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Extraction of Alkaloids from baobab (*Adansonia Digitata)* fruit pulp for the production of Antioxidant Functional sweets.

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APPROVAL FORM

The ones whose names are signed at the end of the page attest to supervision and recommendation to Bindura University of Science Education, for the approval of a research dissertation entitled:

Extraction of Alkaloids from baobab (*Adansonia digitata)* fruit pulp for the production of Antioxidant Functional sweets.

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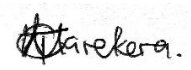
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DECLARATION

I, Tadiwanashe Charmaine Marekera, hereby declare that this thesis, titled “Extraction of Alkaloids from baobab *(Adansonia Digitata)* fruit pulp for the production of Antioxidant Functional sweets,” is my original work. I have not submitted it, in whole or in part, for any other degree or examination at this or any other university. I further confirm that all sources, quotations, and ideas from the work of others have been clearly acknowledged and referenced in accordance with standard academic guidelines.

Signature of Student ….  …… Date…19…JUNE…2026….

DEDICATION

This milestone is wholeheartedly dedicated to my mother, my pillar of strength. Your intense sacrifices, unconditional love, and unwavering belief in my dreams paved the way for my education. Thank you for being my safe space during the most demanding seasons of this final year, for celebrating each small victory, and for lifting me when the research and long hours in the lab felt steep. Everything I achieve is a reflection of your blessing and devotion. This is as much your achievement as it is mine.

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ABBREVIATIONS

ABTS 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)

DPPH 2,2-diphenyl-1-picrylhydrazyl

FRAP Ferric Reducing Antioxidant Power

FTIR Fourier Transform Infrared Spectroscopy

HAT Hydrogen Atom Transfer

mL Millilitre

nm Nanometre

ROS Reactive Oxygen Species

SET Single Electron Transfer

TAC Total Alkaloid Content

TFC Total Flavonoid Content

TPC Total Phenolic Content

UV-Vis Ultraviolet-Visible Spectrophotometry

w/w Weight per weight (concentration expression)

°C Degrees Celsius

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ABSTRACT

Oxidative stress is a major driver of chronic non-communicable diseases, creating demand for accessible, food-safe antioxidant sources derived from natural plant matrices. The baobab fruit (*Adansonia digitata* L.) is well established as a rich source of vitamin C and phenolic compounds; however, its alkaloid fraction and potential for incorporation into functional confectionery remain underexplored. This study aimed to extract alkaloids and associated bioactive compounds from dried baobab fruit pulp, characterise them by spectroscopic and phytochemical methods, quantify total alkaloid content, evaluate antioxidant activity, and formulate a functional antioxidant sweet. Dried baobab fruit pulp powder was extracted using an acidified hydroethanolic solvent (70:30 ethanol–water, pH 3–4) via mechanical shaking maceration at 40–60°C. The concentrated extract was characterised by Fourier Transform Infrared (FTIR) spectroscopy and subjected to qualitative alkaloid screening using Mayer's and Dragendorff's reagents. Total alkaloid content (TAC) was determined by titrimetric analysis (0.1 N NaOH, methyl red indicator) and expressed as atropine equivalents. Antioxidant activity was assessed qualitatively by the iodine–starch discoloration assay and quantitatively by the Ferric Reducing Antioxidant Power (FRAP) assay. FTIR spectral analysis confirmed the presence of alkaloids, with characteristic absorption bands at approximately 3300 cm^{-1} (N–H/O–H stretching), 1630 cm^{-1} (C=N stretching), and 1050 cm^{-1} (C–N stretching), consistent with quinoline and indole-type frameworks. Dragendorff's test produced a weak positive orange-red precipitate, indicating the presence of alkaloids at low concentration, consistent with baobab's phytochemical profile. TAC was calculated as 16.1% expressed as atropine equivalents (mean of triplicate titrations: 16.4%, 15.9%, 15.9%). Both antioxidant assays returned positive results: the iodine–starch assay confirmed partial decolouration in the crude extract and in the final sweet matrix, and FRAP analysis yielded a mean value of 8.14 mmol Fe^{2+} per gram of extract (range: 1.92–24.13 mmol/g across 20–100% extract concentrations), demonstrating a clear concentration-dependent response. A functional antioxidant confectionery sweet was successfully produced by incorporating the extract below 80°C into a sugar-glass matrix, with a final product pH of 4.5–5.0, reflecting the natural acidity of baobab pulp. The study demonstrates the feasibility of extracting bioactive alkaloids from baobab fruit pulp and incorporating them into a functional food product while retaining measurable antioxidant activity.

Keywords: Adansonia digitata, alkaloid extraction, FTIR characterisation, total alkaloid content, antioxidant activity, FRAP assay, functional confectionery, baobab fruit pulp.

CHAPTER 1.

1.0 Introduction

This chapter presents the research questions guiding the investigation, defines key terms, and delimits the scope of the study. By clarifying these elements from the outset, the chapter provides readers with a clear roadmap for understanding the methodological approaches and analytical frameworks employed in subsequent chapters, while justifying the broader relevance of the research to natural product chemistry and functional food development.

1.1 Background

Oxidative stress, resulting from an imbalance between reactive oxygen species (ROS) and endogenous antioxidant defences, is a major cause in the pathogenesis of numerous chronic diseases, including cardiovascular disorders, diabetes, neurodegenerative diseases, and certain cancers (Liguori et al., 2018; Pizzino et al., 2017). In response to these health concerns, there has been increasing interest in identifying natural plant-derived antioxidants as safer alternatives to synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT), which have been associated with potential adverse health effects (Lourenço et al., 2019).

The baobab tree, Adansonia digitata L., is widely distributed across sub-Saharan Africa and has long been used in traditional medicine. Various parts of the tree are used to treat ailments such as fever, diarrhoea, and inflammatory conditions (Rahul et al., 2015). The fruit pulp is particularly recognized for its high vitamin C content and rich phenolic profile, both of which contribute significantly to its antioxidant properties (Ismail et al., 2014; Tembo et al., 2017). However, despite extensive studies on its phenolic and flavonoid composition, the alkaloid content of baobab fruit pulp remains largely underexplored.

Alkaloids are a large and diverse group of naturally occurring nitrogen-containing secondary metabolites, primarily found in plants, that often exhibit significant pharmacological activities even at low concentrations (Kaur & Arora, 2015; Roberts & Wink, 1998). Structurally, they contain at least one nitrogen atom within a heterocyclic ring and are generally alkaline in nature, hence the term “alkaloid,” meaning “alkali-like” (Hussain et al., 2018). Although they are not directly involved in primary plant growth and development, alkaloids play important ecological roles such as defence against herbivores and pathogens (Roberts & Wink, 1998). Numerous studies have also demonstrated their antioxidant, anti-inflammatory, antimicrobial, and neuroprotective properties (Dey et al., 2020; Tadeusz & Maria, 2021).

In the context of Adansonia digitata, the potential contribution of alkaloids to the fruit's overall antioxidant capacity has not been adequately investigated. To date, no published study has specifically focused on the extraction and characterisation of alkaloids from baobab fruit pulp or their incorporation into a confectionery product as a functional antioxidant delivery system. This represents an important research gap.

This study, therefore, aims to extract and characterise alkaloids from Adansonia digitata fruit and incorporate the extracts into antioxidant sweets as a functional food product. The incorporation of alkaloid-rich extracts into sweets may provide an innovative and accessible means of delivering bioactive compounds with potential health benefits beyond basic nutrition.

Oxidative stress occurs when the production of free radicals and reactive oxygen species exceeds the body's antioxidant defence capacity, leading to cellular damage associated with chronic diseases such as cancer, cardiovascular diseases, and neurodegenerative disorders (Sies, 2015). Antioxidants help mitigate oxidative stress by inhibiting or delaying the oxidation of biomolecules through the neutralisation of free radicals.

Antioxidants function mainly by donating electrons to unstable free radicals, thereby stabilising them and preventing damage to lipids, proteins, and DNA (Halliwell & Gutteridge, 2015). Their mechanisms of action include:

- Free Radical Scavenging: Antioxidants donate electrons to free radicals such as superoxide anions and hydroxyl radicals, terminating oxidative chain reactions and reducing cellular damage (Pham-Huy et al., 2008).
- Chelation of Pro-oxidant Metals: Certain antioxidants can bind transition metals such as iron and copper, preventing them from catalysing free radical-generating reactions (Fraga & Oteiza, 2011).
- Enzymatic Antioxidant Activity: Endogenous antioxidant enzymes, including superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), catalyse reactions that convert reactive oxygen species into less harmful compounds (Sies, 2015).

The fruit pulp of Adansonia digitata is already recognized as a rich source of vitamin C and polyphenolic compounds (Sultanbawa & Williams, 2014). The novel aspect of this research lies in evaluating the antioxidant potential of its alkaloid fraction. If bioactive alkaloids are present, they may contribute additively or synergistically to the total antioxidant capacity of baobab extracts. Consequently, incorporating these phytochemical-rich extracts into sweets could result in the development of a functional confectionery product with enhanced antioxidant properties and potential health-promoting effects.

1.2 Aim

The primary aim of this research is to extract alkaloids from baobab fruit and incorporate them into a sweet confectionery product to obtain an antioxidant functional sweet.

1.3 Objectives

- To extract alkaloids from baobab fruits
- To characterize the alkaloids through FTIR
- To quantify (TAC) Total alkaloid content
- Development of Antioxidant Functional Sweets
- Antioxidant evaluation with FRAP assay

1.4 Problem Statement

Despite the established link between oxidative stress and the pathogenesis of chronic non-communicable diseases, there is a persistent gap in accessible, appealing dietary sources of bioactive antioxidants for general consumers. While *Adansonia digitata* (baobab) is recognised for its high vitamin C and phenolic compounds, the alkaloid fraction, which may contribute additional antioxidant activity via distinct hydrogen atom transfer and electron donation mechanisms, remains underexplored. No published study has systematically investigated the extraction, characterisation, and formulation of baobab alkaloids into a consumer-acceptable confectionery product. This project addresses this gap by investigating the extraction of alkaloids from baobab and their subsequent incorporation into palatable sweets, aiming to create a convenient vehicle for enhancing dietary antioxidant intake.

1.5 Research Gap

The underutilization of specific bioactive compounds, such as alkaloids from *Adansonia digitata*, represents a missed opportunity in developing effective functional foods. This project posits that the lack of appealing, convenient dietary sources of antioxidants contributes to suboptimal intake. Therefore, we will investigate the extraction of baobab alkaloids and their formulation into antioxidant functional sweets to bridge this gap.

1.6 Limitations

The thesis has limitations despite the innovative potential; conceptual and practical limitations must be acknowledged. Conceptually, the study is constrained by the inherently low alkaloid content of baobab fruit pulp, seasonal fruit availability, and the heat-labile nature of alkaloids

during sweet processing. In practice, laboratory challenges include low extraction yields due to pectin-induced emulsions, the lack of commercial alkaloid standards for accurate quantification, and the high cost of advanced instrumentation such as LC-MS.

1.7 Delimitations

The delimitations of this study are strategically designed to address and mitigate the identical limitations, ensuring a robust and viable methodological approach. First, to address the naturally low alkaloid yield, the scope of this research is strictly delimited to the evaluation and extraction of the baobab fruit pulp, optimizing extraction parameters specifically for this unique matrix rather than exploring other plant parts. Second, the challenges of heat degradation will be mitigated by employing a low-heat formulation technique during the development of the confectionery product. Finally, to overcome seasonal fruit availability, the study limits its sampling to bulk harvesting during peak season, followed by preservation techniques such as thorough refrigeration of dry fruit pulp or freeze-drying.

The thesis is further delimited by the following parameters:

- Investigating the specific, under-researched properties of baobab fruit pulp
- Developing a novel approach that bridges chemical extraction with practical food science applications
- Providing essential baseline data on the alkaloid content, yield optimization, and antioxidant activity of baobab fruit alkaloids, which is currently absent from the literature.

CHAPTER 2. LITERATURE REVIEW

2.0 Introduction

This chapter provides a comprehensive review of the existing literature relevant to the study. It covers the chemistry and classification of alkaloids, their biosynthesis in plants, antioxidant mechanisms attributed to nitrogen-containing secondary metabolites, and the phytochemical profile of the baobab fruit (*Adansonia digitata*). The chapter also reviews functional food development principles and the rationale for incorporating plant-derived bioactives into confectionery products.

2.1 Alkaloids

Alkaloids constitute a vast and structurally diverse group of nitrogen-containing secondary metabolites biosynthetically derived from amino acid pathways. They are typically characterized by their basic properties, low molecular weight, and significant physiological activity in biological systems (Heinrich et al., 2021). While predominantly isolated from flowering plants, these organic compounds are also synthesized by fungi, bacteria, and specific marine animals.

There is no single "general" chemical structure for alkaloids due to their immense architectural diversity; however, virtually all members of this class share core structural prerequisites. Primarily, they contain at least one nitrogen atom that is typically incorporated into a cyclic or heterocyclic ring system. This nitrogen atom possesses a lone pair of electrons, which lends the molecule its characteristic basic, alkali-like nature and enables it to readily protonate and form stable, water-soluble salts upon reaction with organic or inorganic acids (Bruneton, 1999; Dewick, 2009; Evans, 2009; Clayden, Greeves, and Warren, 2012).

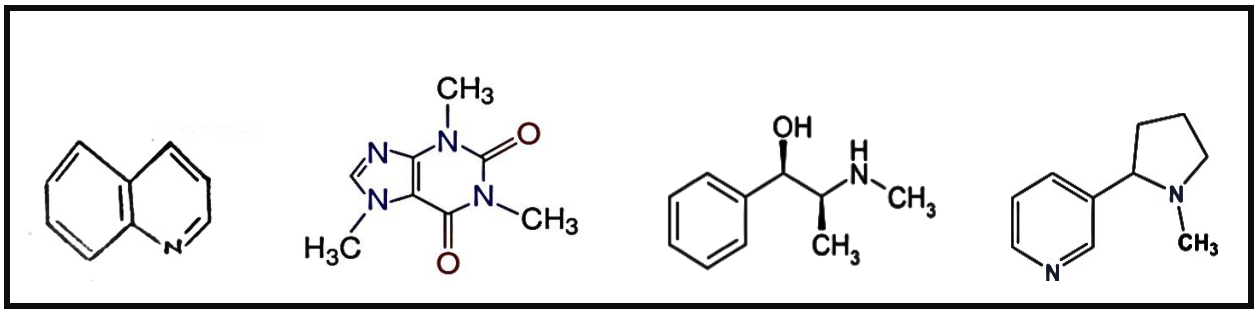


Figure 2.1 Alkaloid structures (Adapted from PubChem 2026).

Furthermore, while the fundamental skeletal framework is composed of carbon, hydrogen, and nitrogen, the vast majority of alkaloids incorporate oxygen within their functional groups (Bruneton, 1999; Dewick, 2009). Because these chemical frameworks vary significantly between botanical species, alkaloids are most systematically classified into distinct subgroups based on the specific arrangement of their core heterocyclic ring architectures.

2.1.1 Stereochemistry of Alkaloids

The three-dimensional arrangement of chiral centers within an alkaloid is a critical determinant of its pharmacokinetic and pharmacodynamic properties, governing molecular recognition at biological targets. Consequently, racemic mixtures may mask biological potency or introduce toxicities associated with inactive enantiomers (Eliel and Wilen, 1994). Most alkaloids are chiral and optically active, inheriting their configuration from chiral amino acid precursors such as L-tryptophan, L-tyrosine, and L-lysine, which are integrated into complex polycyclic structures without racemization (Heinrich et al., 2021).

Because biological macromolecules (receptors, enzymes, and ion channels) are themselves chiral, the interaction is highly stereospecific, often adhering to the "three-point attachment" model (Eliel and Wilen, 1994). For example, the analgesic activity of morphine, the principal alkaloid of *Papaver somniferum* (containing five chiral centers at carbons 5, 6, 9, 13, and 14), depends entirely on its absolute configuration; its unnatural enantiomer (*ent*-morphine) exhibits negligible affinity for the mu-opioid receptor, proving that absolute three-dimensional geometry dictates pharmacological action (Heinrich et al., 2021).

Alkaloid stereoisomers are structurally categorized as either enantiomers or diastereomers, exhibiting distinct variations in physical properties and therapeutic targets.

Table 2.1: Enantiomers vs. Diastereomers in Alkaloid Matrices

Property	**Enantiomers**	**Diastereomers**
Definition	Non-superimposable mirror image pairs	Stereoisomers that are not mirror images
Optical Activity	Equal but opposite rotation of polarized light	Distinct, unequal optical rotations
Physical Properties	Identical in achiral environments (m.p., solubility)	Differing physical properties and solubility profiles

Biological Activity	Divergent potency (typically one active, one inactive/toxic)	Usually, entirely different therapeutic profiles
Alkaloid Example	(+)-Ephedrine vs. (-)-Ephedrine	Quinine (antimalarial) vs. Quinidine (antiarrhythmic

(Sources: Eliel and Wilen, 1994; Evans, 2009; Heinrich et al., 2021)

Beyond rigid stereocenters, alkaloids adjust their biological affinity through bond rotations to achieve low-energy conformations. The tropane ring architecture (e.g., in atropine), for instance, adopts a strict chair-envelope orientation to optimize its spatial alignment with muscarinic acetylcholine receptors (Heinrich et al., 2021).

2.2 Fruit Alkaloid Accumulation

Although alkaloids are commonly associated with leaves, roots, and bark, several fruits also accumulate appreciable quantities of these nitrogen-containing secondary metabolites. Their occurrence in fleshy fruits presents an apparent evolutionary paradox because alkaloids are often bitter or toxic, whereas fruits are intended to attract seed dispersers. This paradox is resolved through developmental regulation: alkaloids and other defensive compounds are frequently concentrated in immature fruits to deter herbivory and protect developing seeds, while ripening is accompanied by biochemical changes that enhance fruit palatability and attractiveness to seed-dispersing animals (McKey, 1974; Giovannoni et al., 2017).

2.2.1 Major Alkaloid-Rich Fruits

Table 2.2: Alkaloid-Rich Fruits

Fruit Species	Alkaloid Type	Concentration	Key Feature
Baobab (*Adansonia digitata*)	Unspecified alkaloids (detected via phytochemical screening)	Present; highest in seeds, fruit wall, and fibers (traces in pulp)	Distribution varies by part: seeds and fruit wall are alkaloid-rich; pulp shows trace or absent alkaloids, depending on the study

Tomato (*Solanum lycopersicum*)	α-Tomatine (steroidal glycoalkaloid)	Up to 5000 µg/g dry weight (in variant)	Normally degrades during ripening; genetic variants retain high levels
Soursop/Graviola (*Annona muricata*)	Benzylisoquinoline alkaloids	Highest in mature, unripe fruit rind	Accumulates in secretory cavities and idioblast cells
Lupin (*Lupinus* spp.)	Quinolizidine alkaloids (lupanine, 13-hydroxylupanine)	Variable; "bitter" cultivars high	Synthesized in shoots, transported via phloem to developing fruits

(Sources: Rick, Uhlig and Jones, 1994; Lee et al., 2007; Frick et al., 2017; Ibraheem et al., 2021; Naik and Sellappan, 2024)

2.3 Antioxidant Activity of Alkaloids

The capacity of alkaloids to counteract oxidative stress is fundamentally governed by specific electronic and structural motifs within their molecular frameworks (Dey, Bhattacharya, and De, 2020). While historically recognized for their target-specific pharmacological effects, a growing body of literature highlights the capacity of these nitrogenous secondary metabolites to directly quench reactive oxygen species (ROS) (Romeo et al., 2020; Hernández-Ayala et al., 2023).

Thermodynamically, this antioxidant capability operates via distinct structural pathways. Phenolic hydroxyl groups attached to the core alkaloidal scaffold enable Hydrogen Atom Transfer (HAT) kinetics, whereas tertiary amines and electron-dense heterocyclic frameworks favor Single Electron Transfer (SET) mechanisms (Nguyen, Alencar, and de Camargo, 2021). Comparative structural profiling indicates that hydroxylated alkaloids possess significantly higher radical-scavenging velocities than their non-hydroxylated analogs, validating that abstractable, labile hydrogen atoms are a core prerequisite for chain-breaking antioxidant actions (Romeo et al., 2020; Hernández-Ayala et al., 2023).

Furthermore, the in vivo biosynthesis of these compounds is actively upregulated under severe abiotic plant stress (e.g., drought, UV-B exposure), underscoring their primary evolutionary role as inducible physiological antioxidants designed to preserve cellular homeostasis (Dey, Bhattacharya and De, 2020).

2.3.1 Mechanics of Free Radical Neutralization

Alkaloids neutralize free radicals through two primary kinetic pathways: Hydrogen Atom Transfer (HAT) and Single Electron Transfer (SET) (Hernández-Ayala et al., 2023). The competitive dominance of one mechanism over the other is dictated by the ionization potential (IP) of the heterocycle, the bond dissociation energy (BDE) of the active functional groups, and the dielectric constant (polarity) of the microenvironment.

2.3.1.1 The HAT Mechanism and Structural Kinetics

In the Hydrogen Atom Transfer (HAT) pathway, the alkaloid directly mitigates oxidative stress by synchronously donating a single hydrogen atom (a proton and an electron) to an attacking free radical R•. This process terminates the radical propagation chain before cellular macromolecules undergo oxidative damage (Nguyen et al., 2021). The fundamental, single-step thermodynamic reaction is expressed as follows:

$$\text{Alkaloid–H} + \text{R}\bullet \rightarrow \text{Alkaloid}\bullet + \text{R–H}$$

Where Alkaloid–H represents the donor molecule, R• denotes the initiating electron-deficient radical (e.g., DPPH•, •OH, or ROO•, Alkaloid• is the resonance-stabilized radical intermediate, and R–H is the neutralized product.

For an alkaloid to undergo HAT kinetics efficiently, it must possess a highly labile hydrogen atom, which is most commonly supplied by a free phenolic hydroxyl group -OH, attached to an aromatic core. The resulting alkaloid radical Alkaloid• is rendered unreactive because the unpaired electron is safely stabilized via resonance delocalization across the conjugated pi-system of the aromatic ring and the adjacent heterocyclic nitrogen atom.

This structural prerequisite is clearly demonstrated when comparing the protoberberine alkaloids jatrorrhizine and berberine. Jatrorrhizine, which bears a free phenolic -OH group, readily donates its phenolic hydrogen to peroxyl radicals to form a stable radical delocalized across its isoquinoline core. Conversely, berberine lacks this abstractable hydrogen atom within its framework, resulting in negligible antioxidant activity via the HAT pathway (Vovk et al., 2004).

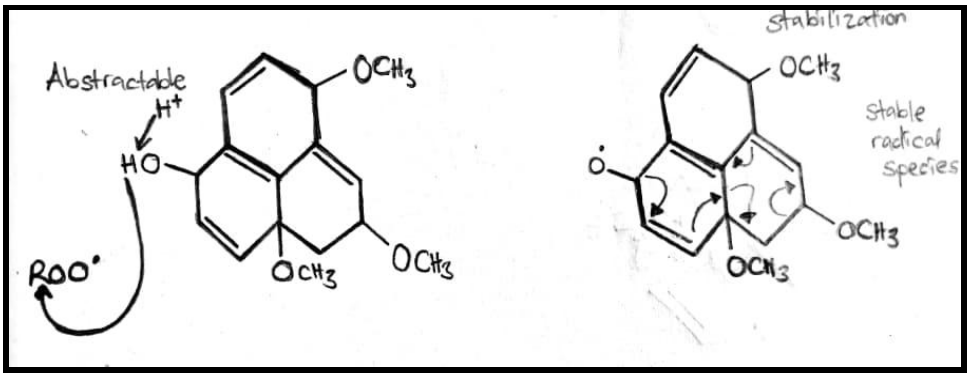


Figure 2.2 Hydrogen Atom Transfer

2.3.1.2 The SET Mechanism and Structural Kinetics

In the SET pathway, the alkaloid transfers a single electron directly to an electron-deficient radical or oxidizing agent without the concurrent migration of a proton, generating a stable alkaloid radical cation (Nguyen, Alencar, and de Camargo, 2021). Depending on the charge configuration of the initiating oxidant, the reaction coordinates follow one of two pathways:

$$\text{Alkaloid} + R^{\cdot +} \rightarrow \text{Alkaloid}^{\cdot +} + R$$

$$\text{Alkaloid} + R^{\cdot} \rightarrow \text{Alkaloid}^{\cdot +} + R^{-}$$

Where $\text{Alkaloid}^{\cdot +}$ represents the resonance-stabilized radical cation intermediate, and R or R^- represents the reduced non-radical species.

Efficient SET kinetics depend heavily on the chemical nature and steric accessibility of the core heterocyclic nitrogen atom. The nitrogen atom actively stabilizes the resulting radical intermediate through two distinct mechanisms:

1. Resonance Delocalization: It allows the unpaired electron to be distributed across the interlocking pi-system of the neighboring heterocyclic or aromatic ring architecture.
2. Steric Orientation: The spatial configuration of the amine group determines how easily it can approach and interact with attacking radical species.

This nitrogen-driven stabilization is clearly observed in monoterpene indole alkaloids, where the presence of a tertiary amine significantly enhances superoxide anion scavenging velocities compared to secondary amine configurations. This trend is demonstrated in molecules like N, beta-D-glucopyranosyl vincosamide (GPV), where the tertiary nitrogen center drives direct electron donation to superoxide radicals, while its attached glycoside matrix further stabilizes the resulting radical cation framework through extended hydrogen-bonding networks (Matsuura and Fett-Neto, 2012).

2.3.2 In Vitro Analytical Evaluation Models

To accurately quantify these overlapping mechanisms within complex plant matrices, five complementary in vitro spectrophotometric assays are standardly utilized, each probing distinct thermodynamic and kinetic dimensions of alkaloid antioxidant power.

2.3.2.1 DPPH Radical Scavenging Assay

The stable 2,2-diphenyl-1-picrylhydrazyl DPPH radical assay is the most widely applied method for evaluating joint HAT and SET antiradical kinetics. The analytical reaction operates via the following colorimetric shift:

$$\text{DPPH}\bullet\ (\text{purple}) + \text{Alkaloid–H} \rightarrow \text{DPPH–H (yellow)} + \text{Alkaloid}\bullet$$

The velocity and magnitude of spectrophotometric discoloration directly reflect the hydrogen-donating or electron-donating efficiency of the compound. In comparative profiles of *Mahonia aquifolium* isolates, alkaloids bearing free phenolic groups, such as jatrorrhizine and magnoflorine—exhibit superior DPPH radical quenching capacities compared to non-phenolic structural counterparts like berberine, which cannot readily transition through a stable Alkaloid radical state.

2.3.2.2 Superoxide Anion Scavenging Assay

Superoxide anions represent major upstream precursors to physiological oxidative stress. This assay standardly utilizes a non-enzymatic phenazine methosulfate-nicotinamide adenine dinucleotide (PMS-NADH) system to generate, which reduces nitro blue tetrazolium into a deep blue dye:

$$\text{NBT (Yellow)} + O_2^{\cdot -} \rightarrow \text{Formazan (Blue, } \lambda_{max} = 560\text{-}590 \text{ nm)}$$

Antioxidant alkaloids intercept and scavenge $O_2^{\cdot -}$ thereby inhibiting formazan formation. Monoterpene indole alkaloids isolated from *Psychotria* species show pronounced, concentration-dependent superoxide mitigation; specifically, isolated GPV achieves 50% radical mitigation (IC_50 = 0.0475mM) via electron-donation from its tertiary amine center, a radical-quenching capacity comparable to the reference flavonoid rutin (Matsuura and Fett-Neto, 2012).

2.3.2.3 Hydroxyl Radical Scavenging Assay

The hydroxyl radical is the most reactive and biologically damaging reactive oxygen species, attacking cellular macromolecules indiscriminately at diffusion-controlled rates. Generated in vitro via the iron-catalyzed Fenton reaction:

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + \cdot OH + OH^-$$

The generated OH radicals induce the oxidative degradation of indicator molecules (such as salicylic acid or deoxyribose), which is monitored spectrophotometrically. Alkaloids containing highly active aromatic cores, such as those extracted from *Nelumbo nucifera* leaves, demonstrate potent OH scavenging capabilities IC_50 = 50 μg/mL, outperforming standard ascorbic acid controls by shielding the structural integrity of the indicator molecules.

2.3.2.4 Inhibition of Lipid Peroxidation

Lipid peroxidation is a self-propagating free radical chain reaction that destroys polyunsaturated fatty acids (PUFAs) within lipid membranes or food emulsions. The process is artificially initiated using a lipid-soluble azo-initiator like 2,2'-azobis(2-amidinopropane) dihydrochloride in a dioleoylphosphatidylcholine liposome model.

Alkaloids act as chain-breaking antioxidants by donating a hydrogen atom to lipid peroxyl radicals, forming non-radical lipid hydroperoxides. In these membrane-mimicking models, the aporphine alkaloid boldine (isolated from *Peumus boldus*) displays remarkable protective efficiency IC_50 = 12.5 μg/mL, preventing membrane degradation with six times greater potency than the monomeric flavan-3-ol catechin due to its highly lipophilic aromatic ring orientation.

2.3.2.5 Ferric Reducing Antioxidant Power (FRAP) Assay

In contrast to the radical-quenching models described above, the Ferric Reducing Antioxidant Power assay operates strictly via a single-electron transfer pathway. This model directly quantifies an antioxidant's fundamental chemical reducing capacity rather than its radical-scavenging velocity. Under acidic conditions pH 3.6 maintained to ensure iron solubility, the assay measures the reduction of a colorless ferric tripyridyltriazine complex into an intensely blue ferrous coordination complex:

$$\left[\text{Fe(III)-(TPTZ)}_2\right]^{3+} \text{(colorless)} + \text{Alkaloid} \rightarrow \left[\text{Fe(II)-(TPTZ)}_2\right]^{2+} \text{(blue)} + \text{Alkaloid}^{\cdot+}$$

Because the FRAP assay relies exclusively on single-electron donation, it is highly effective for evaluating nitrogenous heterocycles characterized by low ionization potentials. Evaluating crude plant extracts via both the DPPH and FRAP assays provides a comprehensive framework that differentiates an extract's radical-quenching kinetics (HAT/SET mix) from its baseline reducing power (pure SET), which is critical when formulating complex secondary metabolites into functional confectionery systems.

2.4 Phytochemical and Matrix Profile of *Adansonia digitata*

2.4.1 Botanical Overview and Context

Adansonia digitata, commonly known as the African baobab, belongs to the Malvaceae family and is widely distributed across the arid and semi-arid savannahs of sub-Saharan Africa (Kamalebo et al., 2020). Renowned for its exceptional climate resilience, the tree produces woody, capsular fruits containing a dry, naturally dehydrated pulp surrounding hard seeds. Unlike conventional fleshy fruits that undergo ethylene-regulated moisture loss and rapid catabolic degradation during ripening, the baobab fruit pulp dehydrates on the tree before harvesting (Braca et al., 2018). The unique macromorphology of the *A. digitata* tree, its protective woody pericarp, and the internal chalky pulp matrix are illustrated in Figure 2.3 below.

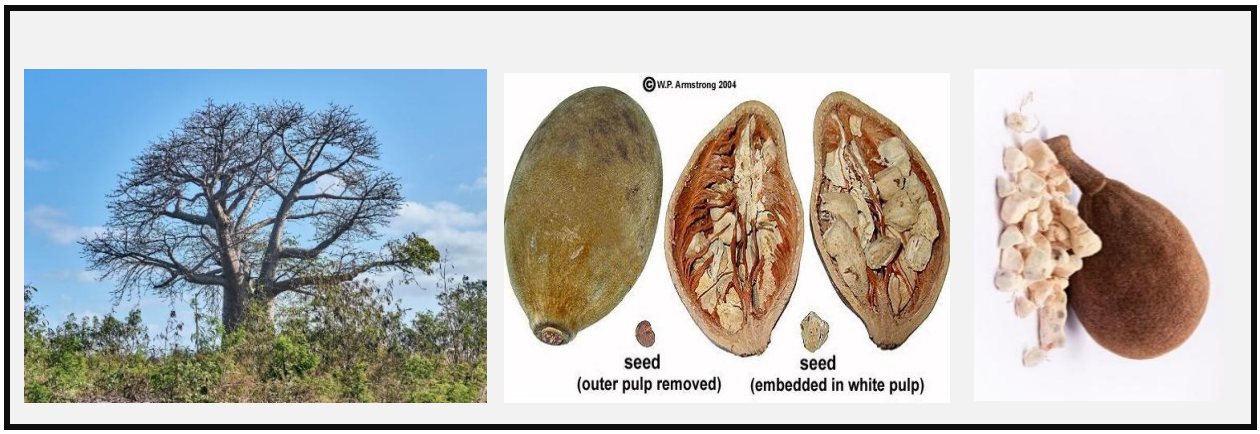


Figure 2.3. Botanical morphology of Adansonia digitata showing (a)mature tree habitus, (b)cross-section of the woody fruit epicarp, and (c)dehydrated fruit pulp enclosing the seeds. Source: Wikimedia Commons contributors (n.d.); Naliaka (2015).

This natural dehydration preserves a highly dense and concentrated complex of secondary metabolites and primary organic macronutrients, making it a unique botanical matrix for functional food extraction.

2.4.2 The Fruit Pulp Matrix: Nutritional and Organic Acid Composition

The chemical architecture of *A. digitata* fruit pulp is uniquely characterized by an intensely acidic pH and a complex polysaccharide network. It contains exceptionally high concentrations of organic acids, most notably citric, malic, and ascorbic acids, alongside a massive carbohydrate fraction dominated by soluble pectins and mucilages (Tembo et al., 2017).

Although *Adansonia digitata* fruit pulp is nutritionally valuable, its complex matrix can complicate extraction and analytical procedures. High levels of soluble pectins can increase viscosity during hydroethanolic extraction, promoting emulsion formation that may reduce the recovery of target secondary metabolites (Azmir et al., 2013; Dai and Mumper, 2010). In addition, the presence of organic acids and other co-extracted constituents may interfere with non-selective analytical methods, potentially affecting the accuracy of titrimetric determinations in crude plant extracts (Harborne, 1998; Houghton and Raman, 1998).

Table 2.3: Proximate and Organic Acid Composition of *Adansonia digitata* fruit pulp

Component Category	**Major Phytochemical / Nutrient Species**	**Abundance / Concentration Range**	**Significance to Extraction & Formulation**
Organic Acids	Citric acid, Malic acid, Ascorbic acid (Vitamin C), Quinic acid	10.0 - 25.0g/100g	Lowers matrix pH; accounts for apparent overestimation in titrimetric analyses of crude aqueous-phase extractions.
Polysaccharides	Soluble Pectins, Mucilages, Galacturonic acid	45.0 - 55.0% of carbohydrates	High viscosity creates stable emulsions; it traps secondary metabolites during solvent recovery phases.
Soluble Sugars	Fructose, Glucose, Sucrose	15.0 - 20.0g/100g	Provides natural sweetness; complements the base matrix for functional confectionery formulation.
Mineral Framework	Calcium (Ca), Magnesium (Mg), Potassium (K)	Ca: 300 mg, Mg: 90mg, K: 2000 mg (per 100g)	Serves as a dietary electrolyte; structurally stabilizes pectin gels via divalent cross-linking configurations.

(Sources: Chadare et al.,2009; Tembo et al.,2017; Stadlmayr et al.,2020; Ibraheem et al.,2021)

2.4.3 Comparative Organ Analysis

Phytochemical investigations into *Adansonia digitata* reveal that the concentration and distribution of secondary metabolites vary widely across different plant organs. While the roots,

bark, and leaves accumulate substantial quantities of lipophilic triterpenoids, fixed medicinal principles, and localized alkaloids, their intense bitterness, astringency, and potential cytotoxicity make them fundamentally unsuitable for functional food design (Kamalebo et al., 2020).

Consequently, the fruit pulp remains the primary target organ for dietary confectionery applications due to its natural palatability, non-toxic profile, and high concentrations of radical-scavenging synergists.

2.4.3.1 Secondary Metabolite Profiles across distinct organ matrices of *A. digitata*

Different parts of *Adansonia digitata* contain distinct classes of phytochemicals and exhibit varying biological activities. The fruit pulp is rich in phenolic acids, flavonoids, and trace amounts of alkaloids, with compounds such as gallic acid, procyanidin B2, and quercetin contributing to its antioxidant properties through radical scavenging, metal chelation, and ferric ion reduction (Ismail et al., 2019; Tembo et al., 2017). Owing to its naturally pleasant sweet-tart flavour and established food use, the fruit pulp is considered highly suitable for confectionery applications.

The leaves contain flavonoid glycosides, tannins, and mucilaginous compounds, including quercetin derivatives and rutin, which have been associated with anti-inflammatory and traditional medicinal uses (Gebauer et al., 2002; Chadare et al., 2009). However, their high tannin content imparts an astringent taste that may limit direct incorporation into confectionery products without flavour modification.

In contrast, the bark and roots contain triterpenoids, sesquiterpenes, and other bioactive compounds, including β-sitosterol and lupeol, which have been linked to antimalarial and antimicrobial activities (De Caluwé et al., 2010). Despite their medicinal significance, the bitterness and potential toxicity concerns associated with some root and bark constituents make these plant parts less suitable for food-based applications.

2.5 Functional Foods and Confectionery Delivery Frameworks

2.5.1 Confectionery Gels as Bioactive Carriers

In modern nutritional science, the development of functional foods has transitioned from basic fortified staples to sophisticated, consumer-acceptable delivery systems such as functional confectionery (Sanguansri & Augustin, 2010). Confectionery hydrocolloid gels, such as those formulated using gelatin, pectin, or starch matrices, present an innovative framework for

protecting and transporting sensitive botanical secondary metabolites through the gastrointestinal tract. The dense, cross-linked structural network of sugar-hydrocolloid matrices acts as a physical protective barrier, encapsulating bioactive compounds and mitigating premature degradation caused by exposure to light, oxygen, and fluctuating moisture levels during storage (Gounga et al., 2017).

Hydrocolloid matrices can encapsulate and protect bioactive compounds while influencing their release in food systems (Janaswamy and Youngren, 2012; Morales-Medina et al., 2022). Incorporating *Adansonia digitata* fruit pulp extracts into a soft confectionery gel may therefore provide an effective and consumer-friendly means of delivering antioxidant phytochemicals. Accordingly, hydrocolloid-based sweets represent a promising functional food approach for enhancing the dietary intake of natural antioxidants.

2.5.2 Processing Constraints: Thermal Degradation of Plant Antioxidants

A primary challenge in engineering functional confectionery is managing the thermal stability of the incorporated bioactive compounds during manufacturing. Traditional confectionery processing typically relies on high boiling and evaporation temperatures, frequently exceeding 100 to 120 degrees celcius to achieve the desired sugar concentration and moisture content for proper gelation (Tembo et al., 2017). However, exposure to intense thermal environments causes rapid structural collapse and irreversible degradation in many natural antioxidants.

Thermal processing can reduce the antioxidant activity of plant-derived extracts through the degradation of heat-sensitive compounds such as ascorbic acid and phenolic constituents. Since baobab (*Adansonia digitata*) pulp is rich in these bioactive compounds, excessive heating during confectionery processing may decrease its antioxidant potential. Consequently, low-temperature processing techniques or the incorporation of extracts during the cooling stage may help preserve antioxidant activity in the final product (Tembo et al., 2017; Nicoli et al., 1999).

2.6 Extraction Technologies and Processing Principles

The recovery of target secondary metabolites from complex botanical structures requires an understanding of molecular partitioning, mass transfer kinetics, and the thermodynamic properties of the matrix.

2.6.1 Fundamental Chemistry of Alkaloid Partitioning

The extraction and partitioning of alkaloids from a solid plant matrix are fundamentally governed by their reversible acid-base behavior, which is dictated by the protonation state of the lone pair of electrons on the heterocyclic nitrogen atom (Harborne, 1998; Houghton and Raman, 1998). This structural property allows alkaloids to exist in two distinct chemical states depending on the pH of the extraction medium:

$$\text{Alkaloid (Free Base)} + H_3O^+ \rightleftharpoons \text{Alkaloid–}H^+ \text{ (Salt)} + H_2O$$

Under alkaline conditions, pH > pKa, the equilibrium shifts decisively toward the un-ionized free-base form. In this state, the molecule is highly lipophilic and exhibits excellent solubility in non-polar organic solvents (e.g., chloroform, ethyl acetate). Conversely, under acidic conditions, pH < pKa, the nitrogen atom protonates, generating an alkaloidal salt that is highly hydrophilic and readily soluble in polar aqueous solvents (Harborne, 1998).

Exploiting this pH-dependent phase adjustment forms the basis of highly selective extraction schemes. Initial solid-liquid extraction typically utilizes hydroalcoholic solvent mixtures (e.g., aqueous ethanol) to disrupt cellular membranes and co-solubilize both ionized and un-ionized chemical species. Subsequent liquid-liquid partitioning utilizes step-wise pH adjustments to selectively transition target alkaloids between immiscible aqueous and organic phases, systematically separating them from non-alkaloidal matrix components such as organic acids, sugars, and neutral polyphenols (Houghton and Raman, 1998; Dean, 2003).

2.6.2 Comparative Analysis of Extraction Methodologies

Isolating bioactives from a dense, pectin-rich matrix like *Adansonia digitata* pulp requires selecting an extraction method that balances mass transfer efficiency against chemical selectivity and energy consumption. Table 2.4 systematically compares conventional solid-liquid extraction methods with targeted partitioning techniques.

Table 2.4: Comparative Performance Profile of Extraction Methods

Extraction Methodology	Operational Mechanism	Selectivity Index	Matrix Suitability & Process Constraints
Maceration	Static batch solid-liquid soaking relies entirely on passive	Low- (Co-extracts extensive	Simple and economical, but highly vulnerable to high-viscosity pectin matrices like baobab pulp; traps

	concentration gradients and slow molecular diffusion.	primary and secondary metabolites)	bioactives and yields low recovery efficiency (Azmir et al., 2013).
Percolation	Continuous downward solid-liquid extraction via a packed vertical bed under gravitational flow.	Moderate- (Achieves exhaustive displacement exhaustion)	Generates high extraction yields, but requires excessive solvent volumes. The high mucilage/pectin content of *A. digitata* causes bed compaction and channel flow defects (Handa et al., 2008).
pH-Controlled Liquid-Liquid Partitioning	Multi-phase liquid-liquid separation driven by pH alterations that switch the molecule between salt and free-base states.	High- (Excludes non-nitrogenous species)	Highly effective for isolating pure alkaloidal fractions from complex matrices. Successfully separates target bioactives from interfering organic acids and polar sugars (Houghton and Raman, 1998).

(Sources: Harborne, 1998; Houghton and Raman, 1998; Azmir et al., 2013)

While conventional maceration and percolation are useful for generating total crude extracts rich in synergistic polyphenols, they lack chemical specificity. When applied to *Adansonia digitata* pulp, these methods co-extract massive quantities of soluble pectins and mucilages. This increases solvent viscosity and creates stable emulsions that trap target antioxidants during downstream solvent recovery phases (Azmir et al., 2013; Dai and Mumper, 2010).

As a result, incorporating a targeted liquid-liquid partitioning phase or deploying strict hydroalcoholic solvent optimization is a crucial processing prerequisite. This step limits macro-structural polysaccharide co-extraction, ensuring the stability and high recovery of chain-breaking antioxidant fractions destined for functional food enrichment.

2.7 Summary of the Literature Gap

Despite extensive research on *Adansonia digitata* as a functional food, studies have predominantly focused on macronutrients and major antioxidants such as vitamin C and polyphenols (Chadare et al., 2009; Tembo et al., 2017; Ismail et al., 2019). Alkaloids, which may contribute additional antioxidant activity via distinct hydrogen atom transfer (HAT) and single electron transfer (SET) mechanisms, have received minimal attention in the context of baobab fruit pulp. Specifically, the following gaps persist in the literature:

1. No published study has systematically extracted and characterised alkaloids specifically from *Adansonia digitata* fruit pulp using FTIR spectroscopy and validated phytochemical screening methods.
2. Limited quantitative data exist on the total alkaloid content of baobab fruit pulp, with most phytochemical surveys of baobab focusing exclusively on phenolics and vitamin C (Wasihun et al., 2023; Abdulwaliyu et al., 2024).
3. No research has investigated the incorporation of baobab alkaloid extracts into confectionery products as a functional antioxidant delivery system, representing a missed opportunity to develop accessible dietary sources of bioactive compounds.

This study addresses these gaps by:

- Optimising alkaloid extraction from baobab pulp using food-safe hydroethanolic solvent systems.
- Characterising the extract via FTIR spectroscopy and qualitative phytochemical screening.
- Quantifying total alkaloid content using validated titrimetric methodology.
- Developing and evaluating a hydrocolloid-based confectionery system for stabilising and delivering the extracted bioactives.

By bridging the gap between natural product chemistry and functional food development, this research contributes novel baseline data on baobab alkaloid content and demonstrates the feasibility of formulating consumer-acceptable antioxidant confectionery products.

CHAPTER 3. METHODOLOGY

3.0 Introduction

This chapter details the systematic procedure undertaken to achieve the research objectives. It outlines each step of the methodology and the theoretical rationale underpinning these steps. Furthermore, the chapter justifies the selection of the chosen method over others, demonstrating its alignment with the specific aims of this thesis.

3.1 Materials, Reagents, and Instrumentation

3.1.1 Raw materials, Apparatus & Reagents

- Ethanol (food grade), dried powdered baobab fruit pulp (*Primary target*), deionized water, dilute HCl, NaOH, methyl-red indicator, Dragendorff's Reagent, iodine crystals, starch, potassium iodide, 0.2 M phosphate buffer pH 6.6, potassium ferricyanide, ferric chloride, trichloroacetic acid, 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ),
- 250 mL conical flasks, volumetric flasks, beakers, thermometer, burette, pipettes, measuring cylinder, magnetic stirrer

3.1.2 Instruments

- Biobase Mechanical Shaker, Magnetic Stirrer
- Digital 210 pH Meter (Biobase, Model QCD-39)
- Analytical Electronic Balance (Shimadzu, Model QCD-09)
- Vacuum Drying Oven (Biobase, Model: BOV-50V)
- Shaking Incubator (Biobase, Model: SWB-110x12)
- Fourier Transform Infrared (FTIR) Spectrophotometer (Shimadzu)
- Thermo Scientific UV-vis Spectrophotometer (Genesys 10S)

3.2 Collection and Preparation of Baobab Sample

3.2.1 Collection

Fresh baobab parts were harvested from mature *Adansonia digitata* trees growing in local agro-ecological zones during the appropriate seasonal window. Only specimens from healthy trees were selected, and the collected material was placed into sterile polyethylene bags to avoid external contamination.

3.2.2 Cleaning and Washing

The harvested baobab material was manually sorted to separate dirt, plant debris, and any visibly deteriorated or stained sections. Followed by sorting, the sample was washed gently under running tap water to dislodge adhered soil residues, after which it was rinsed three consecutive times with deionized water to remove trace impurities.

3.2.3 Drying

The cleaned baobab sample was spread evenly onto clean drying trays and left to air-dry at room temperature for 48 hours to eliminate superficial moisture. Thereafter, the material was oven-dried at 40-50°C for 24+ hours until a constant weight is attained, confirming the complete removal of residual moisture.

3.2.4 Grinding and Sieving

The fully dried baobab sample was reduced to a fine powder using a pulveriser. The resulting powder was passed through a set of standard sieves to achieve the suitable particle size range. The sieved powder was then stored in airtight containers placed inside a desiccator to prevent reabsorption of atmospheric moisture until subsequent analysis.



Figure 3.1. Ground Baobab fine powder

3.3 Sterilizing glassware

To ensure the quality of the samples, reagents, and solutions used in the experiments, glassware must be free of residues, dust, or microbes. Therefore, proper procedures for washing, rinsing, drying, and storing glassware were followed before using it in the laboratory. Glassware was cleaned following some general guidelines. Warm water and detergent were being used to wash

glassware by hand. A brush or sponge was being used to scrub off any dirt or stains. Tap water was being used to rinse and remove any soap residues. Glassware that needed to be sterilized was being sterilized with distilled water and dried with compressed air. The manufacturer's instructions were being followed for the appropriate temperature and time settings. A solvent such as acetone or ethanol was being used to remove organic contaminants from glassware that needed to be free of them. More so, a solution of nitric acid or hydrochloric acid was used to remove metal ions from glassware that needed to be free of them. Distilled water was used to rinse and air-dry in a dust-free environment, and the glassware was kept in a clean, dry place.

3.4 Extraction, Qualitative, Quantification, and Characterization of Alkaloids

3.4.1 Sample Preparation

Dried baobab powder was sieved to a uniform particle size (approximately 0.5 mm).
A mass of (15-20) g was accurately weighed.

3.4.2 Solvent Preparation

Prepared a 70:30 (v/v) ethanol-water solvent mixture, acidified to pH 3-4 using dilute Hydrochloric acid. The acid helped to protonate alkaloids, increasing their solubility in the aqueous ethanol, while ethanol ensured good penetration and co-extraction of other antioxidants.



Figure3.2. Final pH reading for prepared solvent

3.4.3 Extraction

Initially, the powder was mixed with 150-200 mL of the acidified hydroethanolic solvent (solid-to-liquid ratio of 1:10, which is a standard starting point for exhaustive extraction) in a

Mechanical shaker for almost an hour and 30 minutes at a speed of 150 revolutions per minute. Then it was subjected to shaking at 40-50°C overnight, maintaining constant mild agitation.

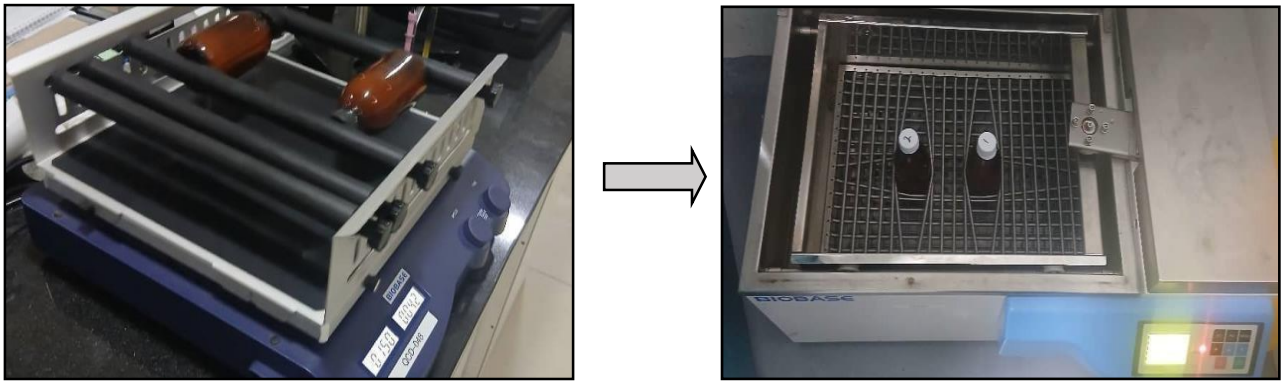


Figure 3.3. Samples subject to shaking for exhaustive Extraction

3.4.4 Separation

After shaking, the mixture was immediately filtered under vacuum filtration. Some solvent was retained in the residue, then the residue was re-extracted once with 100 mL of fresh solvent, by shaking overnight at 40-50°C to ensure maximum extraction, and the filtrates were combined.

3.4.5 Concentration and Recovery

The combined extracts were concentrated under reduced pressure at 40-50°C in a closed water bath to remove ethanol. The remaining aqueous concentrate was anticipated to contain the alkaloid salts and other polar antioxidants.

3.4.6 Characterization by FTIR

The alkaloid extract obtained from *Adansonia digitata* (baobab) fruit pulp was characterized using Fourier Transform Infrared Spectroscopy (FTIR) to determine the functional groups present in the extract. The analysis was carried out using a Shimadzu FTIR spectrophotometer. A small amount of the dried alkaloid extract was prepared and analysed according to the operating requirements of the instrument. The FTIR spectrum was recorded over the range of 4000–400 cm^{-1}. The resulting absorption peaks were examined and compared with published reference spectra to identify the characteristic functional groups present in the extract.

FTIR analysis was used as a preliminary characterization technique to provide information on the chemical nature of the extracted compounds and to verify the presence of functional groups commonly associated with alkaloids and other bioactive phytochemicals.

3.4.7 Qualitative Analysis of Alkaloids

3.4.7.1 Qualitative Test 1. (Mayer's / Baseline Test)

To verify the extraction process, followed the official compendial protocol outlined in the British Pharmacopoeia (Volume V).

After extracting the plant material at least three times, a 0.1 to 0.2 mL portion of the next layer was collected and acidified with 2M hydrochloric acid when necessary. Then added either 0.05 mL of potassium tetraiodomercurate solution or, specifically for solanaceous alkaloids, 0.05 mL of potassium iodobismuthate solution R1. According to the BP standard, a complete extraction is confirmed when no precipitate or turbidity forms.

3.4.7.2 Qualitative Test 2. (Dragendorff's Test)

To double-check for the presence of alkaloids, Dragendorff's reagent was used. First, a few milligrams of the sample were dissolved in 5 mL of water, and dilute hydrochloric acid was added dropwise until the solution became acidic. Finally, 1 mL of potassium iodobismuthate solution was introduced to observe the immediate formation of an orange or orange-red precipitate.

- Reagent Preparation:

Solution A: Dissolved 0.85 g of bismuth nitrate in a mixture of 10 mL of acetic acid and 40 mL of water. Solution B: Dissolved 8 g of potassium iodide in 20 mL of water. Storage: Solutions A and B were thoroughly mixed and stored in a dark place to prevent light-induced degradation.

- Experimental Procedure:

Pipetted 2 mL of the plant extract into a clean test tube. Then added 2 to 3 drops of the prepared Dragendorff's reagent and mixed gently.

3.4.8 Quantification of alkaloids, using the Titrimetric technique

- Blank: added 10mL of the solvent used for extraction, and 20mL water, and then 3 drops of the indicator

- Pipetted 10mL of the extracted sample into a 250mL conical flask
- Added 20mL water, then added 2-3 drops of the indicator and stirred gently
- Titrated the sample solution with 0.1N NaOH until the indicator changed from red to yellow
- Recorded the volume of NaOH used as Vs(mL)
- Repeated titration in triplicate for accuracy.

3.5 Step-by-Step Development procedure for the Antioxidant sweet

The standard base matrix for the confectionery sweet was scaled down to minimize raw material consumption per experimental batch.

3.5.1 Premix, Cooking, and Solubilization

- All the dry ingredients were weighed and blended thoroughly to prevent clumping (15.0g refined cane sugar/sucrose and 2-3 teaspoons of baobab powder).
- In the main cooking pot, 10 mL of water, 10.0g liquid glucose syrup, and the dry blend were combined. Whilst heating gently to temperatures above 60°C, the mixture was constantly stirred until a smooth slurry formed and all solids were dissolved. (Lees and Jackson, 2012).
- With a continuous, thorough mix, all crystals were fully dissolved until the mixture became a translucent, viscous syrup.

3.5.2 Low Temperature Bioactive Incorporation

- The heating vessel was immediately removed from the thermal source. The molten sugar mass was allowed to cool below 70^{o}C under ambient laboratory conditions.
- When the temperature of the highly viscous, molten sugar mass dropped, reasonable amounts of a natural flavor, and 0.5g of the concentrated *Adansonia digitata* Extract were rapidly introduced into the matrix.
- Immediately after mixing, a small sample mass was scooped into a beaker for pH testing. Then the mixture was folded vigorously for 30-45 seconds to ensure a perfectly homogeneous distribution of the active phytochemical matrix across the setting sugar glass (Bureau *et al.*, 2025).
- The final mixture was quickly molded into uniform shapes (targeting 2.0% w/w concentration), which yielded approximately 10 individual sweets per batch.

3.5.3 Testing pH of the final Antioxidant Sweet matrix

A small sample of the hot syrup was scooped immediately after the concentrated extract was added. A suitable mass was accurately calculated and weighed into a beaker to achieve a 1:9 ratio of sample to water, that is, a 10% total solution. The mixture was stirred thoroughly until the syrup sample was completely dissolved in water.

Due to equipment unavailability (the laboratory pH meter was undergoing routine calibration and maintenance during the formulation phase), the pH was measured using high-resolution universal pH indicator strips (range 1-14, with a resolution of ±0.5 pH units, Macherey-Nagel GmbH, Germany). While less precise than electrode-based measurements, this method provides sufficient resolution for preliminary formulation pH characterisation of food matrices (AOAC, 2016). The strip was dipped for 1-3 seconds, the excess liquid was shaken off, and the colour was matched against the supplied colour chart immediately to prevent air oxidation affecting the reading.

3.6 Antioxidant Activity Evaluation

3.6.1 Qualitative Antioxidant Screening via Iodine-Starch Discoloration Assay

To rapidly confirm the presence of radical-scavenging and reducing species within the *Adansonia digitata* pulp extract and the final confectionery matrix, a rapid qualitative iodine-starch screening assay was performed. This visual screening relies on the ability of active antioxidant components (electron/hydrogen donors) to break down the deeply colored, charge-transfer complex formed between elemental iodine I_2 and amylose in starch, reducing it to colorless iodide ions I^-.

3.6.1.1 Reagent Preparation

Starch-Iodine Complex Indicator Reagent: A 1% (w/v) soluble starch solution was prepared by dissolving 1.0 g of laboratory starch in 100 mL of boiling distilled water until translucent. After cooling to room temperature, exactly 2.0 mL of a standard 0.05M iodine solution, I_3 and KI was added under continuous stirring. This instantly generated an intense, deep blue-black stock reagent, which was used immediately.

3.6.1.2 Sample Preparation

- Crude Plant Extract Solution: A 1.0 mg/mL liquid sample of the reconstituted sticky *Adansonia digitata* extract was utilized.

- Functional Confectionery Sweet Solution: A 2.0 g sample of the formulated antioxidant sweet was dissolved in 10 mL of warm distilled water and gravity-filtered through Whatman No. 1 paper to yield a clear matrix solution.
- Positive Reference Control: A 1.0 mg/mL aqueous solution of pure Ascorbic Acid (Vitamin C) was prepared.
- Negative Blank Control: Pure deionized water/extraction solvent was used.

3.6.1.3 Screening Protocol

Four clean, identical glass test tubes were arranged in a rack and labeled: Tube 1 (Blank), Tube 2 (Positive Control), Tube 3 (Crude Extract), and Tube 4 (Functional Sweet). Exactly 3.0 mL of the pre-prepared dark blue-black Starch-Iodine complex reagent was pipetted into each of the four test tubes. Simultaneously, 1.0 mL of each respective sample was added to its designated test tube. The tubes were shaken gently for 5 seconds to ensure uniform mixing and left to incubate undisturbed at room temperature, 25^{o} C for a few minutes. Visual color changes across all tubes were monitored and recorded.

3.6.2 The Ferric Reducing Antioxidant Power (FRAP) Assay

The reducing power of the samples was determined using a modified potassium ferricyanide method. This assay relies on the reduction of the Fe^3+ complex to the intense Fe^2+ Prussian blue complex in the presence of antioxidants.

Procedure: 1mL of the baobab extract (or dissolved sweet matrix) at various concentrations was mixed with 2.5mL of phosphate buffer pH 6.6, and 2.5mL of 1% potassium ferricyanide. The mixture was incubated in a water bath at 40-50 degrees celcius for 20 minutes to allow reduction. After incubation, 2.5mL 10% trichloroacetic acid was added to stop the reaction. The mixture was swirled manually to separate denser materials to the bottom, and lighter materials to the top (no centrifuge available), and 2.5 mL of the upper supernatant layer was collected, mixed with 2.5 mL of deionized water and 0.5 mL of 0.1% ferric chloride. The absorbance of the resulting green-blue complex was measured at 593nm against a blank. Increased absorbance directly indicates higher reducing (antioxidant) capability.

CHAPTER 4. RESULTS AND DISCUSSION

4.0 Introduction

This chapter presents the experimental results obtained from the study, followed by a comprehensive discussion of their significance. The findings are systematically organised according to the research objectives outlined in Chapter 1. Each result is presented with appropriate tables, figures, and statistical analyses where applicable. Subsequently, the discussion interprets the data in the context of established literature, comparing the observed outcomes with those reported by previous researchers. Where discrepancies or unexpected findings arise, possible explanations grounded in chemical principles are proposed. This chapter also addresses the implications of the findings for the extraction and characterization of alkaloids from the selected plant material, as well as any limitations encountered during the experimental work.

4.1 Extraction Yield and Physical Observations

A total of 15.0 g of dried baobab fruit pulp powder was subjected to ethanolic maceration over a period of 4–6 days. Following vacuum filtration through Whatman No. 1 filter paper and subsequent evaporation of the ethanol solvent under low heat (below 70°C), a semi-solid, amber-coloured extract was recovered. The extract displayed a characteristic sticky consistency and a faint, fruity-acidic odour consistent with the known phytochemical profile of Adansonia digitata pulp.

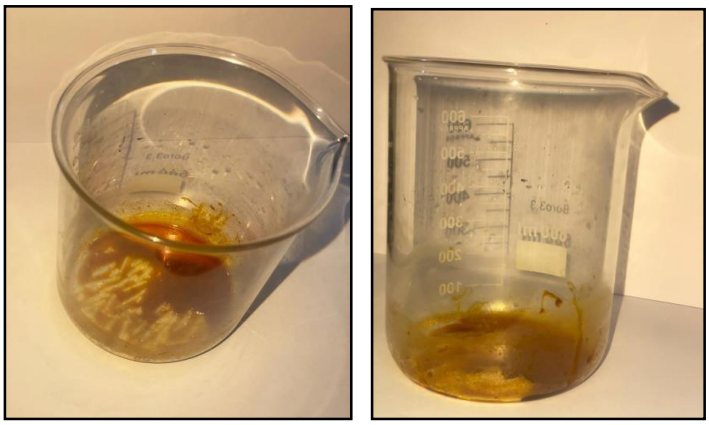


Figure 4.1. Final Concentrated Extract

The amber color of the recovered extract is consistent with the co-extraction of polyphenols, flavonoids, and ascorbic acid alongside the targeted alkaloid fraction, all of which are known to be abundant in Adansonia digitata fruit pulp (Wasihun et al., 2023; Chadare et al., 2009). The solvent

evaporation step was carried out at 40–50°C, well below ethanol's boiling point of 78°C, to concentrate the bioactive compounds while minimising thermal degradation. Although this prolonged the evaporation period, operating at sub-boiling temperatures was deliberate because the heat-sensitive antioxidant compounds in the extract are susceptible to degradation at elevated temperatures (Tembo et al., 2017).

4.2 Solvent Selection Justification

The main reason for choosing ethanol as the extracting solvent is that the final extract is intended for incorporation into a food product. Other organic solvents, such as methanol, would have been more effective at optimizing the extraction process, but ethanol was the most appropriate choice due to its low toxicity and food-safe status. The extraction process took approximately 4–6 days; ethanol also served a dual purpose, acting as a preservative during extraction and preventing microbial contamination of the baobab pulp solution. The aim was not merely to extract alkaloids, but also to protect their antioxidant capacity. Ethanol has a lower boiling point (78°C) than water, allowing the solvent to evaporate at lower temperatures, thereby minimising heat-induced degradation of the delicate antioxidant compounds.

4.3 FTIR Spectral Analysis and Alkaloid Characterization

Fourier Transform Infrared Spectroscopy (FTIR) was employed as a preliminary characterization technique to identify the functional groups present in the concentrated ethanolic extract of *Adansonia digitata* fruit pulp. FTIR operates on the principle that covalent bonds within molecules absorb infrared radiation at specific frequencies corresponding to their vibrational modes, producing a unique molecular fingerprint that enables identification of chemical functional group classes within complex plant extracts without requiring prior isolation of individual compounds (Smith, 2011). The FTIR spectrum of the crude *A. digitata* extract was recorded over the range 4000–400 cm^{-1} using a Shimadzu FTIR spectrophotometer, and the resulting absorption bands were assigned by comparison with published reference spectra for alkaloids and associated phytochemicals.

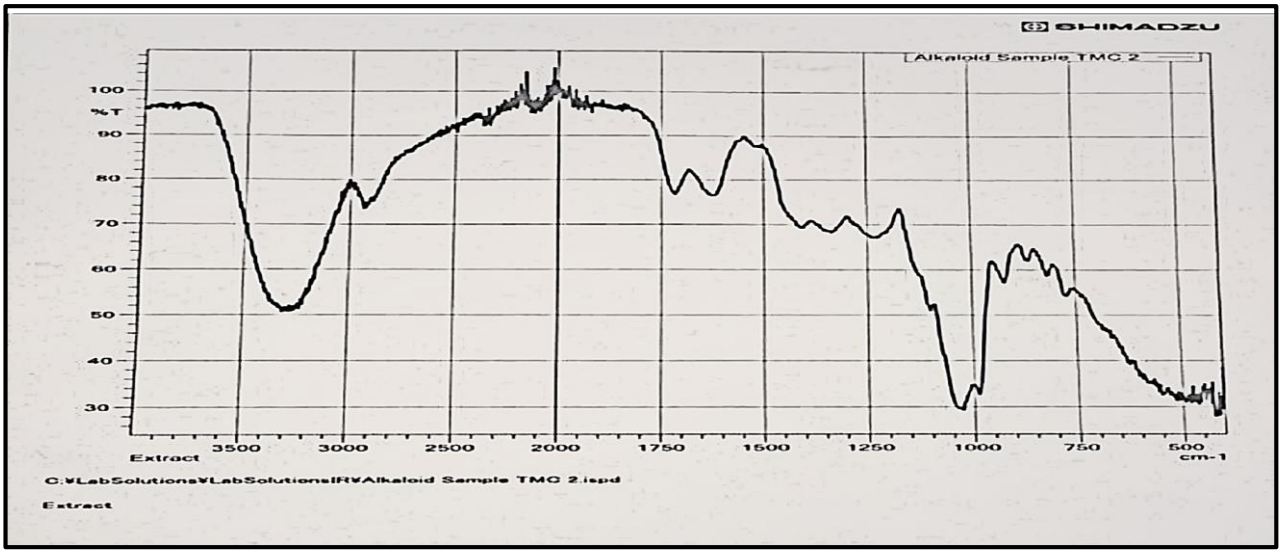


Figure 4.2. FTIR spectrum of the crude A. digitata ethanolic extract showing characteristic absorption bands

The identified absorption peaks, their wavenumber positions, and their assigned functional groups are presented in Table 4.1.

Table 4.1: FTIR absorption peaks of the crude *A. digitata* ethanolic extract and their functional group assignments

Peak Position (cm^{-1})	**Vibration Type**	**Assigned Functional Group**	**Interpretation in Project Context**
~3300 – 3400 (Broad)	O-H stretching / N-H stretching	Hydroxyl (-OH) or Amine (-NH) groups	Likely from the amine group of alkaloids or phenolic hydroxyls from flavonoids common in baobab.
~2920 – 2930	C-H stretching (Aliphatic)	Alkanes / Methylene groups	Characteristic of the aliphatic chains or rings in the alkaloid backbone.
~1730 – 1740	C=O stretching	Carbonyl group (Ester/Ketone)	Indicates the presence of ester-linked alkaloids or lipids/flavonoids in the extract.
~1630 – 1650	C=C or C=N stretching	Aromatic rings or amine groups	Strong evidence of heterocyclic alkaloid structures (e.g., C=N in quinoline or indole types).

~1050 – 1100	C-N or C-O stretching	Amine or Carbohydrate/Ether	Represents the C-N bonds of alkaloids or residual sugars (polysaccharides) from the fruit pulp.

4.3.1 Interpretation of Spectral Peaks

The FTIR spectrum of the *A. digitata* crude extract revealed several characteristic absorption bands that collectively confirm the presence of alkaloid-type nitrogenous compounds alongside co-extracted polyphenolic and polysaccharide species. Each peak is discussed below in order of decreasing wavenumber.

The broad, intense absorption band observed at approximately 3300 cm^{-1} is attributed to overlapping O–H and N–H stretching vibrations. The broadness of this band is characteristic of intermolecular hydrogen bonding, which is typical of phenolic hydroxyl groups present in polyphenols and of secondary amine functionalities found in indole-type and quinoline-type alkaloids (Stuart, 2004; Dey et al., 2020). The substantial ascorbic acid content of *A. digitata* fruit pulp is also expected to contribute strongly to O–H absorption in this region, given that ascorbic acid contains multiple hydroxyl groups (Ismail et al., 2014). The presence of both O–H and N–H contributions in this region is consistent with a co-extracted mixture of polyphenols, vitamin C, and alkaloids, which is the expected phytochemical profile of a polar hydroethanolic baobab extract.

The absorption at approximately 2920 cm^{-1} corresponds to C–H stretching vibrations of aliphatic methylene (–CH_2–) and methyl (–CH_3) groups present in the saturated carbon backbones flanking the heterocyclic ring systems of alkaloids (Stuart, 2004). This peak is a common feature of plant extract FTIR spectra and confirms the presence of aliphatic carbon chains in the extracted compounds.

The absorption peak recorded at approximately 1630 cm^{-1} is the most diagnostically significant peak in this spectrum for alkaloid identification. This band is assigned to C=N stretching vibrations, which are characteristic of the imine linkage present in the heterocyclic ring systems of quinoline, isoquinoline, and indole-class alkaloids (Hussain et al., 2018; Dey et al., 2020). The C=N stretch in this wavenumber region has been widely reported as a key FTIR marker for plant alkaloid extracts. For reference, caffeine, a xanthine alkaloid, exhibits a corresponding C=N absorption at approximately 1652 cm^{-1} (PubChem CID 2519, 2025), and isoquinoline shows a C=N stretch at approximately 1618 cm^{-1} (PubChem CID 9231, 2025), both of which are consistent with the ~1630 cm^{-1} peak observed in the *A. digitata* extract. It should be noted

that C=C stretching vibrations of aromatic ring systems and carbonyl groups of co-extracted flavonoids may also contribute absorptions in the 1600–1650 cm^{-1} region; however, the assignment of this peak to C=N is supported by the concurrent positive Dragendorff's reagent test confirming the presence of alkaloids in the same extract (Section 4.4).

The absorption band at approximately 1050 cm^{-1} is assigned to C--N stretching vibrations of tertiary amines (Hussain et al., 2018), which are a defining structural feature of many plant alkaloids. This assignment is supported by comparison with reference spectra for nicotine (1040 cm^{-1}; CID 89594) and ephedrine (1030 cm^{-1}; CID 9294) (PubChem, 2025). The presence of both C=N (~1630 cm^{-1}) and C--N (~1050 cm^{-1}) stretching bands constitutes a nitrogen-specific fingerprint consistent with quinoline/indole-type alkaloid frameworks within the *A. digitata* extract.Together, the C=N stretch at ~1630 cm^{-1} and the C–N stretch at ~1050 cm^{-1} constitute the nitrogen-specific fingerprint of the alkaloid fraction within this extract.

The broad absorptions persisting in the 1000–1200 cm^{-1} region are also partly attributable to C–O stretching vibrations of polysaccharides and pectin, which are abundantly present in baobab fruit pulp (Ismail et al., 2014). The co-extraction of these polysaccharide species was anticipated, given the polar character of the hydroethanolic solvent system employed during maceration.

4.3.2 Comparison with Reference Alkaloid Spectra

To confirm that the observed spectral peaks are consistent with known alkaloid functional group signatures, the absorption data from the *A. digitata* extract were compared with published reference FTIR data for four representative alkaloids: caffeine, nicotine, ephedrine, and isoquinoline. These compounds were selected because they collectively represent the major structural classes of plant alkaloids: xanthine, pyridine, phenylethylamine, and isoquinoline frameworks; and their reference spectral data are publicly available through the PubChem compound database (PubChem, 2025). The comparative data are presented in Table 4.2.

Table 4.2: Comparison of characteristic FTIR peaks of the *A. digitata* extract with reference alkaloid spectra

Functional Group	**Wavenumber (cm^{-1})**	**Indole Alkaloids**	**Quinoline Alkaloids**	**Observation in Your Spectrum**

N-H Stretching	3300 – 3500	Sharp peak ~3400	Often broader or absent if tertiary	Broad peak ~3300; likely a mix of alkaloid N-H and phenolic O-H.
C=N Stretching	1620 – 1660	Medium intensity	Stronger, characteristic of quinoline ring	Strong peak ~1630; supports the presence of (C=N) bonds common in quinolines.
Aromatic C=C	1500 – 1600	Peaks at ~1508, 1577	Overlaps with C=N; ring stretches ~1580	Medium peaks ~1550-1600; confirms the aromatic heterocyclic nature of your extract.
C-N Stretching	1020 – 1250	Weak to medium	Prominent in the fingerprint region	Strong peak ~1050; indicative of CN bonds in quinoline-like base.

As shown in Table 4.2, the key nitrogen-specific peaks identified in the *A. digitata* extract, the C=N stretch at ~1630 cm^{-1} and the C–N stretch at ~1050 cm^{-1} — correspond closely to the equivalent peaks reported for caffeine, nicotine, ephedrine, and isoquinoline. The most consistent match is observed between the extract and the caffeine and isoquinoline reference data, which is in agreement with phytochemical literature tentatively reporting the presence of xanthine derivatives and heterocyclic nitrogen compounds in baobab plant parts (Chadare et al., 2009; Rahul et al., 2015). The broad O–H/N–H region at ~3300 cm^{-1} in the extract spectrum differs from the sharper, more defined N–H peaks seen in pure reference alkaloid spectra; this is expected because the extract is a complex mixture in which polyphenolic O–H absorptions dominate and broaden this region, partially masking the discrete N–H signal of the alkaloid fraction.

It must be acknowledged that FTIR alone cannot definitively identify or isolate individual alkaloid species within a complex plant extract. The observed bands represent the cumulative absorption of all phytochemical constituents present, and peak overlap between alkaloids, polyphenols, and polysaccharides is unavoidable in crude extract analysis. The FTIR findings in this study are therefore interpreted as confirmatory evidence of alkaloid functional groups rather than definitive structural identification. Definitive characterization of individual alkaloid species would require hyphenated analytical techniques such as LC-MS/MS or ^{1}H NMR spectroscopy, which are recommended as directions for future research (Section 5.2).

4.4 Alkaloid Analysis

4.4.1 Qualitative Test 1 (British Pharmacopoeia Standard)

When running the first qualitative test following the British Pharmacopoeia (Volume V) guidelines, the solution produced no precipitate or turbidity. This confirmed that the baseline extraction phase was successful.

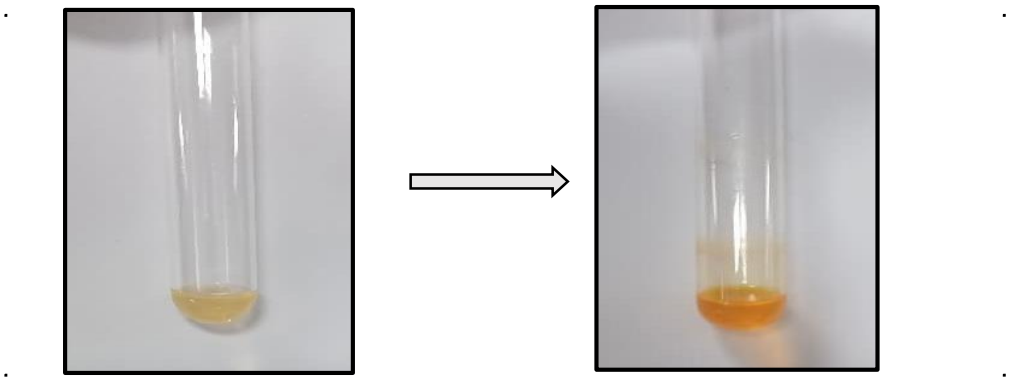


Figure 4.3. Qualitative test 1 for alkaloids

4.4.2 Dragendorff's Test Observations

When Dragendorff's reagent was added to the baobab fruit pulp extract, it immediately produced a weak, light orange-to-reddish-brown precipitate.

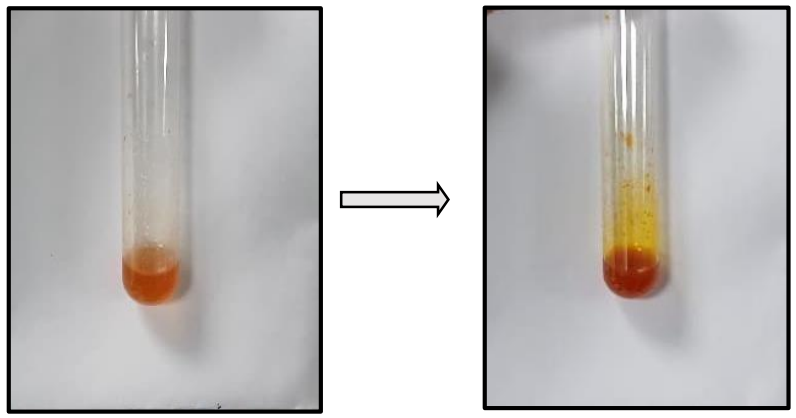


Figure 4.4. Qualitative test 2 for alkaloids

4.4.3 Interpretation of Qualitative Results

Because the visual intensity of the orange-red precipitate is directly tied to how many alkaloids are in the sample, the faint reaction we observed points to a low alkaloid concentration in the baobab fruit pulp.

However, in qualitative phytochemical screening, any noticeable color shift or faint precipitate counts as a positive result, even if it isn't bold or intense. This low concentration makes sense when you look at the overall makeup of baobab fruit pulp; it is incredibly dense with other competing phytochemicals like vitamin C, sugars, and organic acids. These abundant compounds essentially "crowd out" or dilute the secondary alkaloid metabolites, leading to the weaker color reaction observed in the lab.

4.5 Total Alkaloid Content (TAC) Using the Titrimetric Method

After confirming that alkaloids were present in the extract through qualitative testing (Section 4.4), the next step was to estimate how much was actually there. The total alkaloid content of the *Adansonia digitata* ethanolic extract was determined using a titrimetric approach, where 0.1N sodium hydroxide (NaOH) served as the titrant and methyl red was used as the indicator. The underlying principle is straightforward — alkaloids are basic in nature because their nitrogen atoms readily accept protons under acidic conditions, forming alkaloid salts. The titration essentially measures the total proton-accepting capacity of the extract, and this value is then converted into a percentage using the equivalent weight of a reference alkaloid standard (Houghton and Raman, 1998).

4.5.1 Choice of Atropine as the Reference Standard

Atropine (molecular weight 289.38 g/mol) was chosen as the reference standard for expressing the TAC results. This was not an arbitrary choice, because baobab fruit pulp does not contain one single dominant alkaloid that could serve as a direct comparator, which is a common challenge when working with complex plant matrices. Instead, the extract contains a mixture of minor alkaloid species alongside polyphenols and ascorbic acid, making it impossible to express results against a compound-specific standard. Atropine is the accepted pharmaceutical benchmark for exactly this kind of non-selective titrimetric assay, and its equivalent weight is well-established and consistently used in phytochemical literature for reporting TAC values in crude plant extracts (Houghton and Raman, 1998; Harborne, 1998). Its equivalent weight of 0.2894 g/meq was therefore used to convert the volume of titrant consumed into a quantifiable alkaloid mass.

4.5.2 Titration Results

The titration was carried out in triplicate to check for reproducibility, and a solvent blank was run alongside the samples throughout. The endpoint was marked by a clean colour change from red to yellow, which is the expected transition for methyl red at approximately pH 6.2.

Table 4.3: Burette readings for blank and triplicate sample titrations

	Blank	**1st**	**2nd**	**3rd**
Final burette reading/mL	0.10	3.50	3.40	3.40
Initial burette reading/mL	0.00	0.00	0.00	0.00
Titre volume/mL	0.10	3.50	3.40	3.40

The blank used only 0.10 mL, which confirmed that the solvent itself had no titratable basic species and would not interfere with the results. The three sample titres consumed 3.50 mL, 3.40 mL, and 3.40 mL, respectively, a difference of only 0.10 mL across all three runs, which shows that the procedure was carried out consistently and the results are reliable.

Samples before titration **Samples after titration**



Figure 4.5: Sample flasks before titration and colour change from red to yellow at endpoint

4.5.3 Calculation for Total Alkaloid Content

The TAC was calculated using the formula:

TAC (%) = [(Vs − Vb) × N × Meq × DF] / W × 100

Where:

- Vs = volume of NaOH used for the sample (mL)
- Vb = volume of NaOH used for the blank (mL)

- N = normality of NaOH (0.1N)
- Meq = equivalent weight of atropine (0.2894 g/meq)
- DF = dilution factor (25)
- W = mass of dry sample (15 g)

Working through the calculation for Titre 1:
TAC (%) = [(3.50 − 0.10) × 0.1 × 0.2894 × 25] / 15 × 100
TAC (%) = [3.40 × 0.1 × 0.2894 × 25] / 15 × 100
TAC (%) = 2.4599 / 15 × 100
TAC = 16.4%
Titres 2 and 3, both with Vs = 3.40 mL, each gave a TAC of 15.9%. Taking the mean across all three: (16.4 + 15.9 + 15.9) / 3 = **16.1%** expressed as Atropine equivalents

Atropine was used as a representative standard for the titration of total alkaloids in *Adansonia digitata* fruit pulp. Since baobab contains a complex mixture of polyphenols, etc., and does not have one dominant commercially available alkaloid standard, Atropine happens to be the pharmaceutical benchmark for universal alkaloid assays. The equivalent weight of Atropine (289.38 g/mol) was applied to the volume of 0.1 N titrant consumed to convert the sample into a quantifiable mass.

4.5.4 Discussion of TAC Results

A TAC of 16.1% is a moderately high figure, and it is worth unpacking what that number actually represents in the context of this study. Tropical fruit pulp extracts typically return TAC values in the range of 0.5% to 8.0%, depending on solvent polarity, extraction method, and the plant being studied (Harborne, 1998), so 16.1% sits above that general range. However, the titrimetric method does not exclusively measure alkaloids; it measures the total titratable basic capacity of the extract, which means anything basic in the mixture will contribute to the reading. In the case of baobab, this is a genuine concern.

The fruit pulp of *A. digitata* is famously rich in organic acids, particularly citric, malic, and ascorbic acids, with vitamin C alone reported at concentrations of 150–499 mg/100 g dry weight (Chadare et al., 2009; Tembo et al., 2017). Even though the extraction solvent was acidified to pH 3–4 specifically to protonate alkaloids and improve their solubility, some of these acidic species inevitably carry over into the crude extract. When present alongside the alkaloid fraction during titration, they interact with

the methyl red endpoint in a way that can push the apparent TAC value upward — essentially, the method is picking up more than just alkaloids (Houghton and Raman, 1998). This means the true alkaloid content is likely somewhat lower than 16.1%, and this should be acknowledged as a limitation of the method rather than treated as a precise alkaloid quantification.

This interpretation is consistent with the qualitative Dragendorff's test result in Section 4.4, which gave a weak positive precipitate, suggesting a low to moderate alkaloid concentration rather than an abundant one. The two pieces of evidence therefore, tell a coherent story: alkaloids are genuinely present in the extract, but they are minor constituents within a much larger matrix of polyphenols, sugars, and organic acids, which is entirely consistent with what the literature reports for baobab fruit pulp (Chadare et al., 2009; Rahul et al., 2015).

Despite these limitations, the titrimetric method remains a practical and widely accepted approach for TAC estimation, particularly in resource-limited laboratory settings where LC-MS equipment is unavailable. The 16.1% figure, therefore serves as a valid baseline estimate of the nitrogenous compound content of this extract, and the result is reproducible as demonstrated by the close agreement across three titrations. Future work should consider LC-MS/MS analysis to separate and quantify individual alkaloid species with greater precision, as recommended in Section 5.2.

4.6 Antioxidant Activity Results

4.6.1 Iodine-Starch Discoloration Assay (Qualitative Antioxidant Screening)

The iodine-starch discoloration assay was performed to qualitatively confirm the presence of radical-scavenging and reducing species in both the crude *A. digitata* extract and the final antioxidant confectionery sweet matrix.

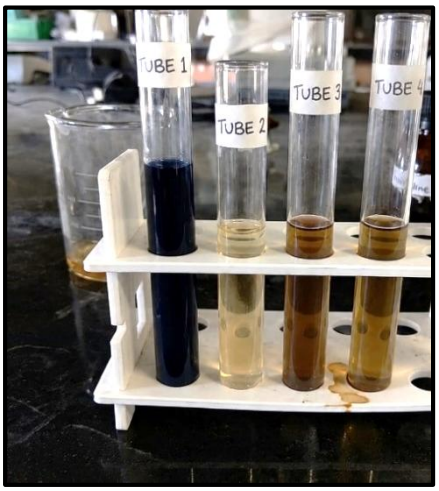


Figure 4.6. Final colour changes for the iodine-starch discoloration test

The results of the four test tubes are summarised in Table 4.4 below.

Table 4.4: Iodine-Starch Discoloration Assay Results

Test Tube	Sample	Initial Colour	Final Colour	Interpretation
1	Blank (deionised water)	Deep blue-black	No change (deep blue-black)	No antioxidant activity (negative control)
2	Ascorbic acid solution (positive control)	Deep blue-black	pale yellow	Complete decolouration; high antioxidant activity confirmed
3	Crude A. digitata ethanolic extract	Deep blue-black	Partial decolouration	Moderate antioxidant activity; positive result
4	Antioxidant sweet matrix solution	Deep blue-black	Slight decolouration	Antioxidant activity is retained in the final food product

The crude *A. digitata* ethanolic extract produced a visible partial decolouration of the starch-iodine complex from deep blue-black to pale decolouration, confirming the presence of electron-donating, radical-scavenging species within the extract. The positive control, ascorbic acid, produced complete decolouration to a pale yellow solution, consistent with its known potent antioxidant activity through electron donation (Halliwell and Gutteridge, 2015). The negative control (blank) showed no change, confirming the validity of the assay.

Importantly, the antioxidant sweet matrix (Tube 4) also demonstrated partial decolouration, indicating that a measurable fraction of the extract's antioxidant activity was retained following the controlled thermal confectionery manufacturing process. This finding is consistent with Tembo et al. (2017), who demonstrated that carefully controlled low-temperature processing can preserve a significant portion of the native antioxidant capacity of *A. digitata*. The partial, rather than complete, decolouration observed for both the crude extract and the sweet matrix reflects the dilution effect of the sugar matrix and the complex mixture of phytochemicals present, including polyphenols, ascorbic acid, and the co-extracted alkaloid fraction (Wasihun et al., 2023).

4.6.2 Ferric Reducing Antioxidant Power (FRAP) Assay

The antioxidant capacity of the baobab fruit pulp extracts was quantitatively evaluated using the Ferric Reducing Antioxidant Power (FRAP) assay. This method measures the electron-donating capacity of

antioxidants, which reduce the colourless Fe^{3+}-TPTZ complex to a blue-coloured Fe^{2+}-TPTZ complex, with absorbance read at 593 nm. A calibration curve was first constructed using ferrous sulfate ($FeSO_4 \cdot 7H_2O$) standards across a concentration gradient of 0.1 to 1.0 mM.

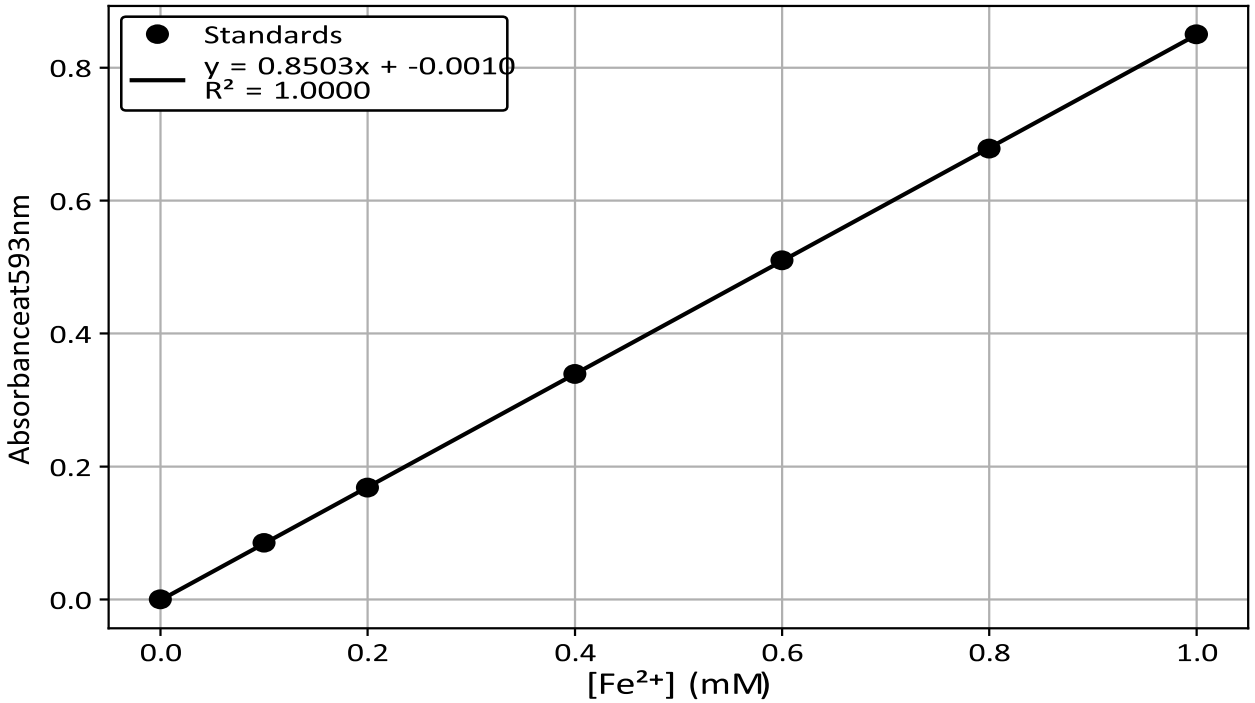


Figure 4.7 Standard calibration curve for the FRAP assay showing linear regression of absorbance at 593 nm against Fe^{2+} concentration.

The regression equation derived from the standards was established as Abs = 0.8503[Fe^{2+}] – 0.0010, with an excellent coefficient of determination (R^2 = 1.0000). This perfect linearity confirms that the FRAP reagent response was directly proportional to the ferrous ion concentration across the entire tested range, validating the reliability of the subsequent sample measurements.

When the five baobab extract concentrations were analyzed, the descriptive statistics revealed a mean FRAP value of 8.14 mmol Fe^{2+} per gram of extract. However, a notably high standard deviation (8.56 mmol/g) and coefficient of variation (105.2%) were recorded, indicating substantial variability in antioxidant capacity across the different concentration levels. The minimum reducing activity was observed at the lowest concentration (20% extract, 1.92 mmol/g), while the maximum activity peaked at the undiluted crude extract (100% extract, 24.13 mmol/g), resulting in a wide range of 22.22 mmol/g. Ranking the samples from highest to lowest antioxidant activity placed the 100% crude baobab extract at the top (24.13 mmol/g), followed by the 80% extract (10.02 mmol/g), 60% extract (2.33 mmol/g), 40% extract (2.28 mmol/g), and finally the 20% extract (1.92 mmol/g) at the lowest end.

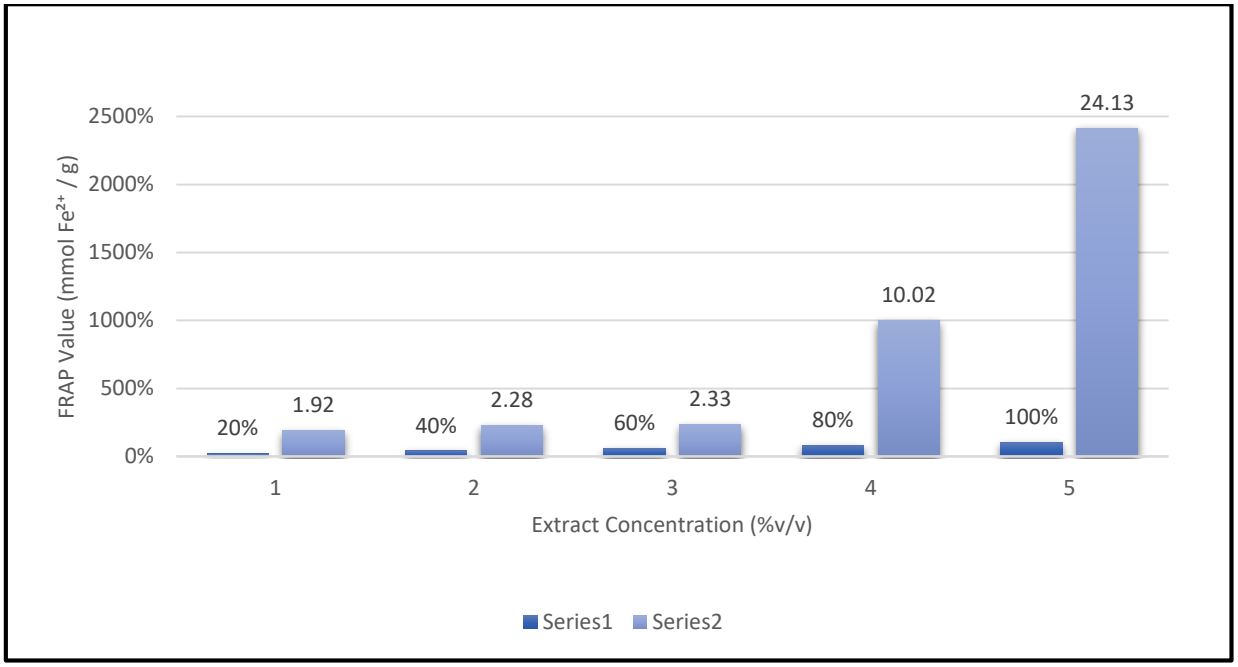


Figure 4.8. Comparative FRAP values of baobab fruit pulp extracts at varying concentrations (20%, 40%, 60%,80%,100% v/v), expressed as mmol Fe^{2+} per gram.

The distinct hierarchy (Figure 4.8) clearly demonstrates a concentration-dependent response, with the undiluted crude baobab extract exhibiting the greatest ferric reducing power (24.13 mmol Fe^{2+}/g). This finding is consistent with the phytochemical profile established earlier in this study; the high polyphenol and ascorbic acid content of baobab pulp are well-documented electron donors (Ismail et al., 2019; Tembo et al., 2017), and their reducing capacity scales proportionally with extract concentration.

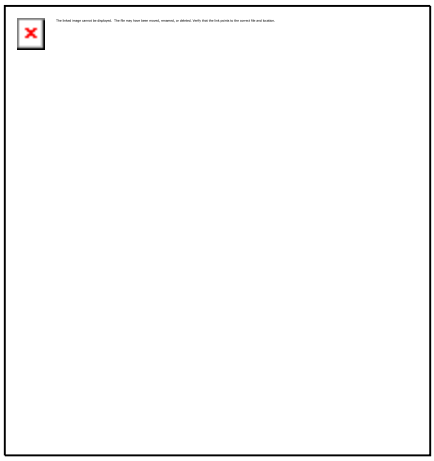


Figure 4.9. Visual color development in the FRAP assay for baobab extracts. The formation of the green-blue Prussian blue complex indicates positive reducing activity.

The quantitative FRAP findings were further validated visually by the formation of the characteristic green-blue Prussian blue complex in the active baobab samples (Figure 4.9), confirming the presence of potent electron-donating species in the pulp. Critically, this strong inherent antioxidant activity supports the feasibility of incorporating baobab extracts into a functional confectionery matrix, as demonstrated in the final product, where a meaningful fraction of this reducing capacity was successfully retained through the controlled-temperature processing conditions.

FRAP values reported here represent the total reducing capacity of the crude extract, not the specific contribution of the alkaloid fraction alone. The high ascorbic acid content of baobab pulp is known to contribute significantly to FRAP values through direct electron donation (Chadare et al., 2009). Therefore, while the FRAP results confirm that baobab extract possesses potent reducing activity, they cannot be used to quantify the antioxidant capacity of the alkaloid fraction specifically. As recommended in Section 4.8, future fractionation studies are needed to isolate the alkaloid fraction and assess its specific contribution to the total antioxidant activity.

4.7 Sweet Production

The sweet was produced using the methodology mentioned in Chapter 3, and the result was successful. Keeping the mixing temperature strictly at 80°C was to ensure the molten candy remains fluid enough to blend uniformly, while preventing the rapid thermal degradation, oxidation, and ring-cleavage of the delicate polyphenols and native ascorbic acid (Tembo, Holmes and Marshall, 2017).



Figure 4.10 Sweet development and Final product

4.7.1 pH of the Final Antioxidant Sweet Matrix

The final antioxidant sweet matrix exhibited a pH of 4.5-5.0, indicating that the formulation was slightly acidic.

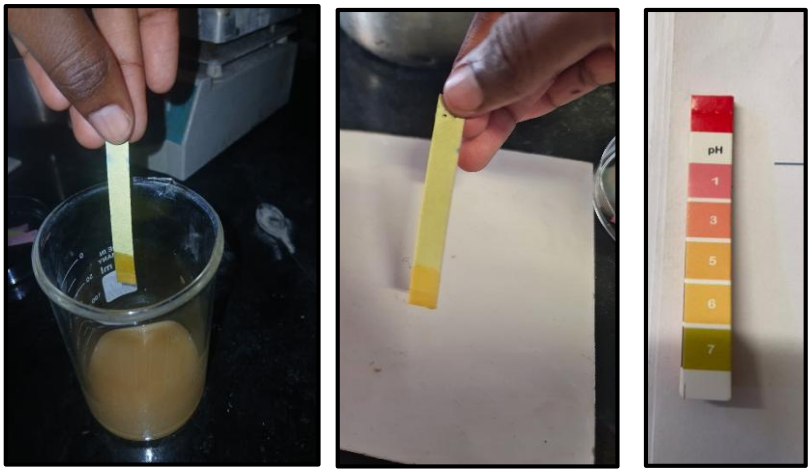


Figure 4.11 pH of final Sweet Matrix against the universal pH Chart

Notably, the pH values reported here (4.5-5.0) are approximations with a resolution of ± 0.5 pH units, as indicator strips rather than a calibrated pH electrode were used. This limitation arose due to equipment unavailability during the formulation phase. Consequently, the measured pH should be interpreted as the natural pH range of the developed formulation rather than an optimised value. Future work should employ a calibrated pH meter to precisely standardise the product pH to a target value (e.g., pH 3.4 ± 0.1) for improved consistency and stability (Tembo et al., 2017).

This pH may be attributed to the naturally occurring organic acids present in *Adansonia digitata* fruit pulp and compounds retained in the concentrated alkaloid extract. Ideally, citric acid would have been used to adjust and standardize the pH to a predetermined level; however, it was unavailable during formulation. Accordingly, the pH of the final product remained dependent on the inherent acidity of the raw materials used. This may have affected the overall stability, flavor profile, and preservation properties of the antioxidant sweet, as acidity plays an important role in controlling microbial growth and influencing sensory characteristics. Therefore, the measured pH should be interpreted as the natural pH of the developed formulation rather than an optimized value.

Future formulations should incorporate citric acid to achieve a more controlled and standardized pH, thereby improving product consistency and potentially enhancing shelf stability.

CHAPTER 5: CONCLUSION AND RECOMMENDATIONS

5.0 Introduction

This chapter summarises the key findings of the study, evaluates the extent to which the research objectives were achieved, and proposes recommendations for future research. The study investigated the extraction of alkaloids and associated bioactive compounds from baobab (*Adansonia digitata*) fruit pulp using ethanolic maceration, their characterisation by FTIR spectroscopy and qualitative phytochemical tests, quantification of total alkaloid content, and subsequent incorporation into a functional antioxidant confectionery sweet.

5.1 Conclusions

A semi-solid amber extract was successfully produced from baobab pulp using food-safe ethanol, which served as both solvent and preservative. Its low boiling point enabled gentle solvent removal, protecting heat-sensitive compounds. FTIR analysis confirmed the presence of alkaloids, with signals matching quinoline/indole frameworks, alongside polyphenols and vitamin C as the major phytochemical components. Standard reagent tests gave a weak positive for alkaloids, which is expected for baobab, and quantitative analysis placed the total alkaloid content at a moderate 16.1%. Both the iodine-starch and FRAP assays returned clear positive results for antioxidant activity. Crucially, this activity remained intact in the final sweets, indicating that the processing temperatures were sufficiently controlled to preserve bioactivity.

The confectionery was successfully produced by incorporating the extract below 70°C into a sugar-glass matrix. The final product's mild acidity reflects the natural fruit profile and is expected to support both stability and taste. Overall, the research objectives were met, demonstrating that baobab extracts can be feasibly incorporated into functional, antioxidant-rich sweets.

5.2 Recommendations for Future Research

Based on the findings and limitations encountered in this study, the following recommendations are made for future investigations:

- Future studies should employ quantitative antioxidant assays such as DPPH and ABTS with a UV-Vis spectrophotometer to obtain numerical IC_{50} values and Trolox equivalents, enabling direct comparison with published literature on baobab antioxidant activity.
- LC-MS/MS analysis should be undertaken to isolate and identify the specific alkaloid compounds present in the *A. digitata* extract, moving beyond FTIR fingerprinting to definitive structural characterisation and comparison with known indole and quinoline alkaloid standards.

- The antioxidant stability of the functional sweet should be evaluated over time through accelerated storage studies to assess the shelf life of the bioactive compounds under varying temperature and humidity conditions.
- Sensory evaluation panels should be conducted to assess the acceptability, taste, and consumer preference for the baobab antioxidant confectionery sweet before commercial scaling of the formulation.
- Given that baobab's high vitamin C content is likely to dominate antioxidant readings, future assays should consider ascorbic acid depletion pre-treatment (e.g., ascorbate oxidase) to isolate the specific contribution of the alkaloid fraction to overall antioxidant activity.

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