ASSESSMENT OF PHYTOCHEMICAL CONSTITUENTS IN LOCALLY MARKETED *Aloe barbadensis Miller* FROM OBA MARKET, BENIN CITY.

BY

Destiny Ifeoma OGOCHUKWU

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NOVEMBER, 2025

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A PROJECT SUBMITTED TO THE

DEPARTMENT OF MEDICAL BIOCHEMISTRY, SCHOOL OF BASIC MEDICAL

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CERTIFICATION

This is to certify that this project work was carried out by Destiny Ifeoma OGOCHUKWU with matriculation number (BMS2006693), of the Department of Medical Biochemistry, School of Basic Medical Sciences, University of Benin, Benin city, in partial fulfillment of the requirements for the award of Bachelor of Science (B.Sc.) degree in Medical Biochemistry

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DEDICATION

I dedicate this work to God Almighty, my source of strength,inspiration, wisdom, knowledge and understanding and to my lecturers who have taught me up to this point in my academic pursuit, equipping me with knowledge for both self and societal development.

ACKNOWLEDGEMENT

would like to express my sincere appreciation to everyone who contributed to the success of this project.

Firstly, I give all glory to God for the grace and strength to successfully carry out this work. I also appreciate my project supervisor, Dr. L.O. Agbontaen for his patient mentorship, insightful corrections for me to grow in both research and writing.

I am deeply thankful to my family, this achievement is as much yours, as it is mine. To my mother, Mrs. Glory Ogochukwu, for her full financial support, prayers, and guidance. I extend my heartfelt appreciation to; My sister Victory Jesse, thank you for listening when I was overwhelmed, for pushing me to high up my game, and for celebrating every little progress with me. And to my brothers who always stood by me financially, Precious Ogochukwu, and Emmanuel Ogochukwu, thank you so much.

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ABSTRACT

This study assessed the phytochemical constituents of *Aloe barbadensis* sold in Oba Market, Benin City. The aim was to identify major phytochemical groups and evaluate their levels relative to standard Aloe samples, providing data for quality control and local herbal use. Methanol extraction was used to obtain the leaf gel, which was analyzed qualitatively and quantitatively. Quantitative results showed high levels of flavonoids (312.62 µg/mL), Terpernoids (294.60 µg/mL), moderate levels of Cardiac glycosides (151.27 µg/mL) and very low level of Steroids (40.43 µg/mL). Qualitative screening confirmed the presence of flavonoids, Terpenoids, Cardiac glycosides, and Steroids, while other groups such as saponins, anthraquinones, alkaloids, phenols, coumarins, and phlobatanins were absent. The results indicate that the market sample retains key bioactive compounds suitable for herbal applications, though some phenolic groups were low. These findings support the need for quality monitoring of Aloe products in local markets and provide reference data for small-scale producers.

CHAPTER ONE

INTRODUCTION

1.0 BACKGROUND OF STUDY

In the world of medicinal plants, the increasing wide reliance on plants can be attributed to the presence of different bioactive compounds known as phytochemicals. Phytochemicals are natural substances found in small amount responsible for therapeutic properties, they stand out for its wide extent in biological activities, which can be linked to its rich phytochemical composition.

There are over 200 varieties of Aloe vera, but it is the Aloe Barbadensis Miller plant species that was collected in the Oba market (Benin city, Nigeria). Commonly called "Burn plant", a perennial xerophyte that can store water in the leaves to survive in dry land. The leaves are thick and fleshy green to gray green in color, with some varieties showing white flecks on their upper and lower stem surface. This plant is usually a combination of two different products, namely the bitter laxative yellow juice, from so-called aloin cells below the leaf rind and the non-bitter inner parenchyma gel (Yates, 2002). It is a drought resistant and able to tolerate wide range of climatic situations, and it was gotten from the local market ring road in Benin city, during the rainy season.

The availability of fresh growing Aloe vera from the traders sold in Oba market is very low, and scarce to find, the reason is because many traders usually sells the extract as an already processed products. Some Aloe vera plants seen in the market were damaged in appearance, which could be because of their poor management or storage which can affect the quality, concentration, and stability of phytochemicals it contains. Phytochemical screening, both qualitative and quantitative, serves as a vital tool in determining the chemical composition in aloe vera plant. Female purchase and utilize it more compared to the male in the market, some plants them around their house to make it accessible.

Phytochemical screening of Aloe vera leaves and gels has revealed their multiple secondary metabolites (Alkaloids, flavonoids, tannins, saponins, glycosides, steroids and anthraquinones) in ethanolic or aqueous extracts. These phytochemicals can be influenced by environmental factors i.e the geography, climate, soil type, sun exposure, grazing stress, seasonal changes (Ganskopp and Bohnert, 2003). Meanwhile benin city is popularly known for its heavy rainfall, which can affect the phytochemicals constituents of the plant.

For the analysis of the phytochemicals in aloe vera, different methods are involved but the widely applied method is the GC-MS (Salisu and Shema, 2020) and HPLC (Mukherjee et al., 2013) to analyse the components in aloe vera. This paper is conducted to increase the awareness and show the scientific evidence of the bioactive phytochemicals present in this Aloe vera plant, verify the quality of plant sold within that ring road market, the

traders information on the usage of such plant, and the consumers attitudes towards demand for such products that contain Aloe vera.

1.1 Aim of study.

The primary aim of this study is to assess the qualitative phytochemical constituents quantitatively present in Aloe vera plant, marketed in Oba market, Benin City. Considering that environmental condition can affect the phytochemicals, with this study we can show the relationship of their use in mankind.

1.2 Objectives of the study.

1. To identify the various types of phytochemicals (qualitative screening) that are present in Aloe vera plants.

2. To measure how much (quantitative screening) of each phytochemicals are in Aloe vera plants.

3. To document thow these phytochemicals relate to the managing benefits for mankind.

1.3 Research questions

The following questions were presented in other to achieve the stated objectives above.

> What are the phytochemical contents in Aloe vera plant sold in Oba market?

> How much of each compounds can be found in Aloe vera plant?

> In what concentrations are these phytochemicals found in Aloe vera samples collected from the market.

> What are the major phytochemical compound found in the Aloe Vera from Oba market?

1.4 Significance of the Study.

This study holds a great value in both the academic and public sector, it highlights scientific evidence on the phytochemical profile of Aloe vera sold in local markets, precisely Oba market, in other to verify its potency, authenticity and quality of this plant. The findings will also serve as a reference for consumers, traders, medical practitioners to understand the possible variations that may occur in marketed Aloe vera samples due to environmental and handling factors. This research encourage informed decision making by identifying and quantifying the phytochemicals present in Aloe vera sold in Benin City.

1.5 Scope of study.

This Aloe vera plant is specifically collected from a particular region, ringroad market in Benin city. Both qualitative and quantitative phytochemical screenings were carried out, to show the presence of

bioactive compounds and it's relations to mankind. Because its specific to one region, there's no comparison of different Aloe vera plants from various region.

1.6 Limitations of the study

Coupled with the verity that aloe vera is a medicinal plant, the study faced some drawbacks, they include:

- The Aloe vera extract for this study was from one particular location, phytochemical readings of Aloe Barbadensis Miller might vary depending on the season, and climate conditions.
- This study does not involve in vivo experiments or clinical trials (human or animal subjects) but literature reviews from other researchers, and also the knowledge from traders in the market which may vary.

CHAPTER TWO

LITERATURE REVIEW

2.0 The Plant Aloe barbadensis

Aloe barbadensis, also known as *Aloe vera,* belongs to the family *Asphodelaceae*. The plant is grown across tropical and subtropical regions and is recognised by several names in different countries. In Nigeria, it is sold in markets for domestic, cosmetic and medicinal use. The plant originates from regions around the Arabian Peninsula but is now generally cultivated across Africa, Asia and the United State of America. It grows well in dry zones and survives on low water. This feature about the plant is advantageous for farmers and home growers to propagate it through off-shoots produced at the base of mature plants (Nakiguli *et al.,* 2022).

The plant grows as a short stemless succulent with thick, fleshy leaves arranged in a rosette. Each leaf has a firm green surface, a moist gel interior and small marginal spines, with mature leaves reaching about 30 – 50 cm in length (Umeoka, 2024). The flowers arise on a tall stalk and form clusters of tubular yellow structures, although flowering is less common in locally cultivated plants due to harvest practices involved. Variants differ in leaf thickness, gel yield and colour, with some cultivars selected for higher gel output or faster growth. Moreover, *A. barbadensis* holds a long record of use in traditional medicine as the gel and latex from the leaves serve different purposes and is useful for wound care, skin irritation and digestive support, while the latex have strong laxative activity (Anwar *et al.,* 2022).

Fig. 2.1 The image shows the plant Aloe vera.

Source : Walter Jose et., 2002

In Africa, local users prepare the gel by cutting the leaf and scraping the inner pulp. Studies have shown that the plant contains phytochemicals such as flavonoids, tannins, alkaloids, saponins, anthraquinones, phenolic acids and glycosides (Shukla *et al.,* 2022; Ejoba, 2020). These compounds have been linked to antimicrobial, antioxidant and anti-inflammatory benefits

(Nalado and Tijjani 2023). Other studies have reported the presence of vitamins, fatty acids, sterols and polysaccharides in the extracted gel. More research from Nigerian and regional markets shows that phytochemical levels vary with location, storage, age of leaf and method of extraction. The plant continues to attract interest from herbal practitioners, traders and researchers due to its versatility and observable biological activity. However, more studies continue to show consistent phytochemical composition across regions, with some variation in antioxidant capacity, allowing for its medicinal multiple applications as a medicinal specie.

2.1 Botanical Description of the Plant

Aloe vera is a perennial herb with a short stem and a firm basal rosette. The plant stays low to the ground, while its leaves sit in a tight spiral and form the main structure of the plant. Each leaf is thick and filled with gel. The outer layer is tough and green to grey-green in colour. The leaf edge carries small teeth that run from the base to the tip. The surface is smooth and marked by faint parallel veins (Grace et al., 2009). Leaves taper to a point and store moisture for long periods. The plant produces a single flowering stalk from the centre of the rosette. This stalk stands well above the leaves. Flowers form along the upper part of the stalk are arranged in a spaced cluster where each flower is tubular and hangs slightly downwards.

The flowers produced are bisexual. They have six tepals joined at the base with six stamens surround a three-lobed ovary. This plant does not show the mix of male and female flowers seen in some genera. Instead, each flower carries both stamens and a single ovary. The six stamens extend beyond the tube when the flower opens. Nectar fills the lower section of the flower and

pollination leads to a dry capsule that splits when mature. Seeds are flat and dark. They form in three sections inside the capsule. The seeds germinate best in warm and dry conditions. The root system is shallow with many fibrous roots. These roots spread outward and anchor the plant in loose soil. Growth starts from the central crown, and new shoots appear near the base. These shoots form small offsets that enlarge with time.

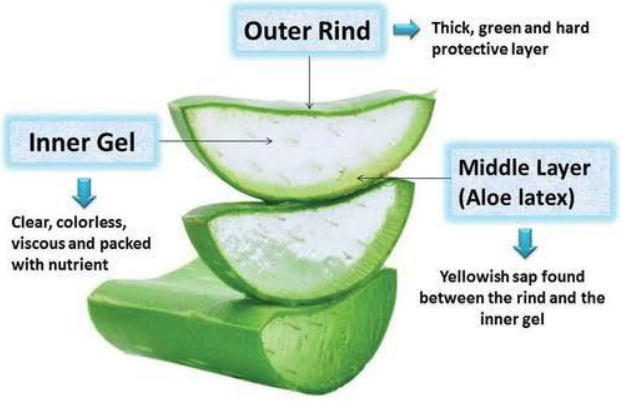

Fig. 2.2 the parts of Aloe vera plants

Source: Amany A et al., 2020

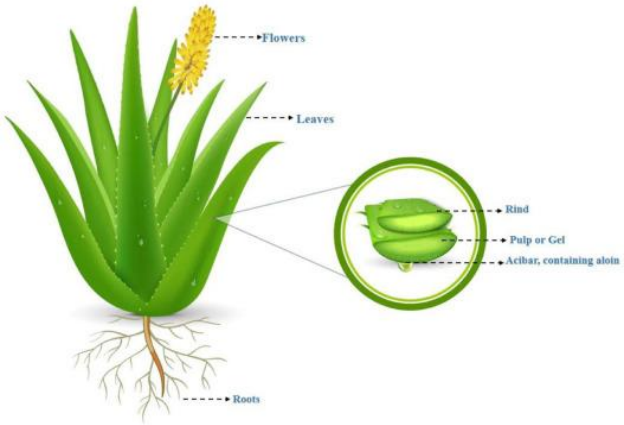

Fig.2.3 Aloe Vera plant having flower stalk.

Source : Walter Jose et., 2002

Aloe vera shows steady growth in warm climates. The rosette remains evergreen. The flowers appear in the dry season in many regions. The plant holds its structure through high temperatures due to its stored moisture. The ovary has a smooth surface and holds several ovules in each chamber. The style rises from the centre and ends in a simple stigma. The flower tube has six segments joined at the base. These segments separate near the top when the flower matures. The stalk holds the flowers at equal spacing, and each flower sits on a short support. After pollination, the ovary enlarges and becomes a dry capsule. This capsule splits into three parts when fully dry. Seeds inside are thin, flat, and dark. They take an angular form and break free when the capsule opens. The seeds carry a small amount of stored tissue and a straight embryo. The reproductive structure forms once a year in warm zones. The stalk rises well above the leaf rosette. The inflorescence stays firm until the seeds drop. This cycle allows the plant to spread through seed and through offsets at the base. A study by Grace et al. in 2009 showed that *A. Barbadensis* maintains a steady flowering pattern and uses both seed production and offsets for its spread.

2.2 Taxonomical Classification

Taxonomical classification refers to the system of naming and organizing organisms into groups based on their similarities and differences. Taxonomy is the branch of biology that deals with this classification, and it's used to help scientists understand the relationships between different organisms. In taxonomy, organisms are classified into groups based on shared characteristics, such as their physical features, genetic makeup, or evolutionary history. For example, all organisms in the same genus share a common ancestor.

Aloe barbadensis has a very specific taxonomic classification.

Kingdom – Plantae

Phylum – Tracheophyta

Class – Liliopsida

Order – Asparagales

Family – Asphodelaceae

Genus – Aloe

Species – barbadensis Miller

2.3 Historical and Medicinal use

Aloe barbadensis dates back to several generations, with oral and written historical accounts attesting to its significance among local herbalists and community healers. Traditionally, it was cultivated within homesteads, community gardens, and market spaces both as a commercial and

subsistence crop, representing a critical nexus between medicinal botany and community health security (Oboh and Ogunleye, 2002). This is because indigenous people have used Aloe vera for a wide array of purposes, integrating it into daily life, ceremonial rituals, and cultural medicine. In the context of health and wellness, the leaves and gel are commonly employed for wound healing, management of skin conditions, and as a soothing agent for burns and insect bites—a common practice believed to have started from generational knowledge passed down through familial lineages (Egharevba and Kunle, 2010). Also, some healers have been known to prescribe Aloe vera preparations in combination with other local produce, thereby maximizing perceived efficacy and spiritual potency through synergistic formulations. However, the therapeutic applications extend beyond simple wound care as traditional healers have documented the use of Aloe vera for treating intestinal ulcers, gynaecological problems, and catarrh, reflecting a sophisticated understanding of the internal medicinal properties of the plant (Ojezele and Abatan, 2011). The root of A. barbadensis have been particularly noted for its use in enhancing libido and treating hypotestosteronemia, demonstrating the comprehensive medicinal repertoire associated with different parts of the plant (Agyakwa et al., 2007).

In ancient Egypt, the Papyrus Ebers (circa 1550 BC) documents aloe-based remedies for both external wounds and internal ailments. The plant was also linked to many beauty and afterlife practices, including Egyptian queens like Cleopatra who reportedly used Aloe vera in their skincare routines (Discovery Journals, 2013).

Greek and Roman physicians were known to have referenced Aloe vera in ‘De Materia Medica’, recommending it for wounds, constipation, hair loss, skin ulcers, and other skin related problems. Pliny the Elder also mentioned its use as a purgative and healing agent (Wake Up World, 2016).

In ancient India, this plant was known as Kumari or Ghrita Kumari which was integrated into Ayurvedic medicine.

2.4 Phytochemical Constituents of Aloe vera plant

Phytochemicals are natural bioactive compounds, found in plants in small amounts. They are not substances that can completely heal an acute disease, but can be protective at low levels against the buildup of degenerative diseases over a lifetime (Dreosti, 1998). The chemicals in Aloe vera plant is divided into two major types: nutritive and non nutritive compounds. The nutritive are carbohydrates, enzymes, vitamins, protein and amino acids, minerals and trace elements, whereas the non nutritive constituents include organic compounds, phytosterol, phenolics, Tannin, saponin and other bioactive compound (Popat et al., 2015). The screening of Aloe vera leaves and gels has revealed their multiple secondary metabolites (Alkaloids, flavonoids, tannins, saponins, glycosides, steroids and anthraquinones) in ethanolic or aqueous extracts.

These phytochemicals confer a wide spectrum of biological activities, such as antioxidant, anti-inflammatory, antimicrobial, laxative, and immunomodulatory effects (Hamman, 2008). The mucilaginous gel from the inner leaf parenchyma is particularly rich in polysaccharides, notably acemannan, alongside vitamins, minerals, and amino acids, which further enhance its health-promoting reputation (Boudreau and Beland, 2006). For the analysis of the phytochemicals in aloe vera, different methods are involved but the widely applied method is the GC-MS (Salisu and Shema, 2020) and HPLC (Mukherjee et al., 2013) to analyse the components in aloe vera.

For a well understanding of the phytochemicals that can be present in Aloe Vera plants, they are:

- Tannins
- Saponin
- Steroid
- Anthraquinones
- Terpernoids
- Cardiac glycosides
- Flavanoids
- Phenol

2.5.1 Alkaloids

Alkaloids are a diverse group of naturally occurring compounds that contain basic nitrogen atoms and are renowned for their physiological and pharmacological activities. Has led to the development of powerful pain killer medications (Kam and Liew, 2002). Alkaloids are used as analgesics, stimulants, anesthetic, hallucinogens, and antibacterial agents (Balbaa, 2006). In Aloe barbadensis, alkaloids comprise a minor yet significant portion of its phytochemical profile. While not present in high concentrations compared to other plant families, the alkaloids detected in Aloe vera contribute to its therapeutic range, particularly in analgesic and anti-inflammatory applications (Hamman, 2008).

Quantitative analysis has revealed alkaloid concentrations of approximately 32.12g/100g in Aloe vera leaf extracts, indicating a substantial presence despite their classification as secondary metabolites (Boudreau and Beland, 2006). The extraction and detection of alkaloids in Aloe vera typically involve solvent-based approaches, with phytochemical screening, revealing their presence via standard alkaloid reagents and spectrometric analyses (Ajila et al., 2013). The concentration and profile of alkaloids can be affected by environmental factors such as soil composition, plant age, and harvesting practices (Chen et al., 2025).

2.5.2 Flavanoids

Flavanoids are large compounds that occur ubiquitously in plants, it is a pigment response for the color on the leaves, also reduce bleeding tendency and plays a role on the permeability of capillaries in the body (Balbaa, 2006). Flavanoids sometimes act as glycosides with several Phenolic hydroxyl groups on their ring structure. Some flavonoids are antioxidants and have been proved to exhibit a wide range of biological activities like antimicrobial, anti- inflammatory, antiangionic, analgesic, anti-allergic, cytostatic and antioxidant properties (Hodek et al., 2002). This widely distributed group of polyphenolic compound act as antioxidants, anti-inflammation, free radicals, and aggregate platelets; it also prevents allergies, ulcers, hepatoxins, virus and tumors. That means any leaves of herbaceous plants, trees, shrubs and ornamentals which contained flavonoids are potential antioxidants.

These secondary metabolites are distributed primarily in the leaf rind and, to a lesser extent, in the gel, imparting a range of biological effects that are important to both healers and scientific communities (Ajila et al., 2013). Flavonoids have antioxidant and free radical scavenging abilities, harnessed for the treatment of inflammatory disorders, skin eruptions, and wounds.

Phytochemical screenings of Aloe vera have confirmed the presence of various flavonoid subtypes including quercetin, kaempferol, and isorhamnetin glycosides. Moreover, the leaf skin of Aloe vera have been found to contain significant concentrations of phenolic compounds including sinapic acid, catechin, and quercetin, as determined by RP-HPLC analysis (Bhalla et al., 2015). These compounds are well documented for their role in modulating inflammatory responses, supporting vascular integrity, and providing ultraviolet (UV) protection, thereby extending its applications into skincare and cosmeceuticals (Boudreau and Beland, 2006). The levels of flavonoids in Aloe vera are found to vary according to plant age, environmental stress, and post-harvest handling.

2.5.3 Tannins

Tannins, a diverse group of polyphenolic compounds, are well recognized for their astringent, antimicrobial, and antioxidant effects. Tannins are present primarily in the leaf rind with lesser amounts detected in the gel. Their concentration and composition are influenced by factors such as soil quality, climate, plant age, and processing methods (Hamman, 2008). It has the ability to react with protein to form stable water (soluble phenolic compounds). They occur in almost all vascular plants, to precipitate proteins

form aqueous solution. There are two different types of tannins; the hydrolysable tannins and condensed tannins (proanthocyanidins) which differ in their nutritional and toxic effects.

Nevertheless, the tree and shrub leaves contain the two types, The condensed tannins have a more digestibility-reducing effect than hydrolysable tannins, wherein the hydrolysable tannins, may cause varied toxic manifestations due to hydrolysis in rumen. Tannins has antimicrobial property. It is suggested that the consumption of tannins-containing beverages is encouraged as it is believed to cure or prevent variety of diseases. Tannin and saponin together in the feed lead to increase intake of undegradable protein and reduction in blood cholesterol respectively. The astringent property of tannins is specifically utilized in remedies for diarrhea, where they help contract the gut lining, reduce intestinal secretions, and restore normal digestive function (Oboh and Ogunleye, 2002). Also, tannins have been shown to disrupt protein synthesis in bacterial cells and are particularly useful in the management of intestinal disorders as well as ulcerated or inflammatory conditions. Phytochemical analyses confirm the presence of both condensed and hydrolysable tannins in Aloe vera, including catechins and gallic acid derivatives (Adnan et al., 2017). These compounds not only grant the plant its therapeutic properties but also contribute to its role in local preservation practices, such as extending the shelf life of traditional remedies and foods (Ajila et al., 2013).

2.5.4 Saponins

Saponins are glycosidic compounds characterized by their structural capacity to form soap-like foams in aqueous solutions. Saponins are among the important phytoconstituents, contributing to the medicinal attributes and applications within traditional herbal practice (Hamman, 2008).

Quantitative analysis has revealed saponin concentrations of approximately 11.52g/100g in Aloe vera leaf extracts, confirming their significant presence (Ashour et al., 2019). Extracts from Aloe leaves, particularly the latex, are employed in the preparation of topical solutions designed to address skin complaints such as fungal infections, rashes, and boils (Egharevba and Kunle, 2010).

The foaming property of saponins is believed by local healers to symbolize purity and efficacy in expelling toxins or pathogens that enhances their role in health related issues. Saponins also exhibit anti-inflammatory and immunostimulatory properties, which are harnessed in Aloe vera-based treatments for wounds, sprains, and muscular discomfort. The phytochemical screenings of Aloe vera reveal that saponin content may fluctuate based on environmental stressors, harvesting time, and method of extraction. Studies utilizing various extraction solvents including petroleum ether, chloroform, and ethanol through Soxhlet extraction have confirmed the presence of saponins in the leaf exudate (Manimegalai et al., 2015).

Their properties include; the formation of foams in aqueous solution, haemolytic activity and cholesterol binding properties and bitterness. They cause gastroenteritis (dysentery, diarrhea), serve as natural antibiotic (fight infections and microbial invasion). When they reduce cholesterol absorption

and increase its excretion, blood pressure will reduce which is a good potential use in heart disease, also cancer cells.

2.5.5 Anthraquinones

Located in the leaf lining (latex, resin or sap) are twelve anthraquinones, a phenolic compound that has stimulating effects on the bowels and antibiotics. The most important are the aloin and emodin, they are potent stimulant laxatives, antibacterial, antiviral, analgesic. Aloin binds with the receptor of paraietal cel (produce hydrochloric acid), and buildup it's inhibitory effects of gastric juice. This phytochemical breakup Aloe vera residue, pus and lifeless cells, bring blood to the region and flush out material form the wound and ulcer (Saumendu et al., 2013).

2.5.6 Steroids

Steroids represent an important yet often understated category of phytochemicals found in Aloe vera. These naturally occurring compounds, including phytosterols such as campesterol, sitosterol, and lupeol, contribute to the plant's anti-inflammatory, analgesic, and immunomodulatory properties. Detailed phytochemical analysis have identified various steroidal compounds in different parts of the Aloe vera plant. The leaf gel yields phytosterols including cycloartanol, lophenol, 24-ethyl-lophenol, 24-methyl-lophenol, and 24-methylene-cycloartanol, isolated through trichloromethane and methanol extraction followed by column chromatography and NMR analysis. Fatty acids identified in the leaf gel,

including hexadecanoic acid, octadecanoic acid, and 9-octadenoic acid, work in concert with sterols to enhance dermatological benefits of the plant (Bawankar et al., 2013).

2.5.7 Glycosides

Glycosides are non reducing substances composed of two parts; the non sugar part of the molecule (Aglycone), and the sugary part called the Glycone (Kaur et al., 2023). However, the non sugary part, Aglycone is often anthraquinones derivatives found in aloe vera extracts. The are responsible for most biological activities i.e strong antibacterial activities (Balbaa, 2006). Cardiac glycosides have long served as the main medical treatment to congestive heart failure and cardiac arrhythmia. Though they are present only in trace amounts in Aloe vera extracts, cardiac glycoside are not the major type of glycosides responsible for its therapeutic actions, so they are available in trace amount in Aloe vera.

These compounds, notably anthraquinone and cardiac glycosides, contribute substantially to its medicinal efficacy, particularly in purgative, antimicrobial, and anti-inflammatory roles (Malik et al., 2016). Anthraquinone glycosides have been identified in Aloe vera including, homonataloin, aloesin, aloenin, barbaloin, aloinosides A and B, and aloesone, detected through exudation into methanol followed by TLC analysis.

2.5.8 Phenols

They are major group of compounds acting as primary antioxidants or free radical scavenger. These compounds are present in both the leaf gel and rind, and are responsible for neutralizing free radicals, mitigating oxidative damage, and enhancing tissue regeneration (Ajila et al., 2013; Hamman, 2008). Phytochemical investigations have confirmed a robust profile of phenolic acids such as cinnamic and ferulic acids, flavonols, and other structurally diverse antioxidants, which work collectively in disease prevention and cellular protection. Specific phenolic compounds identified in the leaf skin include sinapic acid, catechin, and quercetin, as determined through RP-HPLC analysis (Sun et al., 2023). Additionally, the leaf gel have been found to contain pyrocatechol, ascorbic acid, coumaric acid, and p-coumaric acid, identified through cold maceration extraction followed by GC-MS analysis.

2.6 Non medicinal benefits

In local households, it is valued as a protective and purifying plant. It is commonly placed at the entrances of homes, market stalls, and shrines, believed to guard inhabitants against evil spirits, misfortune, and negative energies (Agyakwa et al., 2007). Aloe vera is also utilized in beauty and personal care practices. Its gel is recognized for its soothing and hydrating qualities, used as ingredient in homemade cosmetic formulations such as skin moisturizers, hair conditioners, and face masks (Ojezele and Abatan, 2011).

Aloe vera for beauty purposes traces back to ancient civilizations, with Egyptian queens Nefertiti and Cleopatra incorporating it into their regular beauty regimens. These non-medicinal uses underscore an enduring knowledge of Aloe vera in maintaining skin and hair health, particularly in response to the hot and humid climate typical of Southern Nigeria (Oboh and Ogunleye, 2002). The gel stimulates blood circulation on the scalp, promotes hair growth, and rid the scalp of impurities through natural enzymes, making it a popular ingredient in traditional hair care formulations.

Women have reported using Aloe vera to treat stretch marks developed during pregnancy, applying the gel to affected areas with notable success (Egharevba and Kunle, 2010). Economically, Aloe vera have gained substantial traction as a marketed commodity, where both fresh leaves and processed products like lotions, creams, and ointments are sold (Ojezele and Abatan, 2011).

3.0 FACTORS AFFECTING PHYTOCHEMICALS OF ALOE VERA PLANTS

The phytochemical profile of Aloe barbadensis is affected by a variety of environmental, biological, and post-harvest influences. These factors determine the potency, effectiveness, and safety of Aloe-based remedies and products.

a. Environmental Factors:

Plant grown in nutrient-rich, well-drained soils produce higher levels of polysaccharides, flavonoids, and phenolic compounds, while environmental stressors such as drought can trigger increased accumulation of protective antioxidants. Aloe vera thrives best in light sandy soils with good drainage, as the plant is highly susceptible to water-logging (Aprilia et al., 2025).

The optimal pH range extends up to 8.5, allowing cultivation across a variety of soil conditions, though the best results are achieved in well-drained loamy or sandy-loam soils with pH values between 7.0 and 8.5 (Serra et al., 2024).

Temperature and climate considerations are particularly important, aspathways Aloe vera is very sensitive to extreme cold conditions and cannot tolerate frost (Kumar et al., 2016). The tropical climate in Nigeria are generally favorable, though seasonal variations necessitate adaptive cultivation practices.

b. Plant Growth and Maturity:

Mature leaves generally contain high concentrations of anthraquinones, glycosides, and phenols than younger shoots (Robert et al., 2024). Fully developed mature leaves should be harvested for extraction of juice, with commercial yields typically available from the second year to the fifth year of transplanting. The shallow root system of Aloe vera, which typically does not extend more than 7.87 to 11.81 inches (20-30 cm) influences its

nutrient uptake patterns and subsequent phytochemical production (Owoade et al., 2016).

c. Cultivation and Harvesting Techniques:

Traditional cultivation practices such as intercropping, organic amendments, and timing of harvest can modulate phytochemical composition. Proper land preparation is essential to ensure Aloe plants have optimal root development and nutrient uptake conditions, the soil should not be disturbed during preparation given its shallow root system.

4.0 Review of previous related case study

1. Adibe et al., 2009 conducted a survey among the staff and students of university of Nigeria, Nsukka. Two departments were selected, giving a total of 250 questionnaire. Generally, younger and unmarried and highly educated respondents were likey using Aloe vera more than older, married and less educated respondents. Out of 220 respondents, 57.37% had used it for hair treatment, skincare (46.35%), scar removal was 36.37% but less than 10% has used the palbt for arthritis (5.16%), pain (6.25), diabetes (6.83), and ulcer (8.43%).

2. A landmark field study conducted by Egharevba and Kunle (2010) explored the medicinal uses and phytochemical screening of Aloe vera sourced from Southwestern Nigeria markets, applying standard qualitative

and quantitative protocols to evaluate levels of anthraquinones, saponins, flavonoids, and tannins. Their analysis demonstrated that the majority of freshly harvested Aloe vera leaves exhibited high concentrations of saponins and moderate phenolic compounds, supporting their observed antimicrobial and anti-inflammatory activity in folk remedies.

3. Oboh and Ogunleye (2002) investigated traditional use and market preferences for Aloe vera in Benin City. They found considerable variability in phytochemical content, attributed primarily to market sourcing, leaf age, and post-harvest practices. Their study emphasized the importance of vendor experience in selecting and preparing high-quality Aloe vera, aligning empirical observations with laboratory results.

4. Erhabor et al. (2017) conducted a significant study at the University of Benin investigating the aphrodisiac potentials of ethanol extract of Aloe barbadensis root. This research validated traditional claims of its use in enhancing libido, demonstrating increases in mounting frequency, intromission frequency, and testosterone concentrations in experimental models.

CHAPTER THREE

MATERIALS AND METHODS

3.0 MATERIALS AND METHODS

3.1 Reagents

- Distilled water
- Methanol
- Hydrochloric acid
- Sulphric acid (H2SO4)
- Glacial acetic acid
- Ferric chloride
- Tripyridyl Triazine (TPTZ)
- Molybdate
- Sodium hydroxide (NaOH)
- Methylene red indicator
- Diethyl ether
- Petroleum ether

3.2 Apparatus and equipment

- Test tubes
- Test tubes racks

- Beaker (50ml, 100ml, and 200ml)
- Pipette
- Micro pippet
- Masking tape
- Funnels
- Sieve
- Whatman filter paper
- Electronic sensitive weighing balance
- Foil paper
- Glass stirrer
- Spatula
- Concentration jars
- Universal bottles
- Incubator
- Refrigerator
- Mortal and pestle
- Spectrometer
- Gloves
- Face mask
- EDTA bottle

3.3 METHODS

3.3.1 Collection and Identification:

Fresh leaves of *Aloe barbadensis* as obtained from Ring Road, Oba Market, Benin City, Nigeria. The plant was identified and botanically authenticated by Prof. H. A Akinnibosun of the Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, Benin City, Nigeria, and herbarium authentication number UBH- () was obtained from the same department.

3.3.2 Sample Preparation

The collected leaves were washed with tap water, then with distilled water, to remove any form of contaminants. The leaves were then cut into small pieces and weighed to 5kg with an analytical balance. It was homogenised in 100mL of methanol using a mortar and pestle till it was finely smooth liquid. The same was then turned into a 100mL flat bottom flask and then kept at room temperature till it was required for analysis.

3.3.3 Qualitative Phytochemical Screening Procedure

Phytochemicals are bioactive constituents of medicinal plants which are not nutrients but very useful to the plants. Some bioactive constituents of ethanolic extract were analysed qualitatively for Flavonoids, Tannins, Cardiac Glycosides, Saponins, Steroids, Terpenoids, Phenols, Phlobatanins, Coumarin, Anthraquinone and Alkaloids. Phytochemical screening was carried out on the samples after undergoing methanol extraction, using standard procedures to identify the secondary metabolites (Harborne 1973; Trease and Evans, 1989; Sofowora 1993).

1. **Solvent extract preparation:**

Pulverised sample (150g) was measured and placed in bottles. 500mL of absolute Ethanol was used to soak the samples, subsequently stirred 3 times daily and kept in dark room for 72 hours. Supernatant was filtered using a muslin cloth and the solvent evaporated using a rotary evaporator. The dried extracts were weighed and kept in sterile universal bottles for freezer at a temperature of -4˚C for some time.2g of the crude extract was weighed and used for phytochemical screening.

2. **Test for flavonoids:**

5mL of 10% ammonia was added to 1ml portion of an aqueous filtrate of the extract. Then 1ml concentrated sulfuric acid was added. Observed yellow colour indicates the presence of flavonoids.

3. **Test for tannins:**

1mL of (0.5g/5mL) Ethanol extract was boiled in 2ml of water in a test tube and filtered. A 3drops of 0.1% ferric chloride was added and observed for brownish green to a blue-black colouration.

4. **Test for cardiac glycosides (Keller-Killiani test):**

1mL of 0.5g/5ml aqueous extract was treated with glacial acetic acid containing one drop of ferric chloride solution. 1mL of concentrated sulfuric was gently added. A browning at the interface indicated the presence of a deoxysugar characteristic of cardenolides. Hence, the presence of cardiac glycosides.

5. **Test for saponin (Frothing test):**

The ability of saponins to produce frothing in aqueous solution was used as a screening test for saponins. 1mL of extract (0.5g/5mL of distilled water) was mixed with 5mL of distilled water and shaken vigorously for a stable persistent froth, indicating the presence of saponin. This was further confirmed by adding 3 drops of olive oil and shaking vigorously after which it was observed for the formation of an emulsion.

6. **Test for steroids**

2mL of concentrated acetic anhydride was added to 0.5mL of (0.5g/5mL) Ethanol extract of each sample with 2mL concentrated sulfuric acid. The colour changed from violet to blue or green colouration was positive for steroids.

7. **Test for terpenoids (Salkowski test):**

1mL of the extract in a test tube was mixed with 2mL of concentrated chloroform and 3ml of concentrated sulfuric acid. Reddish brown coloration at the interface confirmed the presence of terpenoids.

8. **Test for phenols:**

3 drops of 10% aqueous FeCl3 solution were added in a test tube to 5mL of (0.5g/5mL) Ethanol extract. Formation of blue or green coloration indicated the presence of phenols.

9. **Test for phlobatanins:**

3mL of (0.5g/5mL) Methanol extract was added to 2mL of 1% HCl and the extract was boiled. Deposition of a red precipitate was taken as an evidence for the presence of phlobatanins.

10. **Test for Coumarin:**

5mL of (0.5g/5mL) Ethanol extract was dissolved in 2mL of hot distilled water and divided into two parts. Half of the volume was a control; the other part 0.5ml of 10% NH4OH was added.

11. Test for alkaloids:

Mayer's test: 1mL of (0.5g/5mL) ethanol extract was mixed with 3drops of Mayer's reagent. Cream coloured precipitate formation confirmed the presence of alkaloids.

12. Test for anthraquinone:

5mL of benzene was added to 1mL of (0.5g/5mL) Methanol extracts in a test tube and shaken vigorously in 2.5mL concentrated NH3. Formation of pink-red colouration at the lower phase was indicative of the presence of free Anthraquinone.

3.2.4 Quantitative Determination of Phytochemicals

1. Estimation of alkaloids (Madhu *et al*., 2016)

To 1ml of test extract, 5ml of pH 4.7 phosphate buffer was added and 5ml BCG solution and shake a mixture with 4ml of chloroform. The extracts were collected in a 10ml volumetric flask and then diluted to adjust volume with chloroform. The absorbance of the complex in chloroform was measured at 760nm against blank prepared as the above with extract. Atropine was used as a standard and compared the assay with atropine equivalent.

2. Flavonoids (Madhu *et al*., 2016)

Total flavonoid content was determined by Aluminum chloride method using Quercetin as a standard. 1ml of test sample and 4ml of water were added to a volumetric flask (10ml vol.). After

5mins, 0.3ml of 5% Sodium nitrite and 0.3ml of 10% Aluminum chloride were added. After 6mins incubation at room temperature, 2ml of 1M Sodium hydroxide was added to the reaction mixture. Immediately, the final volume was made up to 10ml with distilled H2O. The absorbance of the reaction mixture was read at 510nm against a blank spectrophotometrically.

3. Steroids (Madhu *et al.*, 2016)

1ml of extract of steroid solution was transferred into 10ml volumetric flask. Sulphuric acid (4N, 2ml) and Iron (III) chloride (0.5% w/v 2ml) were added, followed by potassium hexacyanoferrate (III) solution (0.5% w/v, 0.5ml). The mixture was heated with occasional shaking and diluted to the mark with diluted water. The absorbance was measured at 780 nm against the reagent blank. Stigmasterol was used as standard.

4. Terpenoids (Alessandra *et al.,* 2020)

To 75ul plant extract, 250ul of vanillin solution (50mg/ml) and 500ul of Sulphuric acid (99.5%). The tube was heated in a water bath (60oC) for 20mins and then transferred into an ice bath followed by the addition of 2500ul of acetic acid (99.5%). The resulting solution was cooled for 20mins and absorbance was measured at 548nm. Beta-sitosterol was used as a standard.

5. Coumarin (Ameen *et al.* 2021)

A 0.5ml of 5NNaOH was added to the solution of 1 ml of the extract (0.5g in 1 ml methanol). The mixture was heated at 80oC for 5mins. After cooled, 0.75ml of 5N H2SO4 was added and mixed thoroughly, then, 0.25g of anhydrous NaHCO3 was also added and transferred to the extractor and made up to 50 ml with pet-ether for 3hrs. About 20ml of H2O was added to the pet-ether extract and carefully evaporate the pet-ether in water bath at 50-55oC. The aqueous solution was transferred to a volumetric flask and made up to 50ml with mixing. 25ml of

aqueous solution was pipetted into a flask and 1%. Na2CO3 solution was added and heated in a water bath at 75% for 15mins and cooled. 5 ml of the diazonium solution was added and stand 2 hours. The absorbance at 540nm against reagent blank. Esculin was used as standard.

6. Phenols (Tofighi *et al.,* 2016)

The methanol solution of each sample (0.2 - 100ug/ml) was mixed with folin-ciocalteu reagent (2 ml, 1:10 diluted with distilled H2O). After 5mins, saturated NaHCO3 solution (1.5ml, 60g/L distilled water) was added. The mixture were allowed to stand for 90mins at room temperature and absorbance of the solution was measured at 725nm. The same procedure was repeated for different concentrations of gallic acid solution (0.2-1.0ug/ml).

7. Cardiac glycosides (Tofighi *et al.,* 2016)

10% extract was mixed with 10ml of freshly prepared Baljet's reagent (95ml of 1% picric of 5ml of 10%NaOH). After an hour, the mixture was diluted with 20ml distilled water and the absorbance was measured at 495nm. Securidaside was used as standard.

8. Tannins (Chandran and Indira, 2016)

Tannins was determined by folin ciocalteu method 0.1ml of sample extract was added to volumetric flask (10ml) containing 7.7ml of distilled water. The mixture was shaken well and kept at room temperature for 30mins, a set of reference standard solutions of tannic acid (20, 40, 60, 80, and 100ug/ml) in the same manner as described for sample extract. Absorbance for test and standard solutions were measured against reagent blank at 700nm.

CHAPTER FOUR

RESULTS

Table 4.1: Qualitative Phytochemical Screening of *Aloe barbadensis*

Phytochemicals	
Flavonoids	+
Tannins	-
Cardiac glycosides	+
Phlobatanins	-
Steroids	++
Terpenoids	+
Anthraquinone	-
Saponins	-
Coumarin	-
Alkaloids	-
Phenols	-

Key + positive, - negative.

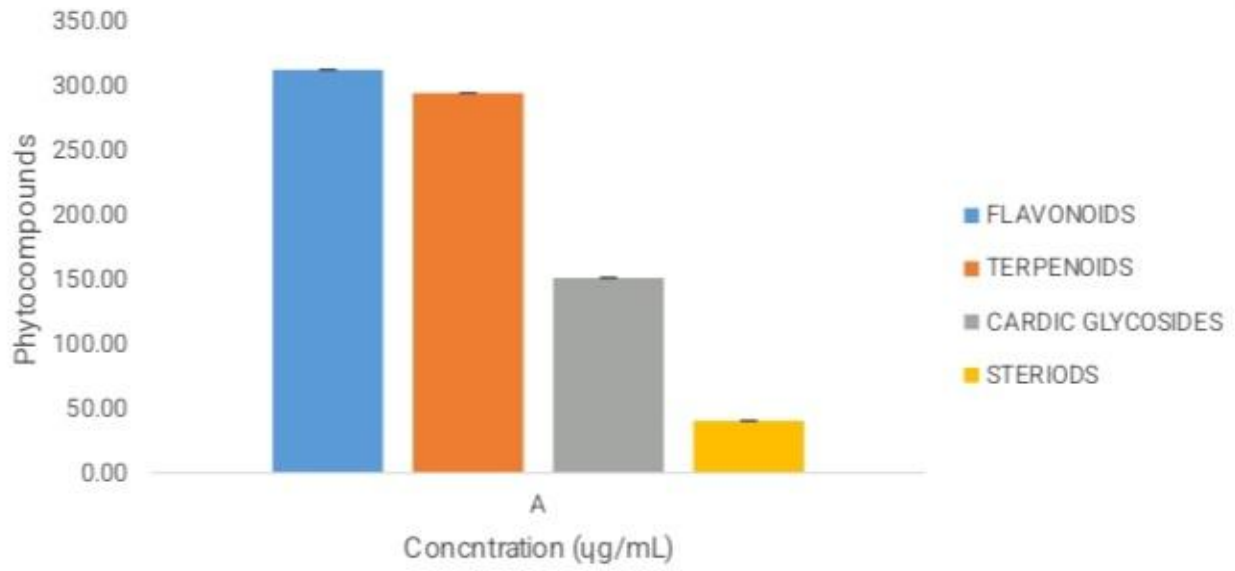

Figure 4.1: Quantitative Phytochemical Screening of *Aloe barbadensis*

CHAPTER FIVE

DISCUSSION

5.1 Discussion

Table 4.1 shows the phytochemical screening of Aloe barbadensis sample where flavonoids, cardiac glycosides, steroids and terpenoids were found present, suggesting the leaf still held bioactive compounds at the time of purchase. Meanwhile, other groups such as tannins, phlobatanins, anthraquinones, saponins, coumarins, alkaloids and phenols were not detected. The presence of flavonoids agrees with many reports on Aloe gel which supports antioxidant and anti-inflammatory activity, hence the wide use of Aloe vera for skin care. Also, cardiac glycosides were present, usually common in the Aloe latex, indicating that the extraction process pulled materials from both gel and other parts of the rind. Steroids were found in low amounts (40.43 µg/mL) from the sample, holding plant steroids in the gel matrix. These compounds support membrane stability in plant cells, signalling that the structural parts of the leaf were well preserved. Moreover, terpenoids were detected at a moderate level. The absence of tannins, phlobatanins and anthraquinones shows limited extraction of phenolic fractions. This can result from solvent choice, short extraction time or mild processing conditions. Likewise, many Aloe samples only show strong anthraquinone signals when the latex is fully extracted. No saponins were detected as some Aloe samples show low saponin signals depending on plant age and hydration status. This is why young plants or plants stored under high heat often lose saponin strength. Coumarins, alkaloids and phenols

were also absent as these groups vary across locations. Their absence is common when the plant is harvested early or when the inner gel is used as the main material.

However, from Figure 4.1, methanol extract of Aloe barbadensis showed a limited phytochemical profile where only flavonoids, cardiac glycosides, steroids and terpenoids were detected. Other groups such as tannins, phlobatanins, anthraquinones, saponins, coumarins, alkaloids and phenols were absent. It was observed that flavonoids had the highest concentration at 312.62 µg/mL extract. Flavonoids are antioxidants and anti-inflammatory agents, its high level suggests the sample retains strong biological activity and may support therapeutic uses such as wound healing or skin care. This findings align with studies reporting high flavonoid content in Aloe gel (Surjushe et al., 2008). This means that high flavonoid level shows that market handling did not degrade this bioactive compound. In addition, terpenoids were also high at 294.60 µg/mL which contribute to the pharmacological potential of Aloe, including its effects on cell metabolism. Its presence indicates the extract captured both gel and residual latex compounds, suggesting that a proper methanol extraction method was employed. Meanwhile, steroids measured the lowest concentration at 40.43 µg/mL. Steroids are structural components of plant cell membranes and support anti-inflammatory activity. Its concentration indicates that leaf tissues were intact and the plant was mature and well handled, preserving its structural compounds. Whereas, cardiac glycosides were recorded as 151.27 µg/mL. This particular compound contributes to antimicrobial activity, aroma, and leaf bitterness. Its moderate level

indicates normal secondary metabolite production in the plant. Tannins were not found present in the Aloe sample. Tannins are phenolic compounds that bind proteins and have antioxidant activity. Its absence may result from the gel-dominated extraction, plant age, or environmental stress before harvest.

Moreover, the phytochemical profile of this plant indicates the sample retains major bioactive compounds useful for local herbal use, including high flavonoid and cardiac glycosides content supporting its antioxidant and therapeutic properties. Moderate terpenoids and low steroids confirm structural and antimicrobial properties, while tannin levels guide producers to consider leaf maturity and storage in order to maintain phenolic strength. Also, methanol is known to pull both polar and mid-polar compounds, so the presence of flavonoids aligns with reported Aloe chemistry. Flavonoids appear in Aloe gel in moderate amounts and support antioxidant activity. Studies report similar findings, with methanol extracts showing clear flavonoid signals in Aloe tissues (Surjushe et al., 2008). Their presence suggests the sample retained its basic antioxidant value despite market handling. Cardiac glycosides were present. These compounds are commonly associated with Aloe latex. Their detection shows that the extract pulled materials from both the gel and parts of the leaf rind. Past studies note that methanol solvents extract cardiac glycosides effectively from Aloe leaves (Hamman, 2008). This supports the idea that the sample had not lost its latex-linked compounds. Plant steroids occur in Aloe gel and contribute to structural integrity in the leaf. Their presence matches reported Aloe profiles where methanol extracts recover sterol components

at measurable levels (Eshun and He, 2004). Terpenoids influence plant aroma and antimicrobial activity. Aloe leaves contain several terpenoid fractions that respond well to methanol extraction, as noted by Ajab et al. (2010).

Aloe gel holds less phenolic compounds than the latex, and methanol extracts often show reduced tannin levels when the leaf is not fully aged (Sánchez-Machado et al., 2017). This pattern suggests low phenolic intensity rather than a technical failure. Likewise, Anthraquinones were not detected. Anthraquinones such as aloin are normally found in the latex, implying the plant may have been handled in a way that reduced latex flow or the sample came from a younger leaf. There are reports showing that storage and frequent washing by vendors reduce anthraquinone traces in marketed Aloe leaves (Shelton, 1991). Aloe species vary in saponin levels, and methanol does not always pull saponins strongly unless the leaf is mature. Low or absent saponins have been noted in Aloe samples from humid environments with inconsistent light exposure (Boudreau and Beland, 2006).

5.2 Conclusion

The study showed that Aloe barbadensis sold in Oba Market contains key bioactive phytochemicals. Flavonoids and terpenoids were present at high levels, while cardiac glycosides were moderate, and steroids were low. Other phytochemical groups, including tannins, saponins, anthraquinones, alkaloids, phenols, coumarins, and phlobatanins, were absent. The results indicate that the market sample retains compounds with antioxidant, anti-inflammatory,

and antimicrobial potential. This highlights its suitability for local herbal use and small-scale production. The data also provides a reference for quality control, emphasizing the need for proper harvesting, handling, and storage to preserve phytochemical content

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