DEVELOPMENT AND ANTIOXIDANT SCAVENGING CAPACITIES OF BISCUITS MADE FROM CARDABA BANANAS, BEETROOT, TIGER NUTS, SOYBEANS AND *Justicia carnea* LEAVES AS A PROBABLE ANTIHYPERTENSIVE SNACK

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SCHOOL OF BASIC MEDICAL SCIENCES

UNIVERSITY OF BENIN

BENIN CITY, EDO STATE

DECEMBER, 2025

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A PROJECT SUBMITTED TO THE DEPARTMENT OF MEDICAL BIOCHEMISTRY, SCHOOL OF BASIC MEDICAL SCIENCES IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF BACHELOR OF SCIENCE, B.Sc. (HONS) MEDICAL BIOCHEMISTRY, OF THE UNIVERSITY OF BENIN, BENIN CITY.

DECEMBER, 2025

CERTIFICATION

We the undersigned hereby certify that Alfred Omenime IWEKA with matriculation number BMS2101417, performed this research at the department of Medical Biochemistry, School of Basic Medical Sciences, University of Benin and thereby approve in scope and quality, for the award of Bachelor of Science degree (B.Sc.) in Medical Biochemistry.

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External Examiner Date

DEDICATION

This project is dedicated in the first place to God Almighty, acknowledging His constant mercy, grace, favour and kindness in my life. Gratitude is also extended to my family, may God bestow His rich blessings upon each and every one of you, Amen.

ACKNOWLEDGEMENT

I am taking this opportunity to express my heartfelt appreciation to all those who played a crucial role in the accomplishment of this project at various stages. I offer my gratitude to the Almighty for providing unwavering support throughout the project's duration. I extend my sincere thanks to my project supervisor, the great and wonderful Professor (Mrs.) H. A. Oboh, for her invaluable support, mentorship in medical biochemistry, and guidance throughout this research work. I also appreciate my lecturers for their dedication and the knowledge they shared, namely: Dr. N.B. Aguebor-Ogie, Dr. Mrs. Eweka, Dr. Mrs. Eluehike, Dr. S. Oghagbon, Dr. Omorowa, Dr. Agbontaen, Dr. J.C. Anionye, Mr. Aisosa, Mrs. Ukwonu-Ediale Ada and Mrs. Oronsaye-Eseosa Oseghale.

Lastly, I would like to convey special thanks to all my fellow project group members, friends, and classmates who have contributed significantly to the success of this endeavor.

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ABSTRACT

The increasing prevalence of hypertension has led to a greater search for functional foods that offer both nutritional and therapeutic benefits. This study focused on the production and evaluation of functional biscuits made from selected plant materials (Cardaba bananas, beetroot, tigernuts, *Justicia carnea* leaves, and soybeans) to determine their antioxidant scavenging abilities and potential as a natural antihypertensive snack. Composite flours were prepared from these plant materials and incorporated into biscuit recipes; their phytochemical profiles, mineral content, and proximate composition were analyzed for nutritional value and bioactive components. Antioxidant activity was determined by DPPH radical scavenging and Ferric Reducing Antioxidant Power (FRAP) assays. The prepared biscuits contained significant amounts of dietary fiber, potassium, nitrates, betalains, phenolics, flavonoids, alkaloids, and other phytochemical components. These findings indicate that the biscuits developed have significant antioxidant properties and can serve as a functional snack with potential antihypertensive benefits, which supports their role in the management of cardiovascular disease.

CHAPTER ONE

1.0 INTRODUCTION

1.1 BACKGROUND OF STUDY

High blood pressure, also known as hypertension, is one of the most important global health challenges and a major cause of cardiovascular disease, renal disease, and premature death (World Health Organization, 2023; Li *et al.*, 2023). It is well-established that oxidative stress, which involves an imbalance between ROS (reactive oxygen species) and antioxidant defense mechanisms, contributes to the development and progression of hypertension (Adebayo *et al*., 2021; Xie *et al*., 2022). Increased ROS production can disturb vascular homeostasis, resulting in endothelial dysfunction, vascular remodeling, and inflammation, all of which contribute to increased arterial stiffness and hypertension (Sies *et al*., 2022; Al-Shamsi *et al.*, 2024). Oxidative stress also interacts with metabolic factors (obesity, insulin resistance, and salt sensitivity) to enhance the risk of hypertension and its related complications (Aiyegoro and Adebayo, 2023; Li *et al.,* 2023). Functional foods enhanced with bioactive compounds are a promising approach for the dietary management of hypertension because dietary antioxidants can help prevent oxidative damage. Biscuits are a popular, reasonably priced, and adaptable food carrier that provides an easy way to add nutrients. The nutritional and medicinal qualities of such products can be improved by adding plant-based antioxidant sources like fruits, nuts, and medicinal leaves (Shareenie *et al.* 2021; Singh and Sharma, 2023). Ingredients with proven antioxidant properties, such as Cardaba banana *(Musa* × *balbisiana*), beetroot (*Beta vulgaris*), tiger nut (*Cyperus esculentus*), and *Justicia carnea* leaves. Unripe *Musa* × *balbisiana* bananas are rich in resistant starch, phenolics, flavonoids, and vitamins, and studies have shown that their flour enhances antioxidant capacity and improves lipid metabolism in baked goods (Osungbaro *et al.,* 2022; Adebowale and Omotayo, 2024). Beetroot is also a rich source of betalains and dietary nitrates as it helps to facilitate

vasodilation and lower blood pressure by increasing nitric oxide bioavailability and antioxidant activity (Kumar *et al.,* 2023; Onyenekwe *et al*., 2024). Tiger nuts supply dietary fiber, unsaturated fatty acids, vitamin E, and phenolic compounds that enhance the nutritional and sensory properties of functional snacks (Ibrahim *et al*., 2022; Enaohwo *et al*., 2025). Leaves of *Justicia carnea* exhibit high antioxidant activity and have been shown to protect against oxidative stress and hypertension-induced tissue damage in experimental models (Umar *et al*., 2024; Adegbite *et al*., 2023).

1.2 AIM OF STUDY

The aim was to create biscuits using cardaba bananas, beetroot, tiger nuts, and *Justicia carnea* leaves in order to assess their potential as a functional snack for managing hypertension by evaluating their antioxidant scavenging capabilities.

1.3 OBJECTIVES OF STUDY

i. To formulate biscuits using cardaba bananas, beetroot, tiger nuts, and Justicia carnea leaves.

ii. To assess the antioxidant scavenging capacity of the developed biscuits.

iii. To evaluate the potential antihypertensive properties of the biscuits.

1.4 JUSTIFICATION OF STUDY

Functional foods with bioactive properties are increasingly recognized for their role in managing cardiovascular health. This study developed biscuits using cardaba bananas, beetroot, tiger nuts, and *Justicia carnea* leaves, containing locally available ingredients with potential health benefits. Cardaba bananas and tiger nuts provided dietary fibre, potassium, and phytochemicals that support vascular function, while beetroot supplied nitrates that promoted both vasodilation and blood pressure regulation. Also, *Justicia carnea* leaves

contributed antioxidants that counteract oxidative stress. The biscuit's antioxidant scavenging capacity was evaluated, demonstrating its potential as a functional snack with antihypertensive properties.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Functional foods with Antioxidant Potentials

Functional foods are foods that provide benefits beyond basic nutrition due to the presence of bioactive compounds such as polyphenols, flavonoids, carotenoids, vitamins, minerals, resistant starch, and peptides. These compounds exhibit antioxidant, anti-inflammatory, and vasodilatory properties, which are particularly relevant in the prevention and management of hypertension (Ribas *et al.,* 2022; Nwachukwu *et al.,* 2023). The strategic combination of plant-based ingredients such as Cardaba bananas, beetroot, tiger nuts, soybean, and Justicia carnea leaves in snack formulations can significantly enhance their health-promoting potential. Such functional foods not only provide energy and essential nutrients but also mitigate oxidative stress and vascular dysfunction, both key contributors to hypertension.

2.1.1 Nutritional composition: dietary fibre, potassium, and resistant starch of Cardaba banana

Cardaba banana (*Musa acuminata* × *balbisiana*, ABB group) is an important source of resistant starch, soluble and insoluble dietary fibre, and potassium. Resistant starch escapes digestion in the small intestine, undergoes fermentation in the colon, and produces short-chain fatty acids (SCFAs), which improve gut microbiota composition and enhance metabolic health (Sun and Wang, 2023). Dietary fibre contributes to reduced serum cholesterol, improved glucose metabolism, and delayed postprandial glucose absorption, all of which indirectly support cardiovascular health (Kumar *et al.,* 2021). Potassium is essential for vasodilation, sodium excretion, and maintenance of fluid balance, directly contributing to lower blood pressure and reduced risk of stroke (Adedokun and Adeeyo, 2022; Whelton *et al.,*

2023). These properties make Cardaba banana flour an ideal ingredient for functional snacks aimed at hypertension management.

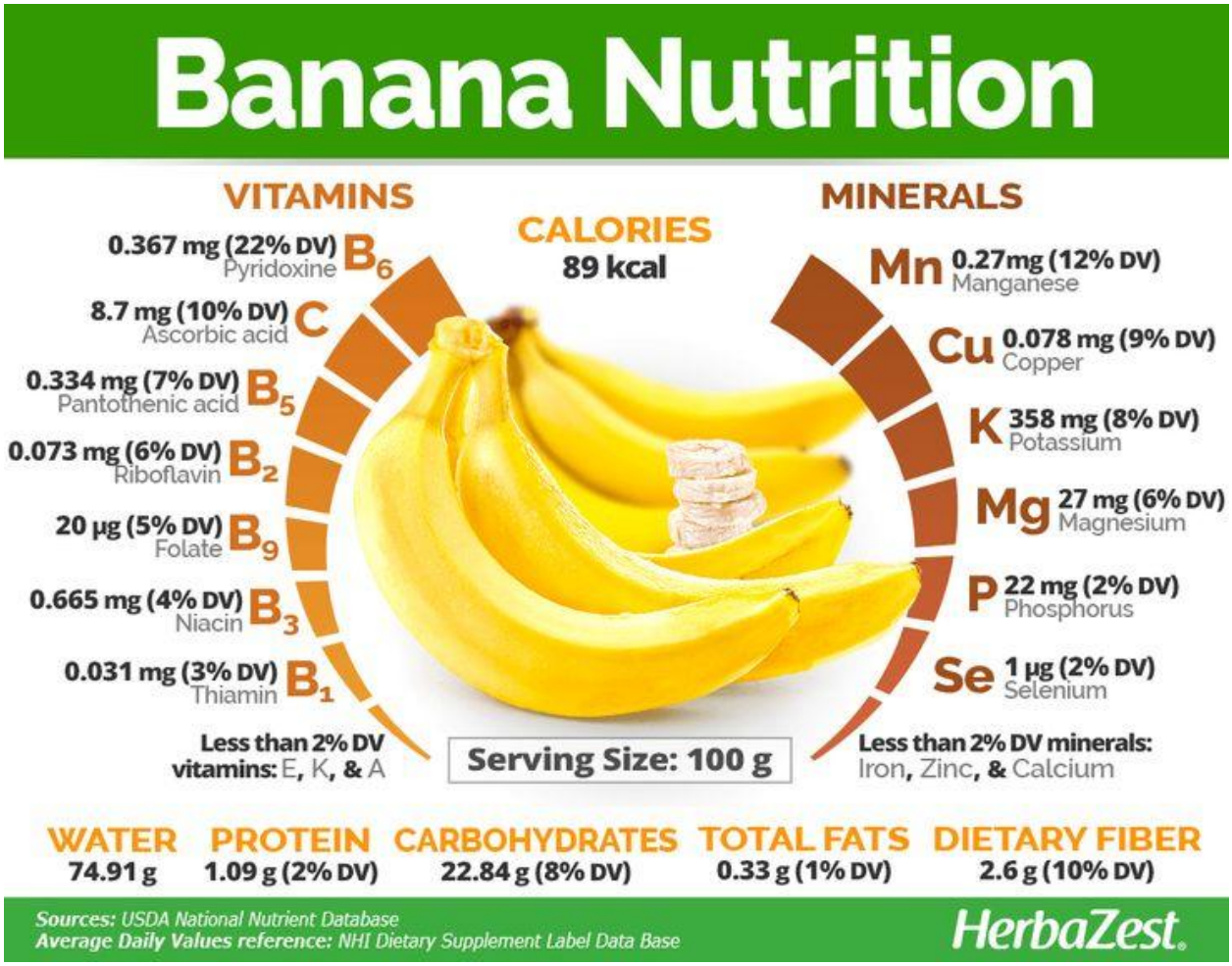


Figure 2.1: Various nutritional compositions of banana (*Musa spp.*)

2.1.2 Antihypertensive Properties of Cardaba Banana

The antihypertensive potential of Cardaba banana is primarily attributed to its high potassium content and polyphenolic compounds. Potassium enhances vasorelaxation by modulating vascular smooth muscle tone and suppressing the renin–angiotensin–aldosterone system (RAAS), resulting in reduced systolic and diastolic blood pressure (Adedokun and Adeeyo, 2022). Additionally, polyphenols such as catechins and flavonoids in bananas exert

antioxidant and anti-inflammatory effects by scavenging reactive oxygen species (ROS) and inhibiting pro-inflammatory mediators, protecting the endothelium from oxidative damage. The inclusion of unripe Cardaba banana in snack products could provide sustained antihypertensive benefits due to its low glycaemic index and high resistant starch content.

2.1.3 Nutrient Composition: Nitrates, Betalains, and Vitamins of Beetroot

Beetroot (*Beta vulgaris*) is a root vegetable rich in dietary nitrates, betalains, polyphenols, vitamins, and minerals. Dietary nitrates are converted in vivo into nitric oxide (NO), a potent vasodilator that regulates vascular tone, inhibits platelet aggregation, and improves endothelial function (Clifford and van der Veer, 2020). Betalains, a class of nitrogen-containing pigments, possess strong antioxidant properties, capable of scavenging free radicals and reducing lipid peroxidation (Domínguez *et al.,* 2021). Vitamins such as vitamin C and minerals like magnesium and potassium further contribute to cardiovascular protection. Incorporating beetroot powder into functional snacks provides a synergistic combination of bioactive compounds that may reduce hypertension risk.

	Raw	Cooked, boiled	Canned	Fresh juice
Water, g	87.58	87.06	90.96	–
Energy, kcal	43	44	31	30
Protein, g	1.61	1.68	0.91	1.02
Total fats, g	0.17	0.18	0.14	0
Carbohydrate, g	9.56	9.96	7.21	6.6
Fiber, g	2.8	2	1.8	0
Sugars, g	6.76	7.96	5.51	6.6
Calcium, mg	16	16	15	0
Iron, mg	0.8	0.79	1.82	0
Magnesium, mg	23	23	17	–
Phosphorus, mg	40	38	17	–
Potassium, mg	325	305	148	–
Sodium, mg	78	77	194	93
Zinc, mg	0.35	0.35	0.21	–
Vitamin C, mg	4.9	3.6	4.1	0
Thiamin, mg	0.031	0.027	0.01	–
Riboflavin, mg	0.04	0.04	0.04	–
Niacin, mg	0.334	0.331	0.157	–
Folate, µg	109	80	30	–
Total phenolic content[a]	255	238	192	225
Total flavonoid content[b]	260	261	173	126

Figure 2.2: Nutrient Composition of Beetroot

2.1.4 Antioxidant and Anti-inflammatory Properties of Beetroot

Beetroot's antioxidative capacity is attributed to betalains, flavonoids, phenolic acids, and vitamin C, which collectively neutralize ROS and enhance endogenous antioxidant enzyme activity (Mirmiran *et al.,* 2020). The anti-inflammatory effect is mediated via downregulation of pro-inflammatory cytokines such as TNF-α and IL-6, and inhibition of NF-κB signaling pathways. Dietary supplementation with beetroot has been shown to reduce markers of oxidative stress, improve endothelial function, and lower systolic and diastolic blood pressure

in clinical and experimental studies, highlighting its potential as a functional ingredient for antihypertensive snacks.

2.1.5 Cardiovascular Benefits of Tiger Nuts

Tiger nuts (*Cyperus esculentus*) are tubers rich in dietary fibre, monounsaturated fatty acids (MUFA), arginine, and vitamin E. MUFAs improve lipid profiles by lowering LDL cholesterol and increasing HDL cholesterol, while arginine serves as a substrate for nitric oxide synthesis, promoting vasodilation (Okpala and Igwe, 2020). Vitamin E and phenolic compounds provide potent antioxidant effects, reducing oxidative stress in the vascular endothelium (Kagami *et al.,* 2022). Regular consumption of tiger nuts has been associated with improved endothelial function and reduced cardiovascular risk, supporting their use in functional food formulations targeting hypertension.

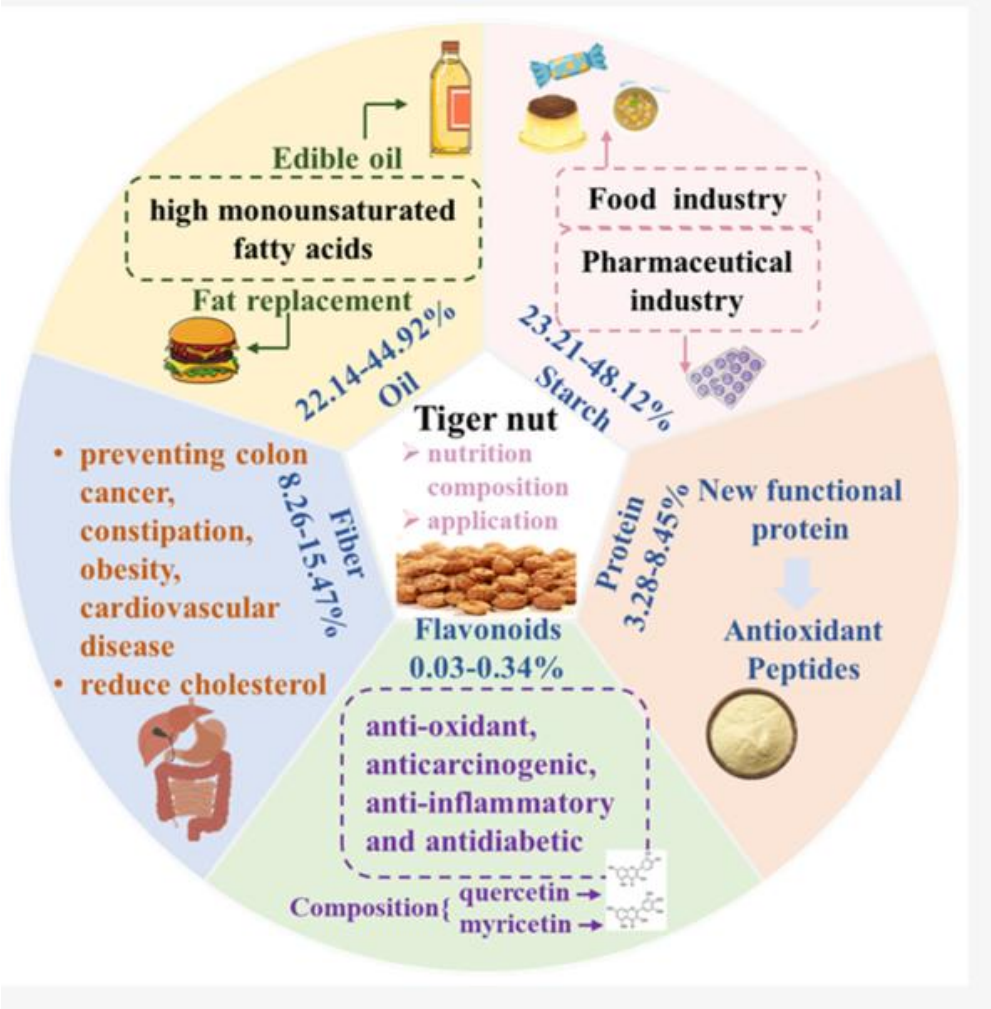


Figure 2.3 : Health Benefits of Tiger Nuts: Nutrition, Uses and Side Effects

2.1.6 Phenolic Compounds and Antioxidant Properties of Tiger Nuts

Tiger nuts are particularly rich in phenolic acids, flavonoids, and tannins, which contribute to their antioxidant activity. These compounds scavenge free radicals, inhibit lipid peroxidation, and protect against oxidative damage to vascular tissues (Adejuyitan, 2021). The inclusion of tiger nut flour in composite biscuits increases the total antioxidant capacity and enhances the functional properties of the snack, making it suitable for hypertensive individuals.

2.1.7 Medicinal Uses of *Justicia carnea* Leaf

Justicia carnea is widely used in African traditional medicine for its haematinic, anti-inflammatory, and organ-protective effects (Okeniyi *et al.,* 2021). The leaf extract has been reported to improve blood circulation, reduce inflammation, and mitigate oxidative damage in various experimental models. Incorporating *Justicia carnea* into food matrices, such as biscuits, provides a natural source of bioactive compounds with cardiovascular benefits.

Vitamin constituents	Quantities
Vitamin B1 (mg/g)	1.37 ± 0.40
Vitamin B2 (mg/g)	2.50 ± 0.50
Vitamin B6 (mg/g)	0.04 ± 0.00
Vitamin B12 (mg/g)	0.05 ± 0.00
Vitamin B9 (mg/g)	0.77 ± 0.03
Vitamin A (µg/g)	2.97 ± 0.05
Vitamin C (mg/g)	36.69 ± 0.83
Vitamin E (mg/g)	0.30 ± 0.00
Each value is expressed as mean ± standard deviation (n=3)	

Figure 2.4 Nutritional Properties of *Justicia carnea*

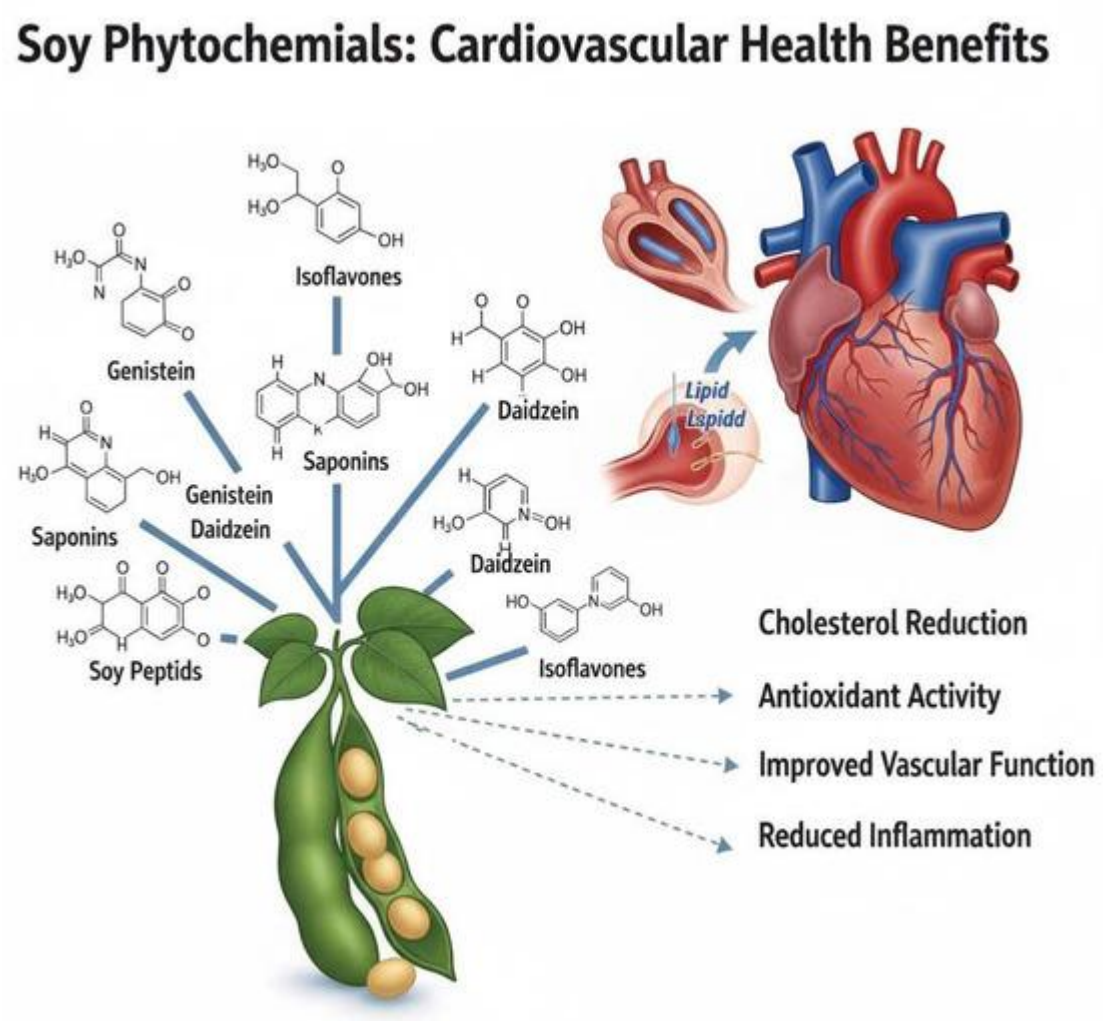
2.1.8 Phytochemical Profile: Flavonoids, Phenols, and Alkaloids of *Justicia carnea*

Phytochemical analysis of *Justicia carnea* leaves shows significant content of flavonoids, phenolic acids, saponins, alkaloids, and tannins, which confer antioxidant and anti-inflammatory activities (Akinyemi *et al.,* 2023). These bioactives enhance endothelial

function, modulate nitric oxide bioavailability, and reduce ROS, making the plant an ideal candidate for functional foods aimed at hypertension management.

2.1.9 Anti-inflammatory and Vasodilatory Effects of Soybean

Soybean (*Glycine max*) contains isoflavones, polyunsaturated fatty acids, bioactive peptides, and fibre, which have documented vasodilatory, antihypertensive, and antioxidant effects (Bolla *et al.,* 2020). Isoflavones like genistein and daidzein enhance nitric oxide production, improve endothelial function, and reduce oxidative stress. Clinical trials have demonstrated that soy protein consumption lowers systolic and diastolic blood pressure, improves lipid profiles, and reduces inflammation (Zhang *et al.,* 2021). Soybean inclusion in functional biscuits not only improves protein content but also contributes to cardiovascular health.



(Zara F.,*et al.,* 2025)

Figure 2.5: Therapeutic Efficacy of Soy-Derived Bioactives

2.2 Oxidative Stress and Hypertension

Hypertension, a major cardiovascular risk factor, is closely linked with oxidative stress, which contributes to vascular dysfunction, inflammation, and end-organ damage. Oxidative stress arises from an imbalance between reactive oxygen species (ROS) production and the antioxidant defense system. Understanding the mechanisms of oxidative stress and its role in

hypertension is essential for developing functional foods that mitigate these pathological processes.

2.2.1 Definition and Classification

Hypertension is defined as a sustained elevation of blood pressure above normal levels, typically ≥130/80 mmHg, according to the American Heart Association (AHA, 2023). Hypertension is classified into:

1. Primary (essential) hypertension – accounts for 90–95% of cases, with no identifiable cause but strongly associated with genetics, diet, lifestyle, and oxidative stress (Williams *et al.,* 2021).
2. Secondary hypertension – results from underlying conditions such as renal disease, endocrine disorders, or medication-induced hypertension (Kearney *et al.,* 2022).

Oxidative stress contributes to both types by impairing endothelial function, enhancing vascular smooth muscle contraction, and promoting inflammation, which collectively raise blood pressure.

2.2.2 Prevalence and Epidemiology of Hypertension

Hypertension is a global public health concern, affecting over 1.3 billion adults worldwide (WHO, 2021). Recent epidemiological data indicate:

- The prevalence in sub-Saharan Africa is rising, estimated at 30–45% of adults (Mensah *et al.,* 2022).

- In Nigeria, approximately 28–32% of adults are hypertensive, with higher prevalence in urban populations due to lifestyle factors, diet, and stress (Okafor *et al.,* 2023).
- Hypertension significantly increases the risk of stroke, myocardial infarction, heart failure, and chronic kidney disease, largely mediated by oxidative stress and endothelial dysfunction.

The high prevalence underscores the need for preventive strategies, including dietary interventions and functional foods rich in antioxidants.

2.2.3 Role of Oxidative Stress in Hypertension

Oxidative stress is a major pathophysiological factor in hypertension. Excess ROS, such as superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radicals (OH•), interact with nitric oxide (NO), reducing its bioavailability and impairing endothelium-dependent vasodilation (Forbes *et al.,* 2021). Mechanisms include:

1. Endothelial Dysfunction: ROS react with NO to form peroxynitrite ($ONOO^-$), decreasing NO-mediated vasodilation and increasing vascular resistance.
2. Activation of Pro-inflammatory Pathways: Oxidative stress activates NF-κB, promoting cytokines (TNF-α, IL-6), adhesion molecules, and vascular inflammation.
3. Renin–Angiotensin–Aldosterone System (RAAS) Dysregulation: ROS upregulate angiotensin II, which stimulates NADPH oxidase, generating more ROS and creating a vicious cycle of oxidative stress and hypertension.
4. Vascular Remodeling: ROS stimulate smooth muscle proliferation and collagen deposition, leading to arterial stiffness and persistent hypertension (Touyz and Montezano, 2020).

Thus, controlling oxidative stress through dietary antioxidants is critical for hypertension prevention and management.

2.2.4 Dietary Approaches to Hypertension Management

Dietary interventions targeting oxidative stress have shown promise in hypertension management. Key strategies include:

1. Increased Intake of Fruits and Vegetables: Rich in polyphenols, vitamins (C, E), and nitrates, which reduce ROS and improve NO bioavailability (Clifford and van der Veer, 2020).
2. Incorporation of Plant-based Functional Foods: Foods containing bioactive compounds such as flavonoids, betalains, isoflavones, and phenolic acids reduce oxidative stress and inflammatory mediators (Ribas *et al.,* 2022).
3. Reduced Sodium and Saturated Fat Intake: Excess sodium and trans fats exacerbate oxidative stress and vascular dysfunction.
4. Dietary Nitrates and Potassium-Rich Foods: Beetroot, bananas, and tiger nuts provide nitrates and potassium, enhancing vasodilation and sodium excretion.
5. Bioactive Peptides from Soy: Soy protein peptides modulate RAAS activity and enhance antioxidant enzyme expression, contributing to blood pressure reduction (Zhang *et al.,* 2021).

Functional foods formulated as snacks, such as biscuits enriched with Cardaba banana, beetroot, tiger nuts, soybean, and *Justicia carnea*, can serve as practical dietary interventions for hypertensive individuals, combining nutrient density with antioxidant and vasoprotective effects.

2.3 Plant-based Ingredients in Biscuit Formulation

The development of functional biscuits has gained attention in recent years as a practical strategy to deliver bioactive compounds, antioxidants, and nutrients in a convenient food form. Plant-based ingredients such as Cardaba bananas, beetroot, tiger nuts, soybean, and *Justicia carnea* leaves can be incorporated into biscuits to enhance nutritional quality, antioxidant capacity, and health-promoting potential, especially for hypertensive individuals.

Functional biscuits are designed not only for sensory acceptability and shelf stability but also for health benefits, integrating ingredients that modulate oxidative stress, improve vascular function, and supply essential nutrients (Ribas *et al.,* 2022; Nwachukwu *et al.,* 2023). The formulation process involves careful selection of flours, sweeteners, fats, and functional powders to optimize bioactive retention, textural properties, and sensory appeal.

2.3.1 Role of Cardaba Banana Flour in Biscuit Formulation

Cardaba banana flour is rich in resistant starch, dietary fibre, and potassium, making it an ideal functional ingredient for biscuits (Sun and Wang, 2023). Its inclusion improves glycaemic control, satiety, and blood pressure regulation, while maintaining a favorable texture when partially substituted for wheat flour (Kumar *et al.,* 2021). The high resistant starch content contributes to prebiotic effects, promoting gut health, which is indirectly linked to cardiovascular function through modulation of the gut–vascular axis (Adedokun and Adeeyo, 2022).

Substitution of wheat flour with 10–30% banana flour has been shown to improve antioxidant capacity, fiber content, and functional properties of baked products without compromising sensory quality.

2.3.2 Incorporation of Beetroot Powder

Beetroot powder, rich in dietary nitrates, betalains, polyphenols, and vitamins, is widely used to fortify biscuits with antioxidant and vasoprotective properties (Clifford and van der Veer, 2020; Domínguez *et al.,* 2021). Betalains are heat-stable pigments that retain antioxidant activity during baking, although their concentration may be partially reduced depending on processing temperature and time.

In biscuit formulation, beetroot contributes color, flavor, and bioactive compounds, enhancing both visual appeal and functional health benefits. Studies suggest that 5–10% substitution of wheat flour with beetroot powder can increase total phenolic content and DPPH radical scavenging activity, thereby improving the antioxidant potential of biscuits (Mirmiran *et al.,* 2020).

2.3.3 Role of Tiger Nut Flour

Tiger nut flour is an excellent source of healthy fats, dietary fibre, arginine, and vitamin E, and can partially replace wheat flour in biscuits (Okpala and Igwe, 2020). Its lipid profile supports cardiovascular health, while phenolic compounds enhance antioxidant activity, contributing to oxidative stress reduction in hypertensive individuals (Kagami *et al.,* 2022).

Tiger nut flour also improves moisture retention and texture of biscuits due to its high fiber and fat content. The arginine content serves as a precursor for nitric oxide synthesis, further reinforcing the vasodilatory and antihypertensive potential of the functional biscuit.

2.3.4 Soybean Flour in Biscuit Formulation

Soy flour contributes high-quality protein, isoflavones, bioactive peptides, and fibre to biscuits (Bolla *et al.,* 2020). Isoflavones exert antioxidant, anti-inflammatory, and vasodilatory effects, which are beneficial in managing hypertension. The inclusion of soy

flour improves protein content, amino acid balance, and functional properties, such as water absorption and dough binding, which are critical for biscuit texture and shelf stability.

Additionally, soy protein peptides may inhibit angiotensin-converting enzyme (ACE), further contributing to blood pressure reduction.

2.3.5 Incorporation of *Justicia carnea* Leaf Powder

Justicia carnea leaves, after drying and grinding, can be used as powdered functional additives in biscuits. These leaves are rich in flavonoids, phenolic acids, and alkaloids, which enhance antioxidant capacity and anti-inflammatory potential (Akinyemi *et al.,* 2023). Careful incorporation ensures bioactive retention during baking, while also contributing unique flavor notes and phytochemical diversity.

The synergistic effect of *Justicia carnea* with other plant-based ingredients may improve vascular health and oxidative stress modulation, making the biscuit a targeted functional snack for hypertensive individuals.

2.3.6 Synergistic Effects and Functional Significance

Combining these plant-based ingredients in biscuits leverages their nutrient and bioactive complementarity. For instance:

- Cardaba banana provides resistant starch and potassium.
- Beetroot contributes nitrates and betalains for NO-mediated vasodilation.
- Tiger nuts offer healthy fats and arginine for endothelial support.
- Soy adds isoflavones and bioactive peptides for ACE inhibition.
- Justicia carnea enhances phenolic content and antioxidant defense.

Together, these ingredients form a multifunctional snack capable of reducing oxidative stress, supporting vascular function, and supplying essential nutrients, while being sensory-acceptable and convenient.

2.3.7 Considerations in Biscuit Formulation

Key considerations for incorporating these plant-based ingredients include:

1. Substitution Level: Excessive substitution may adversely affect texture, color, and sensory acceptance. Optimal substitution ranges between 5–30% depending on the ingredient.
2. Thermal Stability: Heat-sensitive bioactives (e.g., betalains) may degrade during baking; process optimization is critical.
3. Bioavailability: Interactions among phytochemicals, proteins, and carbohydrates can influence bioavailability of antioxidants and minerals.
4. Sensory Acceptability: Color, flavor, and texture must be balanced to ensure consumer acceptance.

Research indicates that careful formulation and ingredient optimization can yield biscuits with enhanced antioxidant activity, antihypertensive potential, and consumer appeal (Ribas *et al.,* 2022; Nwachukwu *et al.,* 2023).

2.4 Significance of Functional Foods with Antioxidant Properties in the Management of High Blood Pressure

Hypertension is a multifactorial disease, with oxidative stress, inflammation, endothelial dysfunction, and dietary factors playing major roles in its pathogenesis (Forbes *et al.,* 2021;

Touyz and Montezano, 2020). Functional foods with antioxidant properties are increasingly recognized for their potential to mitigate these risk factors and support cardiovascular health.

2.4.1 Antioxidant Functional Foods and Vascular Health

Functional foods containing polyphenols, flavonoids, betalains, isoflavones, and vitamins exert beneficial effects on vascular function by:

1. Scavenging Reactive Oxygen Species (ROS): Reducing oxidative stress prevents NO degradation and enhances vasodilation.
2. Improving Endothelial Function: Bioactive compounds improve endothelial nitric oxide synthase (eNOS) activity, leading to increased NO production and reduced vascular resistance.
3. Reducing Inflammation: Antioxidants downregulate pro-inflammatory cytokines such as TNF-α, IL-6, and CRP, which are implicated in vascular remodeling and hypertension (Ribas *et al.,* 2022).

For example, beetroot nitrates and betalains, soy isoflavones, and phenolic compounds in tiger nuts and Cardaba banana synergistically reduce oxidative damage while improving vascular tone and arterial compliance.

2.4.2 Blood Pressure Reduction through Dietary Antioxidants

Multiple clinical and preclinical studies demonstrate that antioxidant-rich foods can contribute to lowering systolic and diastolic blood pressure:

- Beetroot supplementation has been shown to reduce systolic BP by 4–10 mmHg in hypertensive adults, largely due to nitrate-mediated NO production (Clifford and van der Veer, 2020; Mirmiran *et al.,* 2020).
- Soy protein and isoflavones improve endothelial function and reduce ACE activity, resulting in decreased vascular resistance and improved blood pressure (Zhang *et al.,* 2021).
- High-potassium foods, such as Cardaba banana, promote sodium excretion and vasodilation, further contributing to BP regulation (Adedokun and Adeeyo, 2022).

Regular consumption of functional biscuits enriched with these plant-based ingredients can provide a convenient strategy to deliver antioxidant bioactives and improve cardiovascular outcomes.

2.4.3 Mechanistic Insights

The antihypertensive effect of functional foods is mediated through several molecular and cellular mechanisms:

1. Nitric Oxide Pathway: Nitrates and arginine-rich foods increase NO bioavailability, improving vasodilation.
2. ACE Inhibition: Soy-derived peptides inhibit angiotensin-converting enzyme (ACE), reducing angiotensin II levels and vascular constriction.
3. Modulation of Oxidative Stress: Polyphenols and flavonoids activate antioxidant defense enzymes (superoxide dismutase, catalase, glutathione peroxidase), reducing ROS accumulation.

4. Anti-inflammatory Effects: Downregulation of NF-κB signaling decreases cytokine-mediated vascular inflammation.
5. Gut Microbiota Interaction: Resistant starches (from Cardaba banana) act as prebiotics, enhancing SCFA production, which influences blood pressure regulation via immune and hormonal pathways (Sun and Wang, 2023).

These mechanisms highlight the multifaceted role of functional foods in hypertension management, emphasizing both direct vascular effects and indirect systemic modulation.

2.4.4 Practical Implications for Snack Development

Incorporating antioxidant-rich ingredients into convenient snack forms like biscuits allows for:

- Regular intake of bioactives without altering habitual diet significantly.
- Synergistic effects of multiple functional ingredients (beetroot + banana + tiger nuts + soy + *Justicia carnea*).
- Enhanced adherence to dietary interventions, especially for hypertensive individuals seeking practical, portable options.

By integrating these ingredients, functional biscuits serve as a dual-purpose food, providing both nutrition and cardiovascular protection, making them suitable for preventive and complementary dietary strategies.

2.5 Antioxidant Evaluation Methods

Antioxidant evaluation is a critical component in the development of functional foods, as it provides quantitative and qualitative information on the free radical scavenging capacity and

overall antioxidant potential of food products. Various in vitro methods are employed to assess the efficacy of bioactive compounds in neutralizing reactive oxygen species (ROS) and preventing oxidative damage (Gul *et al.,* 2021).

Functional biscuits enriched with Cardaba banana, beetroot, tiger nuts, soybean, and *Justicia carnea* can be evaluated for antioxidant capacity using standardized assays such as DPPH radical scavenging activity and Ferric Reducing Antioxidant Power (FRAP). These methods are widely adopted due to their reliability, simplicity, and reproducibility.

2.5.1 DPPH Radical Scavenging Activity

The DPPH (2,2-diphenyl-1-picrylhydrazyl) assay is a widely used method for evaluating free radical scavenging activity of plant-based extracts and food products (Brand-Williams *et al.,* 2020). DPPH is a stable nitrogen-centered free radical with a deep violet color in solution, which becomes yellow upon reduction by an antioxidant.

Principle

- Antioxidants donate hydrogen atoms or electrons to DPPH radicals, neutralizing them and causing a color change from purple to yellow.
- The degree of discoloration is proportional to the radical scavenging activity of the sample.

Procedure Overview

1. Prepare DPPH solution in methanol or ethanol.
2. Mix with different concentrations of the biscuit extract or plant powder.
3. Incubate in the dark at room temperature for 30 minutes.
4. Measure absorbance at 517 nm using a spectrophotometer.

5. Calculate percentage radical scavenging activity:

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

Significance

- It provides a rapid estimate of antioxidant potency.
- Also suitable for screening multiple samples in functional food development.
- Higher DPPH activity in biscuits indicates greater ability to neutralize free radicals, which correlates with potential antihypertensive and anti-inflammatory effects (Gul *et al.,* 2021; Wang *et al.,* 2022).

2.5.2 Ferric Reducing Antioxidant Power (FRAP)

The FRAP assay measures the reducing power of antioxidants by their ability to reduce ferric (Fe^{3+}) ions to ferrous (Fe^{2+}) ions under acidic conditions (Benzie and Strain, 2020). This assay complements DPPH by assessing electron-donating capacity, another mechanism of antioxidant action.

Principle

- The Fe^{3+}-TPTZ (2,4,6-tripyridyl-s-triazine) complex is reduced to Fe^{2+}-TPTZ, forming an intense blue color.
- The absorbance at 593 nm is directly proportional to the total reducing capacity of the sample.

Procedure Overview

1. Prepare FRAP reagent (acetate buffer, TPTZ solution, $FeCl_3$).
2. Mix with the sample extract and incubate at 37°C for 30 minutes.

3. Measure absorbance at 593 nm.
4. Express results as μmol Fe^{2+} equivalents per gram of sample.

Significance

- FRAP provides an estimate of the total antioxidant power of food matrices.
- Useful in comparing the relative potency of plant-based ingredients in functional biscuits (Domínguez *et al.,* 2021; Gul *et al.,* 2021).
- High FRAP values indicate strong reducing ability, which is beneficial in mitigating oxidative stress linked to hypertension.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 SAMPLE MATERIALS / RAW MATERIALS

1. Beetroot
2. Cardaba Banana
3. Justicia carnea leaf
4. Soybean
5. Tigernut
6. All-purpose flour
7. Butter
8. Cinnamon
9. Oil
10. Egg albumin
11. Date paste
12. Honey
13. Skimmed milk
14. Baking powder

3.1.1 APPARATUS AND EQUIPMENT

1. Century Electric Oven; COV-8320-C
2. Food Dehydrator
3. Kenwood Blender; UK Standard, KC-241B
4. Weighing balance
5. National Spread (NSBP-150K) Refrigerator

6. Knife
7. Scissors
8. Hand gloves
9. Buckets
10. Bowls
11. Trays
12. Towel
13. Sponge
14. Airtight containers
15. Foil paper
16. Sieve
17. Rolling pin
18. Measuring cups
19. Ziplock bags
20. Cookie cutter
21. Black tarpaulin cover
22. Hand mixer

3.1.2 CHEMICALS AND REAGENTS

1. Sodium metabisulfate ($Na_2S_2O_5$)
2. Methanol
3. Ascorbic acid
4. Potassium ferricyanide
5. Phosphate buffer
6. Trichloroacetic acid

3.2 SOURCING AND PREPARATION OF RAW MATERIALS / INGREDIENTS

3.2.1 Beetroot Powder

Fresh beetroot tubers were sourced locally from Hausa Market in Benin City, Edo State, Nigeria. A total of 6 kg of fresh beetroot tubers was purchased. The beetroot samples were thoroughly washed under running water to remove dirt and other extraneous materials. The outer skin was carefully peeled using a sterile knife, after which the beetroot tubers were sliced into thin uniform pieces. The slices were arranged on trays and oven-dried at 50°C until a constant weight was achieved. The dried slices were then milled using a blender to obtain a fine beetroot powder. The powder was sieved to ensure uniform particle size, transferred into an air-tight container, and stored under dry conditions until further use.

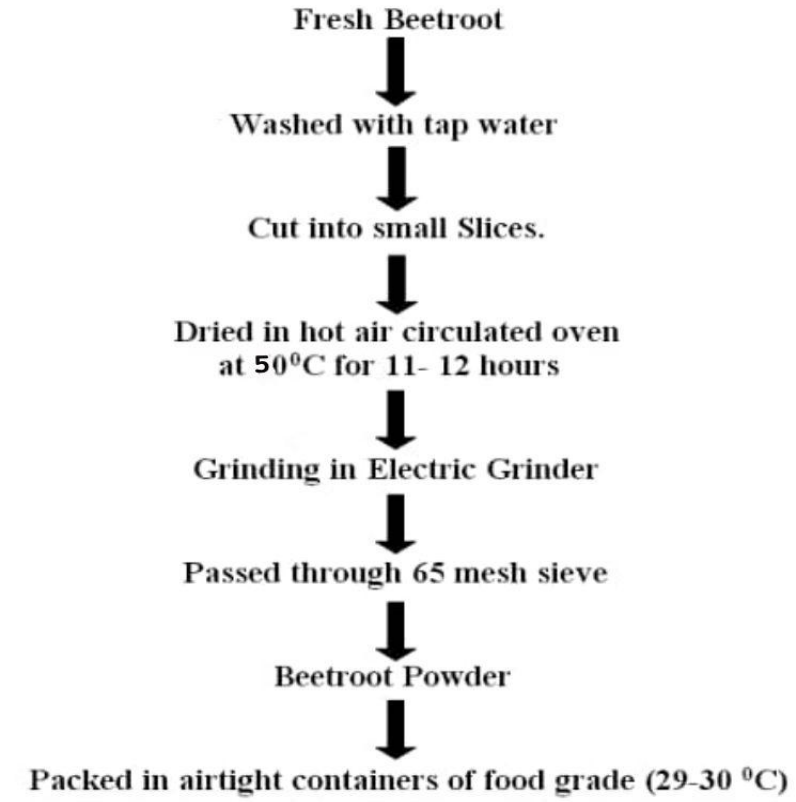


Figure 3.1: Flowchart for the preparation of beetroot powder

3.2.2 Cardaba Banana Flour

Unripe Cardaba bananas used for this study were procured from Efeyi Market located in New Benin, Benin City, Edo State. The bananas were washed, peeled, and sliced into thin, uniform pieces. The slices were then immersed in a solution containing 10 liters of water and 5 grams of sodium metabisulfite, which served as a preservative, and allowed to soak for 30 minutes. After soaking, the slices were drained using a filter to remove excess moisture. They were then placed in an oven and dried at a temperature of 60°C until complete dehydration was achieved. The dried slices were subsequently milled into fine flour and stored in an airtight container until required for use.

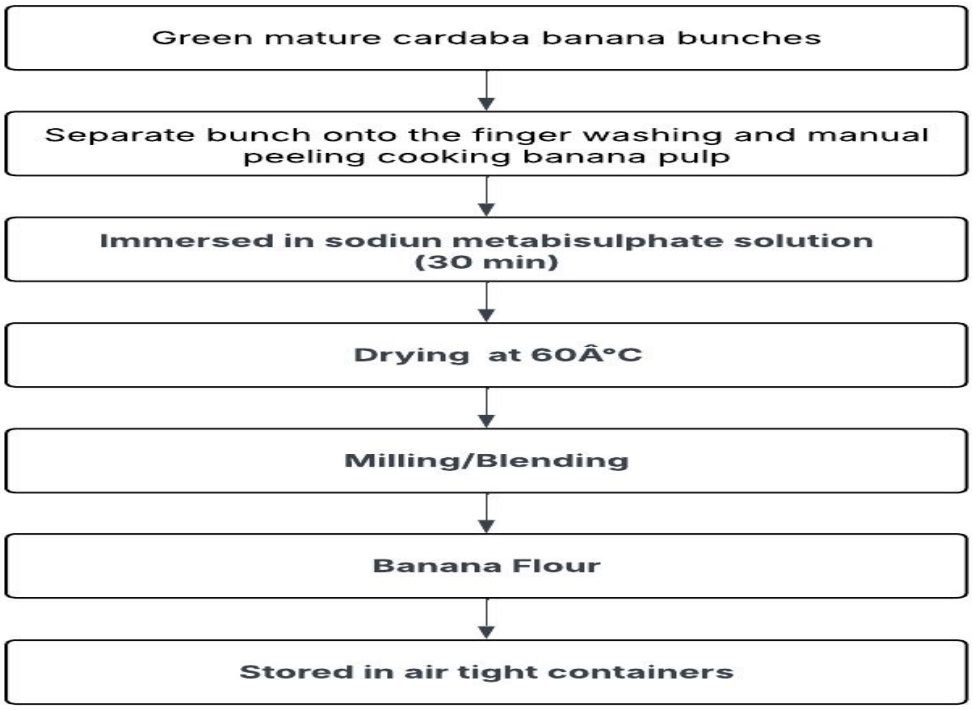


Figure 3.2: Flowchart for the preparation of cardaba banana flour

3.2.3 Justicia carnea Leaf Powder

Fresh *Justicia carnea* leaves were sourced from a garden located within the Supply and Transport (SandT) Barracks, opposite Uselu Market, Benin City, Edo State, Nigeria. The leaves were carefully handpicked and rinsed thoroughly in clean water to remove dirt and other foreign particles. The washed leaves were then placed in a filter to drain excess water and patted dry using a clean napkin. The leaves were subsequently air-dried in the laboratory for approximately two weeks until complete dehydration was achieved. The dried leaves were then milled using a blender to obtain a fine powder, which was sieved to ensure uniform particle size and stored in an air-tight container until further analysis.

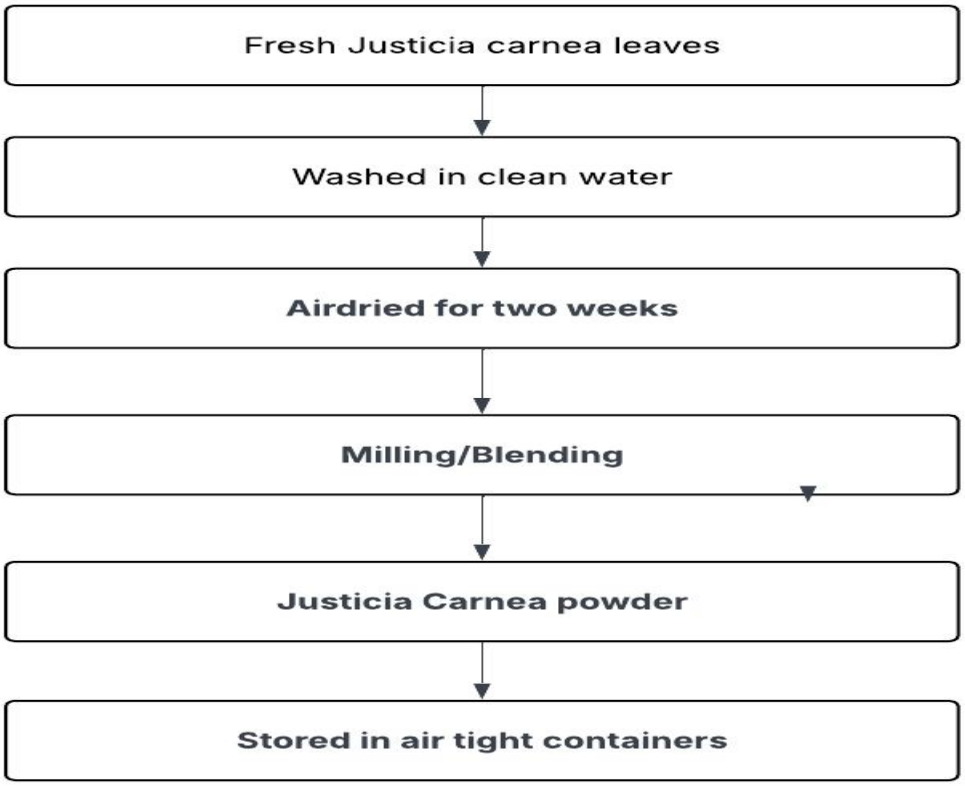


Figure 3.3: Flowchart for preparation of *Justicia carnea* leaf powder

3.2.4 Soybean Flour

The soybeans used in this study were sourced locally from New Benin Market, Benin City, Edo State, Nigeria. The soybeans were manually sorted to remove stones, dirt, and other foreign materials. The cleaned seeds were then soaked in clean water for 24 hours and subsequently boiled for 30 minutes. After boiling, the soybean seeds were thoroughly washed to remove the seed coats (husks). The dehulled seeds were then oven-dried at 80°C until a constant weight was achieved. The dried seeds were milled using a blender to obtain a fine soybean flour, which was sieved to ensure uniform particle size. The resulting flour was stored in an air-tight container until further use.

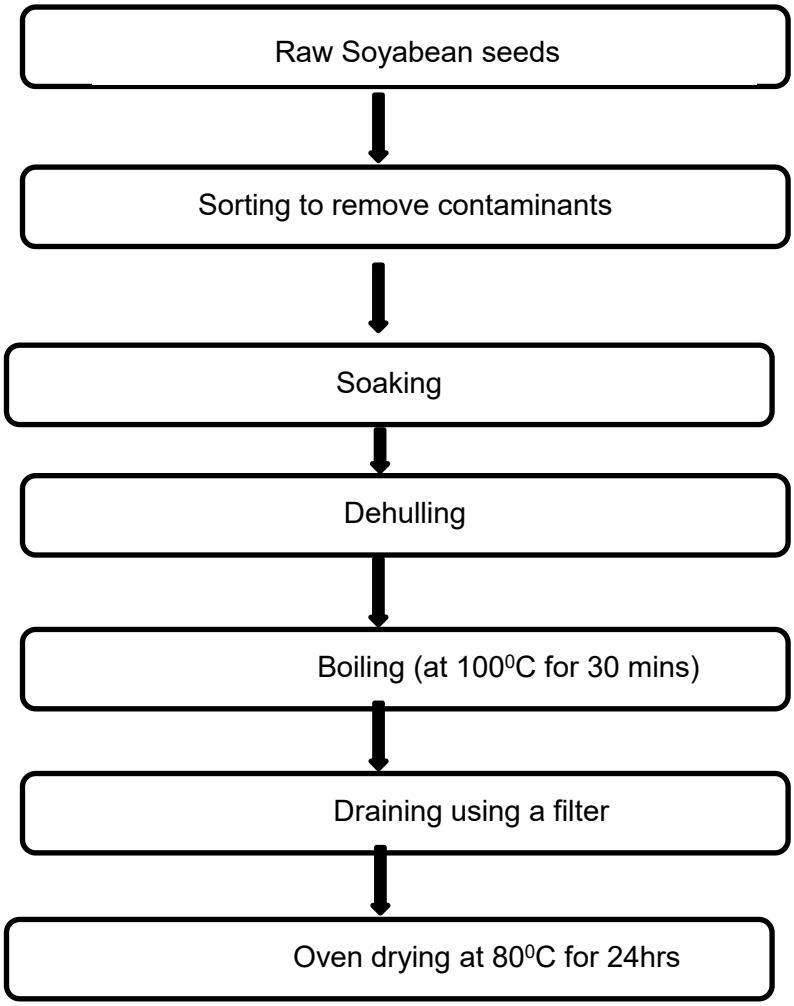


Figure 3.4: Flowchart for preparation of soybean flour

3.2.5 Tigernut Flour

The tigernuts used in this study were obtained from Lagos Street, Benin City, Edo State, Nigeria. The tigernuts were carefully sorted to remove stones, debris, and other impurities. They were then washed thoroughly with clean water and sieved to drain excess water. The cleaned tigernuts were spread on drying trays and oven-dried at 80°C until fully dried, which required approximately 10 hours. The dried tigernuts were then milled using a blender to obtain a fine flour. The resulting flour was sieved to ensure uniform particle size and subsequently stored in an air-tight container until further use.

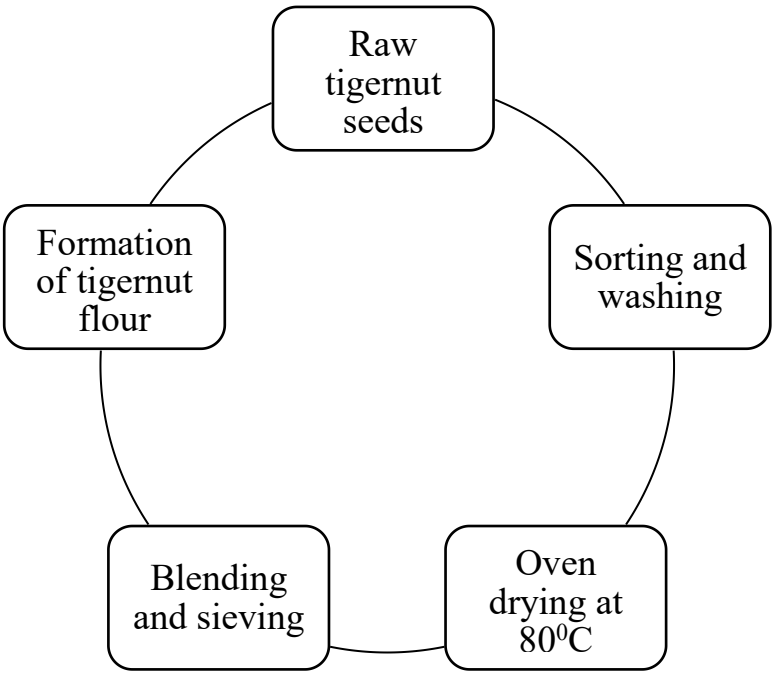


Figure 3.5: Flowchart for the preparation of tigernut flour

3.3 FORMULATION OF SNACK BAR

Snack bar formulations were prepared using composite flours and powders produced from beetroot, cardaba banana, tigernut, soybean, and *Justicia carnea*. The ingredients were measured and combined in varying proportions to create snack bars with different nutritional profiles and textural attributes. Each formulation differed in composition, weight, and size, enabling a comparative assessment of their quality and acceptability.

Ingredients Weighing and Sorting

↓

↓

Mix Dry Ingredients
(Banana flour, beetroot, tiger nut flour, soybean flour, *Justicia carnea* + cinnamon + baking powder)

↓

Add Wet Ingredients
(Egg white + butter + Date paste + skim milk)

↓

Knead into Dough

↓

Roll Out Dough and Cut into Shapes

↓

Preheat the oven before baking
Bake at 160°C for 15–20 mins

↓

Cool to Room Temperature

↓

Packaging (airtight bags or jars)

↓

Storage (Ambient: up to 1 month)

Figure 3.6: Flowchart for preparation of baked snack

Table 3.1: Compositions of the raw materials in the different samples of the probable anti hypertensive snack

Ingredients	**Control**	**Sample A**	**Sample B**	**Sample C**
All-purpose flour	60g	--	--	--
Cardaba Banana	---	60g	77g	90g
Beetroot	37g	37g	25g	27g
Tigernut	52g	52g	52g	40g
Soyabeans	27g	27g	27g	24g
Oil	15g	15g	10g	10g
Justicia carnea	2g	2g	2g	2g
Date	5g	5g	5g	5g
Baking powder	2g	2g	2g	2g
Total	200g	200g	200g	200g

3.4 DETERMINATION OF ANTIOXIDANT CAPACITY

3.4.1 DPPH Radical Scavenging Assay

The free radical scavenging capacity of the plant extracts against 1, 1–diphenyl–2–picrylhydrazyl (DPPH) free radical was determined by a slightly modified method of Brand-Williams *et al.* (1995). Briefly, 0.5 mL of 0.3 mM DPPH solution in methanol was added to

2 mL of various concentrations of the extracts (0.2 - 1.0 mg/mL). The test tubes were shaken and incubated in the dark for 15 min at room temperature, and the absorbance was read at 517 nm. All tests were performed in triplicate. Ascorbic acid (vitamin C) was used as control, with similar concentrations as the test samples. A blank containing 0.5 mL of 0.3 mM DPPH and 2 mL methanol was prepared and treated as the test samples. The radical scavenging activity was calculated as shown in Equation 15:

$$DPPH\ radical\ scavenging\ activity\ (\%) = \frac{A_o - A_1}{A_o} \times 100 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ (15)$$

where A_o was the absorbance of DPPH radical + methanol; A_1 was the absorbance of DPPH radical + sample extract or standard.

3.4.2 Reducing Power Assay

The reducing power (RP) of extract was determined according to the method described by Lai *et al.* (2001). Briefly, 1 mL of different concentrations of extracts (0.1- 1.0 mg/mL) in water was mixed with 2.5 mL of 0.2 M phosphate buffer, pH 6.6 and 2.5 mL of 1 % potassium ferricyanide. The mixture was incubated at 50 ºC for 20 min. Thereafter, 2.5 mL of trichloroacetic acid (10 %) was added to the mixture to stop the reaction. Distilled water (2.5 mL) and 0.5 mL of 0.1 % $FeCl_3$ were then added and the absorbance was read at 700 nm. Higher absorbance values indicated higher reducing power. Ascorbic acid served as the control.

3.4.3 Ferric Reducing Antioxidant Power (FRAP)

A modified method of Benzie and Strain (1996) was used for the FRAP assay. The principle behind this assay is the ability of the sample to reduce ferric tripyridyltriazine (Fe (III)- TPTZ) complex to ferrous tripyridyltriazine (Fe (II) - TPTZ), which at low pH produces an intense blue colour that can be read at 593 nm. Briefly, 1.5 mL of freshly prepared FRAP solution (25 mL of 300 mM acetate buffer pH 3.6, 2.5 mL of 10 mM 2,4,6-tripyridylstriazine (TPTZ)

in 40 mM HCl, and 2.5 mL of 20 mM ferric chloride ($FeCl_3 \cdot 6H_2O$) solution) was mixed with 1 mL of varied concentrations of the extracts (0.2 - 1.0 mg/mL). The reaction mixtures were incubated at 37 ºC for 30 min and the absorbance was read at 593 nm. Ascorbic acid served as the control, while $FeSO_4$ was used for calibration and values expressed as mmol $FeSO_4$ equivalents per gram of sample.

3.5 Statistical Analysis

All data regarding mineral properties were analyzed statistically using SPSS (Version 25.0). The results are expressed as the mean ± standard deviation (SD). Differences among sample means were assessed using one-way Analysis of Variance (ANOVA). Significant differences were identified using Duncan's Multiple Range Test (DMRT) at a 5% probability level ($p < 0.05$).

CHAPTER FOUR

4.0 RESULTS

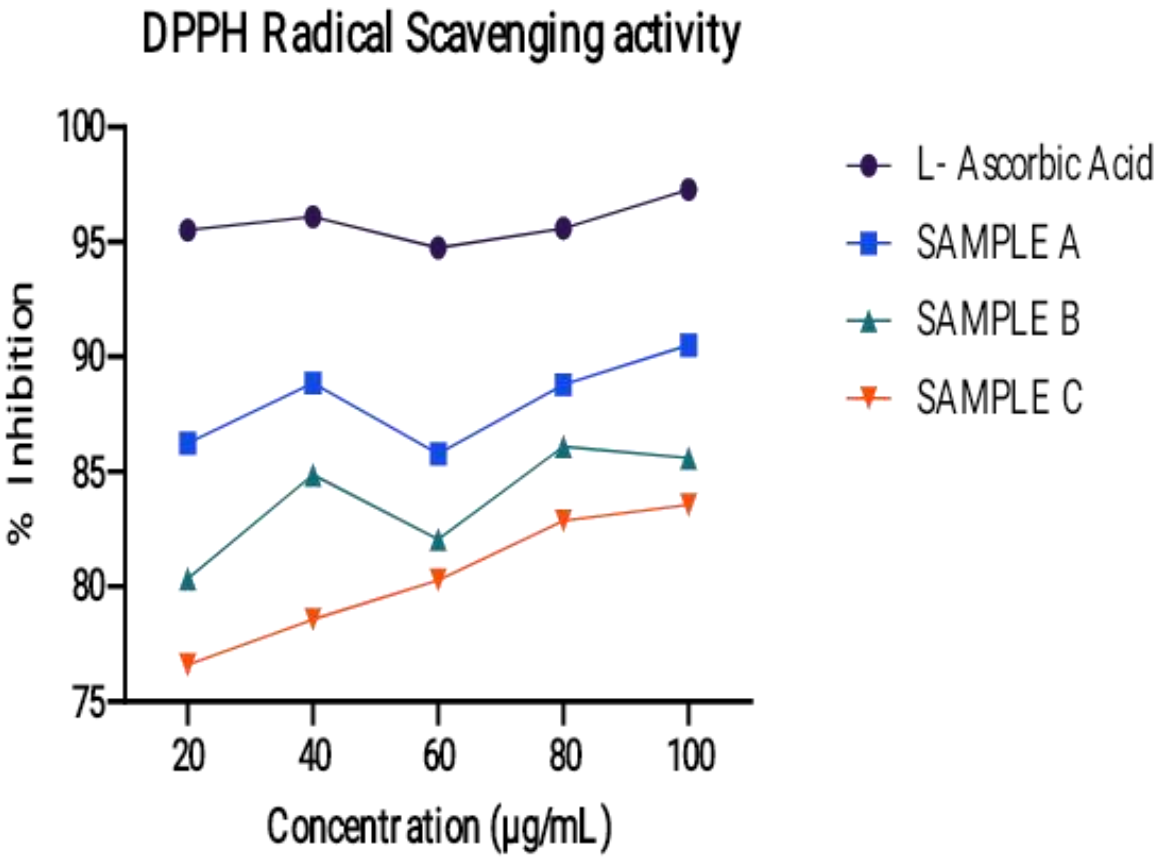


Figure 4.1: Showing DPPH radical scavenging activity

Table 4.1: DPPH Radical Scavenging Activity (IC50) of Sample Formulations

Sample Formulation	IC50 Value (mg/mL)	Goodness of Fit (R2*R*2)	Antioxidant Potency
Control (Vitamin C)	< 0.0001	0.14*	Standard
Sample A	0.0006	0.37*	Very Strong
Sample B	0.031	0.62	Strong
Sample C	0.288	0.94	Moderate

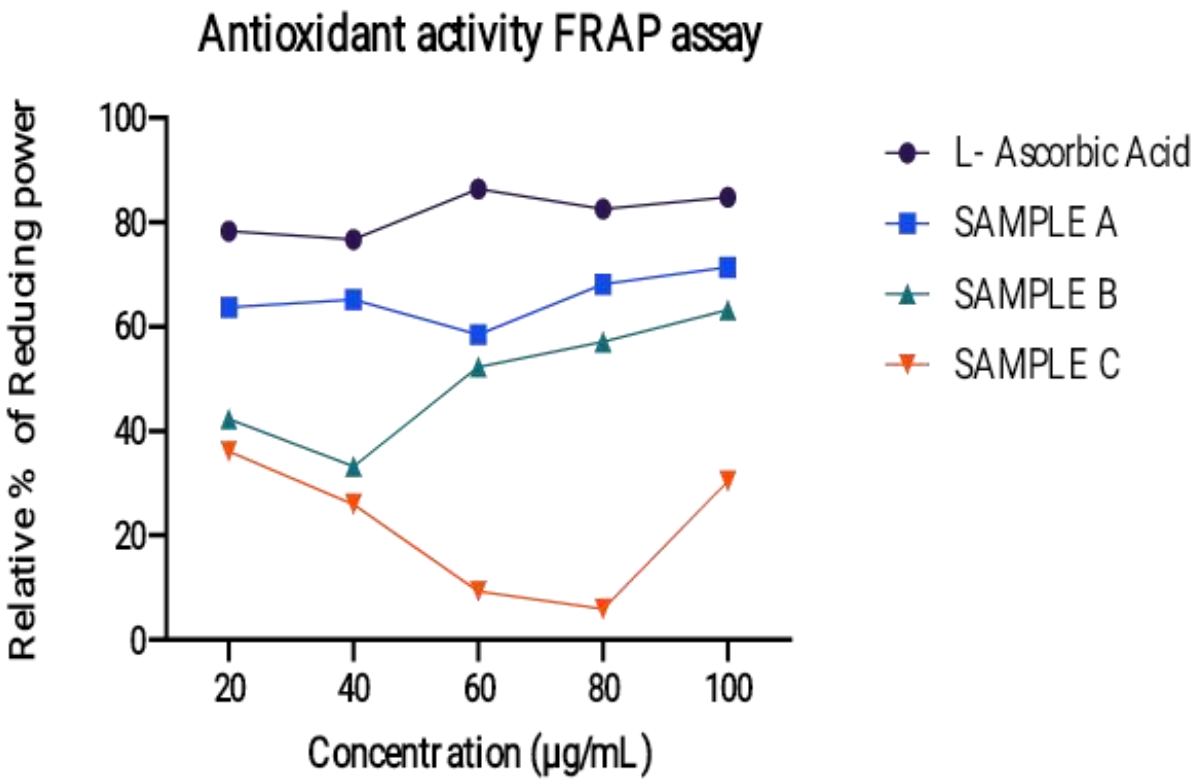


Figure 4.2: Antioxidant activity FRAP assay

CHAPTER FIVE

5.0 DISCUSSION AND CONCLUSION

5.1 Interpretation of DPPH Radical Scavenging Activity

According to the graph and table results in figure 4.1 and table 4.1, it shows the tested samples A,B and C in comparison to L-Ascorbic Acid.

L-Ascorbic Acid (Control): L-Ascorbic Acid, the standard used, consistently demonstrated a higher DPPH radical scavenging activity. Its inhibition rate remained very high, always above 95% across all the tested concentrations ranging from 20 µg/mL to 100 µg/mL . This high efficacy is further supported by an exceptionally low IC50 value of less than 0.0001 mg/mL, categorizing its antioxidant potency as 'Standard' . This simply indicates that L-Ascorbic Acid requires a very small concentration to achieve 50% inhibition of DPPH radicals.

Sample A: This sample exhibited very strong DPPH radical scavenging activity. Its inhibition percentages were also consistently high, ranging from approximately 89% at 20 µg/mL to about 90% at 100 µg/mL . The IC50 value for Sample A was 0.0006 mg/mL, which is close to that of the standard and it is classified as a 'Very Strong' antioxidant potency . This suggests that Sample A is a highly effective scavenger of free radicals.

Sample B: Sample B showed strong DPPH radical scavenging activity. Its inhibition rate started at approximately 85% at 20 µg/mL, peaked around 86% at 40 µg/mL, and remained around 86% at 100 µg/mL . The IC50 value for Sample B was 0.031 mg/mL, which is categorized as 'Strong' antioxidant potency . While it was effective, it required a higher concentration than Sample A to achieve 50% inhibition.

Sample C: The sample showed moderate DPPH radical scavenging activity. Its inhibition percentages increased with concentration, from approximately 77% at 20 µg/mL to about

83% at 100 µg/mL . The IC50 value for Sample C was 0.288 mg/mL, indicating a 'Moderate' antioxidant potency . This suggests that Sample C is the least potent DPPH radical scavenger among the tested samples, requiring a significantly higher concentration to achieve 50% inhibition.

5.2 Interpretation of Antioxidant Activity FRAP Assay

L-Ascorbic Acid (Control): Consistent with also the DPPH results, L-Ascorbic Acid showed the highest reducing power in the FRAP assay. Its relative % of reducing power was constantly above 80% across the entire concentration range from 20 µg/mL to 100 µg/mL . This confirms that it posseses a robust electron-donating capacity.

Sample A: This sample also exhibited strong reducing power, maintaining a relative % of reducing power consistently above 60% across the concentrations from 20 µg/mL to 100 µg/mL This reinforces its 'Very Strong' classification and it also indicates its ability to reduce ferric ions, thereby acting as an antioxidant.

Sample B: For Sample B, it showed a more variable and generally moderate reducing power. It started around 40% at 20 µg/mL, but then dropped significantly to approximately 10% at 60 µg/mL, before recovering to about 60% at 100 µg/mL . This fluctuating pattern suggests that its reducing power might be highly dependent on specific concentrations or that its active components might be less stable or effective at certain concentrations.

Sample C: The sample C displayed the lowest and most inconsistent reducing power. Its relative % of reducing power started around 35-40% at 20 µg/mL, sharply decreased to below 10% at 60-80 µg/mL, and then increased to approximately 35% at 100 µg/mL . This indicates it has a weak and unstable reducing capacity, further supporting its 'Moderate' antioxidant classification.

5.3 Conclusion

L-Ascorbic Acid consistently served as the superior benchmark or simply the standard used for antioxidant activity across both DPPH radical scavenging and FRAP assays. Sample A stood out as the most potent among the tested formulations, demonstrating 'Very Strong' antioxidant properties in both assays. Sample B exhibits 'Strong' antioxidant activity, particularly in DPPH scavenging, but shows more variability and inconsistency in its reducing power in the FRAP assay. Sample C is characterized by 'Moderate' antioxidant activity and the weakest reducing power overall. These findings collectively provided a comprehensive understanding of the antioxidant potential of the different formulations, highlighting Sample A as the most promising in terms of antioxidant efficacy.

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APPENDIX

Table 1: DPPH *In vitro* Antioxidant Assay of the Baked Composite Samples

SAMPLE		DPPH		Aver			DPPH
ID	Abs 1	Abs 2	Abs 3	Abs	A0	%	% Inhib
A1	0.394	0.361	0.351	0.3686666667	1.626	100	77.32677327
A2	0.326	0.283	0.321	0.31	1.626	100	80.93480935
A3	0.295	0.289	0.294	0.2926666667	1.626	100	82.00082001
A4	0.258	0.262	0.287	0.269	1.626	100	83.45633456
A5	0.251	0.212	0.218	0.227	1.626	100	86.03936039
B1	0.314	0.322	0.323	0.3196666667	1.626	100	80.3403034
B2	0.284	0.292	0.309	0.295	1.626	100	81.85731857
B3	0.29	0.291	0.295	0.292	1.626	100	82.04182042
B4	0.227	0.217	0.234	0.226	1.626	100	86.10086101
B5	0.223	0.245	0.235	0.2343333333	1.626	100	85.58835588
C1	0.378	0.381	0.383	0.3806666667	1.626	100	76.58876589
C2	0.332	0.358	0.355	0.3483333	1.626	100	78.57728577

				333			
C3	0.318	0.332	0.312	0.320 6666 667	1.626	100	**80.2788 0279**
C4	0.29	0.28	0.266	0.278 6666 667	1.626	100	**82.8618 2862**
C5	0.281	0.258	0.263	0.267 3333 333	1.626	100	**83.5588 3559**
A1 ASC (Std)	0.07	0.078	0.071	0.073	1.626	100	**95.5104 551**
A2	0.064	0.068	0.059	0.063 6666 6667	1.626	100	**96.0844 6084**
A3	0.086	0.093	0.078	0.085 6666 6667	1.626	100	**94.7314 4731**
A4	0.068	0.075	0.072	0.071 6666 6667	1.626	100	**95.5924 5592**
A5	0.037	0.051	0.045	0.044 3333 3333	1.626	100	**97.2734 7273**

Table 2: FRAP *In vitro* Antioxidant Assay of the Baked Composite Samples

SAMPLE		FRAP		**FRAP**	**×10-2**	
ID	Abs 1	Abs 2	Abs 3	**Abs at 593 nm**		Abs 1
A1	0.626	0.639	0.65	0.6383333333	**63.8**	0.224
A2	0.574	0.589	0.577	0.58	**58**	0.24
A3	0.452	0.49	0.427	0.4563333333	**45.6**	0.256
A4	0.367	0.322	0.34	0.343	**34.3**	0.274
A5	0.263	0.309	0.301	0.291	**29.1**	0.262
B1	0.566	0.576	0.568	0.57	**57**	0.228
B2	0.508	0.538	0.498	0.5146666667	**51.5**	0.254
B3	0.409	0.401	0.41	0.4066666667	**40.7**	0.253
B4	0.354	0.318	0.318	0.33	**33**	0.262
B5	0.295	0.289	0.3	0.2946666667	**29.5**	0.263
C1	0.595	0.437	0.627	0.553	**55.3**	0.237
C2	0.58	0.566	0.594	0.58	**58**	0.26

SAMPLE		FRAP		**FRAP**	**×10-2**	
ID	Abs 1	Abs 2	Abs 3	**Abs at 593 nm**		Abs 1
C3	0.492	0.472	0.466	0.4766666667	**47.7**	0.271
C4	0.401	0.385	0.392	0.3926666667	**39.3**	0.279
C5	0.366	0.363	0.374	0.3676666667	**36.8**	0.263
A1 ASC (Std)	0.182	0.139	0.104	0.1416666667	**14.2**	0.139
A2	0.142	0.147	0.149	0.146	**14.6**	0.12
A3	0.196	0.192	0.129	0.1723333333	**17.2**	0.121
A4	0.152	0.171	0.146	0.1563333333	**15.6**	0.133
A5	0.169	0.124	0.139	0.144	**14.4**	0.109