	9.53^a	107.40^b	35.70^b	33.25	58.17	24.83	12.00	3.50	1.33
±SEM		0.41	1.09	0.16	0.37	3.80	1.30	0.03	0.83	0.92	0.69	0.24	0.20
P-Value		0.041	0.049	0.019	0.036	0.026	0.028	0.102	0.103	0.160	0.805	1.00	0.917
	Arbor Acre	3.33	26.83	2.49	8.92	115.10	38.20	33.22	59.92	23.83	11.33	3.42	1.50
	Cobb500	2.75	28.83	2.64	9.57	108.90	36.10	33.18	58.42	25.17	11.58	3.58	1.25
	±SEM	0.29	0.77	0.11	0.26	2.70	0.90	0.02	0.59	0.65	0.49	0.17	0.14
	P-Value	0.176	0.086	0.349	0.094	0.116	0.105	0.133	0.091	0.165	0.721	0.490	0.238
Diet X Breed													
A	Arbor Acre	4.33	24.33	2.00	8.07	125.30	41.50	33.14	61.33	22.67	11.00	3.33	1.67
	Cobb500	4.00	25.67	2.15	8.50	121.40	40.20	33.12	60.67	22.67	12.00	3.67	1.00
B	Arbor Acre	2.33	29.00	2.84	9.67	108.35	36.10	33.33	58.33	26.00	11.00	3.33	1.33
	Cobb500	2.67	29.33	2.52	9.73	108.20	35.90	33.18	58.33	25.33	11.33	3.67	1.33
C	Arbor Acre	3.33	27.33	2.56	9.07	113.00	37.50	33.17	60.33	22.33	12.33	3.33	1.67
	Cob 500	2.33	29.67	2.83	9.83	104.70	34.70	33.15	58.00	27.33	10.00	3.67	1.00
D	Arbor Acre	2.33	26.67	2.54	8.87	113.90	37.90	33.24	59.67	24.33	11.00	3.67	1.33
	Cobb 500	2.00	30.67	3.05	10.20	100.80	33.50	33.26	56.67	25.33	13.00	3.33	1.67
	±SEM	0.58	1.55	0.22	0.52	5.40	1.80	0.04	1.18	1.30	0.97	0.33	0.29
	P-value	0.502	0.680	0.327	0.661	0.662	0.675	0.220	0.562	0.167	0.184	0.685	0.253

^{ab} = Means on the same column but different superscripts are statistically significant (P< 0.05); ±SEM = ±Standard Error of Mean; ESR = Erythrocyte Sedimentation Rate; PCV = Packed Cell Volume; RBC = Red Blood Cell; Hb = Haemoglobin; MCV = Mean Cell Volume; MCH = Mean Cell Haemoglobin; MCHC = Mean Cell Haemoglobin Concentration; HET = Heterophil; MON = Monocyte; BAS = Basophil; EOS = Eosinophil; A = Diet with 0.00% CSOP; B = Diet with 2.50%CSOP; C = Diet with 5.00%CSOP; D = Diet with 7.50%CSOP.

Serum Antioxidant profile of Two Breeds of Meat-Type Chicken Fed Dietary Inclusion of Composite Sweet Orange Peels (CSOP).

Table 4 shows the effects of breed, diet and interaction on serum anti-oxidant profile of the two breeds of meat-type chicken fed dietary inclusion of composite sweet orange peel (CSOP). Dietary, breed and interaction effects were not significantly (P> 0.05) different in the three anti-oxidants investigated.

Table 4: Serum Antioxidant Profile of Two Breeds of Meat-type Chicken Fed Dietary Inclusion of Composite Sweet Orange Peel (CSOP)

Diet	Breed	Catalase (U/min/ml)	Superoxide Dismutase (U/ml)	Glutathione (mg/g)
A		7.28	78.47	4.93
B		9.25	78.52	4.85
C		10.67	78.95	4.50
D		10.82	81.90	4.40
±SEM		1.17	2.63	0.69
P-value		0.161	0.615	0.903
	Arbor Acre	9.42	77.10	4.82
	Cobb500	9.59	81.07	4.68
	±SEM	0.83	1.86	0.49
	P-value	0.887	0.152	0.625
Breed X Diet (Interaction)				
A	Arbor Acre	6.53	76.53	5.23
	Cobb500	7.50	81.41	4.63
B	Arbor Acre	8.32	79.87	5.50
	Cobb 500	10.17	77.17	4.20
C	Arbor Acre	11.08	72.17	4.25
	Cobb 500	10.25	81.73	4.50
D	Arbor Acre	11.73	79.83	4.43
	Cobb 500	10.90	83.97	4.57
	±SEM	1.66	3.73	0.97
	P-value	0.636	0.453	0.816

[abc]Means of the same column but different superscripts are statistically significant (P< 0.05); ±SEM = Standard Error of Mean; A = Diet with 0.00% CSOP; B = Diet with 2.50% CSOP; C = Diet with 5.00% CSOP; D = Diet with 7.50% CSOP.

The non-significant effect observed suggests that the birds were not subjected to any oxidative stress as a result of the inclusion of composite sweet orange peel in their diets. This observation agreed with the findings of Abd El Latif *et al.* (2023), who reported that Catalase and Superoxide dismutase activities increased non-significantly as the amount of citrus waste in broiler diets increased, while glutathione activities decreased up to 5% in citrus waste-fed groups. Behera *et al.* (2019) also reported that superoxide dismutase and catalase in broiler birds increased with increase in the level of citrus waste. The sweet orange peels probably contain some allelochemicals that helped to increase the blood antioxidant activity of the birds. Anagnostopoulou, *et al.* (2005) and Abd El Latif *et al.* (2023) reported that the antioxidant action of the orange peel meal could be attributed to the flavones present in them. Tirkey *et al.* (2005) reported that one of the most significant flavanones to be extracted from orange peel - hesperidins, demonstrate diuretic and antioxidant effects.

Conclusion

This study showed that no significant (P> 0.05) breed and interaction effects exist between Cobb500 and Arbor Acre for all the parameters measured. However, a tendentiously higher

numerical values were observed in the Arbor Acre breed in comparison with Cobb500 breed which suggest that Arbor Acre had better health profile than Cobb500. Some haematological parameters like the ESR, PCV, RBC and Hb were significantly ($P< 0.05$) influenced by the dietary inclusion levels of composite sweet orange peel in the diets of the birds. Observed trend showed that dietary inclusions of composite sweet orange peels were beneficial to the health profile of the birds by boosting their RBC content and anti-oxidant status.

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Effect of Blanching on the Antioxidant and Antihypertensive Properties (*In vitro*) of Shining Bush (*Peperomia pellucida* L.) Leaves

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Abstract
The antioxidant and antihypertensive properties of shining bush leaf has been reported but with dearth of information on the effect of culinary techniques. Therefore, investigation of the effect of blanching on the antioxidant and antihypertensive properties of leaf of shining bush (*Peperomia pellucida* L.) *in vitro* is the focus of this study. Aqueous extract (5 mg/ml w/v) of leaf of the shining bush (*Peperomia pellucida* l.) were carried out. Thereafter, the extracts were assayed for their total phenol and flavonoid contents, reducing property, 1,1-diphenyl-2-picrylhydrazyl (DPPH) and 2,2'-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid (ABTS) radicals scavenging abilities, Fe^{2+} chelating ability, and inhibition of Fe^{2+} and Sodium nitroprusside (SNP) induced lipid peroxidation reactions in rat's heart. In addition, the inhibitory effect of the extracts on angiotensin-1-converting enzyme (ACE) activity was also determined. The results showed that the raw leaf extracts contained higher amounts of total phenol and flavonoid compared to blanched leaf extracts. However, all the extracts significantly ($P<0.05$) inhibited ACE activity, scavenged (DPPH and ABTS) radicals, chelated Fe^{2+} and also inhibited Fe^{2+} and SNP-induced lipid peroxidation in rat heart (*in vitro*). Nevertheless, the blanched sample had the higher ACE inhibitory and antioxidant properties. This study has hence shown that blanching enhances the ACE inhibitory effects and antioxidant properties of shining bush leaves despite the reduction in phenol and flavonoid contents.
Keywords: ACE; Blanchiing; Antioxidant; Hypertension; Extract

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Introduction

Blanching also has different effects on the nutritional and bioactive composition of plants. Some studies revealed that blanching reduced the polyphenol and flavonoid contents, but improve the vitamins (A, B1, B2, C) and antioxidant activity (Sunjeet *et al.*, 2022; Thi *et al.*, 2022). Like dry leaves, blanched leaves retain higher carotenoids content, total reducing power, and water absorption capacity as major factors to improve the selection of blanched leaf form as a source of dietary antioxidants (Pierre *et al.*, 2017).

Hypertension is a common global health issue resulting in a rising burden of cardiovascular diseases and other morbidities due to its high prevalence of hypertension that leads to persistently high blood pressure (Ademiluyi *et al.*, 2016). While there are some genetic and environmental factors linked to high blood pressure, higher prevalence of cardiovascular diseases and other morbidities (Ademiluyi *et al.*, 2016). Essentially, the main contributors to high blood pressure are genetic and environmental; diet and exercise play a reversible role. Hypertension, a silent killer and the actual leading cause of death worldwide, is associated with a nearly linear increase of adverse

outcomes even at blood pressure levels below 120/80 mmHg (Ademiluyi *et al.,* 2016). The ideas of hypertension were first record in the early 1800s with reports of cardiac and renal damage caused by increased blood pressure which result to the introduction of diagnostic tools such as cuff sphygmomanometers (Subhana *et al.,* 2021).

Renin-angiotensin system (RAS) serves as a vital component of many physiological processes particularly, blood pressure regulations and fluid balance. ACE-inhibitors, Angiotensin-converting enzyme (ACE) inhibitors, drugs like perindopril and captopril are generally utilized to control the RAS, as well as deal with hypertension and cardiovascular diseases (Edward, 2021). According to research, diabetes is characterized by oxidative stress and disordered trace element metabolism promoted by ACEI or ARBs that contribute to oxidative stress and enhancing trace element levels in hypertensive hearts and renal tissue of rats (Tobias *et al.,* 2021). However, it has been shown that there is not much association between plasma concentrations of ACE2 among patients with cardiovascular disease or risk factors using ACE inhibitors/ARBs. This has led to suspicions regarding the connection between angiotensin receptor blockers (ARB) as well as angiotensin converting enzyme inhibitors (ACEI) with ACE2 which is the entry receptor for SARS-CoV-2. Several studies have confirmed that there is no definite relation between plasma concentrations of ACE2 among patients using Angiotensin Converting Enzyme Inhibitors/ACE inhibitors. (Yang-Ching and Tsen-Fang, 2022).

Also, it has been reported that one of the subunits of the RAS, the ACE2 is implicated in the regulation of pathological processes like hypertension and cardiac dysfunction and its expression and activity are reduced in some diseases such as T2DM and hypertension (Ashwin *et al.,* 2021). Interestingly, various works revealed that several plant extracts have been found to possess ACE inhibitory properties in vitro. Most of this property is due to their phytochemical profile, especially phenol (Zhang *et al.,* 2008). It is also noteworthy that oxidative stress is one of the main factors contributing to hypertension and other cardio vascular diseases. It is caused by an imbalance in the production of free radicals including reactive oxygen and nitrogen species which poses a danger to essential biomolecules comprised of lipids, proteins and DNA molecules (Ajila and Prasada, 2008). This damage has been associated with deterioration and manifestation of different forms of metabolic diseases such as hypertension (Manso *et al.,* 2008). Hence, controlling the oxidative stress is a vital part of handling hypertension and its related cardiovascular issues. Antioxidant consumption is another useful dietary intervention owing to its capability for countering the impact of oxidative stress (Schiffrin, 2010).

The plant known as shining bush or pepper elder scientifically called *Peperomia pellucida* was found by researchers to have relatively high antioxidant potency. The phytochemical composition of *P. pellucida* mainly consists of alkaloids, phenols, flavonoids, saponins, and tannins, with alkaloids being seen most dominant among all the category (Ibe-Diala and Igwe, 2022). It also possesses a high total flavonoid and polyphenolic contents and high antioxidant activities as well (Trann *et al.,* 2022). Studies conducted on the two different solvents and origin of the extract have shown that the plant possess good free radical scavenging activity. Specifically, the methanol and ethyl acetate extracts have provided noticeable antioxidant activities (Islamudin *et al.,* 2022). This makes suggested that *P. pellucida* having natural antioxidant substances, believed to be useful in the management of many diseases (Amirah *et al.,* 2020). The objective of this research is to determine the impact of blanching on the antioxidant and antihypertensive potentials of aqueous extract of the leaf of shining bush (*Peperomia pellucida* L.), which is found in Nigeria.

Materials and Methods

Materials

Sample Collection

Shining bush plants (*Peperomia pellucida* L.) were collected from a farm settlement in Ijan-

Ekiti, Ekiti State, Nigeria. The leaves were detached from their stems and divided into two groups: raw and blanched. The blanched leaves were treated with boiled water for five minutes. Both raw and blanched leaves were then pulverized and milled into powder form for further analysis. The samples were authenticated at the Department of Crop, Soil, and Pest Management, Federal University of Technology, Akure.

Chemicals and Reagents

All chemicals used in this study were of analytical grade and glass-distilled water was used.

Methods

Sample Preparation and Extraction

The leaves of *Peperomia pellucida* were thoroughly washed with running water and dried using a freeze-dryer. The dry samples were ground associated with a fine powder. The powdered samples were extracted with water at a concentration of 5 mg/ml (w/v) through maceration for 24 h at room temperature (Ruttoh *et al.,* 2009; Ogbole *et al.,* 2013) The filtered mixture after maceration comes out of muslin cloth and is a transparent decoction. The filtrate was filtered and then kept in the refrigerator for further analysis (Oboh and Rochas, 2007).

Total Phenol Content Assay

Total phenolics content was quantified as described by Singleton *et al.,* (1999) for the extracts. Generally, oxidations of suitable dilutions of the extracts were performed with 2.5 ml of 10% Folin-Ciocalteau's reagent (v/v), and also neutralized by addition of 2.0 ml of 7.5% sodium carbonate. The reaction mixture was incubated at 45 °C for 40 min and the absorbance was read at 765 nm using JENWAY 6305 model UV/visible spectrophotometer. Antioxidant activity in extracts amounted to total phenol content and was expressed as gallic acid equivalent (GAE).

Total Flavonoid Content Assay

A modified method from Meda *et al.,* (2005) was used for determination of total phenolics content by colorimetric assay. 0.5 ml of an appropriate dilution of the extract was combined with 0.5 ml methanol, 50 µl of 10% $A1C1_3$, 50µl 1 M potassium acetate, and 1.4ml water. After that, incubation was done for 30 min at RT. The absorbance of reaction mixture was measured at 415 nm by the use of UV/visible spectrophotometer [Jenway 6305 model] Then total flavonoid content was estimated in terms of QAE.

Determination of Reducing Property

Reducing power of the extracts was determined by the capacity of the extracts to reduce $FeCl_3$ as described by Oyaizu, (1986). In short, a 2.5 ml aliquot of extract was combined with 2.5 ml of 200 mM sodium phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricyanide. It was proceeded to mix followed by incubated at 50 °C for 20 min, then add 2.5 ml of 10% trichloroacetic acid. Centrifuge operation in 80–3 laboratory centrifuge at 650 rpm for 10 minutes was the parameter used in this study. For determining the total phenolic content, the supernatant (5 ml) was thoroughly mixed with 5 ml of water as well as 1 ml of 0.1% ferric chloride. The absorbance was measured at 700 nm using a UV/visible spectrophotometer (Jenway 6305 model). The ferric-reducing antioxidant property was subsequently calculated and expressed as ascorbic acid equivalent (AAE).

DPPH (1,1-diphenyl-2-picrylhydrazyl) Assay

The free radical scavenging ability of the extracts against DPPH (1,1-diphenyl-2-picrylhydrazyl) was evaluated as described by Gyamfi *et al.,* (1999). Briefly, a 1 ml aliquot of appropriately diluted extract was mixed with 1 ml of 0.4 mM DPPH in methanolic solution. The mixture was left in the dark for 30 minutes, and the absorbance was measured at 516 nm using a UV/visible spectrophotometer (Jenway 6305 model). The DPPH free radical scavenging ability was subsequently calculated.

ABTS^{*+}(2,2′-azinobis(3-ethylbenzthiazoline-6-sulphonate)) Assay

The antioxidant capacity of the extracts was measured as ascorbic acid equivalent antioxidant capacity (AAE) by their ability to scavenge 2,2′-azinobis(3-ethylbenzthiazoline-6-sulphonate) ($ABTS^{\bullet+}$) according to the method of Re *et al.,* (1999). $ABTS^{\bullet+}$ generation: ABTS solution 7 mmol/L was mixed with $K_2S_2O_8$ 2.45 mmol/L in water with 0.700 absorbance at 734 nm (ethanolic solution) by dark incubation for 16 h. 0.2 ml aliquot of suitably diluted extract was added to 2.0 ml of $ABTS^{\bullet+}$ solution, incubated for 15 min, read at 734 nm using a UV/visible spectrophotometer. ascorbic acid equivalent antioxidant capacity-tested was then performed.

Fe^{2+} Chelation Assay

Minotti and Aust (1987) method with a modification by Puntel *et al.,* (2005) was used to estimate the metal Fe^{2+} chelating ability of the extracts. New 500 µM $FeSO_4$ (150 µl) was used in the reaction mixture of 168 µl of 0. Consequently, the reaction contents included: 1 M Tris–HCl (pH 7.4), 218 µl saline, and different volumes of the extracts (0–25 µl). The reaction mixture was allowed to incubate for 5 minutes before adding 13 µl of 0. 25% 1,10-phenanthroline (w/v). The absorbance was then recorded at 510 nm with the help of UV/visible spectrophotometer (Jenway 6305 model) and thus percentage Fe^{2+} chelating ability was determined.

Inhibition of Lipid Peroxidation and Thiobarbituric Acid Reactions

The determination of lipid peroxidation was done according to the method described by Ohkawa *et al.,* (1979) with slight modifications. Lastly, 100 µl of the S1 fraction was mixed with a reaction mixture of 30 µl 0.1 M pH 7. 4 Tris–HCl buffer, 0–100 µl of extract, 30 µl of 250 µM freshly prepared $FeSO_4$ (The same procedure was performed using 5 µM sodium nitroprusside). The mixture was brought to 300 µl with water and then incubated at 37 °C for 1 hour. The color reaction was obtained after the addition of 300 µl of 8.1% sodium dodecyl sulfate (SDS) to the reaction mixture containing S1, acetic acid/HCl (pH 3.8 thiobarbituric acid (TBA). This mixture was incubated at 100 °C for 1 hour. TBARS formed were quantified at 532 nm using a UV/visible spectrophotometer (Jenway 6305 model) and results were estimated as MDA equivalents.

Data Analysis

Data obtained from three experiments were combined, and the results were presented as means ± SD. In the analysis of the results, the mean was analyzed using one-way analysis of variance (ANOVA) and the post treatment was done using Duncan multiple test (Zar 1984). Differences were considered significant at ($P < 0.05$). The EC_{50}, which is the effective concentration of the extract that brings about half maximum inhibition, was determined using GraphPad Prism version 8.20 for Windows through nonlinear regression analysis.

3.0 Results

The result for phenolic content and flavonoid content are shown in Table 1. The shining bush leaf extracts analyzed in this study were found to possess phenolic contents in the range of 19.43 mg GAE/g (blanched) to 23.37 mg GAE/g (raw). The total flavonoid contents in the leaf extracts were determined to range from 13.26 mg QAE/ g (blanched) to 17.05 mg QAE/g (raw). This means that, the raw leaf extracts contained higher amounts of total phenol and flavonoid compared to the blanched leaf extracts.

Table 1: Total Phenol and Flavonoid Contents of Raw and Blanched Leaf Extracts of Shining Bush Leaves

	Total Phenol (mg GAE/g)	Total Flavonoid (mg QAE/g)
Raw Extract	**23.37 ± 2.79^{a}**	**17.05 ± 2.68^{a}**
Blanched Extract	**19.43 ± 2.79^{b}**	**13.26 ± 2.68^{b}**

Values represent means ± standard deviation of triplicate experiments. Values with the same

lowercase letter along the same column are not significantly different (P>0.05)

Table 2. presents all the extracts exhibited DPPH radical scavenging activity in concentration dependent manner (0.42- 1.67 mg/ml) as depicted in Figure 2. However, for the extract, the EC_{50} (Table 4) showed that blanched had the lowest DPPH radical scavenging activity at 0. 35 mg/ml while the raw one had the highest at 0. 20 mg/ml. Thus, the raw had significantly higher (P<0.05) DPPH scavenging ability than the corresponding blanched extracts.

Table 2: EC_{50} Values For DPPH And Fe^{2+} Chelating Ability of Raw and Blanched Extracts of Shining Bush

	EC_{50} (mg/ml)	
	DPPH radical scavenging ability	**Fe2+ chelation**
Raw Extract	0.20 ± 0.01^{a}	0.34 ± 0.15^{c}
Blanched Extract	0.35 ± 0.01^{b}	0.17 ± 0.12^{c}

Values represent means ± standard deviation of triplicate experiments. Values with the same lowercase letter along the same column are significantly different (P<0.05)

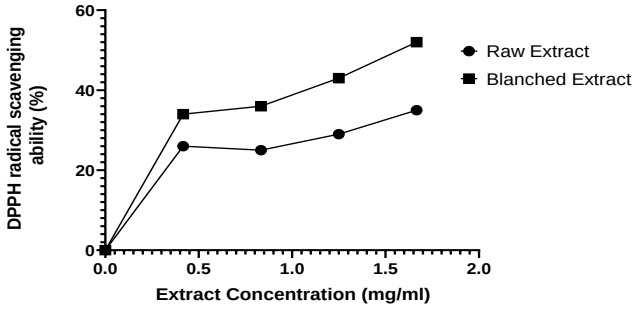

Fig. 1 DPPH Radical Scavenging Ability of Raw and Blanched Extracts of Shining Bush Leaves

The results of Figure 2. (Fe^{2+}chelating ability) showed that all extracts were able to chelate Fe^{2+} in concentration- dependent manner (0–0.5 mg/ml); however, the leaf extract of blanched had the highest EC_{50} values (P<0.05) for chelating ability (Table 2), while the raw extract had the least EC_{50} values of 0.34 mg/ml and was thus less efficient compared to blanched leaf extract with EC_{50} of 0.17 mg/ml as shown by Table 2. Thus, the leaf extract of blanched is a better chelator of Fe^{2+} than the corresponding raw extract.

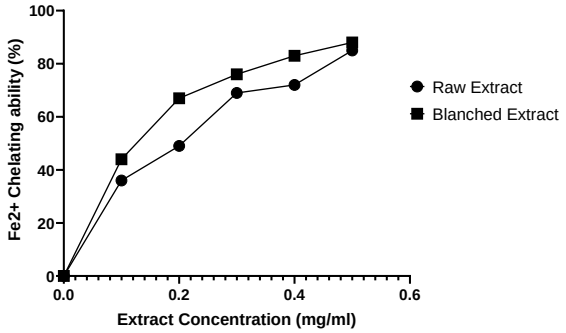

Fig. 2: Fe^{2+} Chelating Ability (%) of Raw and Blanched Extracts of Shining Bush Leaves

Furthermore, the result of the ferric-reducing antioxidant property of the shining bush extracts ascorbic acid equivalent. The results revealed that all the extracts had ferric-reducing antioxidant properties. In terms of reducing ability, raw (1.55 mg AAE/g) had the highest reducing property value while blanched (1.02 mg AAE/g) had the lowest reducing property. For the extract studied, the raw extract had higher ferric-reducing property than the blanched extracts which are significantly different (P<0.05).

The result shown in Table. 3. whereby all the extracts scavenged ABTS• free radicals. For the leaf extracts, the blanched extract (22 mmol TEAC/g) had the least scavenging ability, while the raw (27 mmol TEAC/g) had the highest. The raw extract is a better scavenger of $ABTS^{\bullet+}$ than its corresponding blanched extracts which are significantly different (P<0.05).

The result presented in Table. 3. showed all the extracts vitamin C contents. Comparing the two extracts of shining bush, raw extract revealed the highest Vitamin C content of 6.84 mg AAE/g While the blanched revealed the least amount of vitamin C of 5.41 mg AAE/g. The raw leaf extracts had higher vitamin C contents than the

blanched leaf extracts which are significantly different (P<0.05).

Table 3: Ferric Reducing Antioxidant Properties, ABTS and Vitamin C Contents of Raw and Blanched Leaf Extracts of Shining Bush

	FRAP (mg AAE/g)	ABTS (mmol.AAE/g)	Vitamin C (mg AAE/g)
Raw Extract	**1.55± 0.37[a]**	**27 ± 3.54[b]**	**68.74 ± 10.14[c]**
Blanched Extract	**1.02 ±0.37 [a]**	**22±3.54 [b]**	**54.40 ±10.14 [c]**

Values represent means ± standard deviation of triplicate experiments. Values with the same lowercase letter along the same column are significantly different (P<0.05).

Figure 4 reveals that after incubating the homogenate of rat heart tissue and 25 μM Fe^{2+} for some time, there was significant (P<0.05) elevation in heart malondialdehyde (MDA) content by 106.00 %. However, all the extracts showed concentration dependent inhibition of MDA production in heart tissues (0.42–1.67 mg/ml), where blanched leaf extract had the highest inhibitory effect (20.00%), while raw extracts had the lowest inhibitory effect producing maximum TBARS at its highest concentration which was (37.00%.).

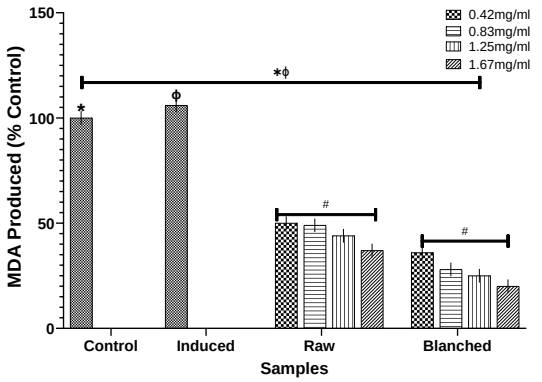

Fig. 3: Inhibition Of Fe^{2+}-Induced Lipid Peroxidation in Rat Heart Tissue Homogenate by Raw and Blanched Extracts of Shining Bush Leaves. Different Symbols (* And Φ) Indicate Significant Differences (P < 0.05) Between Different Concentrations in Raw and Blanched Leaves. Symbol (#) Indicates No Significant Difference (P > 0.05) between different Concentrations in Raw and Blanched Leaves.

In addition, incubation of rat heart tissue homogenates in the presence of 5 μM sodium nitroprusside (SNP) also caused a significant increase (P<0.05). Nevertheless, all the extracts reduced the level of MDA in heart tissue dose-dependently (0.42–1.67 mg/ml). The blanched leaf extract showed the highest inhibitory value of (36.00%), while the raw extract recorded the least value of (46.00%).

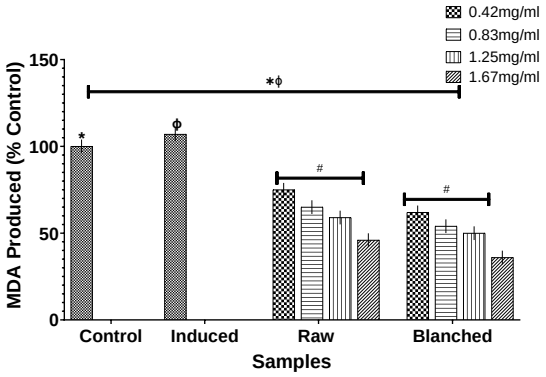

Fig. 4: Inhibition of Sodium nitroprusside-induced Lipid Peroxidation in Rat Heart Tissue Homogenate by Raw and Blanched Extracts of Shining bush. Different symbols (* and ϕ) indicate significant differences (p < 0.05) between different concentrations in raw and blanched. Symbol (#) indicates no significant difference (P > 0.05)

between different concentration in raw and blanched.

Figure 5 showed that all the extracts inhibited ACE activity in a concentration-dependent manner (0-0.1 mg/ml), it was revealed that the blanched extract had the higher inhibitory effect (EC_{50} = 0.02 mg/ml) while the raw extract was the least inhibitory effect (EC_{50} = 0.14 mg/ml). From the extract evaluated in this study, blanched leaf extract is a better ACE inhibitor than raw leaf extract.

Table 4: EC_{50} Values of Angiotensin I Converting Enzyme (ACE) Inhibitory Activity of Raw and Blanched Extracts of Shining Bush Leaves

EC_{50} for ACE inhibition (mg/ml)	
Raw Extract	Blanched Extract
0.14 ± **0.04** [a]	**0.02** ± **0.01** [b]

Values represent means ± standard deviation of triplicate experiments. Values with the lowercase letter along the same column are significantly different (P<0.05)

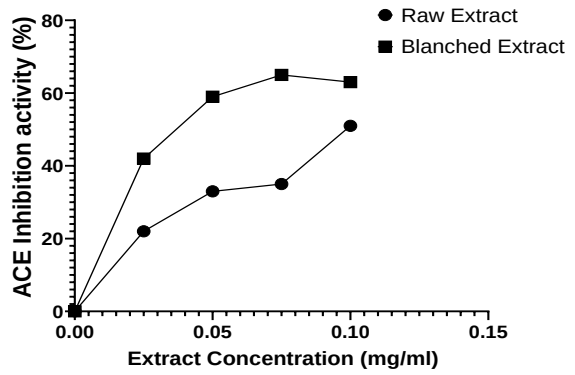

Fig. 5: Angiotensin I Converting Enzyme Inhibitory Effect (%) of Raw and Blanched Leaf Extracts of Shining Bush

Discussion

Phenolics present in fruits, vegetables, and other plant food sources have been described to possess humongous health benefits as a result of their antioxidant actions (Ademiluyi *et al.*, 2015a; Mukherjee *et al.*, 2014). The extents to which phenolics exhibit antioxidant activities include chelation of metal ions, scavenging of free radicals, and activation of endogenous antioxidant enzymes, as well as the suppression of oxidative chain reactions (Ademiluyi *et al.*, 2015a). Similarly, flavonoids, which is the largest group of phenolic compounds have been found to possess antioxidant activities in fruits and other vegetables (Bravo 1998), which gives them the ability to scavenge oxidative stress (Oboh and Rocha 2007). The antihypertensive effects of raw and blanched extracts of shining bush could also be due to their phenolic contents, this is because there are reports on the use of phenolic extracts from different parts of the plant in the management of hypertension (Ademiluyi and Oboh 2013; Quiñones *et al.*, 2013).

Consumption of foods rich in flavonoids has been reported in previous studies to elicit blood pressure-lowering effects (Ademiluyi and Oboh 2013; Quiñones *et al.*, 2013). All the raw and blanched extracts of shining bush have significant amounts of total flavonoid, but the raw leaf extract had significantly higher amounts of flavonoids than the blanched extract which could further enhance the blood pressure-lowering potentials of the leaf extracts. All the extracts showed significantly high ferric-to-ferrous reducing property. In our study, the raw extracts exhibited a high reducing power. This can be explained by the abundance of phenolic hydroxyl groups present in the raw extracts. Phenolic compounds which are abundant in many plant extracts, have hydroxyl groups attached to aromatic rings that can donate electrons or hydrogen atoms to neutralize free radicals and reduce oxidized molecules (Bajpai *et al.*, 2005). The raw extract had the highest reducing property followed by the blanched extracts. However, there was no significant difference between the reductive properties of the raw and blanched extracts. It is however noteworthy that the raw leaf extract showed higher ferric-reducing properties than its corresponding blanched extracts which may be as a result of their higher phenolic contents.

Plant antioxidant activity has been related to their ability to stabilize DPPH radicals by donation of hydrogen or electrons (Arnao 2011). Hence, the ability of the extracts of the shining

bush to significantly scavenge DPPH can be attributed to their rich phenolic contents. This agrees with previous findings which have shown that the DPPH free radical scavenging ability of several foods is directly proportional to their total phenol content (Dastmalchi *et al.*, 2007; Ademiluyi *et al.*, 2015a). However, the leaf extract of the blanched showed higher radical scavenging ability than the corresponding raw extract.

The $ABTS^{*-}$ scavenging abilities of the extracts could therefore be linked to their rich phenolic contents. However, the raw extracts had generally higher scavenging ability than the corresponding blanched extracts. Cardiovascular diseases which include hypertension is known to be associated with persistently high free radicals in the form of reactive oxygen and nitrogen species, which cause damage to vascular endothelial cell, increases vascular contraction, inflammation as well as enhances lipid peroxidation. Some of the researches have been able to show that plant aqueous extracts and antioxidants contain the ability to prevent lipid peroxidation. From this study, it could be concluded that Fe^{2+} causes lipid peroxidation in the rat heart tissue homogenate indicated by the increase in the level of MDA and this may be due to the Fenton reaction that catalyze the generation of highly reactive OH radicals as well as generation of end products including MDA. Therefore, the ability of raw and blanched extract of shining bush to significantly scavenge OH radicals and chelate Fe^{2+} which can both be linked to their rich phenolic contents can possibly explain the observed inhibition of Fe2+-induced lipid peroxidation by the extracts. This agrees with earlier findings by Ogunmefun *et al.*, (2015) that methanol extracts from Phragmanthera incana leaves inhibited Fe2+-induced lipid peroxidation (in vitro) in five organs including the heart; this observation was attributed to the phenolic contents and antioxidant properties of the extracts. Similarly, the observed induction of lipid peroxidation by sodium nitroprusside (SNP) in the rat heart tissue homogenate could be as a result of generation of nitric oxide (NO) by SNP decomposition. SNP is a peripheral vasodilator, acting directly on smooth muscle fibers to elicit blood pressure-lowering effect (Kaisserlian *et al.*, 2005). The release of NO by SNP is proposed to mediate its vasodilatation effect (Chu *et al.*, 2002).

However, at high concentration, SNP has been associated with cytotoxicity as a result of release of NO and cyanide (Kaisserlian *et al.*, 2005; Chu *et al.*, 2002); NO, released is capable of reacting with other reactive oxygen species to form peroxynitrite, contributing to oxidative stress and cellular damage (Calcerrada *et al.*, 2011; Radi 2004). Although the release of NO is a critical factor in the cytotoxicity of SNP, we did not directly measure NO levels in our study. Further investigations could focus on quantifying NO to further elucidate it role in SNP induced cytotoxicity. In addition, decomposition of SNP yields iron which can also propagate the lipid peroxidation chain reaction via Fenton reaction (Wagner *et al.*, 2006). Both Raw and blanched showed significant NO radical scavenging ability which could further explain the inhibition of SNP-induced lipid peroxidation by the extracts; this followed a similar trend as the inhibition of Fe^{2+}-induced lipid peroxidation. Thus, the ability of the blanched extracts of shining bush to significantly inhibit Fe^{2+} and SNP-induced lipid peroxidation could further explain the biochemical rationale behind the use of the shining bush extracts in the management of hypertension as observed in traditional medicine.

The ability of plant bioactive compounds like phenolics to reduce ACE activity has been documented (Zhang *et al.*, 2008; Ademiluyi *et al.*, 2015b). There is literature evidence that phenolic phytochemicals interact directly with the ACE enzyme in a manner depending on their structure, by chelating with the zinc ion or through the creation of hydrogen bridges between the enzyme's active site amino-acid residues and the phenols (Umamaheswari *et al.*, 2012). The extracts of shining bush leaf studied had ACE inhibitory effects in a concentration-dependent manner, and there is a strong correlation between the ACE inhibitory effects of the blanched extract and their total phenolic and flavonoid contents. These could imply that

the phenolic constituents of the extracts could be largely responsible for their ACE inhibitory effects. The reason why the blanched extracts is a better inhibitor of ACE than the corresponding raw leaf extracts remained unclear and subject to further studies; nevertheless, possible synergistic actions of constituent phenolics and/or presence of nonphenolic inhibitors could account for these observations. Therefore, the observed inhibitory effect of the blanched extracts on ACE activity could suggest one of the possible mechanisms of antihypertensive effect of shining bush as reported in traditional medicine.

Blanching is a classic technique of cooking in which food is briefly heated and then soaked in cold water. It is known that such a mechanism decreases the concentrations of certain antioxidants. The research of Dewanto *et al.* (2002), the thermal degradation of heat-sensitive molecules and the resulting leaching of water-soluble antioxidants into the blanching water have the reason causing the reduction in antioxidant levels.

Despite the reduction in antioxidants, blanching has been found to enhance the ACE inhibitory activity of certain plant extracts. This enhancement could be related to changes in the bioavailability of active compounds or the formation of new bioactive peptides and phenolic compounds during the blanching process (Pellegrini *et al.,* 2010).

Iron (Fe) chelation is a crucial factor in understanding this phenomenon. Fe chelation involves binding free iron ions, which reduces their availability to participate in oxidative reactions. Free iron is a catalyst for the formation of reactive oxygen species (ROS) through Fenton reactions, leading to lipid peroxidation (the oxidation of lipid in cell membrane) and subsequent cellular damage (Halliwell and Gutteridge, 1990). By chelating iron, the extracts can effectively reduce oxidative stress and lipid peroxidation.

Our results indicate that blanching enhances the Fe chelation capacity of the shining bush leaf extracts. This increased chelation may be due to the alteration, formation of new phenolic compounds during post-blanching or concentration of phenolic compounds and other chelating agents during blanching. Enhanced Fe chelation can reduce the catalytic activity of iron, thereby lowering reacting oxygen species (ROS) generation and lipid peroxidation. This reduction in oxidative stress can contribute to the improved bioavailability and efficacy of ACE inhibitory compounds present in the blanched extracts (Fuhrman *et al.,* 2002).

The enhanced Fe chelation observed in blanched extracts can also play a role in ACE inhibition. By reducing oxidative stress through effective iron chelation, the stability and activity of ACE inhibitory compounds can be preserved or even enhanced. This could explain the increased ACE inhibitory activity observed in the blanched extracts compared to the raw extracts.

Iron chelation plays a critical role in reducing oxidative stress by binding free iron ions, thereby reducing their availability to participate in Fenton reactions. Fenton reactions generate reactive oxygen species (ROS), which can cause significant cellular damage through lipid peroxidation (Halliwell and Gutteridge, 1990). In our study, the Fe chelation ability of both raw and blanched extracts was assessed. The blanched extracts exhibited significantly higher Fe chelation capacity compared to the raw extracts, suggesting that blanching enhances the ability of the extracts to bind free iron ions and prevent oxidative stress. This finding is consistent with previous studies that have shown thermal processing can increase the bioavailability of chelating agents (Pellegrini *et al.*, 2010).

Lipid peroxidation is a well-known marker of oxidative stress and cellular damage, often induced by iron through the Fenton reaction. The inhibition of Fe-induced LPO by antioxidants can protect cellular integrity and function. Our results demonstrated that both raw and blanched extracts inhibited Fe-induced LPO in rat heart tissue homogenates, with the blanched extracts showing a more pronounced effect. This suggests that blanching not only preserves but may enhance the antioxidant properties of the shining bush leaf, potentially due to the improved availability of phenolic compounds that are effective in preventing lipid

peroxidation. This aligns with the findings of Fuhrman *et al.* (2002), who reported that polyphenolic compounds exhibit strong antioxidant activities, including the inhibition of LPO.

Angiotensin-I-converting enzyme (ACE), a metalloprotein plays a crucial role in the regulation of blood pressure by converting angiotensin I to angiotensin II, a potent vasoconstrictor. Inhibiting ACE activity is a therapeutic target for managing hypertension. Our study found that the ACE inhibitory activity was significantly higher in the blanched extracts compared to the raw extracts. This enhancement might be attributed to the increased concentration or activity of ACE inhibitory peptides and polyphenols released or formed during blanching (Pellegrini *et al.*, 2010). These findings are in agreement with previous research that suggests processing can enhance the antihypertensive properties of plant-based foods (Fuhrman *et al.*, 2002).

The enhanced Fe chelation capacity, inhibition of Fe-induced LPO, and increased ACE inhibitory activity observed in the blanched extracts suggest that blanching may improve the overall health benefits of shining bush leaves. The increased Fe chelation reduces the catalytic activity of free iron, subsequently lowering ROS generation and LPO. This reduction in oxidative stress can further potentiate the effectiveness of ACE inhibitory compounds, leading to better management of hypertension. Therefore, blanching, while reducing some heat-sensitive antioxidants, appears to enhance key bioactive properties that contribute to the antioxidant and antihypertensive effects of shining bush leaves. Future research should aim to quantify these changes more precisely and explore the underlying mechanisms in greater detail to fully understand the benefits of blanching on the functional properties of shining bush leaves.

Conclusion

This study has been able to reveal that extracts of the blanched Shining Bush were able to inhibit angiotensin I converting enzyme (ACE) and had antioxidant properties (in vitro) which were linked to their phenolic contents. The blanched Shining Bush extract exhibited higher ACE inhibitory and antioxidant properties, suggesting a synergistic action of its presence of other ACE inhibitory compounds. Hence, the blanched Shining Bush can be therapeutic target in developing functional foods and nutraceuticals for the prevention and management of hypertension.

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Declaration of Conflict of Interest

None

Author's contribution

BCA: Conceptualization, supervision, methodology and approval of manuscript draft. DIA: Data acquisition and preparation of manuscript draft. OBO: Conceptualization, supervision, data analysis, methodology and proofreading of manuscript draft. GO: Conceptualization, supervision, methodology and approval of manuscript draft.

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Effects of NSPRI Storage Technologies on the Nutritional Qualities of Stored Sweet Potatoes *(Ipomoea batatas Lam.)*

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Abstract
Ipomoea batatas, also known as sweet potatoes, are extremely versatile tuber crops that possess high nutritional value. In this study the effect of two different NSPRI storage technologies on the proximate composition and microbial analyses of sweet potato (*Ipomoea batatas)* was investigated. The sweet potato tubers were harvested, sorted and grouped into two different NSPRI storage structures for a period of three months. Samples were taken monthly for proximate and microbial analyses. The results of the analyses showed significant variations $p>0.05$ across the period of storage. The results of this study after the storage period indicate that the washed sweet potato tubers stored under an improved yam barn retained a significant percentage of its nutritional qualities.

Keywords: Nutritional quality, sweet potatoes, storage, technology, yam barn

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Introduction

Ipomoea batatas, also known as sweet potatoes, are extremely versatile tuber crops that possesses high nutritional value and valuable medicinal properties (Ogliari *et al.,* 2020). It has a large potential to be used as a food security crop because of its short maturity time and ability to grow under diverse climatic conditions (Wireko-manu *et al.,* 2010).

The perishability of sweet potatoes has been attributed to low dry matter content coupled with the thin delicate skin (Tomlins *et al.,* 2012). Some of the methods in elongating the shelf life of sweet potato include keeping sweet potatoes in an area with free air circulation (Wireko-manu *et al.,* 2010). However, all efforts proved inadequate due to fluctuations in atmospheric conditions as well as the need to store the produce for a very long time. NSPRI has developed various storage technologies that can be used for storage of various roots and tuber crops. The technologies include improved yam barn and ventilated yam barn. These storage technologies can be used as a tool in mitigating the loss of nutrient occurring during the storage of roots and tuber crops. Therefore, the objective of this study is to assess the qualities change in nutrient and microbiological properties of the sweet potatoes stored using the NSPRI ventilated yam barn and the NSPRI improved yam barn.

Materials and Method

Sample Collection

This study was carried out in the Nigerian Stored Products Research Institute (NSPRI) Headquarter, Ilorin, Kwara State using NSPRI Ventilated Yam Barn (VYB) and NSPRI Improved Yam Barn (IYB). Fresh Cream fleshed sweet potato cultivars were procured from a farm in Offa, Kwara State; it was sorted, cleaned and grouped into three lots of 30kg each. The first lot (A) washed, the second lot (B) unwashed and lots (C and D) served as treatments control. The roots were arranged on the wooden platform in both structures while the control for the study were kept on a platform outside the barns. Microbial

analysis was determined using (USFDA, 1998) and proximate analysis were carried out according to AOAC (2019) methods of analysis. Data obtained from the analyses were subjected to Analysis of Variance (ANOVA) and tested for significance difference among treatments by New Duncan's Multiple Range F-Test (DMRT) at ($p<0.05$) using SPSS software package version 20.0.0 (IBM Statistics).

Results

Nutritional Composition of Stored Sweet Potatoes

Table 1: Proximate composition of stored sweet potatoes at day 0

Sample	Moisture Content (%)	Ash (%)	Fibre (%)	Fat (%)	Protein (%)	CHO (%)
Washed Control	$64.61^{o}\pm0.01$	$0.79^{k}\pm0.00$	$1.01^{c}\pm0.00$	$1.11^{g}\pm0.01$	$4.85^{f}\pm0.03$	$27.62^{b}\pm0.03$
Unwashed Control	$64.61^{o}\pm0.01$	$0.79^{k}\pm0.00$	$1.01^{c}\pm0.00$	$1.11^{g}\pm0.01$	$4.85^{f}\pm0.03$	$27.62^{b}\pm0.03$
Washed VYB	$64.61^{o}\pm0.01$	$0.79^{k}\pm0.00$	$1.01^{c}\pm0.00$	$1.11^{g}\pm0.01$	$4.85^{f}\pm0.03$	$27.62^{b}\pm0.03$
Unwashed VYB	$64.61^{o}\pm0.01$	$0.79^{k}\pm0.00$	$1.01^{c}\pm0.00$	$1.11^{g}\pm0.01$	$4.85^{f}\pm0.03$	$27.62^{b}\pm0.03$
Washed IYB	$64.61^{o}\pm0.01$	$0.79^{k}\pm0.00$	$1.01^{c}\pm0.00$	$1.11^{g}\pm0.01$	$4.85^{f}\pm0.03$	$27.62^{b}\pm0.03$
Unwashed IYB	$64.61^{o}\pm0.01$	$0.79^{k}\pm0.00$	$1.01^{c}\pm0.00$	$1.11^{g}\pm0.01$	$4.85^{f}\pm0.03$	$27.62^{b}\pm0.03$

Key: *Results show Mean ± SE of triplicate readings (n=3). Means with unshared superscript in the same row are significantly (p<0.05) different. CHO=Carbohydrate*

Table 2: Proximate composition of stored sweet potato for Month 3

Sample	Moisture Content (%)	Ash (%)	Fibre (%)	Fat (%)	Protein (%)	CHO (%)
Washed Control	$55.08^{c}\pm0.02$	$0.52^{d}\pm0.01$	$1.36^{f}\pm0.00$	$1.05^{f}\pm0.01$	$5.99^{j}\pm0.03$	$36.40^{m}\pm0.03$
Unwashed Control	$54.12^{b}\pm0.00$	$0.40^{a}\pm0.00$	$1.76^{j}\pm0.01$	$1.01^{e}\pm0.00$	$5.95^{l}\pm0.03$	$36.77^{n}\pm0.04$
Washed VYB	$57.06^{f}\pm0.04$	$0.48^{b}\pm0.00$	$1.00^{c}\pm0.00$	$1.11^{g}\pm0.00$	$5.02^{g}\pm0.01$	$35.32^{l}\pm0.04$
Unwashed VYB	$58.11^{g}\pm0.01$	$0.40^{a}\pm0.01$	$1.50^{h}\pm0.00$	$1.01^{e}\pm0.01$	$5.75^{k}\pm0.04$	$33.23^{h}\pm0.05$
Washed IYB	$59.51^{h}\pm0.06$	$0.40^{a}\pm0.00$	$2.08^{l}\pm0.04$	$0.68^{a}\pm0.02$	$5.18^{i}\pm0.03$	$32.15^{g}\pm0.04$
Unwashed IYB	$55.94^{e}\pm0.04$	$0.39^{a}\pm0.01$	$2.14^{n}\pm0.00$	$0.70^{a}\pm0.01$	$5.74^{k}\pm0.04$	$35.09^{kl}\pm0.05$

Key: *Results showed Mean ± SE of triplicate readings (n=3). Means with unshared superscript in the same row are significantly (p<0.05) different. CHO=*Carbohydrate

Table 3: Microbial Loads of Potato Tubers Samples

		Day 0		Month 3	
Sample	**Treatments**	**R_{AV}**	**cfu/g**	**R_{AV}**	**cfu/g**
Freshly Harvested	Washed	2.0	**2.0×10^{-3}**	-	**-**
	Unwashed	5.0	**5.0×10^{-3}**	-	**-**
Ventilated Yam Barn	Washed	2.0	**2.0×10^{-3}**	4.0	**4.0×10^{-3}**
	Unwashed	2.0	**2.0×10^{-3}**	4.5	**4.5×10^{-3}**
Improved Yam Barn	Washed	1.0	**1.0×10^{-3}**	2.5	**3.0×10^{-3}**
	Unwashed	3.5	**4.0×10^{-3}**	3.0	**3.0×10^{-3}**
Control	Washed	Nil	**Nil**	1.5	**1.5×10^{-3}**
	Unwashed	3.0	**3.0×10^{-3}**	2.5	**2.5×10^{-3}**

NB: R_{AV} = Average Count of Isolate. The identified fungi isolate includes *A. flavus*, *A. niger*, Penicilium, Mucus and Rhizopus

Discussion

Tables 1 and 2 showed the results of the preliminary and final tests carried out on the fresh and stored sweet potatoes while Table 3 showed microbiological analysis. There were significant ($p < 0.05$) differences in the values of the parameters determined. A significant decreased in moisture content was observed for washed and unwashed stored sweet potatoes. This corroborates the results of Addisu and Gobena, 2014 who reported a decreasing trend in moisture content of potato tubers during storage. The loss of moisture can be attributed to the water loss due to transpiration and evaporation. The ash content range between 0.59% and 0.52%. The general decrease in ash content across the storage period for all the storage technologies was not in conformity with the work of Srivastava and Immanuel, 2012 who recorded an increased in ash content across the storage period which may be as a result of varietal differences. An increase in crude fibre content was observed between the two treatments. This increase agrees with the findings of (Srivastava and Immanuel, 2012). The crude fat content for the two treatments was observed to decrease as storage period increases. However, washed and unwashed sweet potatoes stored under improved yam barn was observed to retain more crude fat content.

The protein content in the freshly harvested sweet potato variety was 4.85% and the protein content of the sweet potato stored range from 3.99% to 5.95%. Generally, increase in crude protein content was observed across the storage structure which may be as a result of biochemical reaction taking place within the tubers during the storage time (Batu and Sen, 2013). The carbohydrate content for the sweet potatoes stored in both storage structures increased along the storage period. The changes observed in carbohydrate content showed no particular trend as the storage period progressed. This corroborates the findings of (Roberts, 2014).

The washed fresh potato samples had lower fungi count (2.0 log10cfu) than the unwashed sample (5.0 log10cfu). Observed increase in fungi count from 1.0 log10cfu in the first month to 3.0 log10cfu in the third of storage while the unwashed sample reduced from 4.0 log10cfu to 3.0 log10cfu for washed potatoes. This report is in line with the work of Kouassi *et al.*, 2019, that moisture content of the sample is a key factor to microbial invasion and multiplication.

Conclusion

This study investigates the effects of NSPRI storage technologies on the nutritional qualities and microbial load of sweet potatoes (*Ipomoea batatas*) over a three-month period. Findings reveals that the choice of storage method significantly influence the retention of nutritional qualities in sweet potatoes. The NSPRI improved yam barn (IYB) proved most effective in preserving the nutritional qualities of sweet potatoes, particularly for washed sweet potatoes. While moisture content decreased across all storage conditions, the IYB maintained the highest moisture retention. Interestingly, protein and carbohydrate content generally increased

during storage, possibly due to biochemical reactions within the tubers. Microbial analysis showed that washed potatoes initially had lower fungal counts, but counts increased over the storage period for all samples. The differences observed between storage conditions highlight the importance of proper ventilation and humidity control in sweet potato storage. These results underscore the potential of well-ventilated structures like the NSPRI improved Yam Barn in enhancing post-harvest management of sweet potatoes. However, further research is needed to optimize storage conditions and minimize quality losses. We recommend conducting extended storage trials to determine the maximum effective storage duration using these technologies. Future studies should also explore combinations of these storage technologies with other preservation methods to enhance long-term storage capabilities. Moreover, examining the economic implications of these storage methods for small-scale farmers and traders would provide valuable insight for practical implementation. By implementing improved storage methods, it may be possible to extend the shelf life of sweet potatoes, reduce post-harvest losses, and ultimately contribute to food security in the regions where sweet potatoes are staple crops. This study serves as a stepping stone towards developing more efficient and effective storage solutions for this important tuber crop.

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Declaration Of Conflict Of Interest
No Conflict of Interest declared by Authors

Author's Contribution
This work was carried out in collaborations with all authors. All authors read and approved the final manuscript.

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Whole Blood Viscosity and Serum Biochemistry of Broiler Birds Fed with Composite Sweet Orange Peel

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Abstract

The poultry industry is in dire need of natural feed additives and ingredients that are cheaper alternatives to the conventional ones which at the same would not pose any threat on the health and welfare of the birds and their consumers. This study thus investigates the impact of dietary composite sweet orange peel (CSOP) on blood viscosity and serum biochemistry of Arbor Acre and Cobb 500 broiler breeds. One hundred and Ninety-two day-old-chicks were used. The experiment was designed and randomised for each of the breeds to have four dietary treatment groups of three replicates with eight birds per replicate in a 2 by 4 factorial experiment. The birds in the first (Control/A), second (B), third (C) and fourth (D) treatment groups were fed dietary inclusion 0%, 2.50%, 5.00% and 7.50% levels of CSOP, respectively. At the end of eight weeks, blood was sampled for blood viscosity and serum biochemistry analyses, using standard procedures. There was no significant ($p>0.05$) treatment, breed and their interaction effects on the blood viscosity of the birds. Total protein, albumin and high-density lipoprotein cholesterol (HDL-C) showed a higher trend in consonance with dietary increase in CSOP, while Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), low density lipoprotein cholesterol (LDL-C) and Triglycerides showed a downward trend. There was however neither breed nor breed versus treatment effects of the use of CSOP on the measured biochemical parameters of the birds. It was concluded that dietary CSOP at the levels used in this trial would not entrain any health and welfare challenges on Arbor Acre and Cobb 500 broiler breeds but would rather improve their meat quality through lowered triglycerides, LDL-C, ALT and AST. The downstream effects of SOP on broilers' meat quality would be imparted on the consumers by way of significant reduction in the bad cholesterol content of the broilers' meat.

Keywords: Broiler meat, composite sweet orange peel, serum biochemistry, blood viscosity.

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Introduction

Atherosclerosis is the underlying factor for several human diseases, contributing significantly to global morbidity and mortality, including ischemic heart disease, myocardial infarction, and stroke (Poznyak *et al*., 2020). The impact of this disease on large vital vessels, such as the carotid and coronary arteries, underscores its potential threat to human health. Notably, the consumption of animal products has long been identified as a major source of dietary fat in humans, playing a crucial role in nutritional health (Monfort-Pires *et al*., 2023). Several kinds of literature have shown concerns about the rising incidence of cardiovascular and degenerative diseases highlighting a functional relationship between the consumption of animal products, animal fat, cholesterol, and the prevalence of heart disease (Monfort-Pires *et al*., 2023). In addressing these concerns, there is a growing consensus among nutritionists that adopting a diet characterised by low salt intake and reduced consumption of animal-derived products, coupled with an increased intake of plant-based foods, is associated with a decreased risk of atherosclerosis (Riccardi *et al*., 2021). The poultry industry has witnessed an increasing interest in using natural additives for poultry feed, with examples including Garlic, Red yeast rice, Berberine, and the Indian bdellium tree (Valli *et al*., 2002). Notably, various plants, such as cinnamon, oregano, cumin, garlic, sumac, cloves, anise, mint, coriander, and ginger, have been extensively studied for their potential

contributions to animal nutrition (Akyıldız and Denli, 2016). The focus of this study is on composite sweet orange peel (CSOP) as a natural additive/ingredient in broiler feed, with a primary emphasis on its health benefits. While CSOP represents an economically viable approach to utilizing all parts of the sweet orange fruit and addresses environmental concerns by reducing waste in landfills, the primary objective is to explore the health implications for broilers. Sweet orange peels have been recognized for their rich nutritional profile, including notable levels of protein (7.15%) and crude fibre (12.79%) (Oyebola *et al.*, 2017). Beyond their nutritional content, orange peels are renowned for their potential health benefits, including antioxidant properties attributed to compounds such as flavonoids and polyphenols. These bioactive compounds have been associated with anti-inflammatory and cardiovascular health-promoting effects (Snyder *et al*., 2011). The presence of bioactive compounds in CSOP may contribute to reducing the risk of atherosclerosis in broilers. Findings by Apostolidou *et al*. (2015) and Zaidun *et al*. (2018) revealed that antioxidants play a crucial role in neutralising oxidative stress, which has been implicated in various cardiovascular diseases. Çiftçi *et al*. (2016) demonstrated noteworthy outcomes, revealing that orange peels supplementation led to a reduction in triglyceride, total protein, glucose, total cholesterol, and uric acid levels in serum. These findings suggest a potential modulation of serum biochemistry, indicating a favourable impact of citrus peel on lipid profiles and glucose metabolism. Furthermore, the dietary fibre content in CSOP may influence blood viscosity, a key parameter in cardiovascular health. Although Çiftçi *et al.,* (2016) did not explicitly investigate blood viscosity, the observed improvements in serum parameters, coupled with the anti-oxidative effects of orange peels, provide a broader influence on cardiovascular health. According to Morand *et al.* (2011), orange juice consumption, which contains hesperidin found in orange peel, has been associated with lower diastolic blood pressure and improved microvascular endothelial reactivity. Hence, these cardiovascular effects may indirectly contribute to factors that influence blood viscosity. However, there is a paucity of information on composite sweet orange peels' influence on broiler chickens' blood viscosity and serum biochemistry. Hence, this study aims to investigate the potential health benefits of CSOP on blood viscosity and serum biochemistry of the broiler chickens.

Materials and Methods

This experiment was carried out at the poultry unit of the Teaching and Research Farm of the Federal University of Technology, Akure, Nigeria. Akure (Latitude 7º18/ N and Longitude 5º10/ E) is located in the humid rain forest zone of western Nigeria which is characterized by two rainfall peaks and high humidity during the raining season. The mean annual rainfall is about 1500 mm and the rains last for about nine months usually March to November of every year. The mean annual relative humidity is over 75 % while the mean annual temperature is about 27ºC. The pen that housed the birds was properly cleaned, washed, disinfected and further partitioned; biosecurity measures were equally taken. The partitions were labelled according to each treatment and it replicates.

Two hundred (200) broiler birds were procured from a reputable hatchery in Ibadan. One hundred (100) of these birds were of Arbor Acre breed while the remaining one hundred (100) birds were Cobb 500 breed. Out of the two hundred broiler birds One hundred and Ninety-two (192) were used. Each of the breeds were randomly distributed to four (4) dietary treatments of three (3) replicates that comprised twenty-four (24) chicks per treatment. The design of the experiment was a 2 by 4 factorial experimental design. The treatment diets: A, B, C and D were formulated and incorporated with 0.00%, 2.50%, 5.00% and 7.50% of composite sweet orange peels (CSOP) respectively at the starter and finisher phases of the experiment. Tables 1 and 2 shows the gross composition of the broiler starter and broiler finishers' diets.

Table 1: Gross Composition (g/100g) of Broiler Starter Diets (0– 4 weeks of age)

Ingredients (%)	**Composite Sweet Orange Peel (CSOP) levels (%)**			
	0.00	**2.50**	**5.00**	**7.50**
Maize	50.00	50.00	50.00	50.00
CSOP	0.00	2.50	5.00	7.50
Wheat Offal	7.50	5.00	2.50	0.00
Groundnut Cake	8.45	8.45	8.45	8.45
Soybean Meal	27.90	27.90	27.90	27.90
Fishmeal	1.30	1.30	1.30	1.30
Premix	0.15	0.15	0.15	0.15
Limestone	1.30	1.30	1.30	1.30
Bone meal	0.70	0.70	0.70	0.70
Methionine	0.10	0.10	0.10	0.10
Lysine	0.10	0.10	0.10	0.10
Salt	0.10	0.10	0.10	0.10
Vegetable oil	2.70	2.70	2.70	2.70
Total	**100.00**	**100.00**	**100.00**	**100.00**
Calculated Nutrient:				
Crude Protein (%)	20.94	20.74	20.55	20.36
Metabolizable Energy (kcal/kg)	3000.43	3019.94	3039.37	3058.84
Crude Fibre (%)	4.10	4.20	4.31	4.41
Calcium (%)	0.80	0.80	0.80	0.79
Phosphorus (%)	0.46	0.46	0.45	0.44
Lysine (%)	1.21	1.19	1.17	1.15
Methionine (%)	0.41	0.40	0.40	0.39

CSOP = Composite Sweet Orange Peel

Table 2: Gross Composition (g/100g) of Broiler finisher Diets (5-8 weeks of age)

Ingredients (%)	**Composite Sweet Orange Peel (CSOP) levels (%)**			
	0.00	**2.50**	**5.00**	**7.50**
Maize	51.40	51.40	51.40	51.40
CSOP	0.00	2.50	5.00	7.50
Wheat Offal	7.50	5.00	2.50	0.00
Groundnut Cake	18.15	18.15	18.15	18.15
Soybean Meal	15.00	15.00	15.00	15.00
Premix	0.15	0.15	0.15	0.15
Limestone	1.55	1.55	1.55	1.55
Bone meal	0.90	0.90	0.90	0.90
Methionine	0.10	0.10	0.10	0.10
Lysine	0.10	0.10	0.10	0.10
Salt	0.15	0.15	0.15	0.15
Vegetable oil	5.00	5.00	5.00	5.00
Total	**100.00**	**100.00**	**100.00**	**100.00**
Calculated Nutrient:				
Crude Protein (%)	18.80	18.60	18.41	18.21
Metabolizable Energy (kcal/kg)	3132.01	3151.48	3170.95	3190.42
Crude Fibre (%)	3.44	3.55	3.66	3.76
Calcium (%)	0.82	0.82	0.82	0.82
Phosphorus (%)	0.45	0.44	0.43	0.43
Lysine (%)	0.98	0.96	0.94	0.91
Methionine (%)	0.37	0.37	0.36	0.35

CSOP = Composite Sweet Orange Peel

The experiment lasted for 8 weeks at the end of which feed was withdrawn from the birds eight hours before slaughtering and collection of blood. Twelve (12) birds were randomly selected per treatment group. The birds were stunned and bled by severing the jugular veins for blood collection. Blood collected for serum biochemical indices were collected in plain bottles with the absence of anticoagulants and was slanted while blood samples to be examined for viscosity were collected in bottles containing EDTA. An automatic viscometer (Baoshishan rotating viscometer, model 220V-NDJI-1, China) was used to run and read the whole blood viscosity of the birds. The Serum biochemical indices determined were Total protein, Albumin, Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), and lipid profiles including Total cholesterol (TC) using commercial kits.

Data were analyzed with Statistical Package for the Social Sciences (SPSS, version 21) in a 2 by 4 factorial experiment while means were separated using Duncan Multiple Range Test of the same statistical package.

Results

Whole Blood viscosity of Two Breeds of Meat-type Chickens Fed Dietary Inclusion of Composite Sweet Orange Peel (CSOP).

Table 3 shows the effects of diet, breed, and their interactions on whole blood viscosity of two breeds of Meat-type chickens. Diets, breed and their interactions were not significantly (P> 0.05) different in all the parameters measured. The treatment effects on whole blood viscosity ranged between 0.38decaPascal - 0.39decaPascal. Breed effect ranged between 0.38decaPascasl (Cobb500) to 0.39decaPascal (Arbor Acre). Interaction effect was highest with a value of 0.39decaPascasl and 0.40decaPascal for Cobb500 and Arbor Acre on diets B (2.5% CSOP) and C (5.00%CSOP), respectively. The lowest value of 0.37decaPascal was observed in Cobb500 on diet C (5.00% CSOP).

Table 3: Whole Blood Viscosity Profile of Two Breeds of Meat-type Chicken Fed Dietary Inclusion of Composite Sweet Orange Peels (CSOP).

Diet	Breed	Blood Viscosity (Decapascal)
A		0.38
B		0.39
C		0.38
D		0.38
±SEM		0.04
P-value		0.29
	Arbor Acre	0.39
	Cobb500	0.38
	±SEM	0.003
	P-value	0.067
Diet Versus	**Breed**	
A	Arbor Acre	0.39
	Cobb500	0.38
B	Arbor Acre	0.38
	Cobb 500	0.39
C	Arbor Acre	0.40
	Cobb500	0.37
D	Arbor Acre	0.38
	Cobb 500	0.38
	±SEM	0.005
	P-value	0.335

A = Diet with 0.00% CSOP; B = Diet with 2.50%CSOP; C = Diet with 5.00%CSOP; D = Diet with 7.50%CSOP; ±SEM = Standard Error of Mean.

Serum Biochemical Profile of Two Breeds of Meat-Type Chicken Fed Dietary Inclusion of Composite Sweet Orange Peels (CSOP).

Table 4 shows the effects of diet, breed and interaction on serum biochemical profile of two breeds of meat-type chicken fed dietary inclusion of composite sweet orange peel (CSOP). The effects of the four diets on the serum biochemical parameters of the birds were significantly ($p< 0.05$) different for Aspartate aminotransferase (AST), Triglycerides (TG) and Low-Density Lipoprotein Cholesterol (LDL-C) but was not significant ($p>0.05$) for Total Protein (TP), Globulin, Albumin, Alanine Transaminase, Total Cholesterol and High-density Lipoprotein Cholesterol (HDL-C). The values for TP ranged (3.63-4.02mg/dl), Albumin (2.17 – 2.68mg/dl), Globulin (1.18 – 1.57mg/dl), Alanine aminotransferase (ALT) (50.27 – 60.11U/L), AST (68.25 – 88.07U/L), Triglycerides (1.54 – 2.25g/dl), Total cholesterol (73.82 – 79.27mg/dl), HDL-C (63.13 – 71.04mg/dl), and LDL-C (7.90 – 10.03mg/dl).

The effects of breed and the interactions between diets and breed were not significantly ($P>0.05$) different for all the serum biochemical parameters measured.

Table 4: Serum Biochemical Profile of Two Breeds of Meat-type Chicken Fed Dietary Inclusion of Composite Sweet Orange Peel (CSOP)

Diet	Breed	TP (mg/dl)	ALB (mg/dl)	GLO (mg/dl)	ALT (U/L)	AST (U/L)	TG(g/dl)	TC (mg/dl)	HDL-C (mg/dl)	LDL-C (mg/dl)
A		3.63	2.17	1.46	60.11	71.15^{b}	1.93^{ab}	73.82	63.13	10.03^{a}
B		4.02	2.68	1.34	59.77	88.07^{a}	2.25^{a}	75.98	67.59	7.93^{b}
C		3.72	2.54	1.18	50.27	68.25^{c}	1.54^{b}	76.25	66.26	9.68^{a}
D		3.97	2.40	1.57	53.83	69.05^{b}	1.62^{b}	79.27	71.04	7.90^{b}
±SEM		0.23	0.19	0.17	4.25	4.29	0.15	5.44	5.41	0.65
P-value		0.522	0.292	0.055	0.057	0.001	0.018	0.915	0.778	0.039
	Arbor Acre	3.64	2.36	1.28	46.66	69.88	1.91	63.31	90.80	9.42
	Cobb500	4.00	2.54	1.47	48.16	62.42	1.76	70.70	88.35	9.59
	±SEM	0.17	0.13	0.50	5.83	3.04	0.11	3.85	0.46	0.83
	P-value	0.134	0.352	0.210	0.856	0.101	0.348	0.210	0.697	0.887
Breed X Diet										
A	Arbor Acre	3.42	2.12	1.30	59.33	81.50	2.00	76.00	64.43	11.17
	Cobb500	3.79	2.21	1.58	60.90	79.50	1.87	71.63	61.83	9.87
B	Arbor Acre	3.65	2.32	1.33	55.70	102.03	2.40	75.17	62.89	10.27
	Cobb 500	4.38	3.05	1.33	63.83	98.68	2.10	81.78	72.30	9.07
C	Arbor Acre	3.67	2.60	1.06	44.20	74.10	1.53	75.40	71.42	9.67
	Cobb 500	3.70	2.47	1.23	46.33	85.07	1.54	85.10	75.09	9.70
D	Arbor	3.80	2.38	1.42	47.40	78.89	1.70	77.53	68.49	8.70

Acre									
Cobb 500	4.15	2.42	1.73	43.63	78.90	1.65	81.00	73.59	7.10
±SEM	0.33	0.27	0.10	11.64	6.07	0.22	7.69	7.65	0.92
P-value	0.077	0.411	0.411	0.473	0.085	0.916	0.522	0.617	0.148

^{abc} = Means of the same column but different superscripts are statistically significant (P< 0.05); ±SEM = Standard Error of Mean; A = Diet with 0.00% CSOP; B = Diet with 2.50%CSOP; C = Diet with 5.00%CSOP; D = Diet with 7.50%CSOP, ALT= Alanine amontransferase, AST= Aspartate Aminotransferase, TC = Total Cholesterol, TP = Total Protein, HDL-C = High Density Lipoprotein Cholesterol, LDL-C = Low Density Lipoprotein Cholesterol and TG = Triglycerides

Discussion

The thickness or stickiness of blood is measured by blood viscosity. It provides an accurate indication of how well blood flows through blood vessels. Higher plasma levels of coagulation factors and fibrinogen, as well as dehydration, can all contribute to increased blood viscosity (Aro, 2014; Aro and Akinlemimu, 2015; Aro, 2018; Aro *et al.*, 2018).

The whole blood viscosity profile in this study was not significantly (P> 0.05) influenced by the diets, breed and their interactions. This observation agrees with the report of Oluwapelumi *et al.* (2022) who reported an insignificant effect in layers whole blood viscosities reared in large furnished, small furnished, and conventional battery cages, and further concluded that stress levels of the birds in the different cages were similar, but differ from the report of Aro (2018) who reported a significant decrease in whole blood viscosity across the treatments relative to the concentration of NaCl in the diets of Isa White and Barred Plymouth Rock cocks. However, the non-significant (P> 0.05) diets, breed and their interactions observed in this study suggest that feeding broiler birds with CSOP as reported in this trial had no deleterious effect on the birds' whole blood viscosity either at dietary treatment levels, between the two breeds or their interactions.

Serum biochemical parameters namely: total protein, globulin, albumin, total cholesterol, high density lipoprotein and Alanine aminotransferase were not significantly influenced by the inclusion of CSOP in the diets of the broiler birds. The non-significant effects observed in these parameters in the control diet versus the CSOP diets suggest that the levels of composite sweet orange peel (CSOP) in the diets did not negatively affect the birds' serum constituents of these parameters, but rather, the consumption of the diets were adequate for the normal serum indices and did not negatively affect the normal liver and other organ functions. This finding agrees with the report of Behera *et al.* (2019) of a non-significant (P> 0.05) effects for total protein, and globulin levels of broiler fed 2.5% to 7.5% of citrus waste. Abassi *et al.* (2015) and Amaga *et al.* (2019) also reported no significant effects on serum enzymes activities with birds fed sweet orange peels and pulps and hence no indication of liver toxicity. The range of 3.63mg/dl to 4.02mg/dl for total protein observed in this study agrees with the range of 3.80mg/dl to 4.36mg/dl reported by Ashom *et al.* (2016) but lower than 5.92mg/dl to 6.93mg/dl reported by (Abd El-Latif *et al.*, 2023)

Aspartate aminotransferase (AST) in the serum was significantly (P< 0.05) different across the four dietary treatments. Birds on diet B (2.50% CSOP) had the highest value for AST and was significantly (P< 0.05) different from birds on other dietary means. Birds on diet A (control) had AST value that is comparable to those on diet D (7.50% CSOP). However, the least value of 68.25U/l for AST was observed in birds on diet C (5.00% CSOP). No clear pattern of variation was observed to suggest that the inclusion of CSOP in the diets of the broiler birds had a deleterious effect on the liver function. The range of 68.25U/l to 88.07U/l observed in this study is higher than 20.70U/l to 59.90U/l reported by Akpe *et al.* (2019) but fell within the normal reference range as reported by Mitruka and Rawnsley (1977).

Triglycerides were significantly influenced by the inclusion of composite sweet orange peel in the diets of broiler birds with no clear pattern of variation among the dietary means. Birds on diet B (2.50% CSOP) had the highest value for triglycerides which is comparable

(P> 0.05) to those on diet A (control). However, birds on diets C (5.00% CSOP) and D (7.50% CSOP) had the least (P> 0.05) values for triglycerides but differ significantly (P< 0.05) from those on diet A and B, respectively. The range of 1.54g/dl to 2.25g/dl observed in this study is within the normal reference range reported by Mitruka and Rawnsley (1977). The observed trend is a lower triglyceride in the blood serum of the birds relative to the increase in the level of CSOP in the diets. This is indicative of the The observed pattern suggests that the inclusion of CSOP in the diet of broiler was able to reduce the level of low-density lipoprotein in the body of the birds. This corroborates findings of Abd El-Latif *et al.* (2023) who reported that citrus peel had a favourable effect in decreasing blood cholesterol, low-density lipoprotein and very-low-density lipoproteins. Abassi *et al.* (2015) also reported a reduced level of low-density lipoprotein and triglyceride in broiler in response to dietary *Citrus sinensis* pulp and concluded that vitamin C and other compound found in the pulp are responsible for the altered metabolites.

Breed and interaction effects were not significantly (P> 0.05) different in the all the parameters measured, this suggest that both breeds were not negatively affected by the inclusion of composite sweet orange in their diet. The non-significant effect observed could suggest that the birds from both breeds were from common haematopoetic linage (Jain 1993; Aro, 2018) and so were able to utilize CSOP at almost the same rate without any damage to the liver and other organ functions of the birds.

Conclusion

This study investigated the physiological response of two breeds of meat-type chicken fed dietary composite sweet orange peels (CSOP) an alternative livestock feed sources.

The study showed that no significant breed and interaction effects exist between Cobb500 and Arbor Acre for all the blood viscosity and serum biochemical parameters measured. However, Arbor Acre breed tends to show a higher numerical value for all parameters measured except for, total protein, total cholesterol, low-density lipoprotein, albumin and globulin which were higher in Cobb500. Arbor Acre thus had better health profile than reduction in the bad cholesterol content in the birds as the level of CSOP increased in the diets.

Low-density lipoprotein (LDL-C) cholesterol was significantly (P< 0.05) influenced by the inclusion of CSOP in the diets, a progressive decrease in low-density lipoprotein was observed as the inclusion of composite sweet orange peels in the diet increased. Birds on diet A (control) had the highest value for LDL-C while the least value was observed in birds on diet D (7.50% CSOP) inclusion level. Cobb500 based on our findings. Serum biochemical parameters like AST, TG and LDL-C were significantly influenced by the inclusion levels of composite sweet orange peel in the diets. Dietary inclusion of composite sweet orange peels was beneficial to the wellbeing of the birds by moderating their whole blood viscosity. Birds on diets C and D showed the least low-density lipoprotein values, and the highest high-density lipoprotein (HDL-C) as pointers to improved carcass quality, lower bad cholesterol content and gingered consumers' preference for lean broiler meat.

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Evaluation of *Corchorus olitorius E*nriched Functional Foods on Biomarkers of Oxidative Stress in N-methyl-nitrosourea (MNU)-induced Toxicity in Male Wistar Rats

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Abstract

Nutritional enhancement with powdered *C. olitorius* to prevent hazards caused by chemical of N-methyl-nitrosourea (MNU)-induced toxicity in male Wistar rats was evaluated in rats divided into six groups of 5 animals each. Following two weeks of acclimatization, all rats except for the control groups were administered MNU intrarectally for 10 weeks alongside treatment with dietary inclusion of C. olitorius, while two control groups received normal saline for the period. The thiobarbituric acid reactive substance level (TBARS) was significantly elevated ($P<0.05$) in both the kidney and liver of the MNU control group compared to the normal control group. In contrast, there was a significant decrease ($p<0.05$) in superoxide dismutase and catalase activity in MNU control group and groups induced with MNU but treated with varying levels of feed enhancement *(C. olitorius*) when compared to the normal control group. Histopathology of the kidney and liver of the MNU control group showed a distorted architecture with distorted blood vessels and hyperchromicity, whereas normalcy increased with increasing the dietary inclusion of *C. olitorius*. Therefore, it can be concluded that *Corchorus olitorius* leaf possesses the ameliorative potential to toxicity induced by MNU.

Keywords: N-methyl-N-nitrosourea; Antioxidant; *Corchorus olitorius;* Haematology

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Introduction

Toxic chemicals from food contaminants can cause organ damage, organ failure, and even death in extreme circumstances. The colon, liver and kidney are the major organs associated with MNU-toxicity. According to donation and transplantation statistics 2019, sadly, each year, 8,000 people die (on average, 22 people per day—nearly one person every hour) from colon cancer, with a total of 102,480 new cases and an estimated mortality rate of 50,830, which connote 50% mortality rate (Siegel *et al.*, 2013), while in Nigeria, the male/female ratio is averagely equal. The peak age remains around 44 years (Irabor *et al.*, 2009). Several animal studies have shown that nonsteroidal anti-inflammatory drug decreases colon carcinogenesis (Rao *et al.*, 1995). It has been proven by Yan*et al*. (2013) that the phenolics of *Corchorus olitorius* showed excellent antioxidant and inflammatory inhibition. Polyphenolic and flavonoid chemicals in the diet have been linked to a reduction in diet-induced obesity and metabolic diseases in humans, such as diabetes and hypertension (wang et al., 2011). *Corchorus olitorius*, among many other plants, contained a reasonable percentage of biologically active cardiac principals which today plays a significant role in the pharmacological reference to cardiovascular activity (Sharaf and Negm, 1969). Several surgical treatments have also been put in place to combat colon carcinogenesis. However, they have posed some adverse effects on individuals, and in many cases, the cost is quite exorbitant and unaffordable to poor patients (Kuipers et al., 2015). In recent times, many foods and medicinal plants have

been investigated for potent antioxidant activities, economic viability, and relative safety (Atawodi *et al.*, 2011) concerning cancer prevention and management

*Corchorus olitorius***,** known by the names 'ewedu' in Yoruba, 'ayoyo' in Hausa, and 'ahuhara' in Igbo language of West Africa, is most often used in some parts of western Nigeria. It is a multipurpose plant that can heal several diseases such as inflammatory diseases, dysentery, and worm infestation (Negmet *et al.*, 1980). Thus, the curiosity to evaluate the preventive effect of *Corchorus olitorius* diet inclusion on MNU-induced colon cancer in male Wistar rats and the accompanying pathophysiological changes.

Materials and Methods

Materials

N-Methyl-N-Nitrosourea and thiobarbituric acid were purchased from Sigma Chemical Company, St. Louis Mo, USA (Catalogue No. N4766-25G). Sigma Aldrich chemical company, St. Louis, USA respectively. Carcinoembryonic antigen assay kit was procured from Diagnostic Automation/cortex Diagnostics (DACD) Incorporation, Calabasas California USA, while other chemicals were of analytical grade and were acquired from Sigma Aldrich chemical company, St. Louis USA.

Animals and Diet

Thirty male Wistar rats weighing 70-90g/Rats were purchased and housed in well-ventilated cages in the Animal House of the Department of Pharmacology, Ahmadu Bello University, Zaria. They were acclimatized for two weeks and fed with standard rat chow and free access to water. The study follows the guidelines of National Institute of Health's involving laboratory animal treatment (NIH publication No. 18-23, 1985). The rats were weighed and randomly divided into six (6) groups of five animals each (n= 5) and were given the basal diet of Vital feed (growers mash), formulated to contain appropriate nutrients of a balanced diet manufactured from Jos, Nigeria. The basal diet was mixed with the dried and homogenized. *C. olitorius* using a blender to ensure uniform distribution with the experimental feed which was given to the treated groups according to the percentage of inclusion (0%, 2.5%, 5%, and 10%). Before feeding, all supplemented diets were moulded into balls and dried under the sun.

Experimental procedure

The experimental rats were given the group's inclusion percentage of basal diet for eight (8) weeks. Afterwards, four (4) groups received an intrarectal dose of 0.1ml of 1% freshly prepared MNU solution thrice a week for 10 weeks while the basal diet control group and the diet control groups received normal saline three times weekly for ten (10) weeks without the stoppage of the different level of inclusion. Animals were fed either control or experimental diets until the end of the research. Feed intake and the body weight of the rats were recorded weekly for the whole ten (10) weeks of the induction. The animals were sacrificed by decapitation following chloroform anaesthesia one week after the end of the induction period. The rats were dissected to harvest two major organs; the liver and kidney. These organs were rinsed in normal saline before being fixed for histopathology analysis using 10% formal-saline. A section of the liver and one of the kidneys were washed immediately with ice-cold saline, with 10% homogenate (100mg tissue/ml buffer) crushed with mortar and pestle in 50mM phosphate buffer pH 7.4 and centrifuged at 10rpm for 10min. The supernatants were used to assay for endogenous antioxidant enzymes (Catalase, Superoxide dismutase) and thiobarbituric acid.

Determination of Feed intake

The feed intake was carried-out according to the methods of Ogungbemi et al., (2017) using the equation shown below:

$$Feed\ intake\ = \left(\frac{Average\ feed\ intake\ (g)}{Average\ Body\ weight\ (kg) of\ rat} \times 1000\right) /10\text{weeks}$$

Biochemical Analysis

Thiobarbituric acid reactive substances (TBARS) Assay

The thiobarbituric acid reactive substances (TBARS) were estimated using the spectrophotometric method published by Sivonnovan et al. (2007) to determine the amount of lipid peroxidation in both the liver and kidney. In the procedure, 1 ml 14% trichloroacetic acid was used to deproteinize 50µl of the supernatant, followed by 1 ml 0.6% thiobarbituric acid. The reaction mixture was then placed in a heating chamber for 30 mins at 80°C. The cooled mixture was then centrifuged at 2000g for 10 mins. A UV spectrophotometer was used to measure the absorbance of the coloured product Thiobarbituric acid reactive substances at 535nm. The level of Thiobarbituric acid reactive substances was extrapolated using the using the formula, A=ΣCL where A=absorbance, Σ=molar extinction C= concentration, and L= pathlength.

Catalase (CAT)

The activity of catalase (CAT) was determined using the Aebi (1979) method, which involved adding 10µL of liver and kidney homogenate to test tubes containing 2.80mL of 50mM phosphate buffer (pH 7.4). The reaction mixture was started by adding 0.1ml of freshly generated 30mM H_2O_2, and the rate of H_2O_2 decomposition was monitored using a spectrophotometer at 240nm after a 5-minute interval. (Jenway 640uv/vis). A molar extinction coefficient (E) of 0.041 mM-1-cm-1 was used to calculate the catalase activity.

$$Catalase\ (\mu mol/min) = \frac{Absorbance}{E}$$

Superoxide Dismutase (SOD)

Superoxide dismutase activity was carried out using the methods described by Martin *et al.* (1987). This involves the autooxidation of hematoxylin repressed by SOD at a PH 7.8. The concentration of the SOD is directly proportional to the level of inhibition. The method can be summarized thus. Exactly 920µL of phosphate buffer (0.05M, pH7.8) was dispensed into clean test tubes; 40µL of sample homogenates were added. A reagent test was also made by substituting 40L of sample dilution buffer for the sample. The mixture was kept at 25ºC for 2 minutes before the addition of 40µL of hematoxylin. The absorbance of the sample test and reagent test were read in duplicate at 560nm immediately after the addition of 40µL of hematoxylin, and compared to the sample blank, which was distilled water.

Exogenous Anti-oxidant

Estimation of total polyphenolic content

Lachman *et al.* (2003) method was adopted in quantifying the total polyphenolic content of the basal diet, pure leaves, and the supplement diets using the Folin-Ciocalteu reagent method. An aliquot of 0.25ml of the extract (10ml of 99.9% methanol was added to 0.2g (0.02g/ml)) of each sample was placed in clean dried test tubes, the tubes were covered with aluminium foil and kept in the water bath at 37ºC for 2 hours shaking every 15 minutes). The mixture was centrifuged for 10 minutes at 3000 rpm, and the supernatant was collected. Test tubes for the blank and standard were set with 2.5 ml of Folin Ciocalteu, and 1M Na_2CO_3 was added and mixed thoroughly; the mixture was allowed to stand for 15 minutes. The absorbance of the blank and samples was measured at 765 nm using a spectrophotometer which was then extrapolated through the standard curve of mg of gallic acid equivalent (GAE) per 100g dry matter.

Determination of total flavonoid content

Total flavonoid content was carried out according to the method of Lachman *et al*. (2003). Procedure: An aliquot of 0.5ml of extract (0.2g of the sample in 10ml of 70% methanol) was placed in tubes. The tubes were allowed to stand in a water bath for 2 hours at 37ºC with occasional shaking every 15 minutes. After centrifugation at 3000rpm for 10min, 1.5ml of 70% methanol was added to the supernatant and mixed thoroughly. 0.1ml of 10% $AlCl_3$ was added to the mixture, followed by 0.1ml of CH_3COOK. The mixture was incubated at 25ºC for 30 minutes, and the absorbance of both blank and samples were taken 415nm. Quercetin was used as a standard at the following concentration: 12.5, 25, 50, 100µg/ml.

Statistical Analysis

A one-way ANOVA was, this is followed by a Bonferroni t-test for multiple comparisons. The data is presented as mean SD with a level of confidence (0.05)

Result

Effect of dietary inclusion of Corchorus olitorius on cumulative feed intake (g/kg body weight) in MNU-treated Wistar rats.

The result in Table 1 shows the evaluation of the effect of C. *olitorius* inclusion on feed intake (g/kg body weight) in MNU-treated Wistar rats. There was no significant difference in the feeding rate of the control group (basal diet), 5% diet inclusion and 10% diet inclusion groups whereas, the MNU control group (3071.18 ± 139.83) and 2.5% diet inclusion (3058.30 ± 90.66) shows a decrease in the feeding rate when compared to the Normal diet group (4138.298 ± 242.81)

Table 1: Effect of Dietary Inclusion of *Corchorus olitorius* on Cumulative Feed Intake (g/Kg Body Weight) in MNU-Treated Wistar Rats for 10 Weeks.

Treatment	Feed intake (g/kg body weight)
Control feed	4138.298 ± 242.81^{b}
MNU + Basal Feed	3071.18 ± 139.83^{a}
10% dietary inclusion Control	4732.31 ± 206.43^{c}
MNU + 2.5% dietary inclusion	3058.30 ± 90.66^{a}
MNU + 5% dietary inclusion	3868.57 ± 187.58^{b}
MNU + 10% dietary inclusion	4222.35 ± 225.47^{bc}

Values with different superscripts down the column are significantly different ($P<0.05$), with n=5.

The Quantitative Analysis of the Total Polyphenolic and Flavonoid Contents Supplemented with *Corchorus olitorius*

The results in Table 2 shows the quantitative analysis of the total polyphenolic and flavonoid content of the leaves of *C. olitorius* supplemented vital feed diet. The total polyphenolic and flavonoid content of the inclusion diet increases with an increasing level of inclusion compared to the normal feed, which shows the lowest polyphenolic and flavonoid content. The basal diet had the lowest crude fibre while the pure leaf had the highest. The crude fibre increases with increasing leaf inclusion.

Table 2: The Quantitative Analysis of the Total Polyphenolic Content (mg Gallic Acid/g of the Sample) of Flavonoid (mg Quercetin Acid /g of Sample of the Leaves of *Corchorus olitorius* Dietary Inclusion

Level of diet inclusion	Total Polyphenols (mg gallic acid/g)	Total flavonoids (mg quercetin acid /g)	Crude fibre (%)
0% Diet inclusion	59.325 ± 0.03	30.977 ± 0.01	7.490
2.5% diet inclusion	71.975 ± 1.42	35.786 ± 3.05	8.340
5% diet inclusion	104.085 ± 3.55	61.133 ± 3.69	8.550
10% diet inclusion	132.504 ± 2.48	82.109 ± 5.54	8.770
100% *C. olitorious*	180.461± 8.34	125.275±11.89	9.890

Values are mean ± SD for the two samples (n=3)

Endogenous Antioxidant

The effect of Dietary inclusion with leaves of Corchorus olitorius on the level of thiobarbituric acid, Superoxide dismutase, and catalase on the Kidney and Liver of MNU-treated Wistar rats

In the result of superoxide dismutase activity carried out on both kidney and liver seen

in Tables 3 and 4, the normal control group, 10% inclusion control group, and the highest inclusion diet group of the kidney showed no significant difference among the groups while other diet inclusion groups showed a lower activity when compared to the normal control group. A similar result was also observed in SOD activity of the liver with the normal control group, diet control group, and 10% inclusion diet giving the highest activity.

The catalase activity of MNU-treated Wistar rats carried out on both kidney and liver in Tables 3 and 4 showed that the normal control group, 10% inclusion control group, and the highest dietary inclusion group of the kidney showed that there was no significant difference amongst the groups while other diet inclusion groups showed a lower activity when compared to the normal control group. A similar result was observed in catalase activity of the liver with the normal control group, diet control group, and 10% dietary inclusion giving the highest catalase activity.

The level of lipid peroxidation in MNU-treated Wistar rats carried out on the kidney and liver in Tables 3 and 4 show that a significant increase was observed in the level of TBARS of the kidney of MNU-treated control group when compared to the normal control group while an observable decrease in the level of TBARS in the other groups with increasing diet inclusion. A similar result was obtained in the level of lipid peroxidation of the liver, too, with the highest level of TBARS seen in the MNU-control group. In contrast, other treated group shows a decrease in the level of TBARS with increasing dosage of the dietary inclusion.

Table 3: The Effect of Dietary Inclusion with Leaves of *Corchorus olitorius* on the Level of Thiobarbituric acid, Superoxide dismutase, and Catalase Activities on the Kidneys of MNU-Treated Wistar Rats

Treatment	TBARS (µmol/mg protein)	Catalase (µmol/min/mg protein)	Superoxide dismutase (U/mg protein)
Control feed	4.03 ± 0.84^{bc}	122.09 ± 7.7^{a}	83.33 ± 7.6^{a}
MNU + Basal Feed	6.65 ± 0.93^{a}	53.738 ± 13.9^{b}	17.25 ± 1.3^{d}
10% diet Control	3.22 ± 0.84^{c}	137.44 ± 11.2^{a}	96.00 ± 9.3^{a}
MNU + 2.5% diet inclusion	5.99 ± 0.4^{ab}	61.13 ± 10.9^{b}	36.00 ± 1.8^{c}
MNU + 5% diet inclusion	5.89 ± 0.4^{ab}	74.00 ± 11.6^{b}	56.00 ± 5.8^{b}
MNU + 10% diet inclusion	4.43 ± 0.58^{abc}	111.10 ± 9.1^{a}	93.50 ± 3.5^{a}

Values with different superscripts down the column are significantly different ($P<0.05$)

Table 4: The Effect of Dietary Inclusion with Leaves of *Corchorus olitorius* on the Level of Thiobarbituric acid, Catalase, and Superoxide dismutase Activities on the Liver of MNU-treated Wistar Rats

Treatment	TBARS (µmol/mg protein)	Catalase (µmol/min/mg protein)	Superoxide dismutase (U/mg protein)
Control feed	6.76 ± 0.96^{b}	137.39 ± 19.90^{a}	98.80 ± 2.97^{a}
MNU + Basal Feed	15.98 ± 2.58^{a}	36.13 ± 10.56^{c}	19.67 ± 1.30^{c}
10% diet inclusion Control	6.29 ± 1.60^{b}	114.97 ± 12.10^{ab}	102.00 ± 7.50^{a}
MNU + 2.5% diet inclusion	10.5 ± 1.65^{ab}	76.18 ± 10.70^{bc}	36.67 ± 9.21^{c}
MNU + 5% diet inclusion	6.17 ± 1.43^{ab}	71.51 ± 10.6^{c}	74.25 ± 7.27^{b}
MNU + 10% diet inclusion	5.92 ± 0.93^{b}	126.5 ± 12.1^{a}	96.50 ± 10.63^{a}

Values with different superscripts down the column are significantly different ($P<0.05$)

Histopathological Analysis

Histology of Kidney & Liver

In the kidney, mononuclear cellular infiltration into intertubular spaces alongside were observed in the MNU control but absent in the normal control group(plate 1a).Whereas, a better architecture was observed when compared to the MNU control group with an increasing level of *C. olitorius* supplementation (plate 1c,1d,1e)

The histology of the liver of the MNU control group shows a congested vein with mononuclear cellular infiltration, when compared to the normal control showing normal architecture, whereas other treated groups show better architecture with increasing supplementation. (plate 3c,3d,3e)

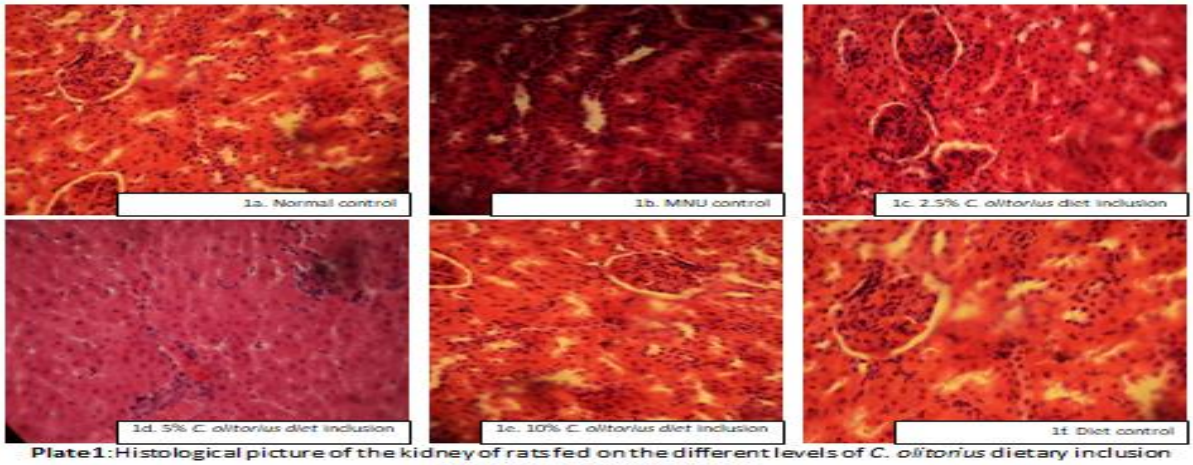

Plate1:Histological picture of the kidney of rats fed on the different levels of *C. olitorius* dietary inclusion MNU-induced toxicity in rats.

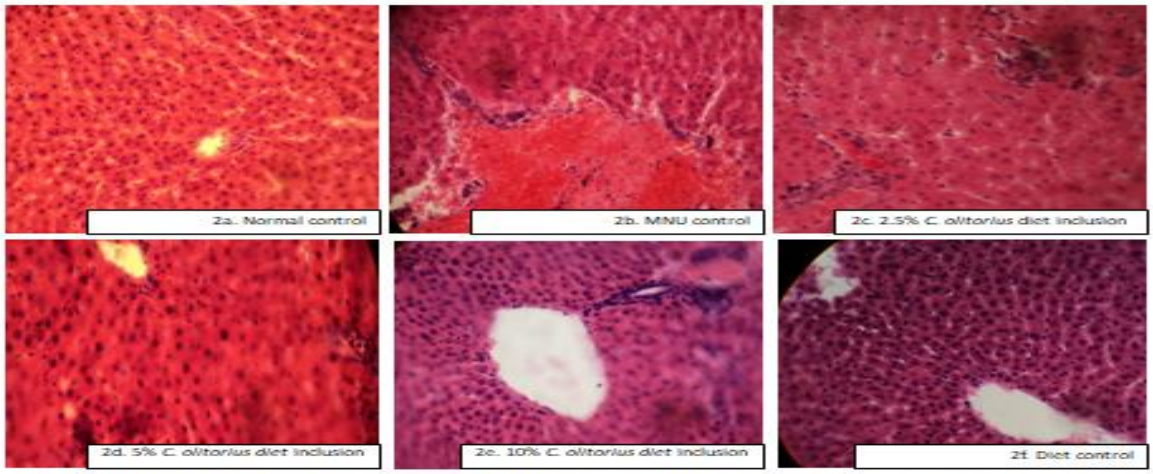

Plate1:Histological picture of the Liver of rats fed on the different levels of *C. olitorius* dietary inclusion MNU-induced toxicity in rats. ((H&E; x200)

Discussion

The use of *Corchorus olitorius* leaves in a regular feed (vital feed growers mash) for male Wistar rats produced significant feed intake results. 10% *Corchorus olitorius* leaf inclusion had the highest feed intake among the MNU-treated groups (Table 1), which can be attributed to the palatability of the *C.olitorius* stimulating the appetite of the rats (Grubben 1997; Fasinmirin and Olufayo 2009; Ogungbemi *et al.*, 2017) which is attributed to the high nutritional constituent associated with weight gains such as carbohydrate, lipids, and minerals.

Because current dietary phenolic and flavonoid consumption is typically insufficient to protect cells from mutagens (either exogenous or endogenous), dietary inclusion as an alternate method is required as demonstrated in this study, with the *Corchorus olitorius* plant having the

highest polyphenol and flavonoid content, followed by a 10% supplemented diet (Table 2). Polyphenolic substances are a wide range of secondary metabolites found in the human diet which contain several chemopreventive substituents that include Hydroxybenzoic acids, resveratrol, curcumin, genistein, and epigallocatechin Hydroxycinnamic acids, among others. This acts as an anti-cancer agent by modulating the immune system and providing antioxidant properties towards Apoptosis induction, also cell cycle arrest suppression of GFR–mediated pathways Suppression of NF-B activation in turn Suppression of angiogenesis. (Ren *et al.*,2003. Fresco *et al.*, 2006, Ghiringhelliy *et al.*, 2012,)

Due to induced toxicity, reactive oxygen species (ROS), byproducts of aerobic metabolism, are elevated in many types of disease. Increased endogenous ROS cause adaptive changes and could be important in toxicogenesis. (Shi *et al.*, 2012), as seen in conformity with the result of the TBARS in (Table 3 and 4) with observable elevated lipid peroxidation in the liver and kidney of the MNU control group and groups fed with the lowest inclusion diet. Also, a means to combat these free radicals are defense machinery which includes endogenous antioxidants such as superoxide dismutase and catalase and the immune system. Superoxide dismutase (SOD) is an essential enzyme that eliminates superoxide radicals (O_2^-) and thus protects cells from damage caused by free radicals. The low SOD activity in diseased rats may render the rats highly dependent on SOD for survival and sensitive to inhibition of SOD (Huang *et al.*, 2000). This result is following the result obtained in (table 3 and 4) with a lowered SOD when compared to the normal, which may be a result of the suppressing potential of the toxicity induced to enable the carcinogen to exert its maximum effect, whereas there was an increase in the SOD level with increase inclusion.

The histopathological finding showed that the MNU control group and the group fed with the lowest supplemented diet had distortion in the colonic architecture, necrosis of the lamina propria, atrophy of the gland, and cellular proliferation with hyperchromacity and cellular pleomorphism, which also represent biomarkers for toxicity in rats while groups fed the higher dosage of the inclusion had improved architecture which can be attributed to the preventive potentials of the plant.

Supplementing the diet with high fibre has been found to protect against the development of a variety of malignancies, particularly those of the colon and breast, in both human and animal studies. (Ferguson and Harris 1999) *C. olitorius* is a rich source of dietary fibres (Phuwapraisirisan *et al*., 2009) that have biomolecules and compositions that suggest they may be cancer-protective (Furumoto *et al*., 2002). In this study, with the polyphenols and flavonoids, dietary fibre of 10% *c. olitorius* inclusion was the highest among the inclusions. Leafy vegetables have been researched to play several medicinal roles in the history of man due to their numerous chemical compounds which have played key roles in biological systems and functions preventing and fighting against organ toxicity At least 12,000 phytochemicals have been isolated such as polyphenols and flavonoids which are said to be medicinal to health (Tapsell *et al.*, 2006). Herbal medicine has been accepted by several African and Asian countries in meeting the need of health challenges affecting about 80% population in their region (Fabricant and Farnsworth 2001). Medicinal plants such as *Corchorus olitorius* have been confirmed as a multipurpose plant having the potentials to heal several diseases such as inflammatory diseases, dysentery, worm infestation (Fasinmirin and Olufayo, 2009; Negm*et al.*, 1980). The result showed that the total polyphenolic content of the pure plant of *Corchorus olitorius* was the highest form (Table 2), whereas the total polyphenolic content in other supplemented diet groups increases with an increased level of inclusion when compared to the normal feed, which shows the lowest total polyphenolic content, a similar result was also obtained for flavonoid (Table 2). Manifold antioxidant phytochemical has been confirmed to plays a function in attenuating toxicity more efficiently when compared with that of a single phytochemical in food. (Narisawa *et al*., 2000). It can therefore be established that leaves of *C. olitorus* possess preventive potentials towards MNU-induced organ toxicity.

Conclusion

The study evaluated the preventive effects of *Corchorus olitorius* enriched functional foods and that there is improvement in the endogenous antioxidant of the liver and kidney with an increase in dietary inclusion of *Corchorus olitorius*. Notably is the improvement of the architecture of the kidney and liver in the highest dietary inclusion. The high content of the polyphenols and flavonoids of *Corchorus olitorius* is seen to prevent toxicity in the liver and kidney. It can be concluded that *Corchorus olitorius* has preventive effect against MNU-induced organ toxicity.

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Declaration of Conflict of Interest

None

Author's Contribution

Ogungbemi, K.: Investigation, Atawode, S.E.: Conceptualization/Supervision, Popoola, S.T.: Writing draft, Fawole, A.O.: Data Curation, Adeniyi, B.M.: Investigation, Ilori, A.O.: Proof reading of draft, Solomon-Ibuwunwa, O.M.: Data analysis, Ajala, O.V.: Writing draft, Akeju, B.M.: Data Curation

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Evaluation of Selected Phyto-Actives in Tea Extracts Used for Cosmetics and Skincare Productions

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Abstract

In this study, the phyto-active contents of six tea brands sold in Nigeria were evaluated. Three are Herbal teas, two are green teas, and one Black tea. These tea brands evaluated are nutritionally valuable, their intake boost the immune system and act as antioxidants protecting the body. Comparing the phyto-actives in the sampled teas, the results showed that there was no significant difference in the mean values of Alkaloid, Saponin, Terpenoid, and Flavonoid with $\rho > 0.05$] extracted. Other phyto-actives with mean values significantly different across the tea types are Tannin [$F(2, 15) = 3.902$; $\rho < 0.05$], Phlobatannin [$F(2, 15) = 6.977$; $\rho < 0.05$], Cardiac Glycosides [$F(2, 15) = 12.415$; $\rho < 0.05$]; Steroids [$F(2, 15) = 8.334$; $\rho < 0.05$]; Reducing Sugar [$F(2, 15) = 9.054$; $\rho < 0.05$] and Phenol [$F(2, 15) = 8.439$; $\rho < 0.05$]. From these results, herbal teas are safe as ingredients in cosmetics and skincare products, while tea types containing cardiac glycosides, such as black and green tea, should be excluded from cosmetics and haircare applications due to potential skin absorption and associated health risks, particularly for individuals with cardiovascular conditions or sensitivity to plant-derived ingredients.

Keywords: **Phyto-actives; Tea; Antioxidants; Skin care; Cosmetics.**

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Introduction

Tea is a healthy and cheap drink used as folk medicine for headache, digestion and immune defense. It is known to contain some phyto-actives with antioxidant properties capable of slowing down and/or prevent oxidative damage to DNA molecules (Krishna et al, 2014). Phyto-actives also known as phytochemicals are natural compounds derived from plants that possess potent medicinal properties and contribute to their therapeutic properties and potential health benefits. A study by Okereke et al, 2015 showed the phytochemical components (phyto-actives) present in the extract of Hibiscus sabdariffa (sobo) as alkaloids, tannins, saponnins, glycosides, phenols and flavonoids and their quantitative result as follows: Tannins (17.0%), saponnins (0.96%), phenols (1.1%), glycosides (0.13%), alkaloids (2.14%) and flavonoids 20.08%). The presence of phyto-actives namely alkaloids, flavonoids, steroids, gallic tannins, catecholic tannins, e.t.c. plays a vital role in the plant defense mechanism (Tariq et al, 2012). A study by Isam Eldin (2015), evaluated the phyto-actives and proximate compositions of black tea (Camellia sinensis Leaves). The phyto-actives screening of the samples revealed the presence of high tannins and saponins content, alkaloids were present in small amount while investigation of the samples showed that flavonoids were absent (Isam Eldin, 2015). In another study by Asaolu et al, (2010), green and black tea are said to have cancer preventive activity (Asaolu et al, 2010).

Cosmetics, as defined in Regulation (EC) No. 1223/2009 of the European Parliament and of the Council dated 30 November 2009

(Parlamento, 2009), encompass a wide range of products intended for personal care, hygiene, and beautification purposes. This definition includes substances or mixtures that are applied externally to various parts of the body, such as the skin, hair, nails, lips, external genital organs, teeth, and mucous membranes of the oral cavity [Cosmetics Europe, 2023]. The primary goal of cosmetics is to enhance the appearance, texture, and overall health of the skin, hair, and nails. These products are utilized for cleansing, moisturizing, protecting, and accentuating the natural beauty of these tissues. Tea plant itself and its extracts together with their centuries-old tradition of use play an important role on the cosmetics market. A lot of work has been done previously describing the activity of specific tea ingredients, e.g., caffeine (Herman et al, 2013). Tea extracts possess a wide spectrum of biological activities, as antioxidants, photo-protectives (against harmful effects of UV irradiation), slimming, improving skin, hair and microcirculation condition properties (Koch et al, 2019). In general, cosmetics products containing tea extracts rich in polyphenols have a positive effect on the skin appearance and ameliorate skin damage, erythema and lipid peroxidation following UV exposure (Arct et al, 2008). There is an increasing number of cosmetics containing tea extracts, especially those produced using green tea infusions, but recently black and herbal teas are in use (Arct et al, 2003 & Gianeti et al, 2013). Applying cosmetics containing tea extract, makes the skin appears more tense and refreshed, as a result of the astringent activity of polyphenols and tannins and their interaction with keratin present in the stratum corneum. This process also leads to a reduction in skin redness, irritation and reduction of swelling. Tea extracts, have a disinfecting, antioxidant and toning effect. They also soothe inflammation, accelerate the healing of wounds and skin eruptions, and also close skin pores by which they reduce their visibility (Możdżeń et al, 2016).

Method: The AOAC method (2015) and the methods described by Osagie and Okwu (Osagie, 2011, Okwu, 2005, Harbone, 1991 and Ejikeme *et al.*, 2014) were used for the analytical screening of the desired phyto-actives in the tea samples.

Sample Collection and Identification: Six tea samples were used for this study. The tea samples were obtained in packs from shops and supermarkets in Ayobo-Ipaja Metropolis, Lagos State, Nigeria. The processed teas were pre-packed in tea bags. The samples were identified at the Department of Chemical Sciences, Faculty of Sciences in Anchor University, Lagos State, Nigeria. H1 was identified as Master Tea Moringa (Herbal Tea), G2 as GoTea (Herbal Tea), M3 as Mejic (Herbal Tea), Q4 as Qualitea Natural (Green Tea), L5 as Legend Green Tea Lemon and Ginger (Green Tea), T6 as TopTea (Black tea).

Figure 1. Pictures of Sample Teas

Table 1: Quality Control Description of the Tea samples analyzed
***NS = Not stated**

SAMPLE CODE	BRAND NAME	TEA TYPE	BATCH NUMBER	MANUFACTURE DATE	EXPIRY DATE	COUNTRY OF PRODUCTION	NAFDAC/ISO NUMBER
T6	TOP TEA	BLACK (*camellia sinensis*)	BN31623GA 11:55	12/11/23	01/06/25	NIGERIA	B1-1551
Q4	QUALITEA	GREEN	D23130	05/2023	05/2026	SRI LANKA	22000:2005
L5	LEGEND	GREEN	NG/02/2023	02/2023	02/2026	SRI LANKA	C1-0816
H1	MORINGA	HERBAL (*moringa oleifera*)	B1-7228L	15/04/24	14/04/26	NIGERIA	08-7228l
M3	MEJIC	HERBAL	NU 2023/01	NS	27/08/2028	NS	A7-4383L
G2	GOTEA	HERBAL	G1001	04/2024	03/2024	NIGERIA	A7-2029L

Results

Table 2: Qualitative Evaluation of the selected Phyto-actives in the tea brands
+ = Present, ++ = Much in abundance, - = Absent

Code	Alkaloid	Tannin	Phlobatannin	Saponnin	Terpenoid	Cardic Glycosides	Steroid	Reducing Sugar	Flavonoid	Phenol
T6	+	++	+	+	+	+	+	+	+	+
M3	+	++	-	+	+	-	+	-	+	+
H1	+	++	+	+	+	-	+	-	+	+
Q4	+	++	+	+	+	+	+	+	+	+
L5	+	++	+	+	+	-	+	-	+	+
G2	+	++	-	+	+	-	+	-	+	+

The statistical software package SPSS Version 23 program was used to analyze all experimental data collected.

Table 3. Mean and standard deviation of the phyto-actives in the tea types

Phyto-actives analyzed	Black Tea	Green Tea	Herbal Tea	Total	Limit
Alkaloid mg/100g	38.46 ± 0.35	32.40 ± 3.07	36.80 ± 7.90	35.61 ± 6.16	20.00
Tannin mg/100g	56.77 ± 0.01	52.65 ± 2.60	43.18 ± 11.45	48.60 ± 9.84	2000.00
Phlobatannin mg/100g	25.52 ± 0.40	24.39 ± 1.27	8.44 ± 12.67	16.61 ± 12.11	Less than 10% of total Polyphenols
Saponin mg/100g	32.48 ± 0.02	36.28 ± 2.08	37.17 ± 10.46	36.09 ±7.46	1500.00
Terpenoid mg/100g	26.55 ± 0.58	26.76 ± 1.09	25.86 ± 0.52	26.28 ± 0.84	1000.00
Cardiac Glycosides mg/100g	18.11 ± 0.61	9.04 ± 9.90	0.00	6.03 ± 8.78	0.10
Steroid mg/100g	20.89 ± 0.01	22.23 ± 0.16	16.80 ± 3.60	19.29 ± 3.59	10.00
Reducing Sugar mg/100g	20.44 ± 0.21	12.72 ± 13.93	0.00	7.65 ± 11.22	20.00
Flavonoid mg/100g	14.64 ± 0.61	16.90 ± 1.72	17.20± 1.55	16.68 ± 1.71	400.00
Phenol mg/100g	23.51 ± 0.30	14.00 ± 1.65	17.72 ± 4.30	17.45 ± 4.50	200.00

Table 3 above shows the mean of selected phyto-actives in the teas samples. It compares the mean of the tea types with the recommended limits given in the table. Table 4 below compares the mean values of the phyto-actives in the three sampled tea types. The table showed that there was no significant difference in the mean values of Alkaloid [$F(2, 15) = 1.358$; $\rho > 0.05$], Saponin [$F(2, 15) = 24.938$; $\rho > 0.05$], Terpenoid [$F(2, 15) = 1.575$; $\rho > 0.05$] and Flavonoid [$F(2, 15) = 7.591$; $\rho > 0.05$] extracted. Other phytochemical properties that the mean values were significantly different across the tea types are Tannin [$F(2, 15) = 3.902$; $\rho < 0.05$], Phlobatannin [$F(2, 15) = 6.977$; $\rho < 0.05$], Cardiac Glycosides [$F(2, 15) = 12.415$; $\rho < 0.05$]; Steroids [$F(2, 15) = 8.334$; $\rho < 0.05$]; Reducing Sugar [$F(2, 15) = 9.054$; $\rho < 0.05$] and Phenol [$F(2, 15) = 8.439$; $\rho < 0.05$].

Table 4. ANOVA of the difference in Mean Values of Tea Types

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Alkaloid	Between Groups	98.863	2	49.432	1.358	0.287
	Within Groups	545.926	15	36.395		
	Total	644.789	17			
Tannin	Between Groups	563.133	2	281.567	3.902	0.043**
	Within Groups	1082.381	15	72.159		
	Total	1645.515	17			
Phlobatannin	Between Groups	1201.607	2	600.803	6.977	0.007**
	Within Groups	1291.594	15	86.106		
	Total	2493.201	17			
Saponin	Between Groups	49.875	2	24.938	.417	0.666
	Within Groups	896.930	15	59.795		
	Total	946.805	17			
Terpenoid	Between Groups	3.151	2	1.575	2.682	0.101
	Within Groups	8.811	15	.587		
	Total	11.962	17			
Cardiac Glycosides	Between Groups	819.086	2	409.543	12.514	0.001**
	Within Groups	490.904	15	32.727		
	Total	1309.990	17			
Steroids	Between Groups	115.152	2	57.576	8.334	0.004**
	Within Groups	103.634	15	6.909		
	Total	218.786	17			
Reducing Sugar	Between Groups	1171.481	2	585.741	9.054	0.003**
	Within Groups	970.366	15	64.691		
	Total	2141.847	17			
Flavonoid	Between Groups	15.182	2	7.591	3.287	0.065
	Within Groups	34.638	15	2.309		
	Total	49.821	17			
Phenol	Between Groups	182.016	2	91.008	8.439	0.004**
	Within Groups	161.770	15	10.785		
	Total	343.786	17			

Discussion

The choice of the right active plant extracts or compounds, the confirmation of their activity, and their stability and synergistic effects in cosmetic products are important factors for the formulation of an effective product (Kusumawati & Indrayanto, 2013). From the analysis above, since there is no significant difference in the mean values of alkaloid, saponin, terpenoid, and flavonoid in all tea samples, the use of such tea types in cosmetics and hair care products is equally safe. However, from the results, there is a significant difference in the amount of the following phyto-actives in the tea samples analyzed, viz-a-viz, tannin (Herbal Tea (G2) has a lower value than the rest; phlobatannin (not detected in Herbal tea (M3) and Herbal tea (G2); cardiac glycosides only detected in Black tea (T6) and Green tea (Q4). Also, there are many variations in the mean values of steroids in the order, green > black > herbal. Reducing sugar is absent in all herbal teas but fairly present in Black tea (T6) and Green tea (Q4). Phenol analyzed in all tea samples is within recommended limit although with much variations.

Figure 2. Pictures of Skincare products containing Herbal teas

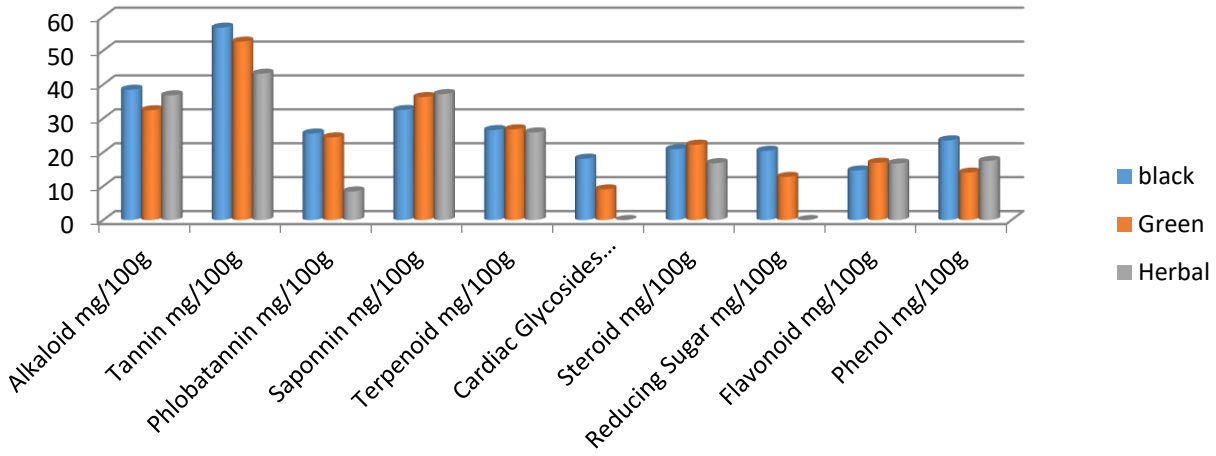

Figure 3. Mean Values of Phyto-actives of Tea samples

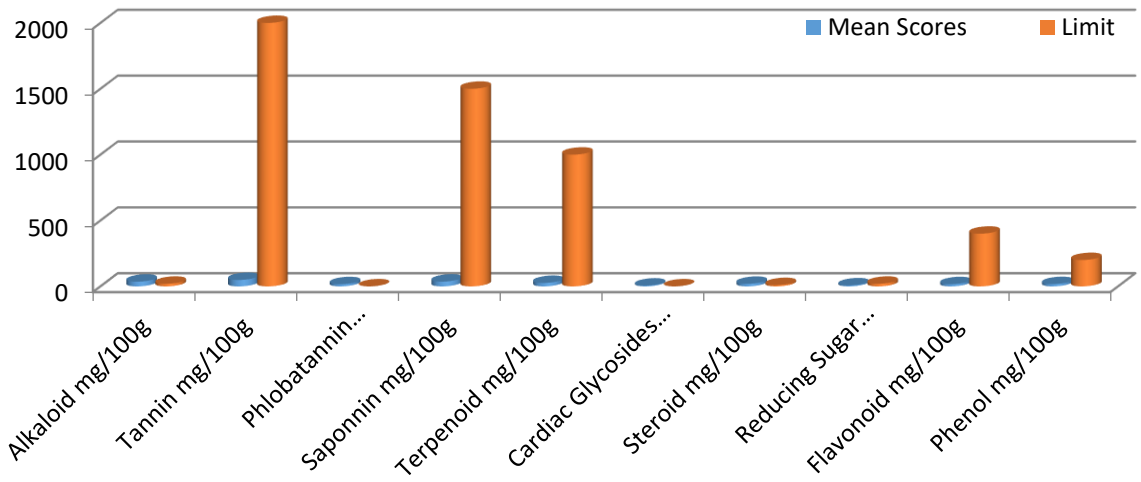

Figure 4. Mean Values compared to Recommended Limits of each Phyto-actives analyzed.

Conclusion

This study reveals that the incorporation of the teas analyzed into cosmetic and haircare products will mitigate inflammation, refine pores, regulate sebum production, enhance skin brightness and uniformity, stimulate collagen synthesis, provide photo-protection, improve skin appearance, and exhibit potent antioxidant properties due to the total polyphenolic content (tannins, phenols, flavonoids, and phenolic acids) in them. However, tea types containing cardiac glycosides, such as black and green tea, should be excluded from cosmetic and haircare applications due to potential skin absorption and associated health risks, particularly for individuals with cardiovascular conditions or sensitivity to plant-derived ingredients. In conclusion, this study recommends using herbal teas in cosmetics and skin care products, highlighting their potential benefits for skin health and appearance.

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Declaration of conflict of interest

The authors declare no conflict of interest.

Authors' contribution

Iretiolu Comfort Lasore: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools, or data; Wrote the paper.

Moses Sunkanmi Oladokun: Performed the experiments; contributed reagents, materials, and analysis tools

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Phytochemistry and Antioxidant Potential of Dried Noni Fruit and Juice

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Abstract
Morinda citrifolia (noni), an underutilized tropical fruit, has drawn interest because of its conceivable health benefits. The objective of this study was to investigate the phytochemistry and free radical scavenging potential of dried noni fruit and juice. Several analytical methods, such as spectrophotometric assays, were used to determine the phytochemical composition of the dried noni fruit and juice samples. The results revealed the presence of various minerals, and bioactive substances, including phenolic compounds, and flavonoids in both the dried fruit and juice. Notably, the dried fruit and juice had varied phytochemical compositions, with K, Na, total phenolics, and flavonoid contents significantly higher ($p < 0.05$) in the juice. The substantial antioxidant activity in the DPPH and FRAP experiments of the dried noni fruit and juice both indicated significant ($p < 0.05$) free radical scavenging capacity. These findings suggest the potential of dried noni fruit and juice as a significant antioxidant source.

Keywords: Bioactive; ,Minerals; antinutrients; Noni fruit; Food processing

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Introduction

According to the Food and Agriculture Organization, consumers are shifting their preferences from processed foods to natural foods of the highest quality that satisfy their nutritional needs and promote health (FAO 2016). The consumption of fruits and fruit products plays a vital role in improving immune function and promoting overall health (Amao, 2018). Fruits contain a variety of vital nutrients, vitamins, minerals, antioxidants, and many other phytochemicals known to reduce the risk of a number of chronic illnesses, including age-related macular degeneration, stroke, gastrointestinal disorders, certain types of cancer, hypertension, and skin conditions (Zhang *et al*., 2015). Fruits offer a natural method to improve diet and encourage optimum health, whether when eaten as snacks, or added to meals (Thirukkumar and Vennila, 2019). *Morinda citrifolia*, commonly known as noni is a tropical fruit that thrives in tropical areas around the world (Sharma *et al*., 2014). Noni is becoming increasingly popular as a dietary supplement, a food functional ingredient, a natural health enhancer, or as a novel food globally (Ali *et al*., 2016). According to Abou Assi *et al*., (2017), fruits, seeds, barks, leaves, flowers, and other parts of *M. citrifolia* are all used separately for their nutritional, but the fruit is thought to contain the most essential chemical compounds. The noni fruit has a wide range of benefits, including antibacterial, antiviral, antifungal, antitumor, anthelmintic, analgesic, hypotensive, anti-inflammatory, and immunological boosting actions (Abou Assi *et al*., 2017; Oly-Alawuba and Iwunze, 2019). Despite the potential health benefits of noni fruit, it has not been widely cultivated or utilized in Nigeria. The phytochemistry and free radical scavenging potential of dried noni fruit and juice, as well as its antioxidant properties, remain underexplored. Addressing this gap in knowledge is crucial to unlocking the full potential of noni as a valuable resource. In this study, the phytochemistry of dried noni fruit and juice was examined, along with its potential as potent free radical scavengers.

Materials and Methods

Five grams of Fresh *Morinda citrifolia* (noni) fruits were obtained at botanical garden of University of Ibadan, Oyo state, Nigeria. The fruits were cleaned and thoroughly washed with water, to remove debris and other impurities. All fruits were carefully sorted into wholesome and unwholesome lots. The wholesome and fully ripened fruits were used. The selected fruits were separated into two equal portions, of 2 g each, for the preparation of the juice extract and fruit powder. Each portion was manually deseeded prior to further processing. All chemicals used were of analytical grade.

Preparation of Juice Extract

The deseeded first portion was mashed using an electric blender to release the juice. Filtration method was then used to separate the juice from the pulp and solids. This was done using a muslin cloth in order to obtain a smooth, clear juice extract and aid in the separation of any solid particles. The resulting juice extract was bottled in sterile glass jars.

Preparation of Fruit Powder

The second portion was thinly sliced into a diameter of 2–5 mm and was air-dried at room temperature for a period of 7 days. An electric grinder was then used to pulverize the dried fruits into powder form, which was then sifted to obtain a fine texture. The resulting fruit powder was stored in sterile containers.

Determination of Mineral Composition

After acid digestion, mineral contents were assessed according to AOAC techniques (2010). While the remaining mineral elements were identified using an atomic absorption spectrophotometer, sodium and potassium were determined using a flame emission photometer.

Determination of Phytochemicals and Free Radical Scavenging Properties

Determination of Total Phenolic Content (TPC)

TPC of the samples was assessed using the Kim et al. (2003) technique and quantified as mg GAE/100 g of gallic acid.

Determination of Total Flavonoid Content (TFC)

Using the method described by Park et al. in 2008, the TFC of the samples was calculated and expressed as quercetin equivalent (mg QUE/100 g).

Determination of Vitamin C

According to a slightly modified procedure described by Patel (2017) the content of vitamin C (ascorbic acid) was measured by redox titration using standardized iodine solution.

Determination of Nitric Oxide (NO)

Using the Mondal *et al*., (2006) approach, the NO of the sample was evaluated and represented as percentage of inhibition.

$$\%\ Inhibition\ of\ NO = \{(Abs\ control - Abs\ sample) / (Abs\ control)\} \times 100$$

Determination of 1, 1-diphenyl- 2-picrylhydrazyl (DPPH) Scavenging Ability

DPPH radical scavenging ability of the samples was measured using the method of Gyamfi et al., (1999). At 520 nm, the absorbance was measured. The percentage of inhibition was calculated as:

$$\%\ Inhibition\ of\ DPPH = \{(Abs\ control - Abs\ sample) / (Abs\ control)\} \times 100$$

Determination of Ferric Reducing Antioxidant Potential (FRAP)

The FRAP of the samples was determined using the method of Benzie and Strain (1996), and the results were represented as (mg Fe^{2+}E/100 g).

Determination of Anti-nutrients Composition

The presence of tannin, saponin, oxalate and phytate was identified by measuring the various absorbances and comparing them to the relevant standard solutions (Jaffe, 2003; Makkar and Becker, 1996; Munro 2000; Reddy and Love (1996). Alkaloids were identified using the approach outlined by Obadoni *et al.*, (2001).

Statistical Analysis

Data were expressed as the mean ± Standard error of mean (SEM) of three measurements, analyzed using ANOVA. The means were separated by the least significant difference ($p < 0.05$), using SPSS Version 20.

Results

The result of the mineral composition of noni juice extract and fruit powder is presented in Table 1. The results revealed that potassium, calcium and sodium were significantly higher ($p < 0.05$) in the juice extract (14.28 mg/100 g, 0.35 mg/100 g and 6.36 mg/100 g) than in the fruit powder (10.56 mg/100 g, 0.19 mg/100 g and 3.33 mg/100 g) respectively.

Table 1: Mineral Composition of Samples

Mineral (mg/100 g)	Sample A	Sample B
K	14.28 ± 2.03	10.56 ± 2.34
Ca	0.35 ± 0.03	0.19 ± 0.005
Na	6.36 ± 0.38	3.33 ± 0.31
Mg	3.40 ± 0.22	2.85 ± 0.081
Fe	0.38 ± 0.031	0.15 ± 0.029

Values are represented as Mean ± Standard error of mean. Values with the same superscript across row are not significantly different ($p < 0.05$). Sample A – juice extract, Sample B – fruit powder

It is observed that the juice extract presented significantly higher potassium, calcium, and sodium contents. However, there were no significant differences in the mean values for magnesium and iron contents.

Table 2 shows the results of the antioxidants of noni juice extract and fruit powder. The findings showed that the juice extract has significantly higher antioxidant activity ($p < 0.05$) than the dried fruit powder. The total phenolic content (TPC) of sample A showed a higher mean value (132.48 mg GAE/100 g) when compared to sample B (84.55 mg GAE/100 g), while 78.50 and 27.56 mg QUE/100 g were observed for total flavonoid content (TFC) in sample A and B respectively. The result of vitamin C followed the same trend, 146.20 mg/100 g for sample A and 94.53 mg/100 g for sample B.

Table 2: Antioxidant Properties of Samples

Bioactive	Sample A	Sample B
TPC (mg GAE/100 g)	132.48 ± 1.64^{b}	84.55 ± 3.11^{b}
TFC (mg QUE/100 g)	78.50 ± 0.306^{a}	27.56 ± 0.32^{a}
Vitamin C (mg/100 g)	146.20 ± 2.21^{c}	94.53 ± 3.00^{c}
NO (% inhibition)	42.49 ± 1.62^{a}	37.87 ± 0.97^{b}
DPPH (% inhibition)	184.99 ± 2.97^{b}	84.24 ± 2.86^{c}
FRAP (mg Fe^{2+}/100 g)	47.54 ± 1.15^{a}	12.78 ± 0.44^{a}

Values are represented as Mean ± Standard error of mean. Values with the same superscript across row are not significantly different ($p < 0.05$). Sample A – juice extract, Sample B – fruit powder

The results of the antinutrient (Table 3) of the juice extract and fruit powder revealed that saponin content of the juice extract (0.45 mg/100 g) was significantly higher than the fruit powder (0.33 mg/100 g).

Table 3: Anti-nutrients Composition of Samples

Antinutrients (mg/100 g)	Sample A	Sample B
Oxalate	0.23 ± 0.033^{a}	$0.27 \pm .032^{a}$
Saponin	0.45 ± 0.034^{b}	0.33 ± 0.042^{a}
Tannin	0.23 ± 0.033^{a}	0.29 ± 0.014^{a}
Alkaloid	3.10 ± 0.11^{c}	5.15 ± 0.46^{b}

Values are represented as Mean ± Standard error of mean. Values with the same superscript across row are not significantly different ($p < 0.05$). Sample A – juice extract, Sample B – fruit powder

The alkaloid content demonstrated a significant difference between the juice extract and fruit powder. Alkaloids in the fruit powder (5.15 mg/100 g) were significantly higher than that of the juice extract (3.10 mg/100 g).

Discussion

The outcome of this study supports what was previously reported by Oly-Alawuba and Iwunze, (2019) regarding the calcium concentration of noni pulp. With the exception of some vegetable species, plants, and fruits typically contain less amount of sodium however, sodium together with potassium, regulates the body's water balance (Slavin and Lloyd, 2012; Callahan *et al.*, 2020). The result which also revealed potassium to be the most abundant mineral in the samples is supported by (Rybicka *et al.*, 2021) which highlighted that the potassium content of noni was the highest mineral. The three crucial minerals—potassium, calcium, and sodium provide the body with important nutritional benefits. Potassium is essential for supporting muscular contractions, controlling blood pressure, and preserving healthy cardiac function (Weaver, 2013). Adequate potassium is linked to a lower risk of kidney stones, osteoporosis, and stroke (Stone *et al*., 2016). Strong bones and teeth, as well as healthy muscle contraction and nerve signaling, ultimately are dependent on calcium intake. Additionally, it helps with hormone secretion and blood coagulation. Osteoporosis, which is characterized by brittle and fragile bones, is a disorder that can be avoided by adequate calcium intake (Vannucci *et al*., 2018). Although excessive sodium consumption is frequently linked to health problems, sodium is essential for maintaining normal fluid balance, neuron function, and muscular contraction (Farquhar *et al*., 2015). Therefore, for overall health and to maintain normal biological processes, it is crucial to balance the consumption of essential minerals through a balanced diet. Owing to the results, both the noni fruit powder and the noni juice extract may be considered as additional sources of sodium, potassium, and calcium that help to balance the mineral composition.

Several of the body's most crucial biochemical functions, including the creation of ATP and the contraction of our muscles, require magnesium (Fiorentini *et al*., 2021). Magnesium is a key component in the relaxation of the muscles lining the airway to the lung, which helps asthmatics breathe more easily. It is believed to be crucial for the structural stability of nucleic acids and intestinal absorption (Yang, 2014). Magnesium deficiency in humans is linked to severe diarrhoea, migraines, hypertension, cardiomyopathy, arteriosclerosis, and stroke (Rosique-Esteban et al., 2018). It also plays fundamental role in the majority of reactions involving phosphate transfer. According to Black and Heidkamp, (2018), iron (Fe) is a crucial component of the diets of pregnant women, lactating mothers, babies, convulsing patients, and the elderly in order to prevent anaemia and other related disorders. Due to its role in distributing oxygen throughout the body, Fe is necessary for both energy and endurance (McMillen *et al*., 2022). Although, the magnesium and iron composition of the samples was not significant, both the juice

extract and the fruit powder may be regarded as additional sources of magnesium and iron.

This also study utilized a variety of antioxidant tests such as NO, DPPH, and FRAP, all having a common mechanism that involves a colour change in which an oxidant is reduced by an antioxidative molecule (Nowak *et al*., 2018). For all of the antioxidant ability assays considered in this investigation, the outcome followed the same trend as that of phenolics and flavonoids. The high antioxidant ability of fresh noni juice when compared to dried fruit powder can be attributed to the moisture content of the juice that helps to maintain the antioxidants' potency and activity. When antioxidants are present in a liquid media, such as phenolic compounds and vitamin C, they are more successfully kept in their active form (Nowak *et al*., 2018; Fontes *et al*., 2023). Furthermore, heat exposure is frequently a part of the drying process used for processing fruit powder, and this might cause heat-sensitive antioxidants to degrade (Roslan *et al*., 2020). This is similar to the findings by Siow and Hui (2013), which suggest that drying guava in a convection oven reduces its antioxidant activity, specifically its capacity to scavenge free radicals. In addition, to the fact that polyphenols may be heat labile and would cause a marked decrease in their ability to scavenge free radicals (Siow and Hui 2013). The antioxidant ability was comparable to that found in the Malaysian seedless. *M. citrifolia* fruit methanol extract reported by Krishnaiah *et al*., (2015) had the highest levels of radical scavenging activity and total phenolic content. The exposure of dried fruit powder to air and oxygen during the drying and powdering process might result in oxidative reactions and the loss of some antioxidants (Guergoletto *et al*., 2020). On the other hand, fresh noni juice is shielded from prolonged oxygen exposure, maintaining its antioxidant capacity. Therefore, due to the lack of water loss and the concentration of components in the liquid form, fresh juice may have a higher concentration of phytochemicals that can be impacted in the dried fruit powder, and the concentration of these chemicals may decrease as a result of drying and direct heat. Noni has strong antioxidant potential, according to Gironés-Vilaplan *et al*. (2014), because of the abundance of flavonoids. The excerpts reveal that ripe fruit functions as a scavenger of nitric oxide and superoxide radicals. According to numerous antioxidant assays, noni fruit juice extract has a strong capacity to scavenge free radicals. Overall, the results of these investigations repeatedly show how effective the noni fruit juice extract is in scavenging free radicals, highlighting its capacity to reduce oxidative stress and shield cells from oxidative damage.

The antinutrient contents of the juice extract and fruit powder also provides some insights. Tannins are known to have negative effects on the digestive system, and their poisonous byproducts (Johnson *et al*., 2012). The exact hazardous dose of tannins required to induce health problems in humans is unknown (Jing *et al*., 2022), however, the levels of these compounds found in this study are quite low and may not cause any harmful effect. Antinutrients have a weak antioxidant effect when present in low concentrations, protecting the body from oxidative stress and lowering the risk of developing certain illnesses like cancer, cardiovascular disease, and neurological disorders (Smeriglio *et al*., 2016). Antinutrients including phytates, tannins, and saponins present in fruits have been demonstrated to decrease the bioavailability of micro- and macronutrients thereby making them inaccessible for absorption in the digestive tract (Faizal *et al*., 2023). Although, these compounds function as antioxidants in low amounts. Antioxidant characteristics are exhibited by antinutrients such as tannins, and saponins which means they have the capacity to counteract damaging free radicals in the body (Hussain *et al*., 2019).

According to research, phytochemicals might lessen the oxidative cell damage that leads to diseases like cancer (Zhang *et al*., 2015). Saponins have also been discovered to possess anticancer characteristics that hinder the growth of cancer cells and lower the chance of developing specific forms of cancer. By lowering cholesterol levels, preventing blood clots, and promoting appropriate blood pressure, saponins can also benefit cardiovascular health (Bachheti *et al*., 2022). Additionally, it has been demonstrated that these substances have antibacterial and antifungal activities, providing defense against a variety of diseases.

Conclusion

This study highlights the potential of this *Morinda citrifoli: an* underutilised crop, notably in the food industry, by shedding light on the phytochemistry and free radical scavenging capacity of dried noni fruit and juice. The presence of numerous bioactive substances was discovered through an investigation of the phytochemical composition, highlighting the abundance of advantageous phytochemicals in noni. Both dried noni fruit and juice have a substantial capacity to scavenge free radicals, which indicates that they can both reduce oxidative stress and benefit general health.

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Declaration of Conflict of Interest: None

Author´s contribution: O.K. Conceptualized the study, involved in collection, analysis aninterpretation of data; **K.** contributed in data collection and statistical analysis; **B.** and **O.** contributed to manuscript writing; **B.T.** and **O.O.** participated in data collection and interpretation of analysed data; **A.O.** edited the manuscript.

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Development of *Carica papaya* L. (Pawpaw) Seeds into Effervescent Tablets for Nutraceutical Application

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Abstract

Pawpaw (*Carica papaya*) belongs to the family Caricaceae and its fruit has high nutritional value. Pawpaw seeds are usually thrown away but it has important medicinal and potent pharmacological properties. This study aims at formulating *Carica papaya* seeds into effervescent tablets which are administered by placing them in water to form a solution of the drug; thus, advancing the use of pawpaw by turning waste into health-promoting products. The formulation into effervescent tablets will encourage improved patient compliance and product presentation. Pawpaw seeds were collected by scraping out of pawpaw fruit, prepared hygienically into powder and stored for usage. The elemental and proximate composition of the powder were evaluated using standard procedures. The powdered seeds were triturated with directly compressible excipients including Microcellac®, Tablettose®, Encompre ss and Avicel 101®, and other important ingredients. Tablets were directly compressed on a carver hydraulic press using two predetermined pressures. Tablets compressed with microcellac® were considered most appropriate as they had the highest crushing strength/friability/disintegration ratio (CSFR/DT). Generally, the tablet parameters ranked Encompress < Avicel 101®< Tablettose® < Microcellac® -The effervescence tablets produced using the seeds of *Carica papaya* have demonstrated acceptable mechanical and release properties, making it a promising source for nutraceutical application.

Keywords: *Carica papaya* seeds; Effervescent tablets; Nutraceuticals; Mechanical properties; Release profiles

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1.0 Introduction

The fruit bearing plant *Carica papaya* Linn. commonly known as “pawpaw” or “papaya”, is from the family Caricaceae. It is native to Central and South America and widely cultivated in tropical and subtropical regions. Papaya fruit is highly valued for its delicious taste, impressive nutritional profile, and diverse range of bioactive compounds (Kaur *et al.,* 2019). Pawpaw has a rich history of use in various traditional cultures including tropical and subtropical regions for its nutritional and medicinal properties. (Tarun and Yash, 2015). In many cultures, the unripe green fruit is used in cooking and is often incorporated into salads, stews, and pickles. In some cultures, papaya leaf extract is used as a treatment for malaria. Its antiviral properties are also used in traditional medicine to treat viral infections, including dengue fever. Studies have also shown that different parts of the plant, including the fruit, leaves, latex and seeds, possess significant medicinal potential including hepatoprotective, nephroprotective, antimicrobial, antimalarial, antiparasitic, antitumor, anti-inflammatory, and wound healing properties (Kadiri *et al.,* 2016).

Embedded in the fruit of pawpaw are seeds which are a rich source of amino acids especially in the sarcotesta (Saran and Ravish, 2013). Pawpaw seeds are often discarded in majority of cultures,

but in few places are sometimes dried and used as a spice with a flavour profile similar to black pepper and are also known for antimicrobial, anti-parasitic, liver and kidney health promotion properties (Mello *et al*, 2008). The seeds just like the latex mentioned earlier, also contain a proteolytic enzyme, *papain*, that literarily rids the body of parasites by targeting parasite eggs and digesting their proteins (Hariono *et al.,* 2022). Papaya seeds have shown immune-modulatory properties which enhances the body's immune response, making it an effective natural remedy for boosting overall immunity and fighting infections (Ugbogu *et al.,* 2023).

Papaya fits in the category of 'Nutraceutical' which is a term derived from nutrition and pharmaceuticals and refers to foods or food products that provide health and medical benefits, including the prevention and treatment of disease (Rajat et al, 2012).

Effervescent tablets are uncoated tablets that generally contain organic acids (such as tartaric or citric acid) and sodium carbonate in addition to the medicinal substance. They react rapidly in the presence of water by releasing carbon dioxide which acts as a disintegrant to produce either a drug suspension or an aqueous solution (Patel and Siddaiah, 2018). Effervescent tablets have multiple applications including dietary supplements, nutraceuticals, herbals, pharmaceuticals. It also affords increase in liquid intake which is good for health promotion. Since effervescent tablets are administered in solution, they are useful in patients with dysphagia who cannot use other types of tablets or capsules. With effervescent tablets, the dose is standardized and the liquid quantity is variable, allowing for taste to be adjusted according to individual preference. It is also a pleasant way of administering a drug substance.

This study has formulated Papaya seeds which are often handled as waste into effervescent tablets which is easy to use, thus offering a convenient form for consumption and helping to reduce waste from the fruit processing industry.

2.0 Materials and methods

The materials used were Citric acid, Sodium bicarbonate, Sorbitol, Flavouring agent, Microcellac®, Tablettose®, Dicalcium Phosphate anhydrous (DCP), Polyethleneglycol (PEG) 4000 and *Carica papaya* seeds. All other reagents were of analytical grade.

2.1 Seed preparation

Carica papaya seeds were obtained from the inner part of the fruit by opening up of the fruit and scraping out the seeds. The seeds were then dried for 5 days at 50^0C until completely dried. The dried seeds were well grinded in a laboratory osterizer and then sieved using 180µm (0.18mm) mesh. The powder was stored in airtight containers until they were ready for use.

2.2 Determination of elemental and proximate content

Carica papaya seeds was evaluated for nine elements using Atomic Absorption Spectrophotometer (AAS, Model 2500 Torontech Inc., Toronto, ON, Canada) and proximate composition (crude protein, ash content, dry matter and moisture content) was determined using established protocols (AOAC 2000).

2.3 Preparation of tablets

Effervescent tablets were prepared by triturating *Carica papaya* seed powder with other excipients according to the formula in Table 1. It was mixed in the ratio of 1/4 (Drug: Excipient) where the powdered seed served as the drug and the different excipients were utilized. The formulation mixture was poured into appropriate containers re-mixed in a tumbling mixer for 5 minutes. A weight of 500 mg of the powder mixture was compressed into tablets for 30 seconds with a single punch Carver hydraulic tablet press (Model C, Carver Inc, Menomonee falls, Wisconsin USA) using a die of 10.5mm in diameter, at two pre-determined compression pressures of 28.31 and 56.62MN/m².

2.4 Tablet evaluation and Data analysis

Tablets were evaluated for mechanical {crushing strength, friability and crushing strength-friability ratio (CSFR)}; release {disintegration time, effervescence time and crushing strength-friability-disintegration ratio (CSFR/DT)} properties, and pH of effervescence solution. All the tests were done using established procedures (Alebiowu and Itiola, 2003; Ajala and Odeku, 2012). The wavelength of maximum absorption for the seed powder was determined using an established method (Majekodunmi, et al., 2008). Drug release was done using appropriate methods as documented by Ajala et al., (2020) and amount released was obtained from a calibration curve. All experiments were performed in replicates and data presented as mean $\pm$ standard deviation except for ratios.

3.0 Results

The findings of this study are presented in this section as Tables and a chart. Table I showed the details of the ingredients used for the formulation of Carica papaya seed effervescence tablets. The seed powder served as the active ingredients while the compression excipients were microcellac®, Tablettose®, Emcompress® and avicel. Sorbitol served as sweetner, the combination of sodium bicarbonate and citric acid will produce the required effervescence, PEG 4000 is required as a wetting agent to assist in tablet dissolution, a flavourant was also included. In Table 2, the different element present in the seed powder were presented, while Table 3 showcased the proximate composition and Figure 1 described the release profiles of effervescence tablets with optimal properties.

Table 1: Details of ingredients in formulated effervescent tablets of *Carica papaya* seed

S/N	Ingredients	$\%^{w}/_{w}$
1	Carica papaya seed powder	20.0
2	Directly compressible excipient	47.2
3	Sodium bicarbonate	15.0
4	Citric acid	15.0
5	Sorbitol	1.5
6	PEG 4000	1.2
7	Pineapple flavour	0.1
Total		**100.0**

Table 2: Elemental composition of *Carica papaya* seed powder

S/N	Elements	g/100g
1	Manganese	0.6100 ± 0.0920
2	Iron	1.4300 ± 0.0340
3	Magnessium	2.5120 ± 0.0010
4	Calcium	3.3400 ± 0.0070
5	Copper	1.9000 ± 0.0060
6	Cobalt	0.1900 ± 0.0250
7	Lead	0.0000 ± 0.0002
8	Cadmium	0.0280 ± 0.0010
9	Nickel	0.1600 ± 0.2350

Table 3: Proximate Composition of Carica papaya seed powder

S/N	Proximate parameter	%
1	Crude protein	14.630
2	Nitrogen free extract	2.341
3	Ash	0.280
4	Moisture content	10.440
5	Dry matter	89.560

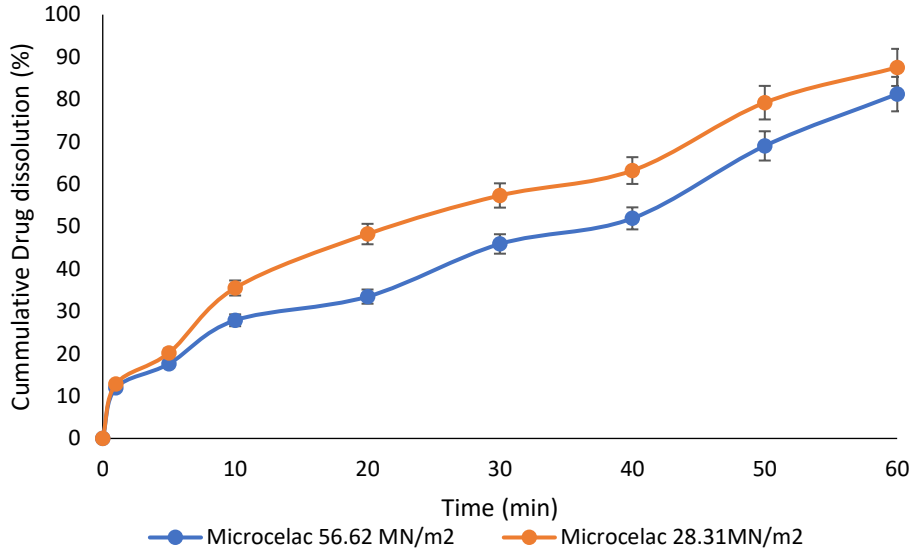

Figure 1: Dissolution profiles of formulated effervescent tablets of *Carica papaya* seeds with optimal mechanical and disintegration properties

Table 4: Properties of formulated effervescent tablets of *Carica papaya* seeds

Excipient	Pressure MN/m^2	Crushing strength (N)	Friability	CS/FR	Disintegration time (min)	CS/FR/DT	Effervescence time	pH of effervescence solution
Microcellac®	28.31	90.50 ± 5.58	0.73	123.97	2.03 ±0.86	61.07	2.52 ± 0.48	5.28 ± 0.03
	56.62	92.80 ± 5.50	0.69	134.49	2.39 ± 0.65	56.27	3.08 ± 0.15	5.41 ± 0.08
Tablettose®	28. 31	45.75 ± 3.04	0.95	48.16	1.69 ± 0.04	28.50	2.37 ± 0.05	5.35 ± 0.08
	56.62	48.90 ± 0.20	0.97	50.41	1.27 ± 0.22	39.69	2.31 ± 1.05	5.19 ± 0.05
Emcompress®	28.31	8.50 ± 1.69	2.17	3.92	0.87 ± 0.11	4.51	0.87 ± 0.27	5.28 ± 0.22
	56.62	9.53 ± 2.20	1.22	7.81	0.71 ± 0.14	11.00	1.22 ± 0.06	5.13 ± 0.07
Avicel 101®	28.31	42.87 ± 5.74	0.96	44.66	1.38 ± 0.06	32.36	3.38 ± 0.06	5.27 ± 0.02
	56.62	70.37 ± 4.07	0.78	90.22	2.34 ± 0.13	38.56	2.38 ± 0.13	5.34 ± 0.09

4.0 Discussion

The elemental content of the seed powder were manganese, iron, magnesium, calcium and copper which are not harmful to the body but plays important role in the maintenance of biological functions. Heavy metals were generally negligible and lead is absent, showing that the seed powder have an acceptable biological profile (Kadiri, 2016). The results of proximate analysis showed the presence of protein and fat with a moisture content of 10.44 % which is less than 15 % stipulated for solid bioactives and excipients. Excess moisture in a material could lead to activation of enzymes and may encourage the growth of microorganisms thereby reducing the shelf life of the formulation. Ash content represents the inorganic residue (minerals) remaining after ignition and complete oxidation of organic matter. Ash content of Carica papaya seed powder was obtained to be 0.28, which signifies that the inorganic component present is low and *Carica papaya* seed powder is high in organic content.

One of the mechanical properties used in assessing and evaluating a tablet is the crushing strength. It is a measure of the bond strength and ability of the tablets to withstand the stress of packaging, transportation and handling. Crushing strength is dependent on the amount of binder used, compression pressure, formulation variable and process parameters. Generally, a crushing strength of 40N is normally considered to be the minimum, for a satisfactory tablet (Ajala and Odeku, 2012). Crushing strength of the tablets increased with compression pressure and varied with excipient type. Encompress yielded least crushing strength while other excipients impacted acceptable hardness. Friability is a disruptive force used to evaluate the ability of tablets to withstand chipping and breakage during use. A maximum weight loss of 1% is usually acceptable for tablets. (Ajala et al., 2020). Generally, all tablets except those prepared with encompress passed the friability test. Crushing strength-friability ratio (CS/FR) is obtainable from the crushing strength and friability tests. The CS/FR provides a measure of tablet strength and weakness and has been described as a useful index for tablet quality (Alebiowu and Itiola, 2003). The CS/FR values obtained were highest for microcellac, thus showing the optimal formulation with respect to mechanical properties. The crushing strength-friability-disintegration ratio (CSFR/DT) provides a good index of tablet quality because it measures tablet strength (CS) and weakness (friability), which are indicators of the bond strength, and simultaneously evaluate any negative effect of these parameters on disintegration time, which is an indicator of disruption of bonds (Alebiowu and Adeyemi, 2009).

Microcellac offered highest CS/FR/DT values still confirming it as the optimal tablet formulation. A higher value of CS/FR/DT indicates a better balance between the tablet's binding and disintegration properties (Alebiowu and Adeyemi, 2009). The pH of the effervescence solutions ranged between 5.13 to 5.35 which are within the acceptable range (2 to 9) for oral solutions (Aulton and Taylor, 2013). Effervescence time of tablet must be less than 3 minutes (Aslani et al., 2013) and most of the tablets passed the test. The release profiles of the optimal tablets are shown in Figure 1, the t_{50} was 22.5 min for tablets compressed at 28.31MN/m^2 and 37.5min for those compressed at 56.62 MN/m^2. The t_{80} was less than 1 hour for the optimal tablets irrespective of compression pressure. Generally, there was a significant difference ($p<0.05$) for the dissolution times and it is considered to be due to compression pressure. The higher compaction forces had tightly packed the particles such that it takes longer time for the dissolution medium to penetrate and achieve drug release. The optimal tablet formulation generally passed all the evaluator parameters.

5.0 **Conclusion**

The seed powder of Carica papaya has elemental composition that is useful for normal biological functions and harmful elements were negligible. The proximate profile showed that the inorganic component is low while the organic content is high. The effervescence tablets were successfully produced from *Carica papaya* seed using appropriate ingredients. The tablets demonstrated acceptable mechanical and release properties

with those from microcellac® having optimal balance of both properties. The effervescence tablets can be further improved for commercial application and benefit of human health instead of eating the seeds ordinarily.

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Declaration of conflict of interest

The authors hereby declare that there is no conflict of interest

Author's contribution

TOA: Concept Design, provision of excipients, data analysis, study supervision and final review

AOT: Literature review, data analysis, writing part of first draft and final review

OMA: Data collection and final review

NAS: Literature review, writing second part of first draft, data analysis, and final review

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Gastroprotective Effect of Cucumber alone and Combination of Cucumber and Cabbage Juice Extracts on Ethanol-Induced Ulcer in Wistar Rats

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Abstract

This study explored the gastroprotective effects of fresh cucumber and cabbage-cucumber juice extracts (CJ and CCJE) in ethanol-induced ulcer in Wistar rats. Sixty male Wistar rats were divided into 12 groups, including control and groups receiving varying doses of CJ, CCJE, omeprazole (20 mg/kg), or cimetidine (50 mg/kg). Treatments were administered twice daily for 14 days, followed by the induction of ulcer with ethanol. The results showed that 2.5 mL doses of CCJE and CJ achieved higher ulcer inhibition rates (65.5% and 59.5%) compared to omeprazole (48.3%) and cimetidine (54%). All treated groups significantly reduced gastric content volume and total acidity, increased pH, and inhibited urease activity. The study revealed that CJ and CCJE exhibited significant gastroprotective effect and may be promising candidates for developing new antiulcer agents.

Keywords: Cabbage; Cucumber; Gastro protective; Juice extract; Ulcer; Urease

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Introduction

Peptic ulcer disease (PUD) is a widespread condition characterized by mucosal breaks in the stomach or duodenum (Sverdén, Agréus, Dunn, & Lagergren, 2019, Kuna, L., Jakab, J., Smolic, R., Raguz-Lucic, N., Vcev, A., & Smolic, M. (2019). Key factors contributing to PUD include *Helicobacter pylori* infection and NSAID use, along with stress, smoking, alcohol, and caffeine consumption. Conventional treatments involve proton pump inhibitors and H2-receptor antagonists (Sverdén, Agréus, Dunn, & Lagergren, 2019, (Kavitt, Lipowska, Anyane-Yeboa, & Gralnek, 2019). Cabbage and cucumber are known for their health benefits, attributed to their phytochemical contents (Favela-González et al., 2020, Uthpala et al., 2019). This study aimed to explore their gastroprotective effect.

Materials and Methods

Cucumber and cabbage were juiced in a 1:1 ratio. Rats were fasted, given ethanol to induce ulcer, and treated with varying doses of cucumber juice (CJ.) combination of cucumber and cabbage juice (CCJE) and standard drugs. The gastric content was analyzed for pH, total acidity, urease activity, and ulcer index/or percentage inhibition Kayode et al (2015).

Statistical Analysis

Data were analyzed using ANOVA and Tukey's test, with significance set at $P < 0.05$. Results are Mean ± S.E.M.

Results

Rats pre-treated with omeprazole, cimetidine, and various doses of CJ and CCJE showed significant ($p<0.05$) inhibition of ulceration, with a dose-dependent increase in percentage inhibition. Ulcer control rats had significantly higher gastric volume than the control group. Pre-treatment with omeprazole and CJ resulted in lower gastric content volume, with the effect varying by dose (Figure 1).

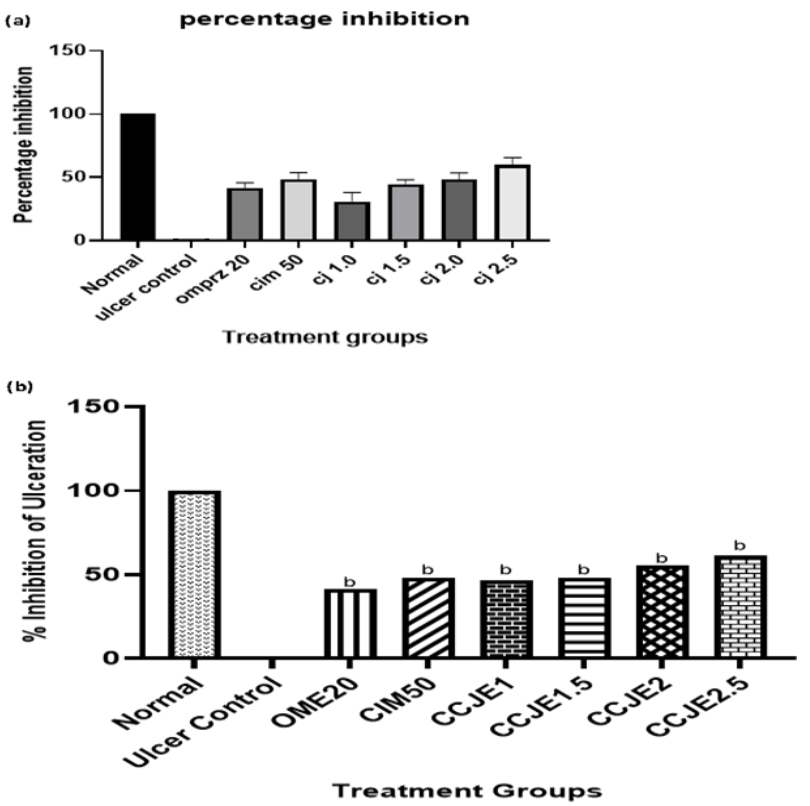

Figure 1: Effect of (a) cucumber juice extract (b) combination of cabbage and cucumber juice extract on percentage inhibition of ulceration.

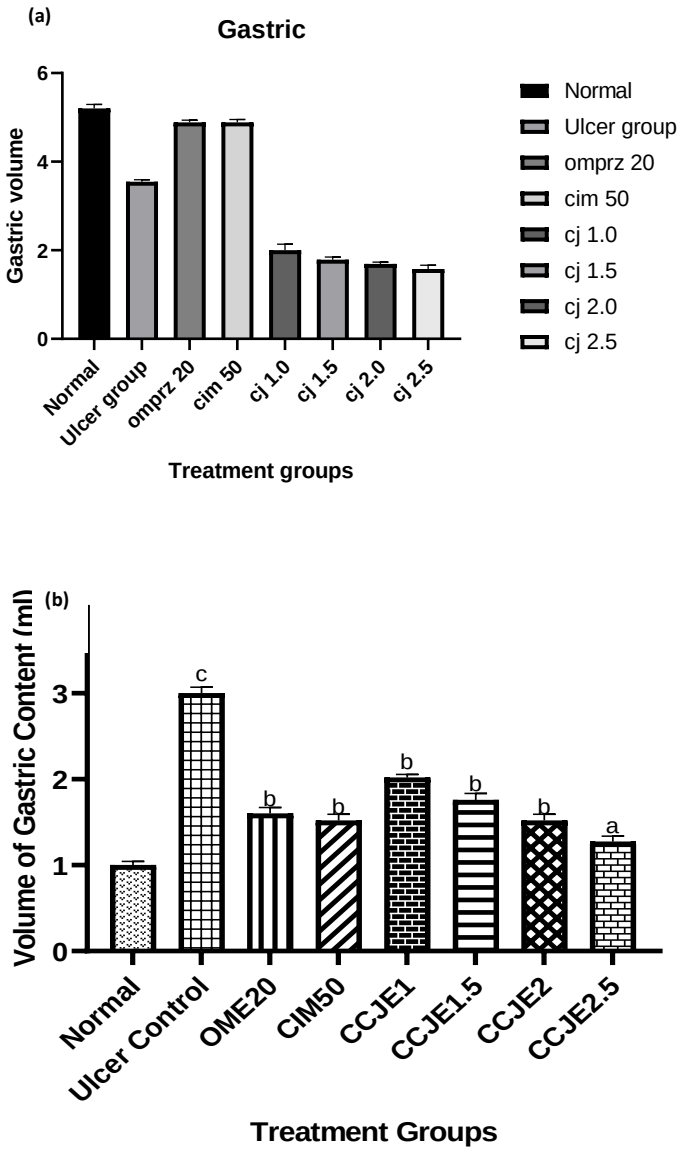

Figure 2: Effect of (a) cucumber juice extract (b) combination of cabbage and cucumber juice extract on volume of gastric content

The untreated ulcer group had significantly higher gastric pH compared to normal. Omeprazole, cimetidine, CJ, and CCJE treatments significantly increased gastric pH ($p<0.05$) (Figure 3). Ulcer control rats had significantly higher total acidity (0.75 ± 0.01 mEq/L) compared to the normal group (0.41 ± 0.01 mEq/L). Rats pre-treated with CCJE, omeprazole, and cimetidine showed significantly lower total acidity values, with the lowest being 0.44 ± 0.02 mEq/L (Figure 4).

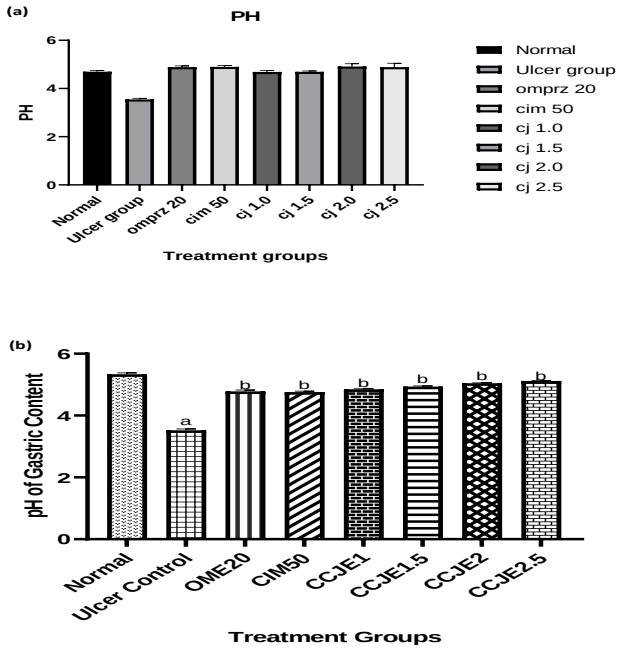

Figure 3: Effect of (a) cucumber juice (b) combination of cabbage and cucumber juice extract on pH of gastric content.

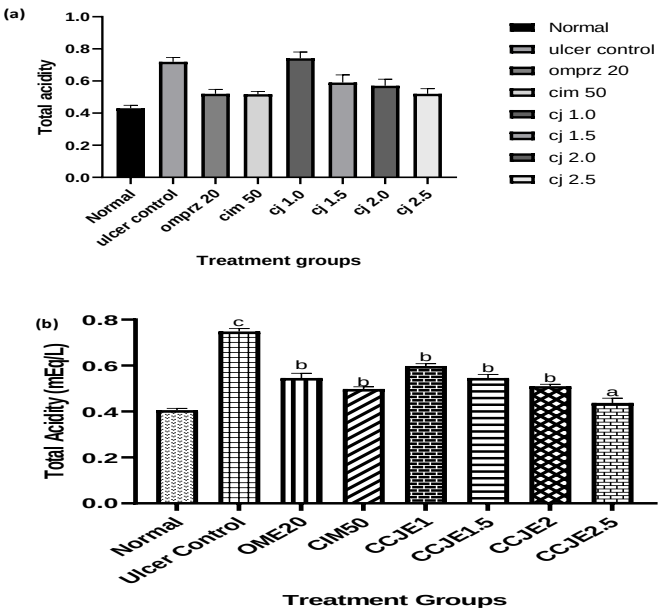

Figure 4: Effect of (a) cucumber juice extract (b) combination of cabbage and cucumber juice extract on total acidity of gastric content

Ulcer control rats had significantly lower urease activity (8.14 ± 0.03 U/L) compared to the normal group (9.51 ± 0.07 U/L). Pre-treatment with cimetidine, omeprazole, and various doses of CCJE resulted in significantly reduced urease activity, with the lowest at 3.69 ± 0.05 U/L (Figure 5).

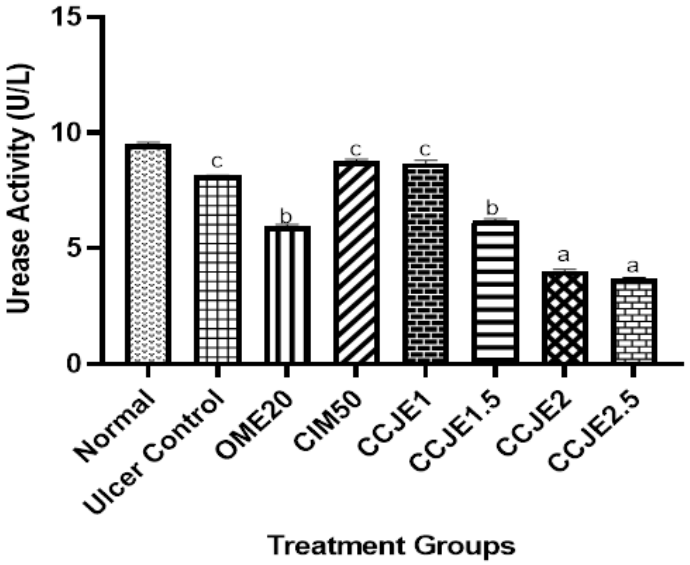

Figure 5: Effect of combination of cabbage and cucumber juice extract on urease activity.

4.0 Discussion

An increase in percentage inhibition was observed in rats pre-treated with omeprazole, cimetidine as well as cucumber, and the combination of cabbage and cucumber juice extract. This showed that CCJE can prevent injury to the mucosal layer of the stomach and inhibit ulceration. The inhibition against ulceration was seen to progress in a dose-dependent manner with rats pre-treated with 2.5 mL CCJE having the highest percentage inhibition of gastric ulcer. This indicated great gastroprotective ability of cabbage and cucumber and goes along with studies which have shown cabbage and cucumber to protect against gastric ulceration (Pradhan, Biswasroy, Singh, & Suri, 2013; Mirzaliev, Kononenko, & Chikitkina, 2019, Okeke, Olayemi, Kayode, 2024).

Ethanol-induced ulcer, similar to peptic ulcer in humans, is known to cause a decrease in pH of gastric content with a subsequent increase in total acidity through the prevention of bicarbonate secretion and the overstimulation of stomach parietal cells ((Mai et al., 2022). These factors lead to an increase in mucosal injury as the highly acidic juice causes slower healing (Arin et al., 2017). Cabbage has been shown to reduce the pH of gastric juice in the past (Hadda, Elsawy, Header, Mabkhot, & Mubarak, 2014) and the present study has shown that fresh juice extract of cabbage and cucumber alters the total acidity and pH of the gastric content in pre-treated rats. The pH of the gastric content of rats pre-treated with CCJE is increased when compared to that of the ulcer control with the total acidity being reduced. This effect was also seen in the standard antiulcer drugs omeprazole and cimetidine with similar effects reported in previous studies (Pagan, Petroski-Rose, Mann, & Hauss, 2020; Sanad, Challan, Marzook, Abd-Elhaliem, & Marzook, 2020, Olayemi, Soremekun, Kayode,

2024). This suggests that the acid output is brought back within normal physiological range upon treatment with cabbage and cucumber juice extract.

Injury caused to the mucosal layer of the stomach can lead to hypersecretion of gastric juice (Phan, Benhammou, & Pisegna, 2015). This proves counter-productive as hyper secretion of acidic gastric juice in turn causes more injury to the mucosal layer (Arin et al., 2017). Ethanol induced ulcer have been reported to increase the total volume of gastric juice (Mousa, El-Sammad, Hassan, Madboli, Hashim, Moustafa,et al. 2019). The findings in this study have shown a decrease in the volume of gastric content for rats pre-treated with CCJE when compared to the ulcer control group. This is similar to what is seen in the groups treated with the standard antiulcer drugs omeprazole and cimetidine with 2.5 mL CCJE being the most effective in reducing the volume of the gastric content. These results showed that CCJE is effective in reducing gastric juice volume after ethanol-induced ulceration and these results are in accordance with a previous study which has shown cabbage to reduce the gastric juice volume after aspirin-induced ulceration (Hadda, Elsawy, Header, Mabkhot, & Mubarak, 2014).

The urease enzyme of the *Helicobacter pylori* is one of great importance in its infection of the stomach and in its causation of gastric ulcer as this is the enzyme that allows the bacteria to survive in the acidic pH of the stomach (Mousa et al., 2019, Olivera-Severo et al., 2017). The rats treated with cabbage and cucumber juice extract showed a great decrease in urease activity with the group given 2.5 mL CCJE having the lowest amount of urease activity. This showed that higher doses of cabbage and cucumber will have greater inhibitory effect on this enzyme. These results are in accordance with other studies which have found cabbage and cucumber to be able to inhibit urease activity on their own (Olech, Zaborska, & Kot, 2014; Kasim et al., 2021). The ability of cabbage and cucumber to inhibit urease activity will in turn reduce the occurrence of *H. pylori* associated peptic ulcer disease as urease is a major virulence factor of the bacterium and is essential for its survival in the acidic medium of the stomach.

5.0 Conclusion

The study found that cucumber and cabbage-cucumber juice extracts have antiulcer properties, reducing gastric content volume and acidity while increasing pH, thus supporting their protective effect against gastric ulcers.

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Declaration of conflict of interest

None

Author´s contribution.

AAAK, developed the main idea and framework of the study (conceptualization), designed the methods and procedures for the research, supervised the research project and managed the overall progress and coordination of the research, AOL and EEJ, conducted experiments and data collection, AOL prepared the initial draft of the manuscript, AOL and EEJ secured financial support for the project, AAAK, AOL and EEJ, analyzed and interpreted the data, AAAK,

AOL, EEJ and OTK, provided materials, equipment, or other resources necessary for the research, AAAK reviewed and edited the manuscript.

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Hormonal Profiles of African Catfish (*Clarias gariepinus)* Broodstock Fed Cocoa Bean Shell Waste and Ascorbic Acid as Additives

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Abstract
Natural products, vitamins and sex hormones played significant roles in growth and reproduction in fish. Cocoa Bean Shell Waste (CBSW) is a by-product of chocolate industries containing both primary and secondary metabolites. Therefore, this study evaluated feeding CBSW and Ascorbic acid (AA) on hormonal profiles of *Clarias gariepinus.* Diets containing CBSW1 (0.00g Control), while CBSW2 (0.0125g) CBSW6 (0.2g) and AA (0.15g) were produced. Eight males and eight females' broodstock (mean weight=362.5±1.80g) were fed 2% body weight for 112 days. Luteinizing hormone (LH) and Testosterone hormone (TH) were determined using quantitative immunoassay technique. LH in the female *C. gariepinus* ranged from 0.01 ± 00-0.016±0.05ng ml-1. Highest LH was from fish fed CBSW4 and lowest jointly from fish fed CBSW2, CBSW3 and AA diets respectively, while in males, there were no significant difference ($p>0.05$) in LH across treatments. TH ranged from 6.42 ± 0.24-8.28 ± 0.4ng ml-1 (males) and 7 .27±0.24-8.53±0.15ng ml-1 (females), with highest from fish fed CBSW4 diet and the lowest from fish fed CBSW1 and AA diets respectively. Fish consuming CBSW diets were able to produce more testosterone and other hormones. However, CBSW4 diet proved more efficient and should be administered to *Clarias gariepinus* brood stock to aid reproduction.

Keywords: Aquaculture; Fish nutrition; Functional foods; Fish reproduction; Natural products

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Introduction

Natural plant-derived substances known as medicinal plants, or phytobiotics, are supplemented to fish diets to enhance growth, health, productivity, and resistance to diseases. Some plants have been shown to support the gonadal growth of some fish species (Al-Khalaifah *et al*., 2020; Mehrim *et al*., 2022). Vitamins often serve as cofactors for enzymes and enable organisms to maintain optimal health and regular metabolic processes, which is why fish need vitamins to survive (Gouda *et al.*, 2020). Furthermore, the vitamin nutrition of catfish has been the subject of numerous researches, most reports especially centered on vitamin C (Ascorbic acid [AA]). Vitamin C, is a water-soluble vitamin that is essential for fish growth and other physiological functions such as reproduction, cartilage and bone formation, and reduction of oxidative stress (Caxico *et al*., 2020).

One part of the fruit from the plant *Theobroma cacao* L. is the well-known cocoa bean, which is the main raw material for chocolate manufacturing. Cocoa production generates substantial quantities of waste. Indeed, only 10% of the total cocoa fruit weight is used for its commercialization, while the remaining 90% is discarded as waste or by-products (Chandrasekaran, 2012). The fundamental distinction between the nutritional composition of cocoa bean shells (CBS) and cocoa beans is that the former has a higher fat content, while the latter have a higher fiber content. Significant quantities of bioactive substances, including as polyphenols, which are known to contribute to the numerous health advantages of cocoa consumption, are also present in CBSW. Industrialized nations are drafting strategic strategies to create a bio-based circular economy in response to the growing attention being paid recently to the bioconversion of food processing

leftovers into valuable goods. Furthermore, other fields have used CBS valuation techniques, and numerous investigations have been conducted to identify novel uses for this by-product. The most popular usage among them might be regarded as novel applications in the food business, animal feed, or industrial uses as a biofuel, absorbent, or composite, among other purposes (Kowalska *et al*., 2017).

Fish reproduction is regulated by a complex system of environmental, social, neurological, endocrine, and metabolic factors. Reproduction is regulated by a series of hormones (Ma *et al*., 2020). Thus, the continuous expansion of aquaculture and perhaps the advocacy for organic farming and minimal use of chemicals in crop and animal productions, due to their negative effects, this made man to engineer a shift from synthetic drugs and chemical to natural plants (neutraceutical) to aid artificial breeding in fishes. Hence medicinal plant/phytochemicals which were then little thought of are now researched, evaluated and developed into additives or drugs with little or no side effects. Medicinal plants and other plant products and by-products are now being used as fertility enhancers in production of catfish species through the boosting of hormonal functions (Adeparusi *et al* 2010). Considering the huge importance of hormones in fish physiology such as controlling reproduction and sexual maturity, growth and metamorphosis regulation (Sahafi *et al*.,2020). African catfish *(Clarias gariepinus)* is commonly cultivated in tropical and sub-tropical areas (Ogunji and Iheanacho,2021) and command a high marketability in Nigeria (Adewole, 2022). Therefore, this study evaluated the hormonal profiles of male and female *Clarias gariepinus* fed Cocoa Bean Shell Waste and Ascorbic acid as additives in sustainable fish production.

Methodology

Experimental Site: The research was carried out at the fish hatchery unit of the Department of Animal and Environmental Biology, Adekunle Ajasin University, Akungba Akoko, Ondo State, Nigeria.

Sources of Experimental Fish, Feed Ingredients, Formulation and Feed Preparations

One hundred and fifty fish were purchased from the Department of Animal and Environmental Biology hatchery unit in Akungba-Akoko, Ondo State. The feed ingredients were purchased from a reputable agro shop at Ugbe-Akoko, Ondo State. While the Cocoa Bean Shell Waste (CBSW) was sourced from Oluji Cocoa Processing Limited, Ile-Oluji, Ondo State, Nigeria. The experimental diets were formulated with 40% crude protein using the Pearson's square method. The feed preparations were according to Adewole (2022). Seven (7) diets containing varying concentrations of CBSW: (CBSW1- 0.00 %, CBSW2- 0.0125%, CBSW3- 0.025%, CBSW4- 0.50%, CBSW5 - 1.00 %, CBSW6 -2.00 %) and Ascorbic Acid of 0.15 % as recommended by Gbadamosi *et al.,* (2013) were used for feeding trial.

Experimental Designs

Broodstocks of *Clarias gariepinus* (n=112) with initial mean body weight of 352±9.00g comprising of 8 males and 8 females per treatment, replicated were stocked in a Completely Randomized Experimental Design and randomly selected into seven rectangular transparent 10 x 90 x 120 (cm) plastic tanks containing 800 litres of water. The fish was acclimatized for 2 weeks and fed commercial diet. During experimental trial, the fish were fed 2% body weight twice daily between 8:00am and 6:00pm for 16weeks. The changes in weight and length were recorded by selection of four fish from each tank bi-weekly in duplicate. The experimental tanks were inspected daily to remove dead fish and to check the activeness of the fish as observed by Adewole, (2022).

Blood Samples Collection and Hormonal Assays

The fish blood samples were collected by cardiac puncture then centrifuged to obtain the serum. The serum gotten from centrifuged blood was used for the assay of testosterone, and other hormones investigated during the study using radioimmunoassay by Rinchard *et al* (1993). Quantitative immunoassay was used to detect the

level of these hormones in the plasma by measuring the precipitation formed by the complex protein and antiserum according to (Sahafi *et al.,* 2020).

Statistical Analysis

Data obtained were analyzed by the one-way analysis of variance (ANOVA) at (α0.005). While within the group comparisons were done using Duncan's Multiple Range Test (DMRT) with SPSS 22.0, SPSS Inc., Chicago, Illinois, USA.

Results

The effects of feeding CBSW and AA on Follicle Stimulating Hormone (FSH) from the female broodstock *C. gariepinus* ranged from 0.10 ± 0.00- 0.16 ± 0.05ng ml-1, with the highest jointly from the fish fed diets CBSW1, CBSW3 and CBSW5; while the lowest was from fish fed diet CBW6 respectively (Table 1). Luteinizing hormone (LH) in the female *C. gariepinus* broodstock fed the experimental diets ranged from 0.01 ± 00-0.016±0.05ng ml-1. The highest LH was obtained from the fish fed CBSW4diet and the lowest was observed from the fish fed CBSW2, CBSW3 and AA diets respectively (Table 1). The testosterone levels from *C. gariepinus* fed the tested diets varied significantly from 7.27 ± 0.24-8.53 ± 0.015ng ml-1. The highest value was obtained from the *C. gariepinus* fed CBWS4 diet and the least was from fish fed AA diet respectively (Table 1).

Table 1: Effect of Cocoa Beans Shell Waste and Ascorbic Acid on the Female Hormones of *Clarias gariepinus*

	Treatments						
Parameters (ng ml-1)	**CBSW1**	**CBSW2**	**CBSW3**	**CBSW4**	**CBSW5**	**CBSW6**	**AA**
Follicle stimulating hormone	0.16±0.05[a]	0.13±0.05[a]	0.16±0.05[a]	0.16±0.05[a]	0.16±0.05[a]	0.10±0.00[a]	0.10±0.00[a]
Luteinizing hormone	0.13±0.00[a]	0.10±0.00[a]	0.10±0.00[a]	0.13±0.05[a]	0.16±0.05[a]	0.11±0.05[a]	0.10±0.05[a]
Testosterone	7.70±0.89[a b]	7.67±0.61[a b]	7.67±0.70[a b]	8.53±0.15[b]	7.72±0.61[a b]	7.90±0.05[a b]	7.27±0.24[a]

Note: **CBSW= Cocoa bean shell waste**
AA= Ascorbic Acid
Data with the same superscript are not significantly different (p<0.05)

There were no significant differences (p>0.05) in Luteinizing hormone and Follicle stimulating hormone across the treatments, however, the testosterone hormone varied significantly (p<0.05) among the treatments. The highest value of 0.16±0.05ng ml-1 was jointly from fish fed diets: CBSW1, CBSW2, CBSW4 and CBSW5 for FSH and fish fed CBSW5 diet for LH respectively, while the lowest was jointly from the fish fed AA diet for both FSH and LH respectively. The highest testosterone hormone value of 8.53±0.15ng ml-1 was the fish fed CBSW4 diets, while the lowest value of 7.27±0.24ng ml-1 was from the fish fed AA diet (Table 2).

Table 2: Effect of Cocoa Beans Shell Waste and Ascorbic Acid on the Male Hormones of *Clarias gariepinus*

	Treatments						
Parameters	**CBSW1**	**CBSW2**	**CBSW3**	**CBSW4**	**CBSW5**	**CBSW6**	**AA**
Testosterone	6.42±0.38^{a}	6.63±0.23^{a}	7.40±0.86^{ab}	8.28±0.48^{b}	7.90±0.10^{a} ^{b}	7.55±0.05^{a} ^{b}	7.70±0.30^{ab}
Luteinizing hormone	0.10±0.00^{a}	0.15±0.05^{a}	0.10±0.00^{a}	0.10±0.00^{a}	0.10±0.00^{a}	0.10±0.00^{a}	0.15±0.05^{a}

Note: **CBSW= Cocoa bean shell waste**
AA= Ascorbic Acid
Data with the same superscript are not significantly different ($p<0.05$)

Discussion

Gonadotropin-releasing hormone (GnRH), which is released by the hypothalamus in fish and all other vertebrates, controls reproduction (Hatef and Unniappan, 2019). The neuroendocrine control of reproduction is largely dependent on this hormone (Hatef and Unniappan 2019; Ma *et. al*., 2020). Also reports on growth as an important activity in living organism, therefore, Biologist and Physiologist have often laid emphasis on the relationship between growth rate to hormonal control as essential processes in living organism (Weidner *et. al.*, 2020). The authors further study a range of poor to rich environments and observed that the level of food availability in the environment resulted in the different evolutionary optimal strategies of hormone levels. It was acertained that with more food available, the higher levels of hormones are optimal, resulting in higher food intake, standard metabolism and growth.

The results obtained from this study showed that at higher inclusions of cocoa bean shell waste some hormones had no higher hormonal optimization (e.g .the testosterones in males *C. gariepinus* did not follow the pattern of higher optimization, while the Lutenizing hormone showed no hormonal optimization as the quantity of CBSW increases. However, in the female *C. gariepinus*, the Follicle Stimulating Hormone, had the lowest hormonal optimization at the highest inclusions of CBSW (CBSW6 diet), while Lutenizing and testosterone hormones, did not follow a definite pattern of hormonal optimization at the highest level of CBSW. The differences between these different hormonal optimizations, showed the differences between the sexes of the *C. gariepinus* to utilization and metabolization of the active metabolites present in the CBSW diets to elicits higher food intake, standard metabolism and growth rate. It may also may be due to changes in the environment compared and fish speciation. The values of testosterone in this study for the male and female *C.gariepinus* were higher than the values 0.84±0.003 $ngml^{-1}$ and 0.11±0.003 $ngml^{-1}$ also recorded for both male and female Rohu carp *Labeo rohita* broodstock from Khuzestan, Iran.

The higher levels of the mentioned hormones in gravid species, may be responsible for a shift in the activity of key enzyme involved in steroidogenesis as follicle are prepared for synthesis of progestogens (Nagama,1978). Furthermore, higher testosterone level in goldfish *Carassius auratus* was obtained when gametes are fully mature and ready to ovulate (Kobayashi *et al.*, 1987). The differences in the hormones may be due to the different diets (food availability), sex, age and environment factors such as variation in temperature, salinity, photo period as well as social parameters induce changes in the equilibrium of the fishes (Shirinabadi *et al.*, 2013).

Conclusion

In conclusion, an insignificant effect of cocoa bean shell waste and ascorbic acid on the hormones of *Clarias gariepinus were* observed in this study suggested its antioxidant properties thus, validates the theory that ascorbic acid plays an important role in protection against oxidative stress cause by toxicants on various tissues and protects sper from oxidative damage. Also, there is no remarkable effect of cocoa bean shell waste on the hormones observed compared with normal control. This suggests that fishes consuming CBSW4 and other CBSWs diets will produce more testosterone. Thus, improving reproduction and promoting the use of natural products in sustainable fish farming in Nigeria.

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Author´s contribution

Adewole, A.M. supervised the undergraduate projects that culminated into this manuscript. Also, he was virtually involved in all the aspects of the manuscripts.

Ojo, O. A. carried out the practical aspect of the research project as an undergraduate student and typed the project that was used in the development of the manuscript.

Omokinde, O. S. was also involved in the research project as an undergraduate

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Aqueous Extract of *Momordica charantia* Seed Modulates Activity of Carbohydrate-metabolizing Enzymes in High Fat Diet/Streptozotocin-induced Diabetic Rats

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Abstract
Type 2 diabetes is a metabolic disorder disease characterized by significant insulin failure which could lead to tissue damage, cardiovascular and neurological problems. This study sought to assess the anti-hyperglycemic effect of an aqueous extract of *Momordica charantia* seed (MCSE) in high-fat diet/streptozotocin (HFD/STZ)-induced diabetic rats. Thirty-two male Wistar rats were divided into four groups (n = 8). All groups were fed a high-fat diet except the control group and were administered a single dose of streptozotocin (35 mg/kg). Group 3 received 25 mg/kg of Metformin, while Group 4 received 200 mg/kg (MCSE). The experiment lasted 14 days and the animals were sacrificed 24 h after the last treatment. Thereafter, the pancreas was excised, weighed, homogenized in cold phosphate buffer, and centrifuged to obtain a clear supernatant used for biochemical analyses. The α-amylase and α-glucosidase activities were assessed as well as the pancreatic antioxidant enzymes (superoxide dismutase and catalase) activity and malondialdehyde (MDA) level. The results showed a significant ($p< 0.05$) increase in α-amylase and α-glucosidase activities with reduced antioxidant activities. However, treatment with Metformin and *Momordica charantia* seed extract significantly lowered α-amylase and α-glucosidase activities and improved pancreatic antioxidant status by lowering MDA level. Thus, *Momordica charantia* seed could be a natural agent and/or alternative therapy for managing type-2 diabetes.
Keywords: Momordica; Diabetes; Aqueous extract; Hyperglycemia

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Introduction

Diabetes mellitus is a major global health condition considered one of the five leading causes of death in the world. In 2019 the International Diabetes Federation (IDF) estimates that 79% of the total numbers of adults with diabetes (463 million) worldwide have type II diabetes and that number is projected to grow to 84 % nearly 700 million 2045(Smokovski., 2021). It is a metabolic disease syndrome that typically results from a mix of environmental and genetic factors, raising blood sugar levels abnormally (hyperglycaemia). It is caused by the immune system attacks on the insulin-producing cells (beta cells) located in the islets of the Langerhans in the pancreas. There are two major types of diabetes mellitus, the first being type 1 diabetes, it is characterized by pancreatic islet inflammation (insulitis) and an auto-immune attack on the pancreatic beta cells (Marroqui *et al*.,2021), resulting in the overall decreased production of insulin. The most common version of this metabolic disorder is type 2 diabetes mellitus, described by increased insulin release to compensate for insulin resistance and progressive decline in islet secretory function within the pancreas, thus causing overall insulin deficiency.

Supplements are used in complementary and alternative medicine as an alternative to traditional Western medical care (Jones & Brown, 2022). According to a recent study, up to 30% of diabetic patients also take complementary and alternative medicines (Chen *et al*., 2024). Bitter gourd has a long history of use as a hypoglycemic agent in Asia, Africa, and Latin America, where the plant extract has been referred to as bitter insulin. It has long been a part of many Asian traditional medical systems. Bitter gourd fruits have long been used as a traditional medicine to treat a

wide range of ailments, including diabetes, cancer, rheumatism, gout, worms, liver and spleen diseases, and skin disorders. The bitter melon is a robust, high-nutrient plant comprising a wide range of advantageous substances. These include antioxidants, vitamins, minerals, and bioactive compounds, all of which add to its extraordinary adaptability in treating a variety of ailments. *Momordica charantia* contains a collection of biologically active plant chemicals including triterpenes, and proteins. Steroids, alkaloids, saponins, and flavonoids, gives the plants its anti-fungal, anti-bacterial, anti-parasitic, anti-viral, anti-fertility, anti-tumorous, and anti-carcinogenic properties (Bamgbose, *et al*., 2021). Thus, present study aimed at assessing the effect of *Momordica charantia* seed extract on the activity of carbohydrate-metabolizing enzymes (α-amylase and α-glucosidase) and antioxidant status in high-fat diet/streptozotocin-induced diabetes in Wistar rats.

Materials and methods

Sample collection and preparation

Momordica charantia fruit was purchased from a local herb store in Akure and identified at the Department of Biological Sciences, Joseph Ayo Babalola University, Nigeria, with Voucher Number CANS/PL/IDN/0365. The seeds were separated from the fruits and rinsed properly with clean water. The bitter gourd seed was oven-dried till it achieved a constant weight. The sample was pulverized to a fine powder using a domestic blender after which 4g of the powdered sample was weighed and dissolved in 400 mL of water. The mixture was stirred on an orbital shaker for 24 hours after which the homogenate was filtered using No. 1 Whatman filter paper to obtain a clear filtrate, which was freeze-dried and later reconstituted with distilled water to the desired concentration. The prepared concentration was stored in refrigerator for further analysis.

Chemicals and reagents

All chemicals used were of analytical grade. Streptozotocin (STZ), PNPG, GSH, DNSA Saline phosphate buffer, phosphate buffer (pH 7.0), Dichromate acetic acid, carbonate buffer, adrenaline, Metformin was purchased from Matador Pharmaceutical Ltd. (Akure, Ondo State, Nigeria).

Experimental design

Male Wistar albino rats were randomly divided into four (4) groups of eight rats each weighing between (174-200 g). The animals were procured from the Department of Biochemistry breeding colony, Joseph Ayo Babalola University, Nigeria, and acclimatized for 2 weeks. The animals were kept in clean cages under standard laboratory conditions (12h light/12 h dark cycle) and placed on free access to feed and water.

Table 1: Feed formulation for normal control (NC) and High-fat diet (HFD)

	Normal control	High fat diet
Skimmed milk	500	500
Lard	-	300
Rice bran	200	90
Corn starch	160	70
Premix	40	40
Ground nut oil	100	-
Total	1000 g	1000 g

Induction of diabetes with STZ in high-fat diet fed rats (Type 2 diabetic rat model)

All the rats were fed viable pellets before being fed with a formulated diet. After acclimatization, the rats were grouped into two dietary sections: the normal control (NC) and the high-fat diet (HFD). After two weeks of dietary intake, the rats fed HFD were injected with STZ intraperitoneally at a dose of 35 mg/kg according to their average body weights (Huo et al., 2021). Two days after induction, food was withdrawn for a period of 12 hours before checking for their blood glucose levels. Blood samples of the rats were obtained through the tail vein and the fasting blood sample was analyzed using an automatic auto-analyzer (fine test Auto-coding ™). The different group was placed on different treatments using *Momordica charantia* seed extract and Metformin as standard drug.

. The rats were grouped as follows.

- Group 1: Normal control
- Group 2: HFD/STZ-induced diabetic control group.
- Group 3: HFD/STZ-induced diabetic rat + 25 mg/kg Metformin.

- Group 4: HFD/STZ-induced diabetic rat + 200 mg/kg *Momordica charantia* seed extract

The extract and standard drug were given orally for 14 days. The choice of dose of STZ (35 mg/kg) was in accordance with the previous work of Bahr *et al*., (2023); while that of *Momordica charantia* extract (200 mg/kg) was as reported by Obiandu *et al*. (2020).

Tissue preparation

After 14 days of treatment, the rats were sacrificed by cervical dislocation. The pancreas was isolated and rapidly placed on ice and weighed. Subsequently, it was homogenized in ice-cold phosphate buffer (0.1M, pH 7.0) using a Tefon glass homogenizer. The homogenate was centrifuged for 5 min to yield pellets, which were discarded. The supernatant was taken and kept in refrigerator at 4^0C for further analysis.

Biochemical Assays

Determination of lipid peroxidation

Thiobarbituric acid of 1.2g was weighed and dissolved in 200 mL of 0.08% of NaOH. The blank was prepared by pipetting 300 µL of SDS, 500 µL of acetic acid, and 500 µL of TBA into the test tube and the sample test tube was arranged, 300 µL of tissue homogenate, 300 µL of SDS, 500 µL of acetic acid, 500 µL of TBA and was incubated at 100ºC for one hour. The absorbance was read on a spectrophotometer at 532 nm (Saito, 2021).

Determination of superoxide dismutase activity

The superoxide dismutase (SOD) activity assay was in terms of its ability to inhibit the autoxidation of epinephrine to adrenochrome, which has an absorption maximum of 480nm. This was done by mixing 50 µL of the heart homogenate supernatant, 1000 µL of sodium carbonate buffer (pH 10.2), and 17 µL of adrenaline together. The reaction was carried out in a cuvette for 2 min at 30 second interval, and the absorbance was taken at 480 nm (Carmo de Carvalho e Martins et al., 2022).

Determination of catalase activity

The enzyme assay was carried out by reacting 500 µL of phosphate buffer (0.01M, pH 7.0), 50 µL of the heart tissue homogenate supernatant, 200 µL of 2M H_2O_2, and 1000 µL dichromate acetic acid. The reaction was carried out in a cuvette for 3 min at one (1) minute intervals, and absorbance were measured at 620 nm (Anwar, 2024).

Determination of α-amylase activity

To determine α-amylase activity, 50 µL of distilled water was added to the blank test tube, 50 µL of tissue homogenate was pipetted to each test tubes and 250 µL of 0.02 M saline phosphate buffer (pH 6.9) was added to the blank test tube and the test tubes containing the samples, and the sample was incubated at 25ºC for 10 min. After incubation 50 µL of starch was pipetted into all the sample and was incubated at 25ºC for 10 min, then 200 µL of DNSA was added to all the sample and was incubated at 100ºC for 5 min, 2 mL of H_2O was added and absorbance was read at 540 nm.

Determination of α-glucosidase activity

The activity of alpha glucosidase activity in the pancreas homogenate was determined by pipetting 15 µL of distilled water into the blank test tube, 15 µL of tissue homogenate added, pipetted into each test tubes and 15 µL of GSH was pipetted in to all the test tubes, 445 µL of 0.02 M phosphate buffer (pH 6.9) was pipetted, the samples were incubated at 37ºC for 10 min, 40 µL of PNPG was pipetted and incubated at 37ºC for 20 min, after incubation 1% Na_2CO_3 was added and absorbance was read at 540 nm.

Data Analysis

The results of the replicate were pooled and expressed as mean and standard deviation values. One way analysis of variance (ANOVA) was used to analyze the results with level of significance with the aid of graph pad prism. The level of significance was accepted at ($p<0.05$).

Results

The result of the effects of *Momordica charantia* seed extract on blood glucose level is presented in Figure 1; the result showed that HFD/STZ caused significant increase ($p>0.05$) in blood glucose levels when compared with the control group. However, treatment with metformin and *Momordica charantia* seed caused a significant decrease in blood glucose levels when compared with untreated group.

The superoxide dismutase (SOD) activity of the pancreas homogenate is presented in Figure 2, the result showed that there was significant decrease ($p< 0.05$) in the SOD activity of the HFD+STZ group when compared to the control group. However, there was a significant increase ($p< 0.05$) in the SOD activity in the groups treated with metformin and 200 mg/kg MS when compared to the HFD+STZ (untreated) with 200 mg/kg MS having higher increased SOD activity. Likewise, similar result was observed in the catalase activity, as presented in Figure 3. A significant decrease ($p< 0.05$) in the catalase activity of HFD+STZ group was observed when compared to control groups. The metformin and 200 mg/kg MS-treated group showed a significant ($p>0.05$) increase in catalase activity compared to HFD+STZ groups. The 200mg/kg MS showed higher catalase activity than the Metformin-treated group.

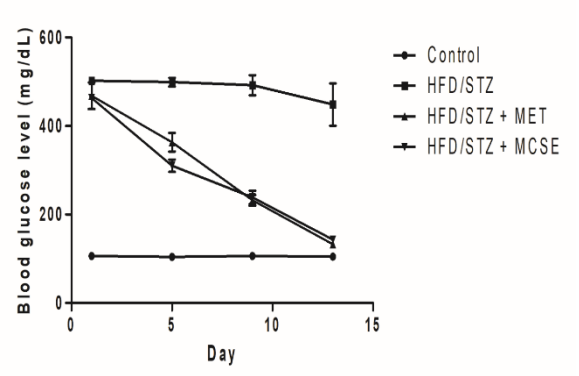

Figure 1: Effects of *Momordica charantia* seed extract on blood glucose level in HFD+STZ-induced diabetic rats.

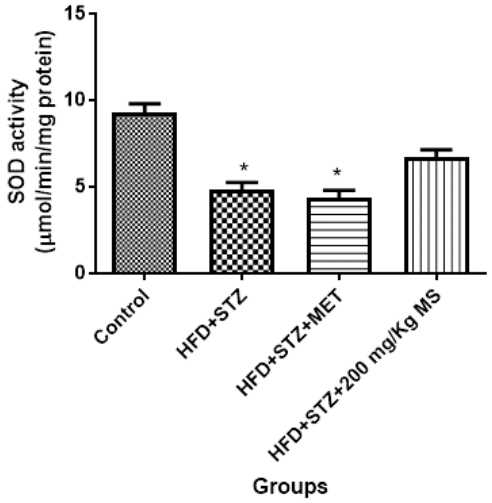

Figure 2: Effects of aqueous extract of *Momordica charantia* seed on the SOD activity in HFD/ STZ-induced rats
Bars represent mean standard deviation (n=8).
HFD/STZ represents the diabetes control group.HFD/STZ+200 mg/kg MS represent the *Mormordica charantia* seed.

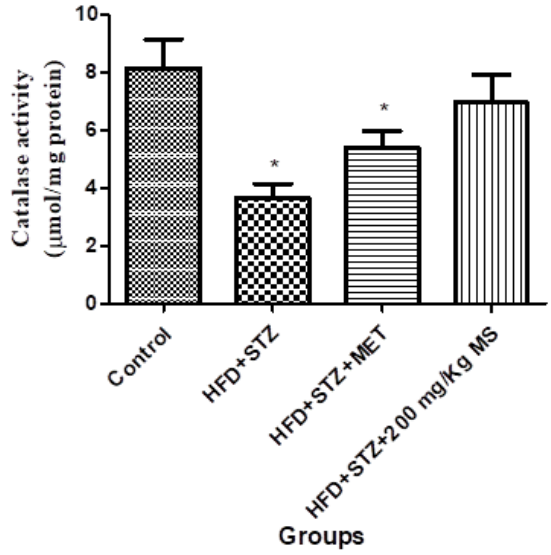

Figure 3 Effects of aqueous extract of *Momordica charantia* seed on the Catalase activity in HFD/STZ-induced rats.
The bars represent mean standard deviation (n=8). The bar with the hashtag is significantly different from the HFD/STZ and control group.
The HFD/STZ represents the diabetes control group, while the HFD/STZ+200mg /kg MS represents the *Momordica charantia* seed.

The result of the malondialdehyde (MDA) content is presented in Figure 4. This revealed a significant ($p > 0.05$) increase in the MDA content of the pancreas in untreated (HFD+STZ) group compared with the control. The metformin-treated group showed no significant decrease ($p < 0.05$) in the lipid peroxidation levels when compared to the HFD/STZ group. However, 200 mg/kg MS showed a significant decrease ($p < 0.05$) in the lipid peroxidation levels when compared to HFD/STZ and metformin group.

The animals' intestinal α-glucosidase activity is presented in Figure 5, the result showed a significant ($p > 0.05$) increase in the intestinal α-glucosidase activity of the untreated group when compared with the control group. However, a significant decrease ($p < 0.05$) was shown in the metformin and 200 mg/kg MS treated group.

Similarly, as presented in Figure 6, is the pancreatic α-amylase activity. There was a significant increase in pancreatic α-amylase activity of HFD/STZ (untreated) group compared with the control. There was a significant decrease ($p < 0.05$) in the observed activity in metformin and 200mg/kg MS treated group.

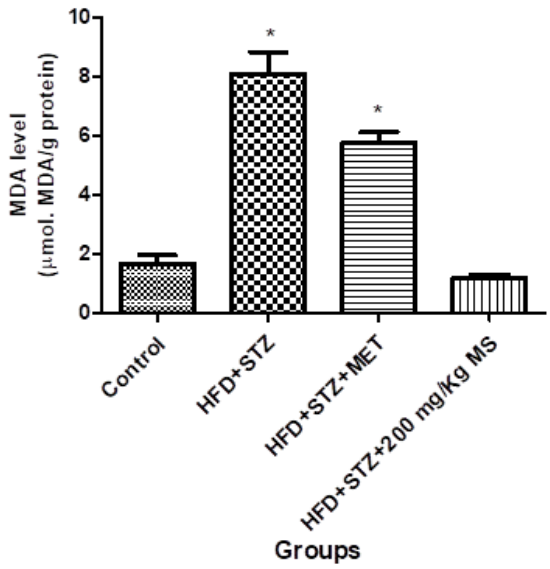

Figure 4: Effects of aqueous extract of *Momordica charantia* seed on Lipid peroxidation in HFD/STZ-induced rats.
Bars represent mean standard deviation (n=8). The bar with asterisk is significantly different from the control group. The bar with an asterisk and hashtag is significantly different from the HFD/STZ and control groups. The bar with an asterisk, hashtag and symbol is significantly different from the control, HFD/STZ and metformin groups.
HFD/STZ represents the diabetes control group. The HFD/STZ+200mg/kg MS represent the *Momordica charantia* seed. MDA represent Malondialdehyde.

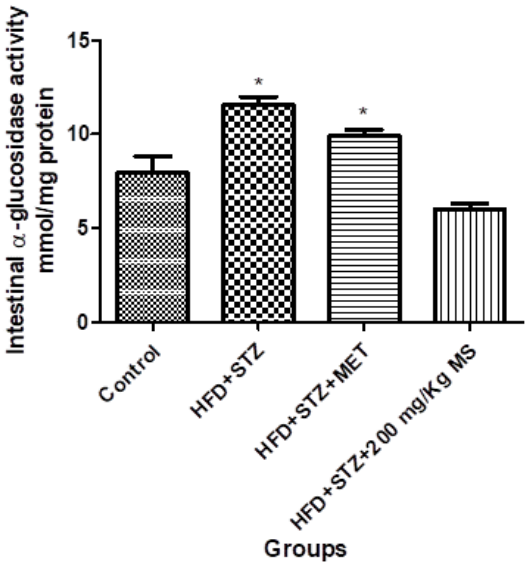

Figure 5: Effects of aqueous extract of Momordica charantia seed on the intestinal activity of α glucosidase in HFD/ STZ-induced rats.
Bars represent mean standard deviation (n=8). The bar with an asterisk is significantly different from the control group. The bar with a hashtag is significantly different from the HFD/STZ and control groups. The bar with an asterisk, hashtag and symbol is significantly different from control, HFD/STZ and metformin groups.
HFD/STZ represents the diabetes control group, and the HFD/STZ+200 mg/kg MS represent the *Momordica charantia* seed.

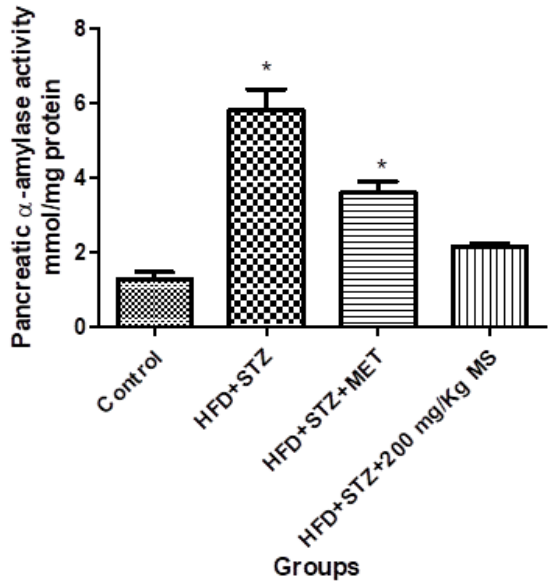

Figure 6: Effects of aqueous extract of *Momordica charantia* seed on the pancreatic activity of α amylase in HFD/ STZ-induced rats.
Bars represent mean standard deviation (n=8). The bar with the hashtag is significantly different from the HFD/STZ and the control group. The bar with an asterisk and hashtag is significantly different from the HFD/STZ and control groups. The bar with the hashtag is significantly different from the control, HFD/STZ and metformin group.
The HFD+STZ represents the diabetes control group, and the HFD/STZ+200 mg/kg MS represent the *Momordica charantia* seed.

Discussion

Several studies have reported the beneficial effects of natural products with antioxidant and anti-inflammatory properties in managing diabetes and other diseases such as cancer, rheumatism, gout, worms, liver and spleen diseases (Chen *et al*., 2021). Medicinal plants have played a crucial role in human healthcare for centuries with their diverse bioactive compounds (Dar, 2023). The plants as foundation in traditional medicine systems will continue to be valuable in modern healthcare for managing and preventing various diseases, including diabetes. The present study comprehensively evaluates the aqueous extract of *Momordica charantia* seeds and its impact on STZ-induced diabetic rats. The administration of streptozotocin (STZ) induces diabetes by causing selective destruction of pancreatic beta cells, leading to insulin deficiency and hyperglycemia. The therapeutic potential of *Momordica charantia*, commonly known as bitter melon, has been well-documented in traditional medicine, particularly for its hypoglycemic properties (Mukherjee *et a*l., 2022). This was shown as the *Momordica charantia* seed extract reduces the blood glucose level which is a first front-line observation in treatment/management of hyperglycemic state in diabetes patients. Findings from this study correlate with another report (Mukherjee *et a*l., 2022) on the use of glucose levels for treatment of diabetes.

Finding from this study centered on some of the biochemical mechanism used by the seed of *Momordica charantia* in the management of diabetes in the folklore, superoxide dismutase (SOD) is an antioxidant enzyme that plays a crucial role in the defense against reactive oxygen species (ROS), specifically superoxide radicals (Saxena *et al*., 2022) It catalyzes the conversion of superoxide anions to hydrogen peroxide (H_2O_2) which is subsequently detoxified by catalase or glutathione peroxidase to oxygen and water. This study revealed that there was a significant increase in the SOD activity level of the *Momordica charantia* seed extract-treated animals when compared to standard drug-treated group (Metformin). The elevated SOD activity indicates that the *Momordica charantia* seed extract enhances the antioxidant defense system in diabetic rats, reducing oxidative stress. This enhanced antioxidant defense likely plays a pivotal role in preserving pancreatic function and reducing diabetes-related complications. This could be as a result of different phytochemical contents of the plant, different from a single compound targeting drug (metformin), hence providing other benefits to the pancreatic cells, thereby boosting support for living and its functional properties.

Reduced catalase activity has been associated with diabetes mellitus, particularly type 2 diabetes, because low catalase activity has been observed in patients with type 2 diabetes mellitus, indicating impaired antioxidant defence. Lower catalase activity is linked to increased oxidative stress, insulin resistance, hyperglylcemia and diabetic complications (Chen *et al*., 2024). This study revealed that there was a significant ($p<0.05$) increase in the catalase activity of the *Momordica charantia* seed extract treated group when compared to standard drug (Metformin) treat group. The observed increase in catalase activity is

complimentary to SOD activity which assist in the complete detoxification of superoxide anions to hydrogen peroxide (H_2O_2). The abundance of phyto-constituent of the seed could the reason for this brilliant response to promote this increased activity better than standard drug.

Findings from this study were in agreement with each other as they provide a sequential line of information regarding the potency of the seed extract. The results of this study is in agreement with other report where oxidative stress was found to cause increased MDA content in the animal tissue through peroxidation of the cell lipid and it accumulation over a period of time could result to damage of the cell membrane and other diseases such as diabetes (Kakkar *et al.*, 2020). *Momordica charantia* seed used in this study significantly demonstrated free radical scavenging potential by significantly reducing the pancreas MDA content. This is one of the vital biochemical mechanisms in the management of diabetes and interestingly, it was able to reduce it better than the standard drug used in this study.

Alpha-glucosidase is an enzyme crucial in carbohydrate metabolism. Inhibition of this enzyme slows down carbohydrate digestion and glucose absorption, thereby blunting postprandial blood glucose spikes. The inhibitory effect of the extract on these enzymes highlights its potential to modulate glycemic control through a mechanism akin to that of pharmaceutical alpha-glucosidase inhibitors, which are commonly used in managing diabetes. Furthermore, the study revealed a significant decrease in the activities of alpha-glucosidase of the *Momordica charantia* seed as compared to the standard drug (metformin) group. Findings from this study showed that the seed extract has low intestinal alpha-glucosidase level inhibitory property, which has been reported to be good in the management of type 2 diabetes, as prolonged inhibition of the enzyme could result in blurting, where fermentation occurred due to prolonged digestion of food substance (Marroqui *et al.*, 2021). Findings from this study showed a mild inhibition of the intestinal alpha glucosidase by the seed extract.

Alpha-amylase is an enzyme, which catalyzes the hydrolysis of starch into sugars and plays a crucial role in carbohydrate digestion. Inhibition of alpha-amylase had been implicated in the reduction of the breakdown of starch into glucose, thereby potentially lowering postprandial blood glucose levels. The findings from this study also shows a significant ($p<0.05$) decrease in the pancreatic alpha-amylase activity by the aqueous extract of *Momordica charantia* seed compared to the standard drug. This correlates with other reports (Jones & Brown, 2022), where alpha-amylase inhibitors have been used for the treatment of diabetes. Hence, findings from this study showed that the seed is a potent amylase inhibitor.

Conclusion

The aqueous extract of *Momordica charantia* seeds demonstrated a significant therapeutic potential in managing diabetes by modulating antioxidant enzyme activities, inhibiting key pancreatic enzymes, and reducing lipid peroxidation, the extract provides a robust defence against oxidative stress and hyperglycemia characteristic of diabetes. These findings pave the way for further research into the development of *Momordica charantia* seed-based therapeutic agents for diabetes treatment/management.

Acknowledgement

Funding

Declaration of Conflict of Interest

Authors have no conflict of interest to declare.

Author's contribution

L.J. Babatola was involved in experimental design and manuscript writeup
A.A. Adebayo was also involved in experimental design and execution of the research
G.P. Akerele was involved in experimental execution and data processing
O.O. Adegoke was involved in biochemical analysis and results collation
A.P. Oluwagbemigun was involved in the biochemical analysis and manuscript preparation
G. Oboh was involved in the experimental design

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Immunomodulatory Impact of Citrus Peel on Broiler Chickens: Boosting Immune Response and Reducing Inflammation

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Abstract

Citrus fruits are widely consumed because of its medicinal values but its waste constitutes about 50-55% of the total fruit mass, the peels which contain 80% of water attract flies and produce toxin if not properly manage causing environmental pollution. Hence harnessing the peels that is rich in polyphenols will be a benefit. A total number of 96-day-old broiler chicks were distributed into a completely randomized design (CRD) which included 3 dietary treatments with 4 replicates per treatment and 8 birds fed in each replicate. The experimental treatment consists of a control group (without the CSOP additive), a group with 2.5% of CSOP, and a group with 5% CSOP supplement in the diet. The results of the study revealed that CSOP caused a significant ($p<0.05$) decreased TNF-α, elevated IL-10 levels in broiler serum both in 2.5 and 5% CSOP. Also, CSOP supplementation caused significantly ($p<0.05$) elevated IgA, IgG, and IgM levels of broiler serum compared to the control. Conclusively, supplementing broiler diets with CSOP as additive boost the chicken immunity,

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Introduction

Due to its status as meat that is widely consumed regardless of cultural or religious beliefs, poultry is currently the fastest animal-producing system (Matuszews *et al* 2020). FAO 2017 predicted that by 2026, poultry meat will account for 45% of world meat consumed. Unfortunately, infectious diseases can affect poultry, just like they can any other animal maintained in close quarters. To satisfy market demands, farmers have a stake in maintaining disease-free flocks that grow larger and quicker, improving performance in poultry production requires a fully working immune system. Though the nutritional needs of chickens are met by the current formulation of poultry feeds, further investigation is required to ascertain whether these feeds sufficiently support the immune system of the bird or whether other substances are required to prevent immunological deficits.

Improving the birds' immune systems should ideally make them more resilient to infectious illnesses. Human immune systems and overall health have been demonstrated to be enhanced by phytochemicals, especially those present in some fruit extracts and fruit peels.

These phytochemicals are rich in antioxidant components, in poultry like broiler chickens, fruits and their extracts could boost immune systems and assist in preventing diseases using entire fruits, plant extracts, and fruit wastes along with their derivatives as supplements to strengthen the birds and the producer's immune systems. The intricate avian immune system is essential for defending birds against infectious and metabolic illnesses. The avian immune system offers both innate and adaptive responses, much like the immune system of mammals. A varied class of proteins or peptides known as cytokines are released by cells and serves to guide

both innate and adaptive immune responses. Chickens under chronic stress, for instance, have higher amounts of IL-1β, IL-2, IL-18, interferon-gamma (IFN-γ), and IL-6 in their thymic tissue as well as granulocyte colony-stimulating factor (G-CSF) and interleukin-2 (IL-2) in their spleen tissue Zaytsoff *et al* 2020. Leukocyte populations, cytokine responses, antibody concentrations, inflammatory markers, phagocytic capacities, and the masses of distinct immune organs are only a few methods to measure the immune response

Materials and Method

Preparation of Serum

At the end of the experiment, birds were sacrificed by cervical dislocation, dissected and blood from the heart was collected into a plain bottle without ethylene diaminetetracetic (EDTA) acid and centrifuged at 5000g for 15 minutes in an MSC bench centrifuge (Benchman Coulter, Fullerton, CA, USA). The clear supernatant obtained C-reactive protein (serum) was used for the estimation of the antibodies and cytokines.

Quantification of Inflammatory Cytokines and Antibodies

The tumor necrosis factor α (TNF-α), interleukin 6 (IL-6), interleukin 10 (IL-10), IgA and IgM were measured by ELISA assay according to the manufacturer's instruction (Quantikine Immunoassay kits, R&D Systems Minneapolis, MN). Briefly, 96 well microplates were sensitized with the primary antibody at room temperature (RT) for 30 minutes; then the sample was added and incubated at 37°C for 30 minutes. After washing, the secondary antibody conjugated with peroxidase was added and incubated at 37^{o}C for 30 minutes. Cytokine and antibody concentration was determined by the intensity of the colour measured by spectrometry in a micro-ELISA reader.

Procedure

Forty microliter of sample was added to sample wells and 10µL anti-(TNF-α, IL-6 and IL-10) antibody was thereafter added.50µL of streptavidin-HRP was added mixed well and the plate covered with a sealer. The mixture was incubated at for 60 min at 37^{o}C. The sealer was removed and plate washed 5 times with wash buffer with well soaked in wash buffer for 30seconds to 1min for each wash. The plates were blotted onto paper towels and thereafter, 50 µL of substrate solution A and 50µL of substrate solution B was added to each well. The plates were covered with a new sealer and incubated for 10min at 37°C in the dark.50µL of acidic stop solution was then added to each well to terminate the reaction with the colour changing immediately from blue into yellow. The optical density (OD value) of value well was immediately determined using a microplate reader set to 45nm within 10min after adding the stop solution.

Statistical Analysis

The general linear model of SAS 8.0, version 9.3, 2018, and Graph pad Prism software version 8.02 was used to analyze the data, and the difference between the mean values was separated by Duncan's multiple range test level of significance at $P<0.05$.

Results

The result for TNF-A pro-inflammatory cytokine level in serum of control and treated broiler chicks revealed no significant difference ($p>0.05$) among the treatment groups (Figure 1a) while in figure 1b, it revealed that there was significant elevation ($p<0.05$) in the level of the pro-inflammatory cytokine-IL-10, in the serum of broiler chicks fed 5% composite sweet orange peel (T3), but not in those fed 2.5% composite sweet orange peel (T2), when compared to the normal control group (T1). Nevertheless, no significant difference ($p>0.05$) was observed in the level of serum IL-10 between T2 and T3 groups.

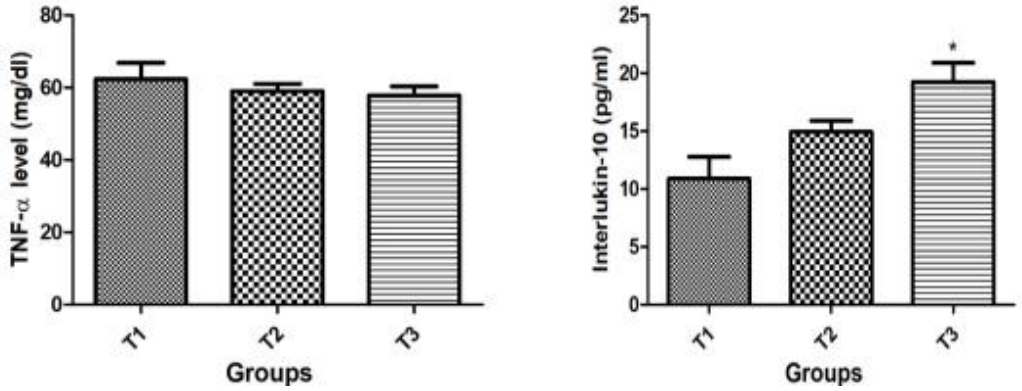

Figure 1. Effect of dietary CSOP supplementation on a) Tumour Necrosis Factor Alpha (TNFα) and b) Interleukin 10 (IL-10) Inflammatory Cytokines in the Serum of Broiler Chicken. T1: Normal Control; T2: 2.5% Composite Sweet Orange Peel; T3: 5% Composite Sweet Orange Peel

Furthermore, figures 2a and 2b showed the levels of serum immunoglobulins (IgM an IgA) in normal and broiler chicks fed 2.5% and 5% composite sweet orange peel. This showed that IgM and IgA levels were significantly ($p<0.05$) elevated in broiler chicks fed 5% composite sweet orange peel, but not in those fed 2.5% composite sweet orange peel (T2), when compared to the control birds. Furthermore, the IgM level in T3 group was significantly higher compared to that observed in T2 group. However, no significant difference in IgA level was observed between T2 and T3 groups.

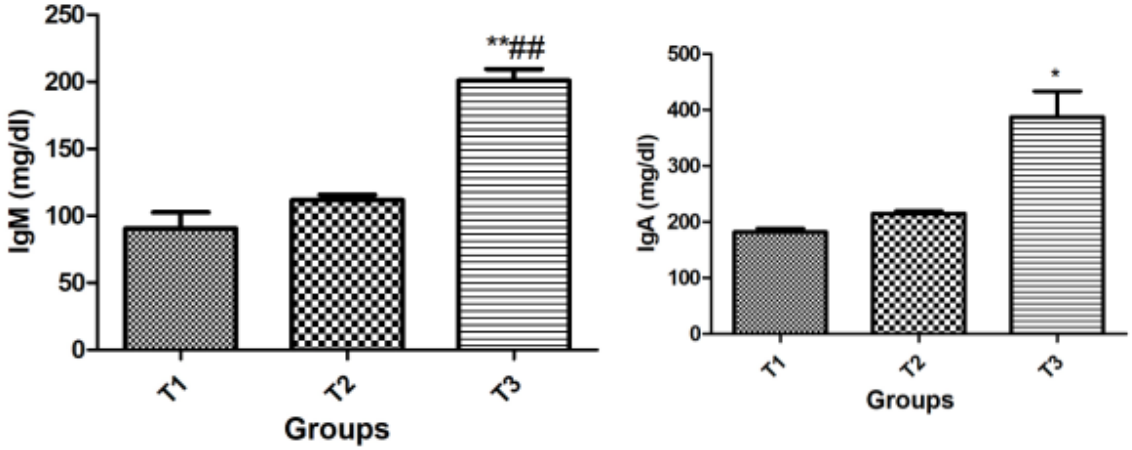

Figure 2. Effect of dietary CSOP supplementation on a) Immunoglobulin M (IgM) and b) Immunoglobulin (IgA) Antibodies in the Serum of Broiler Chicken. T1: Normal Control; T2: 2.5% Composite Sweet Orange Peel; T3: 5% Composite Sweet Orange Peel.

Discussion

Inflammatory cytokines can trigger a cascade of events that promote the recruitment of immune cells and the release of additional inflammatory mediators. This sustained inflammatory environment can contribute to disturbances in lipid metabolism. Tumour Necrosis Factor-Alpha (TNF-α) and Interleukin-1 Beta (IL-1β) are pro-inflammatory cytokines and signalling molecules involved in the inflammatory and immune response. Previous studies reported that acute inflammatory conditions and the rise in the circulating levels of TNF-α resulted in hypercholesterolemia (Basiak *et al*., 2022). Interestingly, the treatment with 5% supplementation of orange peel significantly lowered TNF-α and elevated IL-10 levels in broilers' serum. This is in concordance with previous studies which showed that IL-10 significantly lowered LDL- cholesterol levels and suppressed detrimental inflammation in poultry birds and mammals (Amer *et al*., 2020; Kim *et al* 2021). This denotes that the intervention in IL-10 production can be used to modulate chicken immune responses. More so, interleukin (IL)-10 has been shown to have a pivotal function on B cells, including positively affecting the production of immunoglobulin A (IgA) and IgG (Hummelshoj *et al*., 2006; Yogev *et al*., 2022). The immune system is a complex network influenced by various factors, including diet and nutrition. Citrus peels are rich in phenolic compounds. These compounds perform a pivotal function in supporting the immune system. Captivatingly, the groups treated with the dietary supplementation of citrus peel at varying percentage inclusion (2.5% and 5%) showed significant elevation ($P<0.05$) in the serum immunoglobulins (IgA and IgM) levels. Some studies revealed that the flavonoids present in this peel could be responsible for the stimulation of the immune system via the elevation in IgA and IgM antibody production (Ribeiro *et al*., 2015; Monica *et al*., 2022).

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Exercise-Gallic acid Restored Cognitive Neurochemicals and Oxidative Stress Generated in Diabetic Rats Treated with Acarbose

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ABSTRACT

This study evaluates the influence of swimming exercise (SWEX) and gallic acid (GA) on cognitive neurochemical indices in streptozotocin (STZ)-induced diabetic rats administered with ACA (acarbose). The rats were randomly grouped into normal control (NC), NC + SWEX, STZ + SWEX, STZ + ACA-25 + GA-25, STZ + ACA + GA-25 + SWEX, STZ + ACA + GA-50, and STZ + ACA + GA-50 + SWEX. Acetylcholinesterase (AChE), butyrylcholinesterase (BChE), adenosine deaminase (ADA), NTPdase, 5'-nucleotidase, glutathione peroxidase (GPx), and thiobarbituric reactive species (TBARS) were evaluated. The results showed that SWEX, ACA, and GA treatment reduce enzyme activities and TBARS levels. Still, GPx activity improved compared to that of untreated diabetic rats. The combination of SWEX, ACA, and GA had the highest ameliorative effect. Hence, combined administration of SWEX, ACA, and GA mitigated neurochemical alterations, thus indicating the efficiency of regular exercise with GA-rich diets with antidiabetic drugs in controlling DM.

Keywords: Food-drug interaction; food-bioactive; physical exercise; nootropic; encephalopathy

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Introduction

Insufficient insulin or its insensitivity causes diabetes mellitus (DM) (insulin-dependent DM, insulin non-dependent DM), an endocrine disease of carbohydrate metabolism (Oboh *et al.,* 2018). Structure-related changes or cerebral atrophy, as well as electrophysiological property changes, may be signs of complications caused by diabetes that affect the central nervous system and eventually lead to deficiencies in cognitive function (Adefegha *et al.,* 2020). Alterations in insulin signalling and CNS glucose homeostasis are additional variables that could contribute to diabetes-related cognitive impairment (Nosal *et al.,* 2003). Regionally specific structural abnormalities have been regularly seen in DM, particularly in the cortex's grey matter. N-acetyl aspartate, myoinositol, and choline levels also changed, with magnitudes of approximately 10-15% comparable to those seen in dementia, epilepsy, and Parkinson's disease. (Nosal *et al.,* 2008). Postmortem investigations also supported the results of the neuroimaging experiments. A case study of two diabetic patients who died from diabetic ketoacidosis revealed a significant neuronal loss in the frontal cortex and hippocampus, atrophy of the frontal and temporal white matter, and overexpression of AGP receptors (Jongen *et al.,* 2005).

Phenolic acids are a group of polyphenolic compounds known for their antioxidant properties, which help improve quality of life by reducing oxidative stress. One specific phenolic acid, gallic acid, is naturally found in various plant foods, including Moringa leaves and fruits such as pomegranates and dates (Oboh *et al.,* 2020). Besides its antioxidant benefits, gallic acid has been shown to possess antihyperglycemic properties. It does this by inhibiting enzymes such as α-amylase and α-glucosidase, improving glucose tolerance, and enhancing hepatic insulin sensitivity (Oboh *et al.,* 2020).

Exercise is any physical activity that maintains or improves overall health and wellness (Gavrav *et al*., 2011). It is done for various reasons, including promoting strength and growth, slowing ageing, building up muscles and the cardiovascular system, improving athletic ability, losing or maintaining weight, and enhancing health (Offiong *et al.,* 2019). Engaging in exercise enhances blood glucose regulation in

diabetes. The challenges related to blood glucose management vary with diabetes, activity type, and diabetes-related complications (Smith-Palmer *et al.,* 2014). Therefore, physical activity and exercise recommendations should be tailored to meet the specific needs of each individual. Acarbose is a widely used antidiabetic medication that functions as a starch blocker. It regulates the activity of α-glucosidase, an enzyme that breaks down larger carbohydrates into glucose. The structure of acarbose includes an acrosin moiety with maltose at the reducing terminus (Smith-Palmer *et al.,* 2014). This drug is essential for carbohydrate digestion, specifically targeting α-glucosidase enzymes in the small intestine and pancreatic α-amylase activities. Acarbose counteracts the digestive action of these enzymes (Oyeleye *et al.,* 2024). However, its combination with the ACA and/or effectiveness under physical exercise has not been verified. This study, therefore, aims to evaluate the effect of exercise and/or gallic acid on cognitive neurochemicals and oxidative stress in diabetic rats treated with acarbose.

Materials and Methods

Sample Preparation

Gallic acid and acarbose were dissolved in distilled water, and a solution at a molar concentration was prepared. The solutions were later used for treatment.

Chemicals and Reagents

Ellman's reagent, acetylthiocholine iodide, and adenosine were purchased from Sigma–Aldrich (St. Louis, MO, USA). Kenxin refrigerated centrifuge Model KX3400C and UV–vis spectrophotometer (Model 6305; Jenway, Barloworld Scientific, Dunmow, United Kingdom) were used for centrifuging and the absorbance measurement, respectively. Distilled water (DW) was used to prepare the reagents. All other chemicals used in this experiment were of analytical grade.

Experimental Animals

Sixty-four (64) adult Wistar rats (200-250 g) were used for this study and obtained from the Federal University of Technology, Akure. The animals were maintained in cages at a constant room temperature (25–28 °C) on a 12-dark/light cycle with access to food and water. The animals were allowed to acclimatise under these conditions for 2 weeks before the commencement of the experiment and were kept under the same conditions throughout the study. The animals were housed in standard cages throughout the experiment. All applicable international, national, and/or institutional guidelines for treating and using laboratory animals were followed.

Experimental Design

Before the induction of rats with diabetes, the animals were subjected to overnight fasting. Streptozotocin (STZ) was freshly prepared in citrate buffer (0.1M, pH 4.5) and administered intraperitoneally (i.p.) at a single dose of 50 mg/kg body weight (Hasanein & Shahidi, 2011). Auto-analyser (Fine test Auto-coding™ Infopia Co., Ltd., Kyunggi, Korea) was used to determine the blood glucose level after 72 hours. Blood glucose level was tested to confirm hyperglycemia using a test strip and glucometer. Animals with blood glucose levels ≥ 250mg/dL were considered diabetic and used for this study.

The rats were divided into eight (8) groups (n=8) of animals as follows:

GROUPS	TREATMENTS
I	Normal control rats were administered citrate buffer (0.1M, pH 4.5).
II	Normal control was administered with swimming exercises for 20 minutes (NC + SWEX-20 minutes)
III	Diabetic rats (STZ), Group IV: diabetic rats administered with swimming exercise for 20 minutes (STZ + SWEX-20 minutes)
V	Diabetic rats were administered 25mg/kg of acarbose and 25 mg/kg gallic acid (STZ+ACA-25+GA-25).

VI	Diabetic rats were administered with 25 mg/kg of acarbose, 25 mg/kg of gallic acid and swimming exercise (STZ+ ACA+ GA-25 + SWEX-20 minutes)
VII	Diabetic rats were administered with 25mg/kg of acarbose, and 50mg/kg gallic acid (STZ+ ACA+ GA-50)
VIII	Diabetic rats administered with 25mg/kg of acarbose, 50mg/kg of gallic acid and swimming exercise for 20 minutes (STZ+ ACA+ GA-50+ SWEX-20 minutes).

The treatment lasted 14 days, after which the rats were sacrificed by cervical dislocation and the brain tissues were rapidly isolated, placed on ice, and weighed. This tissue was subsequently rinsed in cold saline solution and later homogenised in phosphate buffer pH 7.4 (1:5 w/v) with 10 up and down strokes at approximately 1,200 rev/min in a Teflon-glass homogeniser. The homogenate was centrifuged for 10 minutes at 3,000xg to yield a discarded pellet, and the assay was carried out.

For the induction of diabetes, the assigned rats were weighed to determine the amount of STZ per group. STZ was dissolved in a 0.01 M citrate buffer, pH 4.5, and injected intraperitoneally. After 72 hours, a blood glucose test was conducted to confirm the diabetic status, and animals with blood glucose levels equal to or greater than 250 mg/dL were identified as diabetic.

Tissue Preparation

The rats were decapitated via cervical dislocation, and the brain tissue was rapidly isolated, placed on ice and weighed. This tissue was subsequently rinsed in cold saline solution and later homogenised in phosphate buffer pH 7.4 (1:5 w/v) with 10 up and down strokes at approximately 1,200 rev/min in a Teflon-glass homogeniser. The homogenate was centrifuged for 10 minutes at 3,000xg to yield a discarded pellet, and the assay was carried out.

Biochemical Assays

AChE and BChE were determined according to Ellman's method, with some modifications to the spectrophotometric method (Rocha *et al.,* 1993), while 5'-nucleotidase and ATPDase activities were evaluated in the brain tissue homogenate using the method described by Olasehinde *et al.* (2022). Adenosine deaminase (ADA) activity was investigated (Oyeleye *et al.,* 2024). The glutathione peroxidase (GPx) and Brain tissue malondialdehyde (MDA) levels were determined by the thiobarbituric acid reactive substances measurement using the method described in the report of Olagunju *et al.* (2020).

Data analysis

Analysis and graph construction were performed using GraphPad Prism version 8.0 (GraphPad Prism Software, Inc.). The results were analysed by one-way ANOVA followed by the Bonferroni multiple range test, and data are presented as mean ± standard error of the mean (SEM).

Result

As shown in Figure 1, it was observed that SWEX decreased the activity of AChE in the NC + SWEX rats' group. Although the untreated DM group exhibited a higher level (****$p < 0.0001$) of AChE activity compared to the normal control and ACA + SWEX + GA-50 mg treated rats. There was a significant difference in the enzyme activity (••••$p < 0.0001$). This result further revealed that SWEX plus 50 mg GA had a higher ameliorative effect on AChE activity (°°°°$p < 0.0001$) in the brain tissue of DM-induced rats. The same result trend was also recorded for BChE activity (Figure 2). The activity of BChE was greatly inhibited (****$p < 0.0001$) in the tissues homogenate of diabetic rats treated with SWEX, ACA+GA-25, ACA+GA-50, ACA+SWEX+GA-25, ACA+SWEX+GA-50. The increase in the activity of the enzyme is well noticed from the treatment with NC+ SWEX only.

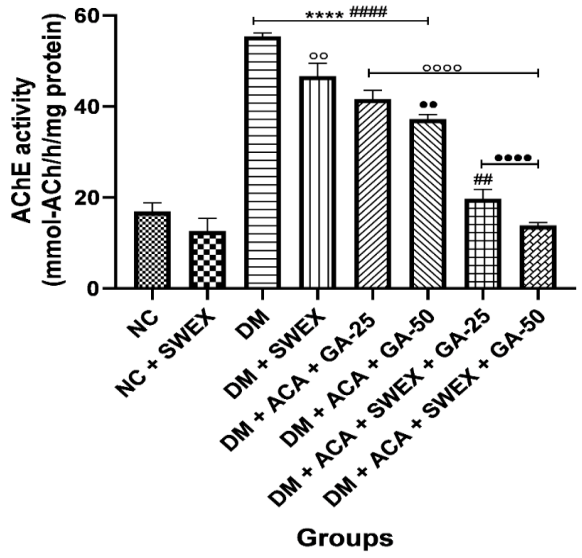

Figure 1: Effect of Exercise-gallic acid on AChE activity in DM-induced rats treated with ACA. NC- Normal Control. SWEX- Swimming Exercise, ACA- Acarbose, GA- Gallic acid.

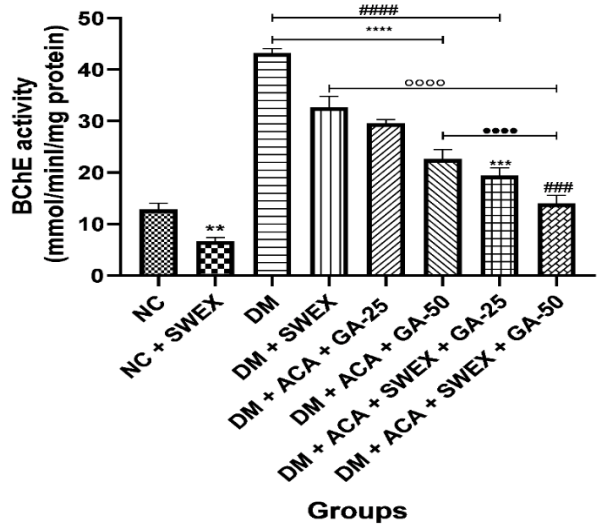

Figure 2: Effect of Exercise-gallic acid on neuronal BChE activity in DM-induced rats treated with ACA. NC- Normal Control. SWEX- Swimming Exercise, ACA- Acarbose.GA- Gallic Acid

Figure 3 revealed a significant increase (****p < 0.0001) in the activity of ATPdase activity in the diabetic rats when compared with NC and NC + SWEX, which was greatly inhibited (°°°°p<0.0001) in the brain tissues of diabetic rats treated with SWEX, ACA + GA-25, ACA + GA-50, ACA + SWEX + GA-25, ACA + SWEX+GA-50. There was a significant decrease in the enzyme activity level when ACA + EX + GA-25/50 mg-treated groups were compared with the untreated DM group. Also, the result revealed that the ACA + GA-50 mg is relatively lower than the ACA + GA-25 mg treated group. Hence, it indicates the importance and role of the higher concentration of GA. Furthermore, it was observed that SWEX drastically reduced ATPase activity compared with the untreated NC group. This result implies that SWEX is responsible for ATPDase reduction in brain tissue.

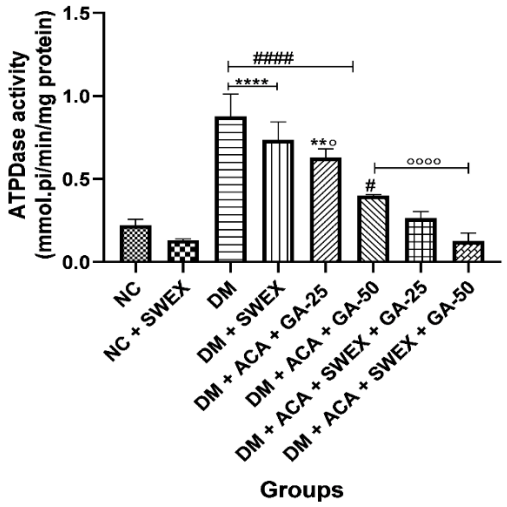

Figure 3: Effect of Exercise-GA on neuronal ATPDase activity in DM-induced rats treated with ACA. NC- Normal Control. SWEX- Swimming Exercise, ACA- Acarbose, GA- Gallic Acid

The activity of the ADA enzyme was greatly inhibited (****p < 0.0001) in the brain tissues of diabetic rats treated with SWEX, ACA + GA-25, ACA+GA-50, ACA+SWEX+GA-25, ACA+SWEX+GA-50. About the same level of effect was recorded across all the ACA+GA-25, ACA+GA-50 and ACA+SWEX+GA-25, ACA+SWEX+GA-50 groups (°°p < 0.01). The effect of SWEX and NC was notably observed (Figure 4).

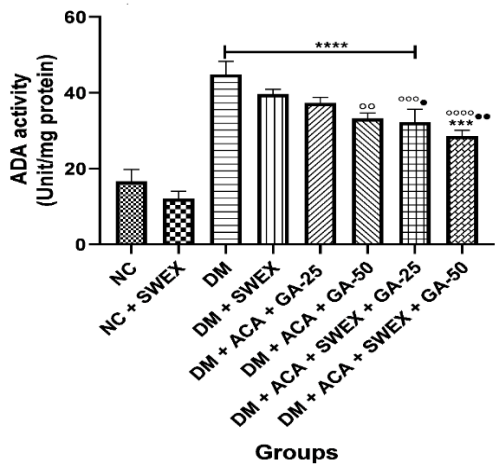

Figure 4: Effect of Exercise-GA on neuronal adenosine deaminase (ADA) activity in DM-induced rats treated with ACA. NC- Normal Control. SWEX- Swimming Exercise, ACA- Acarbose, GA- Gallic Acid

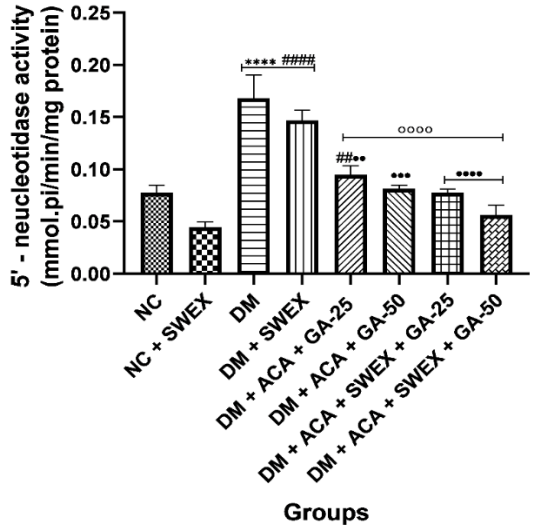

Figure 5: Effect of Exercise-GA on neuronal 5'-nucleotidase activity in DM-induced rats treated with ACA. NC- Normal Control. SWEX- Swimming Exercise, ACA- Acarbose, GA- Gallic Acid

As depicted in Figure 5, the activity of neuronal 5'-nucleotidase was significantly increased (****p<0.0001) in the brain tissues of diabetic rats and DM placed on SWEX. Interestingly, ACA+GA-25, ACA+GA-50, ACA+SWEX+GA-25, and ACA+SWEX+GA-50 treated DM rats had reduced 5'-nucleotidase activity relative to the untreated DM rats. Notably observed (••••p < 0.0001) were ACA+SWEX+GA-25, and ACA+SWEX+GA-50

According to Figure 6, the activity of the GPx enzyme was significantly increased (°°°°p<0.0001) in the brain tissues of diabetic rats treated (NC + SWEX, ACA+GA-25, ACA+GA-50, ACA+SWEX+GA-25, ACA+SWEX+GA-50). No significant difference was noticed across all the NC, SWEX, ACA+GA-25, ACA+GA-50, ACA+SWEX+GA-25, and ACA+SWEX+GA-50 groups. Notably observed (***p < 0.001) was the effect of DM and SWEX+DM.

The level of TBARS was greatly inhibited (p < 0.0001) in the brain tissues of diabetic rats treated with SWEX, ACA+GA-25, ACA+GA-50, ACA+SWEX+GA-25, and ACA+SWEX+GA-50 (Figure 7). The induction of DM gives rise to increased TBARS levels. Therefore, the increase in TBARS levels in the brain tissue of rats is induced by DM. The untreated DM group is relatively higher (°°°°p<0.0001) compared to other groups treated with SWEX+ACA+GA-25/50mg and ACA+SWEX+GA-25/50mg. However, a major reduction was observed in rats administered GA-25/50mg.

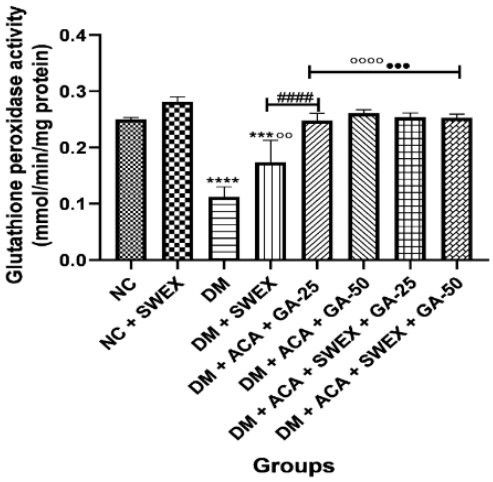

Figure 6: Effect of Exercise-GA on neuronal ATPDase activity in DM-induced rats treated with ACA. NC- Normal Control. SWEX- Swimming Exercise, ACA- Acarbose, GA- Gallic Acid

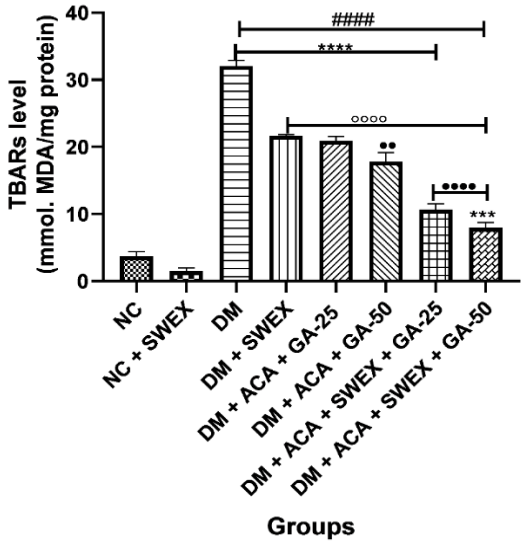

Figure 7: Thiobarbituric acid reactive species (TBARS) level in the brain of diabetic rats treated with gallic acid plus ACA and subjected to SWEX. NC-Normal Control. SWEX- Swimming exercise, ACA-Acarbose, GA- Gallic Acid

Discussion

The condition of hyperglycemia is an aberrant increase in blood glucose levels. It is a precursor to diabetes, an illness brought on by insulin resistance/insufficiency (Jaspinder, 2014). Controlling postprandial hyperglycemia has been demonstrated to be an effective method in the management of diabetes and associated problems brought on by oxidative stress because hyperglycemia plays a significant role in the development of diabetes and diabetic complications. This supports the use of several antidiabetic medications, such as acarbose (Alhaide *et al.,* 2011; Nna *et al.,* 2018) as well as natural remedies, such as polyphenols (Vallejo *et al.*, 2000; Kim *et al.*, 2011).

A cholinergic enzyme called cholinesterase is mainly present in postsynaptic neuromuscular junctions, particularly in muscles and nerves. Acetylcholine (ACh), a naturally occurring neurotransmitter, is quickly hydrolysed into acetic acid and choline by cholinesterase (Thapa *et al.,* 2017). ACh is the main neurotransmitter released by vagus (parasympathetic) nerves that innervate the neuronal cells. Acetylcholine is a chemical that reduces inflammation. Activating cholinesterase may increase inflammation and cause a loss of cognitive function (Allam *et al.,* 2008).

Figures 1 and 2 show that the diabetic rats' AChE/BChE activities were much higher than those of normal control rats. AChE and BChE activities were observed in Figures 1 and 2, respectively, to be very high in diabetic rats compared to the normal control rats. When the rats were treated solely with exercise, there was a significant reduction in enzyme activity. In the diabetic rats placed on SWEX, ACA and GA at 50 mg, the enzyme activities were reduced even more than those of the normal control group. This demonstrates that while the drugs effectively decreased enzyme activity (as observed in the groups treated with DM+ACA+GA-25 and DM+ACA+GA-50), the exercises had an even greater impact.

The study further shows that the activity of the ATPDase enzyme was greatly increased in diabetic rats in the presence of ATP. There was a rather significant reduction in the ATPDase activities in the brain tissue of the diabetic rats treated with exercise in combination with Acarbose and gallic acid (50 mg). This level of reduction in the enzyme activities is less than that of the normal control and is equal to that of the group of normal control rats tasked with only exercise. The diabetic rats treated with the ACA+SWEX+GA-50 group recorded the lowest level of enzyme activities.

The result illustrates that the hydrolysis activity of the 5'-nucleotidase was decreased when acarbose, swimming activities, and gallic acid were combined. This was confirmed when the concentration of gallic acid was 50 mg. This implies that exercise influences the efficacy of the co-administration of acarbose and gallic acid in treating Alzheimer's disease (AD) caused by diabetes by reducing the activity of the hydrolysis of phosphate esterified at carbon 5' of the ribose and deoxyribose portions of nucleotide molecules.

The enzyme adenosine deaminase (ADA), also known as adenosine aminohydrolase, is a key enzyme involved in the metabolism of purines, a

group of molecules essential for various biological functions (Kathiresan *et al.,* 2013). In a recent study conducted with different groups of rats, the researchers aimed to assess the activity levels of ADA across various conditions.

The findings revealed that, in general, the activity of the ADA enzyme exhibited a reduction by the end of the treatment period in all the experimental groups. Specifically, normal control rats subjected to a SWEX regimen showed slightly decreased ADA activity. This suggests exercise might have a minor regulatory effect on healthy individuals' enzymes. In contrast, the diabetic rats in the study displayed a significant increase in ADA activity, which could reduce adenosine levels. This important nucleoside plays an important role in cellular signalling. This heightened activity in diabetic rats could indicate a metabolic alteration due to their hyperglycemic state. Notably, by the conclusion of the treatment, the observed effects were more substantial in the exercised normal control rats compared to the other groups, highlighting the potential impact of physical activity on enzyme regulation and overall metabolic health. This suggests that regular exercise may enhance the body's response in maintaining adenosine levels, especially under normal physiological conditions.

Glutathione peroxidase (GPx) has the biological function of shielding living things from oxidative damage. GPx works biochemically to convert hydroperoxide to the corresponding alcohol and reduce hydrogen peroxide to water (Hayyan, 2016). GPx and catalase both serve comparable purposes. Hydrogen peroxide (H_2O_2) is a substrate for GPx and CAT. While GPx acts at low concentrations, CAT acts at high substrate concentrations. In this study, the GPx activity level was lower in the untreated diabetic rats relative to NC (Fig. 6), thus confirming the successful diabetic model. Furthermore, the administration of the treatment regimens (NC+SWEX, NC+SWEX, ACA+GA-25, ACA+GA-50, ACA+SWEX+GA-25, ACA+SWEX+GA-50) increases the activity of GPx. This confirmed that the ACA + SWEX + GA combination reduced oxidative damage. The decreased activities of GPx enzymes in the brain tissue of untreated diabetic rats could be further substantiated by the overproduction of thiobarbituric reactive species (TBARS); multiple acute and chronic brain illnesses are known to have a harmful mechanism involving lipid peroxidation by reactive oxygen species (ROS). Thiobarbituric acid served as an indicator of lipid peroxidation (Garcia *et al*., 2005). It was observed (Fig. 7) that there was significantly reduction of TBARS in treated rats, especially when acarbose, swimming activities, and gallic acid were combined.

Conclusion

Gallic acid, acarbose (ACA), and exercise (EX) combined therapy effectively treats diabetic encephalopathy. This treatment successfully abated related neurochemicals and oxidative stress by exhibiting strong enzyme inhibitory and antioxidant properties. The therapy could improve cognitive function by reducing enzyme activity, including acetylcholinesterase (AChE) and improving antioxidant status in the diabetic subject. This combo therapy provides a thorough method of treating diabetic encephalopathy and enhancing cognitive function.

Conflict of Interest

The author declares no conflict of interest

Ethical approval

The Federal University of Technology, Akure's ethical committee approved the use of animals in this study.

Data availability statements

The data supporting this study's findings are available from the corresponding author upon reasonable request.

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Momordica charantia Pulp Extract Modulates Neuronal Cholinergic and Antioxidant Enzymes´ Activity in High-fat diet/Streptozotocin-induced Diabetic Rats

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ABSTRACT
Diabetes mellitus has been reported with complications such as cardiovascular, nephropathy and neuropathy. This study investigates the modulatory effects of *Momordica charantia* pulp extract (MCPE) on high-fat diet/streptozotocin (HFD/STZ)-induced diabetic neuropathy in Wistar rats. Thirty-two male Wistar rats were randomly divided into four groups (n = 8). Diabetes was induced via administration of high-fat diet for 2 weeks followed by an intraperitoneal administration of STZ (35 mg/kg). Group 1: Control; Group 2: HFD/STZ-induced diabetic rats; Group 3: HFD/STZ-induced diabetic rats treated with 25 mg/kg Metformin; Group 4: HFD/STZ-induced diabetic rats treated with 200 mg/kg MCPE. The experiment lasted for 14 days. Thereafter, rats were sacrificed via cervical dislocation and the brain was harvested for biochemical analysis. Acetylcholinesterase (AChE) and butrylcholinesterase (BChE) activities, thiobarbituric acid reactive substances (TBARS) level, catalase and superoxide dismutase (SOD) activities of the brain homogenate were assessed. The results showed that administration of HFD/STZ significantly ($p < 0.05$) increased cholinesterase (AChE and BChE) activities and TBARS level, with simultaneous decrease in catalase and SOD activities. However, treatment with MCPE significantly ($p<0.05$) decreases BChE and AChE activities, suggesting a protective effect against diabetic neuropathy. Also, MCPE significantly lowered TBARS levels and enhances catalase and SOD activity; an indication of improved antioxidant status. Findings from this study revealed antioxidant and modulatory effects of MCPE. Hence, *Mormodica charantia* pulp extract could be considered a natural and alternative therapy for the management of neuropathy that could arise from complications of type-2 diabetes mellitus.
Keywords: Momordica; Antioxidant; Pulp extract; Neuroprotection

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Introduction

The brain is a highly intricate organ that acts as the command center of the nervous system in animals, including humans. It coordinates physical actions, processes sensory information, and enables complex cognitive abilities such as thinking, memory, and emotion (Gaiseanu, 2020). The brain's vast network of neurons and supporting glial cells communicate through electrical and chemical signals to perform these functions, regulating everything from basic survival mechanisms to advanced intellectual tasks. Its plasticity allows it to adapt and reorganize throughout life, reflecting its capacity to learn and respond to new experiences (Linne, 2022).

Diabetes mellitus is a chronic metabolic disorder characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. The global prevalence of diabetes has been rising, presenting significant health challenges. One of the severe complications associated with diabetes is diabetic neuropathy, which affects a considerable percentage of diabetic patients and can lead to debilitating pain and sensory deficits (Mukhtar *et al*., 2020). Diabetic neuropathy is a form of nerve damage that occurs due to chronic high blood sugar levels. It primarily

affects peripheral nerves, leading to symptoms such as pain, tingling, and numbness, particularly in the extremities. This condition significantly impacts the quality of life and increases the risk of infections and ulcers, which can lead to amputations (Pang *et al*., 2020). Despite the availability of various therapeutic options, the management of diabetic neuropathy remains challenging, necessitating the exploration of novel treatment approaches. Streptozotocin (STZ) is a naturally occurring chemical that is particularly toxic to the insulin-producing β-cells of the pancreas in mammals. It is widely used to induce diabetes in experimental animal models, primarily rats, due to its ability to mimic the pathophysiological conditions of human diabetes (Zhu, 2022). HFD/STZ-induced diabetic neuropathy in rats serves as a relevant model for studying the efficacy of potential therapeutic agents, including plant extracts. Several studies have explored the effects of various plant extracts on diabetic neuropathy. For instance, an investigation into the use of traditional herbal remedies highlighted the neuroprotective effects of certain plant compounds in diabetic neuropathy (Yang, 2022).

Bitter gourd (*Momordica charantia*), is a plant traditionally used in various cultures for its medicinal properties. It is rich in bioactive compounds, including charantin, insulin-like peptides, and alkaloids, which have demonstrated hypoglycemic effects in both animal models (Valyaie *et al*., 2021). The aqueous extract of the pulp of *Momordica charantia*, in particular, has been studied for its potential to modulate glucose metabolism and improve insulin sensitivity. The therapeutic potential of *Momordica charantia* in diabetes management is attributed to its ability to enhance glucose uptake, promote insulin secretion, and inhibit glucose absorption in the intestine. Additionally, it exhibits antioxidant properties that may protect pancreatic β-cells from oxidative stress, a key factor in the pathogenesis of diabetes (Li *et al*., 2020). The plant's anti-inflammatory properties further support its role in mitigating diabetes-related complications, including neuropathy. The prevalence of diabetic neuropathy is increasing globally, posing a major public health concern. Current pharmacological treatments are often inadequate and associated with adverse effects. There is a critical need for alternative therapeutic strategies that are both effective and safe. Thus, this study aims to investigate the effects of *Momordica charantia* pulp extract on HFD/STZ-induced diabetic neuropathy in rats, providing a potential natural remedy for this debilitating condition.

Materials and methods

Chemicals

Streptozotocin (STZ), acetylcholine thioiodide, butyrycholine thioiodide, adrenaline, hydrogen peroxide, 5,5’ dithiobis-(2-nitrobenzoic acid) (DTNB) and Phosphate buffer were procured from Sigma-Aldrich Co. (Sigma-Aldrich Co., St Louis, MO, USA). Other chemicals used were analytical grade and glass distilled water was used throughout.

Sample Collection and Preparation

The fruit of *Momordica charantia* was purchased from local herb store in Akure and identified at the Department of Biological Sciences, Joseph Ayo Babalola University, Nigeria, with Voucher Number CANS/PL/IDN/0365. The pulps were carefully removed from the fruits, dried and pulverized to fine powder using a domestic blender. Forty grams of the powdered sample was dissolved into 400 mL of water, stirred continuously on an orbital shaker for 24 hours and then filtered. The extract was then stored in a refrigerator for further use.

Methods

Animal care and experimental design

Thirty-two male Wistar rat weighing 136-177 g were procured from the breeding colony of the Department of Biochemistry, Joseph Ayo Babalola University, Nigeria. The animals were acclimatized for two weeks and fed with rat pellet and water. The handling of the animals was in accordance with the guideline of the National Institute of Health (NIH). The animals were housed in stainless steel cage and kept in a room where 12 hours light/dark cycle was maintained throughout the period of experiment.

Following their acclimatization, the rats were divided into two dietary groups: the high-fat diet and the regular diet. After 14-day period of dietary modification, rats given a high-fat diet

were intraperitoneally administered a single dosage of streptozotocin (35 mg/kg body weight). Seventy-two hours following the induction, the rats' blood glucose levels were measured. Using a lancet, the rats' tails were punctured to get a blood sample, and a glucometer was used to measure the blood glucose level. The diabetic rats were divided into three groups based on the treatments they received, while the normal rats served as a control group.

The rats were grouped (n = 8) as follows:

Group 1: Normal control rats;

Group 2: HFD/STZ-induced diabetic rats

Group 3: HFD/STZ-induced diabetic rats + 25 mg/kg Metformin

Group 4: HFD/STZ-induced diabetic rats + 200 mg/kg MCPE

Momordica charantia pulp extract (MCPE) and the standard drug were administered orally for 14 consecutive days. The choice of dose of STZ (35mg/kg) was in accordance with previous work of (Bahr *et al*., 2023); while that of *Momordica charantia* extract (200mg/kg) was as reported by Obiandu *et al*. (2020)

Table 1: Feed formulation for normal control and High Fat Diet groups

	NC diet (g/kg)	HFD (g/kg)
Skimmed milk	500	500
Lard	__	300
Rice bran	200	90
Corn starch	160	70
Premix	40	40
Groundnut oil	100	

Tissue Preparation

The animals were sacrificed via cervical dislocation thereafter the brain was collected, rinsed in cold buffer, weighed and homogenized using phosphate buffer. The homogenate was centrifuged for 10 minutes at 4000 rpm and the supernatant was decanted into the sample bottle and stored in the refrigerator for subsequent analysis.

Biochemical Assays

Acetylcholinesterase (AChE) Assay

This was carried out as described by Lakshmanan, 2021, briefly, a 100 µL of the brain homogenate was mixed with 100 µL of DTNB, followed by the addition of 0.1 M PO^{3-} buffer (pH 8.0). The mixture was incubated at room temperature for 20 minutes before the addition of the substrate, acetylthiocholine iodide. The absorbance was taken at 412 nm for 3 minutes at 3 sec interval and the enzyme activity was subsequently calculated

Butyrylcholinesterase (BChE) Assay

The brain homogenate (100 µL) was mixed with 100 µL of DTNB, followed by the addition of 0.1 M PO^{3-} buffer (pH 8.0). The mixture was incubated at room temperature for 20 minutes before the addition of the substrate, butyryl thiocholine iodide. The absorbance was taken at 412 nm for 3 minutes at 3 sec interval and the enzyme activity was subsequently calculated (Obaid, 2022).

Lipid peroxidation Assay

Thiobarbituric acid of 1.2g was weighed and dissolved in 200 mL of 0.08% of NaOH. The blank was prepared by pipetting 300 µL of SDS, 500 µL of acetic acid, and 500 µL of TBA into the test tube and the sample test tube was arranged, 300 µL of tissue homogenate, 300 µL of SDS, 500 µL of acetic acid, 500 µL of TBA and was incubated at 100 ^{0}c for one hour. The absorbance was read on a spectrophotometer at 532 nm (Saito, 2021).

Determination of superoxide dismutase (SOD) Activity

SOD activity assay was in terms of its ability to inhibit the autoxidation of epinephrine to adrenochrome, which has an absorption maximum of 480nm. This was carried out by mixing 50 µL of the heart homogenate supernatant, 1000 µL of sodium carbonate buffer (pH 10.2), and 17 µL of adrenaline together. The reaction was carried out in a cuvette for 2 min at 30 seconds intervals, and the absorbance taken at 480nm (Carmo de Carvalho e Martins et al., 2022).

Determination of Catalase Activity

The enzyme assay was carried out by reacting 500 µL of phosphate buffer (0.01M, pH 7.0), 50 µL of the heart tissue homogenate supernatant, 200 µL of 2M H_2O_2, and 1000 µL dichromate acetic acid. The reaction was carried out in a cuvette for 3 min at one (1) minute intervals,

and absorbance were measured at 620nm (Anwar, 2024).

Data analysis

The results are expressed as mean ± standard deviation of replicates (n=8). One-way analysis of variance was used to analyze the data followed by post-hoc Dunnet's test for comparing the mean, significance was accepted at $p<0.05$ using GraphPad Prism (PRISMA 5.00) for windows (GraphPad Prism Software, Inc., San Diego, CA, USA).

Results

As presented in Figure 1, HFD/STZ-induction caused a significant ($p < 0.05$) increase in blood glucose level compare to the control group. However, the treated groups showed a significant ($p < 0.05$) reduction in blood glucose level when compared with the untreated group.

The results in Figure 2 showed the effect of *Momordica charantia* pulp extract (MCPE) on the brain tissue homogenate Acetylcholinesterase (AChE) activity, the high fat diet STZ-induced diabetic rats. The untreated group showed a significant increase in AChE activity in the brain homogenate when compared with the control group. However, treatment with Metformin and 200 mg/kg of MCPE cause significant decrease ($p < 0.05$) in the AChE activity with the extract showing a more pronounced decrease in AChE activity. This trend was also observed as presented in Figure 3 as increased level of BChE activities in untreated rat group got significantly decreased on administration of Metformin and 200 mg/kg *Momordica charantia* pulp extract.

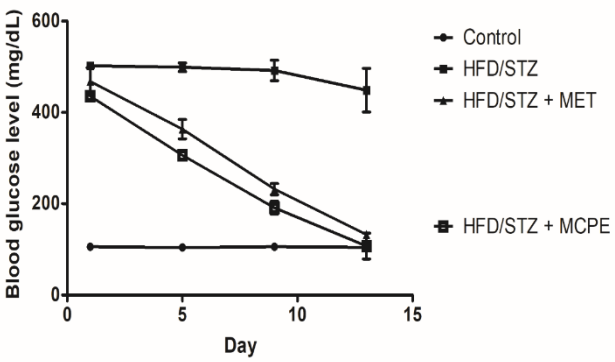

Figure 1: Effect of *Momordica charantia* pulp extract on blood glucose levels in HFD/STZ diabetic rats.

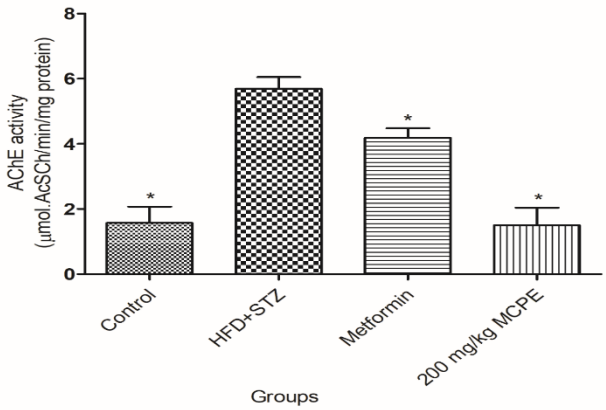

Figure 2: Effect of Momordica charantia pulp extract (MCPE) on brain Acetylcholinesterase (AChE) enzyme level in high-fat diet/streptozotocin (HFD/STZ-induced diabetic rats.

Bars represent mean±standard deviation (n = 8). Bars with asterisk (*) are significantly different at $p < 0.05$.

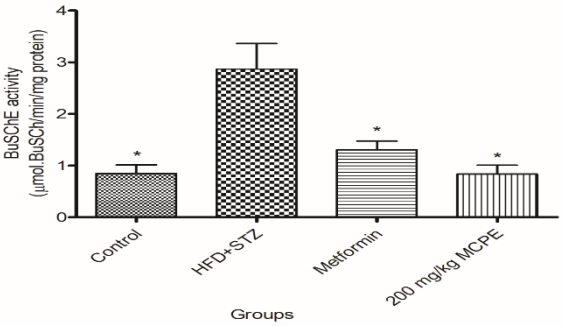

Figure 3: Effect of *Momordica charantia* pulp extract (MCPE) on brain Butyrylcholinesterase (BChE) enzyme level in high-fat diet/streptozotocin (HFD/STZ-induced diabetic rats.

Bars represent mean standard deviation (n = 5). Bars with asterisk (*) are significantly different at $p < 0.05$.

Administration of High fat diet in STZ induced diabetic rats cause a significant increase ($p < 0.05$) in the brain Melondyaldehyde (MDA) content in the brain tissue as shown in Figure 4, however, treatment with Metformin and 200 mg/kg *Momordica charantia* pulp extract causes significant decrease in the MDA content in the brain homogenate.

Likewise, Figure 5 showed that administration of High fat diet in STZ-induced diabetic rats caused a significant ($p < 0.05$) decrease in the activity of Superoxide dismutase (SOD). However, treatment with metformin and 200 mg/kg *Momordica charantia* pulp extract increase SOD activity in the brain tissue homogenate with *Momordica charantia* pulp extract treated group having higher SOD activity.

Also, a significant decrease ($p < 0.05$) in the catalase activity in untreated group was shown (Figure 6) when compared with the control group, however, treatment with Metformin and 200 mg/kg of *Momordica charantia* pulp extract significantly increase ($p > 0.05$) the catalase activity.

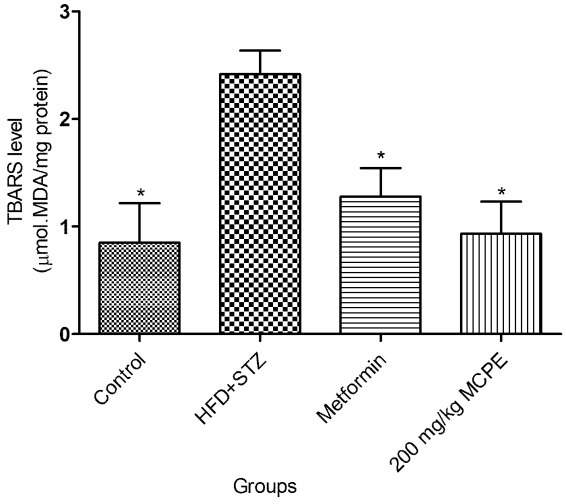

Figure 4: Effect of *Momordica charantia* pulp extract (MCPE) on brain thiobarbituric acid reactive species (TBARS) level in high-fat diet/streptozotocin (HFD/STZ-induced diabetic rats. Bars represent mean±standard deviation (n = 5). Bars with asterisk (*) are significantly different at $p < 0.05$.

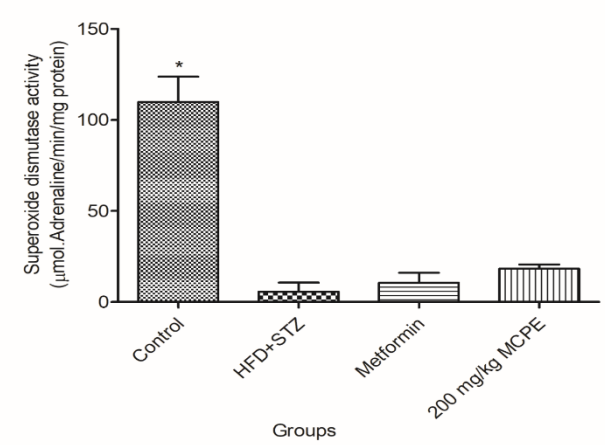

Figure 5: Effect of *Momordica charantia* pulp extract (MCPE) on brain Superoxide Dismutase enzyme level in high-fat diet/streptozotocin (HFD/STZ-induced diabetic rats.

Bars represent mean±standard deviation (n = 5). Bars with asterisk (*) are significantly different at $p < 0.05$.

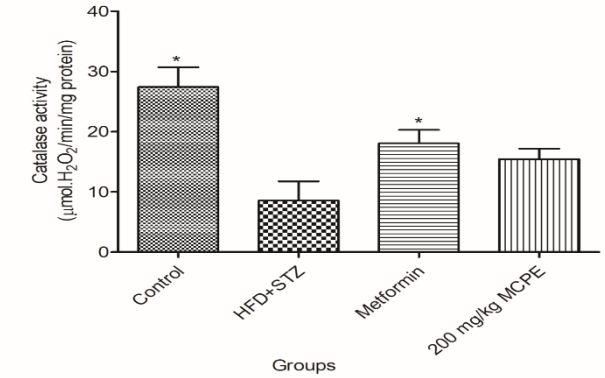

Figure 6: Effect of *Momordica charantia* pulp extract (MCPE) on brain Catalase enzyme level in high-fat diet/streptozotocin (HFD/STZ-induced diabetic rats.

Bars represent mean±standard deviation (n = 5). Bars with asterisk (*) are significantly different at $p < 0.05$.

Discussion

The present study investigated the effects of *Momordica charantia* pulp extract (MCPE) on HFD/STZ-induced diabetic neuropathy in rats. The findings from this study demonstrated that the extract significantly reduced AChE and BChE activities in HFD/STZ-induced diabetic rats. Both enzymes are involved in cholinergic neurotransmission, and their increased activity has been associated with neurodegenerative conditions and diabetic complications (Singh et al., 2023). The inhibitory effect of the extract on

these enzymes suggests a potential mechanism for its neuroprotective effects, which aligns with findings from other studies with similar outcomes using different compounds of natural source (Gopalakrishnan et al., 2023; Zhang et al., 2021). The cholinergic system plays a crucial role in memory and cognition (Chen et al., 2022). The decline of the cholinergic system is one of the major causes of neurodegeneration. Thus, AChE and BChE inhibitors are commonly used first-line therapy in the management of neurodegeneration (Walczak-Nowicka, 2021). Interestingly, *Momordica charantia* pulp extract ameliorated increased AChE and BChE activities observed in HFD/STZ-induced diabetic rats used for this study.

Oxidative stress is one of the major mechanisms through which diabetes damage tissues or organs. An imbalance between free radical production and protective mechanism conferred by antioxidants is one of the culprits in pathogenesis and progression of several neurodegenerative diseases (Akanji et al., 2021). SOD is a critical antioxidant enzyme that converts superoxide radicals into hydrogen peroxide (Ahmed et al., 2020). In agreement with other studies where increased SOD activity were found following treatment with various antioxidants (Liu et al., 2022; Wang et al., 2023), our study revealed that aqueous extract of *Momordica charantia* contain bioactive compounds capable of reducing the organ free radicals thereby aiding the SOD activity to enhance anti-oxidation capability in the body. The SOD has been reported as first line of defense antioxidant enzyme and its lower activity has been implicated in degenerative disease such as neuronal disorders. The increased activity of this enzyme in the aqueous extract of *Momordica charantia* pulp treated group suggest it has been potential therapeutic agent for diabetic neuropathy as revealed by finding from this study.

Furthermore, we observed a significant increase in catalase activity in the brains of the treated rats. Catalase is a crucial antioxidant enzyme that decomposes hydrogen peroxide into water and oxygen, thereby protecting cells from oxidative damage (Anwar, 2024). The enhancement of catalase activity by the extract indicates a bolstering of the brain's antioxidant defense system, which could be a vital factor in mitigating diabetic neuropathy. This finding is supported by previous research showing the beneficial effects of enhanced catalase activity in diabetic models (Chen et al., 2021; Patel et al., 2023).

Conclusion

Conclusively, this study revealed that *Momordica charantia* pulp extract exhibits significant a neuroprotective effect in HFD/STZ-induced diabetic neuropathy in Wistar rats by reducing oxidative stress through reduction in the brain tissue homogenate MDA content and cholinesterase activity with improved SOD and catalase activity. These findings highlight the potential of *Momordica charantia* pulp extract as a natural therapeutic agent that could be useful in the management/treatment of diabetic neuropathy.

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Declaration of Conflict of Interest

Authors have no conflict of interest to declare.

Author's contribution

L.J. Babatola was involved in experimental design and manuscript writeup
A.A. Adebayo was also involved in experimental design and execution of the research
G.P. Akerele was involved in experimental execution and data processing
O.O. Adegoke was involved in biochemical analysis and results collation
I.S. Adejumo was involved in the biochemical analysis and manuscript preparation
G. Oboh was involved in the experimental design

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