EXTRACTION, OPTIMIZATION, AND CHARACTERIZATION OF BIOACTIVE COMPOUNDS FROM LOCAL PLANTS (CLOVES, MORINGA, AND ROSEMARY) FOR THE MANAGEMENT OF ANDROGENETIC ALOPECIA

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A RESEARCH PROJECT ON THE EXTRACTION, OPTIMIZATION, AND CHARACTERIZATION OF BIOACTIVE COMPOUNDS FROM LOCAL PLANTS (CLOVES, MORINGA, AND ROSEMARY) FOR THE MANAGEMENT OF ANDROGENETIC ALOPECIA

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A PROJECT SUBMITTED TO THE DEPARTMENT OF CHEMICAL ENGINEERING IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF BACHELOR OF ENGINEERING (B.ENG) DEGREE OF THE UNIVERSITY OF BENIN

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CERTIFICATION

This is to certify that this research project was carried out by Muhammad-Ali Firdaus Ominyokhoshe with matriculation number ENG2002055 in the Department of Chemical Engineering, University of Benin, Benin City, Edo State Nigeria.

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DEDICATION

This work is dedicated to Almighty Allah, the Most Gracious and Most Merciful, for His boundless blessings, wisdom, and strength that guided me through my academic journey and made the successful completion of this research possible.

I also dedicate this project to my loving parents and siblings, whose unwavering love, prayers, and sacrifices have been my greatest source of motivation and support. Their constant encouragement and belief in my potential inspired me to persevere through every challenge.

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ABSTRACT

Androgenetic alopecia (AGA), a gradual form of hair loss driven by oxidative stress and hormonal imbalance, remains a major dermatological issue. Conventional synthetic treatments often lead to undesirable side effects, creating the need for safer, plant-based alternatives rich in bioactive compounds that can promote hair regrowth and scalp health. This study aimed to investigate the extraction, optimization, and characterization of bioactive compounds from *Syzygium aromaticum* (clove), *Rosmarinus officinalis* (rosemary), and *Moringa oleifera* (moringa) for potential application in natural formulations targeting androgenetic alopecia.

Extraction was optimized using a mixture design approach in Design Expert® software, where the proportions of the three plant materials were systematically varied to maximize total phytochemical yield. The experimental data were fitted into a quadratic model that exhibited strong predictive accuracy with an R^2 value of 0.9633, while the predicted and adjusted R^2 values were closely aligned, 0.9268 and 0.9560 respectively, confirming the model's reliability and significance ($p < 0.0001$). Optimization results showed that the best formulation was gotten using 3.553 g of cloves, 2.389 g of rosemary, and 4.057 g of moringa, yielding 15.108 with a maximum desirability value of 1.000. Phytochemical screening and quantitative analysis revealed that the optimized blend possessed very high concentrations of phenols, flavonoids, terpenoids, and steroids. Fourier Transform Infrared (FTIR) spectroscopy further confirmed the presence of crucial functional groups such as hydroxyl (–OH), carbonyl (C=O), and C–O linkages, characteristic of polyphenolic and terpenoid compounds.

The results indicated that the combined extract showed synergistic phytochemical enrichment, suggesting improved bioactive potency. The dominance of phenolic and flavonoid compounds implies strong antioxidant and 5α-reductase inhibitory potential, thereby reducing dihydrotestosterone (DHT)-induced follicular shrinkage. Terpenoids and steroids were also found to contribute to follicular nourishment and stimulation of keratinocyte activity, enhancing overall hair growth. In conclusion, the optimized mixture of *Syzygium aromaticum*, *Rosmarinus officinalis*, and *Moringa oleifera* extracts exhibited promising bioactive and functional properties, supporting its potential as a natural therapeutic formulation against androgenetic alopecia.

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LIST OF SYMBOLS AND ABBREVIATIONS

Abbreviation	Meaning
AGA	Androgenetic Alopecia
ANOVA	Analysis of Variance
°C	Degree Celsius
DMSO	Dimethyl Sulfoxide
DES	Design Expert Software
DNA	Deoxyribonucleic Acid
FTIR	Fourier Transform Infrared Spectroscopy
GAE	Gallic Acid Equivalent
G	Gram
H_2SO_4	Sulfuric Acid
HCl	Hydrochloric Acid
H	Hour
IR	Infrared
mL	Millilitre
mg/mL	Milligram per Millilitre
mg GAE/g	Milligram Gallic Acid Equivalent per Gram
min	Minute
mV	Millivolt
Nm	Nanometre
OD	Optical Density

Abbreviation	Meaning
R^2	Coefficient of Determination
rpm	Revolutions per Minute
RSM	Response Surface Methodology
UV–Vis	Ultraviolet–Visible Spectroscopy
v/v	Volume per Volume
w/v	Weight per Volume
µL	Microlitre
µm	Micrometre
%	Percentage

CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND OF STUDY

Androgenetic alopecia (AGA), usually referred to as male or female pattern hair loss, is a progressive condition characterized by hair follicle miniaturization driven by genetic susceptibility and androgen (dihydrotestosterone, DHT) mediated effects on susceptible follicles (Choi et al., 2024). AGA negatively affects quality of life, self-image, and psychosocial wellbeing, which has driven interest in safe, affordable, and locally sourced treatments (Choi et al., 2024; Kesika et al., 2023). Current pharmacological options such as topical minoxidil and oral 5α-reductase inhibitors (finasteride) can be effective but are associated with side effects, cost, and limited long-term adherence, motivating exploration of plant-derived alternatives with anti-androgenic, antioxidant and anti-inflammatory activities (Shafqat et al., 2021).

Plant phytochemicals such as phenolics, flavonoids, terpenoids, and phytosterols have been shown to modulate biological pathways relevant to hair biology, including inhibition of 5α-reductase (reducing conversion of testosterone to DHT), attenuation of oxidative stress, and modulation of inflammatory signalling in the follicular microenvironment (Kesika et al., 2023; Shafqat et al., 2021). Recent reviews highlight that certain phytochemicals can act via multiple mechanisms: direct enzyme inhibition, antioxidant scavenging, and improving scalp microcirculation mechanisms that collectively support hair follicle survival and prolong the anagen (growth) phase (Kesika et al., 2023; Shafqat et al., 2021).

Among locally available botanicals, rosemary (*Rosmarinus officinalis*) has attracted clinical interest after a randomized comparative trial showed that rosemary oil produced hair count increases comparable to 2% minoxidil over six months, suggesting clinically meaningful hair-growth effects with a favourable tolerability profile (Panahi et al., 2015). Mechanistic studies implicate rosemary constituents such as rosmarinic acid, carnosic acid and ursolic acid in antioxidant, anti-inflammatory and potential 5α-reductase inhibitory activity which are attributes of direct relevance to AGA treatment (Kesika et al., 2023; Panahi et al., 2015).

Clove (*Syzygium aromaticum*) is rich in eugenol and a spectrum of phenolic acids, which exhibit strong antioxidant and anti-inflammatory properties in vitro and in vivo; eugenol in particular has been widely reviewed for its pharmacological activities including modulation of oxidative stress and inflammation that are implicated in scalp pathology (Nisar et al., 2021). These activities make clove extracts promising candidates for topical formulations aimed at protecting follicles from oxidative damage and inflammatory insult, both contributors to follicle miniaturization in AGA (Kesika et al., 2023; Nisar et al., 2021).

Moringa *(Moringa oleifera*) contains multiple bioactive classes such as flavonoids (quercetin, kaempferol), phenolic acids, and phytosterols such as β-sitosterol that have been associated with antioxidant, anti-inflammatory and putative anti-androgenic effects (Pareek et al., 2023). β-Sitosterol and related plant sterols have been investigated for 5α-reductase inhibitory properties and are proposed as natural alternatives to synthetic inhibitors; thus, moringa's phytosterol content makes it especially relevant to an anti-AGA strategy (Pareek et al., 2023; Shafqat et al., 2021).

Extraction method and extract composition are critical: solvent polarity, extraction technique, and processing conditions substantially influence the yield and profile of phytochemicals

recovered from plant matrices (Fitri et al., 2025). For engineering-oriented projects, optimization of the extraction step (yield, reproducibility, solvent economy) and rigorous characterization of the extract (qualitative and quantitative phytochemical screening, UV-Vis, FTIR) are central deliverables because they determine downstream formulation quality and the plausibility of biological activity claims (Altemimi et al., 2017; Fitri et al., 2025). Recent reviews recommend a workflow that couples reproducible, scalable extraction protocols with targeted analytical characterization and, when feasible, bioactivity correlation (Kesika et al., 2023; Shafqat et al., 2021).

In the Nigerian context, where these plants are widely available and inexpensive, developing validated extraction and characterization workflows for rosemary, clove and moringa could enable low cost, locally sourced topical products for individuals affected by AGA, while also contributing to ethnopharmacological documentation and value-chain development (Pareek et al., 2023). Furthermore, establishing robust, reproducible extraction and analytical procedures (using accessible instrumentation such as UV-Vis and FTIR) strengthens the scientific credibility and future scalability of any promising formulations (Altemimi et al., 2017; Kesika et al., 2023)

This project therefore focuses on extracting and characterizing the bioactive compounds from cloves, rosemary and moringa, optimizing a practical extraction workflow compatible with available laboratory resources, and identifying marker phytochemicals linked in the literature to anti-androgenic, antioxidant and anti-inflammatory mechanisms relevant to androgenetic alopecia. By concentrating on extraction optimization and analytical characterization rather than in-house bioassays, the study stays within a chemical engineering remit (process design

and standardization) while producing data that can support later biological testing and product development.

1.2 STATEMENT OF THE PROBLEM

Androgenetic alopecia (AGA), a progressive form of hair loss characterized by follicular miniaturization, remains a major dermatological and psychosocial concern worldwide. Current pharmacological treatments such as minoxidil and finasteride are limited by adverse effects, poor compliance, and relapse after discontinuation (Adil & Godwin, 2017). Therefore, there is increasing interest in identifying safe, effective, and sustainable plant-based alternatives that can inhibit dihydrotestosterone (DHT) activity and stimulate follicular regeneration.

Several studies have investigated individual medicinal plants such as Moringa, Rosemary, and Cloves for their hair growth-promoting properties due to their rich profiles of phenolic, flavonoid, and terpenoid compounds (Dahiru et al., 2006). However, most of these investigations focus on isolated plant extracts rather than synergistic combinations that could enhance efficacy through multiple bioactive interactions. Furthermore, many existing works provide limited chemical characterization or lack optimization of the extraction process to maximize yield and compound recovery.

Given that extraction is a critical step influencing the quantity and quality of bioactive compounds, the absence of optimized protocols for blended herbal systems represents a major gap in current literature. In addition, the majority of available research emphasizes pharmacological assays rather than process optimization from a chemical engineering perspective. This creates a need to establish an experimentally sound, reproducible extraction

protocol capable of producing a potent multi-plant extract blend for androgenetic alopecia treatment.

This study, therefore, seeks to bridge this gap by developing and optimizing the methanolic extraction of clove (*S. aromaticum*), rosemary (*R. officinalis*), moringa (*M. oleifera*) using a reflux system. The optimized extract will be characterized for its major bioactive constituents relevant to androgenetic alopecia. By focusing on extraction optimization and chemical characterization, this work provides a novel, engineering-oriented contribution toward the development of safe, plant-based anti-alopecia formulations.

1.3 AIM AND OBJECTIVES

AIM OF THE STUDY

The primary aim of this study is to optimize and characterize the methanolic extraction of bioactive compounds from a blended formulation of clove, rosemary, and moringa for potential application in the management of androgenetic alopecia.

OBJECTIVES OF THE STUDY

To achieve the above aim, the study seeks to:

1. Prepare the methanolic extraction of *clove*, *rosemary*, and *moringa* blend using a reflux extraction setup under controlled conditions.
2. Optimize the extraction process by adjusting parameters such as solvent volume, extraction time, and temperature to achieve maximum yield and process efficiency.

3. Characterize functional groups present in the optimized extract using FTIR analysis to identify key chemical bonds associated with bioactive compounds.
4. Quantitatively determine selected bioactive constituents in the extract using UV-Visible spectrophotometry to assess compound concentration and purity.
5. Conduct phytochemical screening of the combined extract to identify the presence of major secondary metabolites such as flavonoids, phenols, terpenoids, and steroids linked to anti-androgenic and hair growth-promoting activity.
6. Evaluate the synergistic potential of the optimized multi-plant extract formulation by relating quantified bioactive compounds to biochemical pathways involved in androgenetic alopecia particularly dihydrotestosterone (DHT) inhibition and follicular stimulation, based on supporting literature evidence.

1.4 SIGNIFICANCE OF THE STUDY

1. Addresses the global rise in androgenetic alopecia (AGA) cases by exploring safer, plant-based alternatives.
2. Synthetic medications like finasteride and minoxidil have adverse side effects such as hormonal imbalance and scalp irritation.
3. Highlights the potential of medicinal plants as eco-friendly and biocompatible treatments for hair loss.
4. Employs a methanol-based reflux extraction technique using chemical engineering principles to optimize yield and reproducibility.
5. Uses Fourier Transform Infrared (FTIR) spectroscopy to identify important functional groups in major bioactive compounds.

6. Detects *eugenol*, *rosmarinic acid*, and *quercetin*, associated with DHT inhibition, antioxidant defense, and scalp microcirculation.
7. Proposes an extraction method that can be scaled industrially, advancing natural and sustainable cosmetic production.
8. Bridges traditional herbal knowledge with modern analytical chemistry through the study of *Syzygium aromaticum*, *Rosmarinus officinalis*, and *Moringa oleifera* for potential AGA therapy.

1.5 SCOPE OF THE STUDY

1. The research aims to explore potential treatments for androgenetic alopecia through the extraction, optimization, phytochemical screening, and spectroscopic characterization of bioactive compounds from *clove*, *rosemary*, and *moringa*.
2. The study emphasizes compound identification and process optimization rather than biological or clinical evaluation.
3. A methanol-based reflux extraction method is utilized to ensure efficient and repeatable yields.
4. Extraction is conducted under defined conditions of time, temperature, and solvent volume to maintain consistency and reproducibility.
5. Qualitative phytochemical tests confirm the presence of phenols, flavonoids, terpenoids, and steroids compounds linked to:
    - 5α-reductase inhibition
    - Antioxidant activities
    - Scalp stimulation

6. Fourier Transform Infrared (FTIR) analysis identifies key functional groups supporting the presence of active bioactive compounds.
7. The research excludes bioassays and clinical testing, focusing only on laboratory-scale chemical analysis.
8. The findings provide a foundation for future studies involving biological validation and formulation of standardized, plant-based hair growth products.

1.6 LIMITATIONS OF THE STUDY

1. The study was limited to extraction, phytochemical screening, and FTIR characterization of *clove*, *rosemary*, and *moringa* extracts.
2. Lack of GC–MS and HPLC analyses restricted detailed profiling and quantification of phytochemicals.
3. Due to financial and logistical limitations, no in vitro or in vivo assays were performed, preventing confirmation of biological efficacy.
4. Using only methanol under reflux improved reproducibility but limited extraction diversity across different polarities.
5. Reliance on commercially obtained dried plant materials may have introduced variability in phytochemical content.
6. Limited laboratory equipment prevented complementary analyses such as antioxidant and DHT-inhibition testing.
7. The study lays groundwork for subsequent studies focusing on biological validation, formulation development, and optimization of the characterized plant extracts.

CHAPTER TWO

LITERATURE REVIEW

2.1 INTRODUCTION

Hair loss, also known as alopecia, is a major condition that affects people around the world. It leads to psychological distress and reduced quality of life. Although conventional treatments like minoxidil and finasteride are available, they can come with side effects and may lose effectiveness over time. As a result, there has been growing interest in alternative therapies, especially those derived from plants, due to their perceived safety and holistic benefits (Choi et al., 2024).

Recent reviews highlight the potential of plant extracts to prevent hair loss and support hair growth. These natural products contain bioactive compounds such flavonoids, terpenoids, and phenolic acids, which have antibacterial, anti-inflammatory, and antioxidant qualities. Choi et al. (2024) have noted that several plant extracts have demonstrated effectiveness in promoting hair growth by extending the anagen phase of the hair growth cycle and encouraging the proliferation of dermal papilla cells.

The extraction of these bioactive compounds is a critical step in utilizing their medicinal potential (Sasidharan et al., 2010). Traditional methods that use organic solvents can be less effective and harmful to the environment. However, modern techniques like supercritical fluid extraction, ultrasound-assisted extraction, and microwave-assisted extraction are being increasingly employed. These methods do not only enhance the purity and yield but also support sustainable practices.

Characterization of the extracted compounds is equally important to identify the key active ingredients responsible for promoting hair growth (Sasidharan et al., 2010). Techniques such as Fourier-transform infrared (FTIR) spectroscopy, high-performance liquid chromatography (HPLC), and gas chromatography-mass spectrometry (GC-MS) are commonly used for this purpose (Sasidharan et al., 2010). These analytical methods help in understanding the chemical composition of the extracts, facilitating the standardization and quality control of plant-based formulations for hair growth.

This chapter reviews extraction and characterization methods used to isolate these bioactive compounds and examines their mechanisms in promoting hair regeneration. It also identifies key areas that need further exploration, such as the underutilization of specific indigenous plant species, the synergistic effects of combined plant extracts, the need for standardized extraction procedures, and thorough characterization of active components. These areas highlight the significance of this study, which aims to optimize plant-based treatments for alopecia by addressing the gaps and improving their safety, effectiveness, and scientific validation (Choi et al., 2024).

2.2 HAIR FOLLICULAR BIOLOGY

Serving as the primary defense against external environmental stressors, the skin is structured into three distinct layers. The outermost layer, the epidermis, is made up of continuously renewing keratinocytes that migrate outward and differentiate into dead, cornified cells, forming a protective barrier against environmental damage and excessive moisture loss. Beneath the epidermis lies the dermis, which houses various skin appendages such as

sebaceous glands, hair follicles, eccrine glands, and apocrine sweat glands; the nails, located at the tips of the digits, originate from this layer. The innermost layer, known as the subcutaneous tissue, is composed mainly of adipose and connective tissues that provide insulation and anchor the skin to underlying structures. This layer contains blood vessels and nerves that supply capillaries and nerve endings extending into the dermis, where they interact with the skin appendages (Lin et al., 2022; Stephens & Fox, 2024).

Hair follicles are among the most essential skin appendages and play a critical role in maintaining skin function as well as supporting skin regeneration processes (Lin et al., 2022). In mammals, the hair follicle represents a specialized skin structure that functions as a mini-organ formed through dynamic interactions between the epidermal and dermal layers. Composed of multiple components with intricate microstructures, hair follicles exhibit a remarkable capacity for self-renewal and undergo a cyclical pattern of growth that persists throughout the lifespan of mammalian organisms (Lin et al., 2022). They are enriched with diverse stem cell populations that not only drive hair growth and regeneration but also actively participate in skin repair following injury. Consequently, hair follicles serve as valuable models for investigating tissue regeneration and systems biology (Ma et al., 2017). The growth of hair follicles and the activity of their resident stem cells are tightly controlled by multiple signalling pathways. Additionally, hair growth is influenced by factors such as climate, age, environmental conditions, and overall health status, which may also affect the development of hair follicle tumours, alopecia areata, and other associated disorders (Lin et al., 2022).

2.2.1 STRUCTURE OF HAIR FOLLICLES

As the largest organ in the human body, the skin is primarily composed of epidermis and dermis (Souto et al., 2022). The epidermis is further organized into several sublayers which, from the outermost to the deepest, include the stratum corneum, stratum lucidum, stratum granulosum, stratum spinosum, and stratum basale. Located directly beneath the stratum basale is the dermis, which is subdivided into the papillary and reticular layers. Beneath the dermis lies the subcutaneous tissue, also known as the hypodermis, which mainly consists of loose connective tissue and adipose tissue. Although hair follicles vary in size and shape depending on their anatomical location, they share a common fundamental structure (Morita et al., 2021). Hair follicles are oriented obliquely within the skin. On the obtuse side of the follicle, a bundle of smooth muscle known as the arrector pili muscle (APM) connects the hair follicle to the papillary dermis. The APM is innervated by the sympathetic nervous system, and its contraction causes hair erection and stimulates secretion from the associated sebaceous gland (SG). Structurally, the hair follicle is divided into three regions from top to bottom: the infundibulum, isthmus, and inferior segment, which includes the suprabulbar region and the bulb. The infundibulum extends from the follicular opening to the opening of the SG, while the isthmus spans from the SG opening to the attachment site of the APM. Below the isthmus lies the suprabulbar region, beginning at the APM insertion and ending in the enlarged, spherical bulb (Carrasco et al., 2019).

The cellular composition of the upper portion of the hair follicle, comprising the infundibulum and isthmus, remains relatively stable. However, the isthmus contains a population of stem cells that play a role in epidermal regeneration during wound healing. Research has identified $Gli1^{+}Lgr6^{+}$ cells within the isthmus that contribute to wound epithelium formation and serve

as a source of long-term epithelial progenitors in repaired epidermis (Huang et al., 2012; Snippert et al., 2010). Hair follicle stem cells (HFSCs) exhibit classic stem cell properties, demonstrate high proliferative capacity, and are essential for sustaining hair growth and regeneration. Evidence suggests that periodic hair follicle growth depends on the preservation of HFSCs, which participate in hair follicle formation, maintenance of SGs, and renewal of the epidermis (Hsu et al., 2011). For instance, krt15^{+} HFSCs located in the bulge region can generate progeny that rapidly repopulate wound epithelium and facilitate epidermal repair (Yu et al., 2020). Additionally, studies have shown that adipogenesis in vivo is synchronized with HFSC activation, with subcutaneous adipose precursor cells reaching peak numbers during periods of HFSC activity (Festa et al., 2011). In contrast, the lower region of the hair follicle exhibits greater structural variability and includes differentiated epithelial cells, the hair matrix, and the dermal papilla (DP) (O'Sullivan et al., 2021). The bulb, situated at the base of the follicle, serves as the primary site of active hair growth. Hair follicles are embedded obliquely within the dermis, which plays a crucial role in providing nutrients required for follicular growth and development (Lee et al., 2020). The dermal papilla appears as an upward-directed indentation at the base of the bulb and is formed by connective tissue invagination, containing an abundant network of capillaries and nerve endings (Park et al., 2018).

The dermal papilla (DP) provides essential nutritional support required for hair growth and follicular maintenance. It is a multicellular structure formed by the aggregation of dermal cells and plays a central role in initiating and regulating hair growth (Ge et al., 2020). Dermal stem cells, a population of skin stem cells with self-renewing capacity, reside within the dermal cup and lower dermal sheath; during the hair cycle, these cells regenerate the dermal sheath and replenish the DP cell population (Rahmani et al., 2014). Hair follicle injury has been shown to enhance the recruitment of dermal stem cell progeny, which subsequently differentiate into DP

cells (Sparks et al., 2019). Hair matrix cells are positioned above and along the lateral aspects of the DP, with melanocytes scattered among them. Through interactions with adjacent hair matrix cells, dermal papilla cells (DPCs) stimulate the formation of epithelial structures, including the inner root sheath (IRS) and the medulla of the hair shaft (Limbu & Higgins, 2020). From the upper region of the bulb toward the upper portion of the follicle, the hair follicle exhibits a concentric, layered organization consisting of three components arranged from the center outward: the hair shaft (HS), the IRS, and the outer root sheath (ORS). The HS, which emerges above the skin surface, is composed primarily of keratinocytes and consists of the medulla, cortex, and cuticle layers arranged from inner to outer regions (Watanabe et al., 2021). The IRS is organized into the IRS cuticle, Huxley's layer, Henle's layer, and the companion layer from inside to outside (Watanabe et al., 2021). The ORS originates from the Malpighian layer of the epidermis (Nilforoushzadeh et al., 2020). The IRS and ORS constitute the epithelial sheath, representing the epidermal component of the hair follicle. Surrounding this is the connective tissue sheath, also referred to as the dermal sheath, which is derived from the mesenchymal dermal component of the follicle and is composed of three layers of collagen fibers oriented in different directions (Martino et al., 2021). This connective tissue sheath provides essential structural support for the maintenance and regeneration of the dermal papilla and is indispensable for hair follicle regeneration (Heitman et al., 2020). SGs are situated within the dermis, and their ducts open into the hair follicle between the isthmus and the infundibulum (Geueke & Niemann, 2021).

2.2.2 HAIR GROWTH CYCLE

Hair follicles undergo continuous and orderly growth cycles throughout the lifespan, characterized by cyclical changes that primarily involve alterations in the morphology and

structure of the dermal papilla at the base of the follicle, the generation of new HSs, and the shedding of aged hair. This cyclical process is conventionally classified into three distinct phases: anagen, catagen, and telogen (Shin et al., 2020). Under normal physiological conditions, the duration of each phase is relatively stable and tightly regulated. For instance, in C57BL/6 mice, the timing of anagen, catagen, and telogen phases is highly predictable, with newborn mice entering catagen during the second postnatal week, telogen in the third week, and anagen in the fourth week (Chen et al., 2020). In adult human scalp hair, the anagen phase typically persists for approximately three years, followed by a catagen phase lasting about three weeks and a telogen phase of roughly three months (Grymowicz et al., 2020; Oh et al., 2016). Nevertheless, the progression and duration of these stages can be influenced by a range of factors, including environmental conditions, sex-related factors, genetic background, nutritional status, and other physiological variables (Müller-Röver et al., 2001). Variations in hair growth cycles have been observed among different mouse strains, and in C57BL/6 mice, skin pigmentation changes in accordance with specific stages of the hair follicle cycle. External factors such as temperature and light exposure also affect follicular growth; notably, red light at a wavelength of 650 nm has been shown to stimulate proliferation of human hair follicle cells and significantly delay the transition from anagen to telogen (Yang et al., 2021). Sex-related influences on the hair growth cycle are largely mediated by hormonal regulation, with androgens exerting a particularly strong effect on hair growth and cycling dynamics (Grymowicz et al., 2020). Also, the maintenance of a normal hair growth cycle depends on adequate nutritional support and proper regulation by the peripheral nervous system surrounding the hair follicles (J. Zhang et al., 2021).

2.2.2.1 ANAGEN

The anagen phase represents the most biologically active stage of the hair follicle cycle, during which rapid hair growth occurs and a fully developed HS is produced (Suen et al., 2020). The beginning of anagen is initiated by the proliferation of secondary hair germ cells in proximity to the dermal papilla (DP), followed by the downward extension of the hair follicle into the subcutaneous tissue. During this phase, bulb cells undergo vigorous proliferation, while cells of the HS and inner root sheath (IRS) begin to differentiate, accompanied by increases in the size and structural complexity of both the dermal papilla cells (DPCs) and the bulb. From a histological perspective, anagen hair follicles appear elongated and straight, with an oblique orientation that allows the hair shaft to lie flat against the skin surface. Keratinocyte progenitor cells within the hair matrix migrate upward along the follicle and differentiate into HS and IRS lineages. As HS cells undergo terminal differentiation, they associate tightly with cysteine-rich hair keratins to form filamentous bundles approximately 10 nm in diameter, conferring the characteristic tensile strength and elasticity of the hair shaft. The IRS also undergoes keratinization, providing structural support and directional guidance for the developing HS during its differentiation. Throughout anagen, highly proliferative stromal cells exhibit a cell cycle duration of approximately 18 hours (Harland, 2018). The overall length of the hair is determined by the duration of this growth phase, which depends on sustained proliferation and differentiation of stromal cells at the base of the hair follicle (Morgun & Vorotelyak, 2020).

2.2.2.2 CATAGEN

When hair follicles transition into the catagen phase, several characteristic changes occur, including cessation of HS elongation, a marked reduction in cellular proliferation and differentiation, the initiation of apoptosis, and rapid follicular regression. Programmed cell death predominantly affects epithelial cells of the hair matrix and the ORS, while the DP undergoes a noticeable reduction in size (Nicu et al., 2020). In contrast, DPCs exhibit resistance to apoptosis, largely due to their expression of the anti-apoptotic protein BCL-2 (Nan et al., 2020). The degenerative events of catagen are highly regulated, with extensive apoptosis occurring among keratinocytes within the hair follicle (Bak Ho et al., 2020). During this stage, melanogenesis ceases, and melanocytes in certain follicles also undergo apoptotic cell death(Bejaoui et al., 2020). By the end of catagen, the hair follicle becomes markedly atrophic, and DPCs condense and migrate upward toward the lower region of the bulge. Failure of DPCs to reposition beneath the bulge during catagen disrupts subsequent cyclic growth of the follicle, ultimately leading to hair loss, a phenomenon observed in both humans and mice carrying hair loss–associated gene mutations (Y. Zhang et al., 2021). In humans, the initial entry into catagen occurs during foetal development, whereas in mice, this transition typically takes place approximately 17 days after birth (Paus & Foitzik, 2004).

2.2.2.3 TELOGEN

Following the catagen phase, the hair follicle transitions into telogen, a stage characterized by minimal biological activity and the shedding of the HS. Despite this reduced activity, the expression and function of key regulatory factors involved in controlling the follicular growth

cycle are markedly upregulated in preparation for the subsequent anagen phase. During telogen, DPCs relocate to the lower region of the bulge, enabling direct interaction with bulge-resident stem cells. These DPCs play a crucial role in activating stem cells and triggering the initiation of a new hair growth cycle. Once activated, HFSCs undergo proliferation, and upon reaching a critical population threshold, the follicle re-enters the anagen phase (H. Kim et al., 2022). In murine hair cycles, the initial telogen phase is extremely brief, lasting only one to two days, whereas the second telogen phase extends for more than two weeks and begins approximately 42 days after birth (Hwang et al., 2021).

2.3 UNDERSTANDING ALOPECIA

2.3.1 DEFINITION OF ALOPECIA

Alopecia is a disorder characterized by the loss of hair from the scalp or other areas of the body where hair normally grows (Rambwawasvika, 2021). This distressing condition often results in reduced self-esteem and has significant psychological and social impacts on affected individuals(Upton et al., 2015). Alopecia presents in several forms, with androgenetic alopecia (pattern baldness), alopecia areata, and chemotherapy-induced alopecia being the most prevalent types (Falto-Aizpurua et al., 2014). The etiology of alopecia is multifactorial and includes factors such as stress, genetic predisposition, hormonal imbalance, nutritional deficiencies, underlying illnesses, and the use of certain medications, particularly those employed in cancer therapy (Biran et al., 2015; Hagenaars et al., 2017). Although only two drugs, finasteride and minoxidil, have been approved by the U.S. Food and Drug Administration for the treatment of alopecia, numerous unapproved agents are promoted as hair loss remedies (Khidhir et al., 2013). However, many of these products lack convincing

evidence from controlled scientific studies, limiting their acceptance and commercial application. Hair growth occurs in a cyclical pattern consisting of four distinct phases: anagen, catagen, telogen, and exogen These phases repeat continuously as long as hair follicles retain their ability to generate hair. The anagen phase, which represents the most active stage of hair growth, typically lasts between two and seven years, with approximately 90% of hair follicles on a healthy scalp existing in this phase (Rambwawasvika, 2021). With successive cycles, the duration of anagen gradually shortens, leading to the production of finer and weaker vellus hair. The phases following anagen are associated with hair regression and are comparatively shorter in a healthy scalp (Geyfman et al., 2015).

In general, all types of alopecia disrupt the normal hair growth cycle and result in hair loss through two primary mechanisms. The first involves a reduction in the duration of the anagen phase, a hallmark of androgenetic alopecia, which decreases the normal anagen-to-telogen ratio from approximately 6:1 to as low as 2:1, thereby prolonging the telogen phase (Orasan et al., 2016). The second mechanism is the reduction in the size of the dermal papilla, a structure essential for hair follicle growth and cellular differentiation through nutrient delivery. This reduction is often caused by vasoconstriction of blood vessels that supply oxygen and nutrients to the hair follicle (Orasan et al., 2016). Consequently, hair fibres undergo changes in both diameter and appearance, transitioning abruptly from thick, pigmented hair to thin, depigmented vellus hair.

2.3.2 TYPES OF ALOPECIA

Alopecia encompasses a wide range of forms, reflecting the diverse etiological factors underlying the condition. Common variants include androgenetic alopecia, alopecia areata,

chemotherapy-induced alopecia (CIA), anagen effluvium, telogen effluvium, traction alopecia, and trichotillomania. Broadly, alopecia can be categorized into two main groups: scarring alopecia, which results from inflammatory responses that damage hair follicles, and the more prevalent non-scarring alopecia, which arises from factors such as hormonal imbalance, medication use, nutritional deficiencies, and certain medical conditions (Rambwawasvika, 2021).

2.3.2.1 ANDROGENETIC ALOPECIA

Androgenetic alopecia, commonly referred to as pattern baldness, affects both males (male pattern baldness) and females (female pattern baldness). The condition is more prevalent in men than in women, largely due to higher levels of the male sex hormone testosterone in males. In women, androgenetic alopecia manifests differently, typically presenting as diffuse thinning of scalp hair rather than the frontal hairline recession observed in men (Semalty et al., 2011). Testosterone is metabolized in the gonads and other organs, including the liver and brain, by the enzyme 5-alpha reductase to form dihydrotestosterone (DHT), a more potent androgen. Approximately 10% of circulating testosterone is converted into DHT (Dhariwala & Ravikumar, 2019). DHT, like testosterone, is a steroid hormone but exerts a stronger effect on hair follicles due to its higher binding affinity for androgen receptors, remaining bound for approximately 53 minutes compared to 35 minutes for testosterone. The interaction of androgens with hair follicle receptors leads to progressive follicular miniaturization and weakening, ultimately resulting in the destruction of follicular cells. This androgen–follicle interaction shortens the anagen phase while extending the telogen phase of the hair growth cycle. Androgenetic alopecia affects approximately 70% of men and about 40% of women over

their lifetime (Urysiak-Czubatka et al., 2014). Men who do not experience baldness generally exhibit lower levels of 5-alpha reductase activity. Additionally, androgenetic alopecia is more common among individuals of Caucasian descent compared to other ethnic groups. Variations in prevalence across gender and race are presented in Table 2.1 (Dhariwala & Ravikumar, 2019; Kaliyadan et al., 2013; Kelly et al., 2016).

Table 2.1: Prevalence of Androgenic Alopecia Across Gender and Race

RACE	African	Asian	Caucasian	Chinese
MALE %	14.5	60.0	79.9	21.4
FEMALE %	3.5	-	45.0	6.0

2.3.2.2 ALOPECIA AREATA

Alopecia areata is a non-scarring autoimmune disorder that causes hair loss on the scalp or other hair-bearing areas of the body (Rambwawasvika, 2021). The exact etiology of the condition remains unclear, although some evidence suggests it may involve a hair follicle-specific immune response mediated by lymphocytes (Broadley & McElwee, 2020). Genetic predisposition and environmental factors have also been implicated in disease onset (Simakou et al., 2019). Clinically, alopecia areata is marked by the appearance of discrete, patchy bald areas, most commonly on the scalp, which may enlarge if the condition is untreated. Both males and females are affected equally, and the disorder is frequently observed in infants (Alsantali, 2011).

Severe manifestations include alopecia totalis, characterized by complete scalp hair loss, and alopecia universalis, which involves the loss of hair across the entire body. Typically, alopecia areata does not destroy hair follicle cells, allowing for potential hair regrowth following resolution of immune activity. Various interventions, including zinc, corticosteroids, dithranol, tretinoin, azelaic acid, systemic cortisone, minoxidil, and immunosuppressive therapies, have been employed with limited success, indicating a continued need for effective treatments. Additional contributing factors include genetics, stress, medications, hormonal imbalances, post-illness complications, systemic disorders, local skin conditions, and nutritional deficiencies (Rambwawasvika, 2021).

2.3.2.3 CHEMOTHERAPY INDUCED ALOPECIA

Hair loss is a well-known and distressing adverse effect of chemotherapy, sometimes causing patients to delay or refuse treatment (Ishida et al., 2015). Chemotherapeutic agents target rapidly dividing cells, not only malignant ones, which results in the destruction of rapidly proliferating hair matrix keratinocytes in the anagen phase (Keum et al., 2016). Hair matrix cells in anagen are among the fastest dividing cells in the body, with approximately 60% remaining in the S (synthesis) phase. Chemotherapy triggers premature entry of anagen follicles into a dystrophic catagen phase, leading to hair breakage and shedding (Yeager & Olsen, 2011).

Following chemotherapy, about 90% of hairs in anagen transform to telogen and shed as club hairs without immediate replacement, lengthening the telogen phase and shortening anagen in the hair cycle (Rambwawasvika, 2021). In most cases, hair regrowth occurs once treatment ceases, as follicular stem cells are generally preserved and capable of regenerating new hair. Maintaining follicle integrity during chemotherapy would require strategies such as telogen

arrest and catagen inhibition, potentially through agents that block keratinocyte apoptosis. Unfortunately, no clinically approved therapies currently exist for this purpose, highlighting the need for approaches that protect hair during chemotherapy (Keum et al., 2016; Rambwawasvika, 2021).

2.3.2.4 ANAGEN EFFLUVIUM

Anagen effluvium refers to the abrupt loss of hair in the anagen phase (anagen arrest) (Kanwar & Narang, 2013). Similar to chemotherapy-induced alopecia, it can be triggered by chemotherapeutic agents or other medications (Ghias et al., 2020).). The condition results in the prolonged retention of hair in the telogen phase for the duration of treatment. Medications such as anticoagulants, oral contraceptives, diuretics, and drugs for acne have also been implicated in its onset (Rambwawasvika, 2021). While hair regrowth is typically observed within three months, in some cases, the condition may become irreversible, causing psychological distress due to its impact on appearance (Phillips et al., 2017).

2.3.2.5 TELOGEN EFFLUVIUM

Telogen effluvium is defined as non-scarring hair loss resulting from the premature transition of hair follicles into the resting (telogen) phase (Phillips et al., 2017). It is commonly observed in older adults, individuals experiencing physical or emotional stress, and patients with thyroid or other hormonal disorders (Fatani et al., 2015; Jain et al., 2017). The severity of shedding depends on the intensity and duration of exposure to the triggering factor rather than the specific agent. Telogen effluvium may present as acute (less than six months), chronic (more than six months), or recurrent chronic forms (Fatani et al., 2015).

2.3.2.6 TRACTION ALOPECIA

Traction alopecia is primarily caused by mechanical stress on the hair, leading to breakage (Billero & Miteva, 2018). Middle-aged women are disproportionately affected due to frequent styling practices intended to maintain a polished appearance. Hairstyles such as tight braids, ponytails, and repeated chemical treatments like hair bleaching or dyeing are the most common contributing factors (H.-S. Kim et al., 2019). Traction alopecia occurs across diverse populations and is largely influenced by personal hair care and styling habits.

2.3.2.7 TRICHOTILLOMANIA

Trichotillomania is characterized by repetitive self-pulling of hair from the scalp, resulting in hair loss or follicular damage. It is most frequently observed in children or individuals with psychiatric disorders Management typically involves psychiatric intervention in adults, while in children, the behaviour may resolve naturally with age (Falkenstein et al., 2016).

2.3.3 CAUSES OF ALOPECIA

According to Rajabi et al. (2018) and Sankhwar & Khan, (2025), alopecia arises from a variety of causes, which can be categorized as follows:

- Genetic factors: Androgenetic alopecia is largely hereditary and influenced by follicular sensitivity to androgens, particularly dihydrotestosterone (DHT).
- Autoimmune mechanisms: In alopecia areata, the body's immune system erroneously targets hair follicles, resulting in patchy hair loss.
- Hormonal fluctuations: Hair loss may be triggered by physiological changes such as pregnancy, menopause, thyroid dysfunction, and other hormonal imbalances.

- Physical or psychological stress: Events such as major surgery, systemic illness, abrupt weight loss, childbirth, or emotional stress can induce telogen effluvium.
- Medications and medical interventions: Chemotherapy, radiation, certain pharmaceutical agents (e.g., corticosteroids, antidepressants), and systemic conditions (e.g., lupus, renal failure) may lead to hair loss.
- Nutritional deficiencies: Insufficient intake of essential nutrients, including iron, zinc, and protein, can contribute to hair thinning.
- Infections and dermatological disorders: Scalp infections such as tinea capitis, psoriasis, and other skin conditions can cause localized alopecia.
- Mechanical or chemical trauma: Excessive hairstyling, heat exposure, harsh chemical treatments, and tightly pulled hairstyles may result in traction alopecia or structural hair shaft damage.

2.3.4 PREVALENCE AND IMPACT ON INDIVIDUALS

Gupta et al. (2025) reported that alopecia is a prevalent condition globally. Androgenetic alopecia affects approximately 50% of men and women by the age of 50, increasing to nearly 80% by age 70. The lifetime risk of alopecia areata is around 2%, and it can manifest in individuals of any age, sex, or ethnic background. Central centrifugal cicatricial alopecia is particularly prevalent among Black women. The psychological and social impacts of alopecia are considerable, as the loss of hair can profoundly affect self-perception. Gandhi et al. (2023) observed that patients with alopecia areata often experience a range of psychological symptoms, including depression, anxiety, anger, social withdrawal, embarrassment, and reduced self-esteem.

2.3.5 CONVENTIONAL TREATMENTS AND THEIR LIMITATIONS

Rambwawasvika (2021) highlighted various conventional approaches for managing alopecia:

2.3.5.1 MINOXIDIL (ROGAINE)

Minoxidil, commercially known as Rogaine, is the only FDA-approved topical agent for alopecia. Initially developed as an oral antihypertensive, its hair growth-promoting effect was discovered incidentally (Rambwawasvika, 2021; Rossi, Mari, et al., 2012).). Minoxidil is typically formulated as a 5% solution for men and 2% for women. Although its precise mechanism in promoting hair regrowth remains unclear, it is recognized as a potassium channel opener that hyperpolarizes cell membranes, inducing vasodilation, angiogenesis, and enhanced nutrient and oxygen delivery to hair follicles (Maekawa et al., 2019; McElwee & Shapiro, 2012). Minoxidil also facilitates the transition of follicles from telogen back to anagen and may prolong the active growth phase (Rambwawasvika, 2021). Side effects include scalp irritation, dryness, itching, flaking due to excipients like alcohol or propylene glycol (Rossi, Cantisani, et al., 2012), and hypertrichosis in unintended areas due to systemic absorption or improper application (Majzoub & Moris, 2025).

2.3.5.2 FINASTERIDE (PROPECIA)

Finasteride, a synthetic 4-azasteroid compound, is another FDA-approved medication for alopecia. Like minoxidil, its hair growth effects were discovered accidentally during its initial use for treating benign prostatic hyperplasia (Lucia et al., 2007). Finasteride is primarily prescribed for men, as it inhibits 5-alpha reductase, preventing the conversion of testosterone to dihydrotestosterone (DHT), which is implicated in follicular miniaturization. Its efficacy is

greatest when administered early, before permanent follicular damage occurs. Use in women, particularly during pregnancy, is contraindicated due to teratogenicity (Kaur et al., 2013).

Finasteride can slow hair loss and stimulate regrowth, but it does not address the genetic basis of androgenetic alopecia, so hair loss may recur. Adverse effects include sexual dysfunction such as decreased libido, erectile difficulties, and reduced ejaculation volume (Irwig & Kolukula, 2011), with some patients experiencing persistent symptoms post-treatment, referred to as post-finasteride syndrome. Psychological effects, including mood changes, have also been reported, although causal links remain uncertain (Pompili et al., 2021).

2.3.5.3 SURGICAL HAIR TRANSPLANT

Hair transplantation involves relocating hair-bearing scalp tissue from donor areas to balding regions, primarily to treat male pattern baldness (McElwee & Shapiro, 2012; Olsen et al., 2005). Donor sites are typically the temporal and occipital regions, which are less sensitive to androgens. Common techniques include follicular unit strip surgery (FUSS) and follicular unit extraction (FUE) (Leavitt et al., 2005; Navarro et al., 2018). The procedure requires skilled surgeons, and success is not guaranteed, as transplanted follicles may eventually enter telogen. Risks include excessive bleeding, infection, and postoperative pain, which may deter patients, highlighting the need for alternative therapeutic strategies (Perez-Meza & Niedbalski, 2009; Rambwawasvika, 2021).

2.3.5.4 PSYCHOLOGICAL AND BEHAVIORAL THERAPY

Patients with alopecia may require counselling to manage the psychosocial burden of hair loss, including low self-esteem and, in severe cases, suicidal ideation (Lemieux et al., 2008). Counselling is particularly important for adolescents and young women, for whom hair loss

may impact perceptions of sexuality and social acceptance (Li et al., 2018). Studies have shown that chemotherapy patients experiencing hair loss exhibit increased stress, poor body image, and reduced self-esteem compared with those without alopecia (Lemieux et al., 2008; Rivitti, 2005). Psychological support, coupled with behavioural interventions such as wigs, scalp tattoos, or hats, can improve coping and quality of life (Strazzulla et al., 2018).

2.4 MEDICINAL PLANTS FOR HAIR GROWTH

2.4.1 CLOVES (*Syzygium aromaticum*)

Cloves have abundant antioxidants, particularly eugenol, which neutralize free radicals and mitigate oxidative stress. Oxidative stress can harm hair follicles, contributing to hair thinning and loss. The antioxidant content of cloves may help prevent hair loss and premature greying by protecting follicles from oxidative damage (Chen et al., 2023). Eugenol and other bioactive compounds in cloves may improve scalp blood circulation, enhancing delivery of oxygen and essential nutrients to hair follicles, which is critical for healthy hair growth; however, direct evidence linking cloves to increased scalp perfusion remains limited (Cintya et al., 2025). Cloves also contain potent anti-inflammatory compounds, including eugenol and gallic acid, which may alleviate chronic scalp inflammation associated with conditions such as seborrheic dermatitis, dandruff, and psoriasis, all of which can impair hair growth (Pandey et al., 2024). Additionally, cloves exhibit antifungal and antibacterial properties, potentially preventing scalp infections caused by pathogens like Candida and *Tinea capitis*, which can hinder hair growth and contribute to hair loss (Chen et al., 2023). Eugenol, a major constituent of clove essential oil, has demonstrated androgenic activity and may stimulate hair follicle roots, making it a promising agent for anti-hair loss formulations (Shahtalebi et al., 2016).

2.4.2 MORINGA (*Moringa oleifera*)

Moringa oleifera seed oil has been shown to promote hair growth in animal models. It modulates the expression of genes involved in hair growth regulation, including upregulation of VEGF and downregulation of TGF-β1 and 5α-reductase, both of which influence the hair growth cycle and hair loss (Junlatat & Sripanidkulchai, 2022). In rabbits with DHT-induced alopecia, moringa seed oil demonstrated anti-alopecia effects, with higher concentrations producing increased hair length and weight comparable to finasteride, a standard treatment (Korassa et al., 2023). Moringa is also rich in antioxidants, such as quercetin, vitamin C, and beta-carotene, which scavenge reactive oxygen species and protect cells from oxidative damage. Although certain studies focus on ototoxicity, the general antioxidant properties are relevant to hair follicle health and cellular integrity (Broderick et al., 2021).

2.4.3 ROSEMARY (*Rosmarinus officinalis*)

Rosemary leaf extract has been reported to inhibit testosterone 5α-reductase, the enzyme responsible for converting testosterone to DHT, a key androgen in androgenic alopecia. Inhibition of this enzyme helps reduce DHT levels, supporting hair regrowth (Murata et al., 2013). The extract, particularly its active compound 12-methoxycarnosic acid, can also prevent DHT from binding to androgen receptors, enhancing its anti-androgenic effect (Murata et al., 2013). Experimental studies, including randomized comparative trials, have demonstrated that topical rosemary oil can stimulate hair growth in animal models, with some evidence suggesting effects comparable to minoxidil, a conventional hair loss therapy (Hashem et al., 2024; Murata et al., 2013). Rosemary also possesses antioxidant activity, protecting hair follicles from oxidative stress, and is thought to improve scalp vascularity, thereby promoting follicular regeneration and overall hair growth (Murata et al., 2013).

2.5 BIOACTIVE COMPOUNDS

Bioactive compounds are chemical constituents present in small quantities in plants and certain foods, which have been shown to exert a variety of beneficial effects on human health. Often referred to as phytochemicals or secondary metabolites, these substances are not essential for fundamental nutritional requirements but play significant roles in disease prevention and health promotion. They largely account for the therapeutic properties of many plants, including those utilized for hair loss management. This section examines the nature and significance of bioactive compounds (Teodoro, 2019).

Bioactive compounds are naturally occurring plant-derived molecules capable of interacting with cells, tissues, or organisms to elicit specific biological responses (Gonçalves et al., 2026). Unlike primary metabolites, such as carbohydrates, proteins, and lipids, that are directly involved in plant growth and development, bioactive compounds are often produced as defence mechanisms against pathogens, herbivores, and environmental stress. Their structural diversity enables them to exert various physiological effects in the human body (Chen et al., 2022).

2.5.1 MAJOR CLASSES OF PLANT BIOACTIVE COMPOUNDS

Bioactive compounds can be broadly classified into several major categories, each exhibiting distinct chemical structures and biological activities:

- Polyphenols:

This group is defined by multiple phenolic hydroxyl groups and is abundant in fruits, vegetables, cereals, teas, and spices. They include flavonoids (e.g., anthocyanins, flavanones,

flavanols, isoflavones, catechins), phenolic acids (e.g., chlorogenic acid, caffeic acid), stilbenes (e.g., resveratrol), and lignans. Polyphenols possess antioxidant, anti-inflammatory, anticancer, and cardioprotective properties, influencing enzyme activity, receptor signalling, and gene expression, thereby contributing to the prevention of chronic conditions such as cardiovascular disease, diabetes, and cancer (Chen et al., 2022). For instance, flavonoids like quercetin promote wound healing by enhancing collagen synthesis and reducing inflammation (Sharma et al., 2021).

- Terpenoids:

Terpenoids are a diverse class of compounds constructed from five-carbon isoprene units and are responsible for the characteristic aromas of many plants. This class includes monoterpenes (e.g., eugenol in cloves, 1,8-cineole in rosemary), diterpenes, triterpenes, and carotenoids. They demonstrate antioxidant, anti-inflammatory, antimicrobial, and anticancer activities (Chen et al., 2022). Carotenoids, such as beta-carotene, lutein, and zeaxanthin, are notable for their antioxidant function and roles in vision and immune health. Regarding hair loss, terpenoids like eugenol show androgenic activity, and rosemary diterpenes (e.g., carnosic acid, carnosol) can inhibit 5α-reductase (Murata et al., 2013).

- Alkaloids:

These nitrogen-containing compounds possess complex and diverse structures and often exhibit potent pharmacological activities. Examples include berberine, caffeine, and nicotine, which display analgesic, anti-inflammatory, antimicrobial, and anticancer effects (Chen et al., 2022).

- Glucosinolates:

Sulphur-rich compounds predominantly found in cruciferous vegetables (e.g., broccoli, cauliflower). Their hydrolysis products are associated with anticancer properties (Baldelli et al., 2025).

- Saponin:

Glycosides that form foam in aqueous solutions. Some saponins exhibit anti-inflammatory, cholesterol-lowering, and anticancer effects (Chen et al., 2022).

2.5.2 ROLE OF BIOACTIVE COMPOUNDS IN HUMAN HEALTH

Plant bioactive compounds contribute to human health through multiple mechanisms, including:

- Antioxidant Activity: Neutralizing free radicals and reducing oxidative stress, which can damage cells and contribute to aging and chronic diseases, including hair follicle damage (Chen et al., 2022).
- Anti-inflammatory Effects: Modulating inflammatory pathways to reduce chronic inflammation linked to various conditions (Chen et al., 2022).
- Antimicrobial Activity: Inhibiting the spread of bacteria, fungi, and viruses, thereby preventing or mitigating infections (Dang et al., 2024).

- Enzyme Modulation: Altering enzyme activity involved in metabolic pathways, with therapeutic implications; for example, rosemary's inhibition of 5α-reductase is key to its hair loss prevention effect (Chen et al., 2022; Murata et al., 2013).

- Immune System Modulation: Enhancing or regulating immune responses to improve disease resistance (Negi et al., 2025).

- Gene Expression Regulation: Influencing gene expression to affect cell growth, differentiation, and apoptosis, which may have applications in cancer prevention and treatment (Chen et al., 2022).

2.6 EXTRACTION

Medicinal plants have gained substantial attention due to their unique characteristics as a rich source of therapeutic phytochemicals, which hold potential for the development of novel pharmaceutical agents. Many plant-derived compounds, including phenolics and flavonoids, have been reported to confer health benefits and possess chemo preventive properties (Venugopal & Liu, 2012). Research on medicinal plants typically begins with pre-extraction and extraction procedures, which are crucial for isolating bioactive constituents from plant materials. Traditional techniques, such as maceration and Soxhlet extraction, are widely employed at small-scale research or Small Manufacturing Enterprise (SME) levels. Significant advancements have been made in extraction technologies, including modern methods such as microwave-assisted extraction (MAE), ultrasound-assisted extraction (UAE), and supercritical fluid extraction (SFE), which aim to enhance yield while reducing cost. Continuous refinements and modifications of these techniques are being developed. Given the diversity of

methods, selecting an appropriate extraction strategy requires careful consideration. This review outlines the principles, advantages, and limitations of commonly used extraction methods, with examples from recent studies to guide method selection (Nn, 2015).

2.6.1 PRE-EXTRACTION PREPARATION

The initial step in medicinal plant research involves the preparation of plant materials to preserve bioactive molecules prior to extraction. Samples, including leaves, bark, roots, fruits, and flowers, may be obtained from fresh or dried plant material. Pre-processing steps, such as grinding and drying, influence the retention of phytochemicals in the final extracts (Nn, 2015).

- Fresh vs. dried samples: Both fresh and dried materials are utilized, although dried samples are often preferred due to their convenience in experimental planning (Nn, 2015). Comparative studies on fresh and dried Moringa leaves indicated no significant difference in total phenolic content, while flavonoid levels were higher in dried samples (Vongsak et al., 2013).
- Grinded vs. powdered samples: Reducing particle size increases the contact area between the solvent and plant material, enhancing extraction efficiency. Grinding produces coarse, smaller particles, whereas powdering generates a more uniform and finer particle size, improving solvent penetration (Conservation et al., n.d.). Efficient extraction requires particle sizes smaller than 0.5 mm, typically achieved using mortar and pestle, electric blenders, or mills (Nn, 2015).
- Air-drying, microwave-drying, oven-drying and freeze-drying (lyophilisation) of plants samples: Air-drying, microwave-drying, oven-drying, and freeze-drying (lyophilization) are commonly employed. Air-drying exposes tied plant material to

ambient air for several days to months, preserving heat-sensitive compounds but requiring longer times and presenting contamination risks (Nn, 2015).

- Microwave-drying shortens drying time using electromagnetic radiation, though it may degrade certain phytochemicals (Nn, 2015).
- Oven-drying applies thermal energy to remove moisture efficiently but may affect thermolabile compounds such as sinensetin and rosmarinic acid (Abdullah et al., 2012).
- Freeze-drying uses sublimation to preserve phytochemicals, yielding higher phenolic content than air-drying; however, it is expensive and primarily reserved for delicate, high-value plant materials (Nn, 2015).

2.6.2 EXTRACTION METHODS

Extraction is the process of separating bioactive constituents from plant material using selective solvents and standardized procedures (Nn, 2015). The fundamental aim of extraction is to isolate soluble phytochemicals from plant materials while removing the insoluble cellular residue (marc). Crude extracts produced through these techniques often contain complex mixtures of diverse plant metabolites, including alkaloids, glycosides, phenolic compounds, terpenoids, and flavonoids. Some crude extracts may be used directly as tinctures or fluid extracts, whereas others require further processing (Nn, 2015). Common extraction techniques include:

2.6.2.1 MACERATION, INFUSION, PERCOLATION AND DECOCTION

Maceration, originally employed in winemaking, involves soaking coarse or powdered plant material in solvent at room temperature for a minimum of three days with intermittent agitation

to release soluble phytochemicals (Nn, 2015). The mixture is subsequently filtered or pressed. Infusion and decoction follow similar principles; infusion uses a shorter soaking period, while decoction involves boiling plant material, typically suitable for heat-stable compounds in hard materials such as roots or bark. Percolation employs a percolator where powdered plant material is macerated with hot solvent at a controlled flow rate, yielding concentrated extracts (Rathi et al., 2006).

STRENGTH AND LIMITATION**:** These methods are simple and cost-effective but require large volumes of solvent, generating considerable organic waste. Modifications, such as altering temperature or solvent choice, can enhance extraction efficiency, as observed with Centella asiatica, where phenolic content and antioxidant activity increased with controlled heating but pH and extract quality were affected (Nn, 2015).

2.6.2.2 SOXHLET EXTRACTION OR HOT CONTINUOUS EXTRACTION

Finely ground plant material is placed in a thimble within the Soxhlet apparatus. The solvent is heated, vaporized, condensed, and allowed to percolate through the material repeatedly (Nn, 2015).

STRENGTH AND LIMITATION: Soxhlet extraction requires less solvent than maceration but poses risks due to flammable solvents, potential toxic emissions, and environmental concerns. It is suitable only for dry, finely divided solids, and parameters such as temperature, solvent ratio, and agitation must be carefully controlled (Amid et al., 2010; Naudé et al., 1998).

2.6.2.3 MICROWAVE ASSISTED EXTRACTION (MAE)

MAE employs microwave radiation to facilitate the transfer of analytes from plant matrices into solvents (Trusheva et al., 2007). Microwaves induce dipole rotation in polar molecules,

disrupting hydrogen bonds, enhancing ion migration, and improving solvent penetration (Kaufmann & Christen, 2002).

STRENGTH AND LIMITATION: MAE reduces extraction time and solvent consumption while improving recovery and reproducibility. However, thermal degradation may occur if conditions are not optimized, limiting applicability to small-molecule phenolics, flavonoids, and resveratrol (Nn, 2015; Trusheva et al., 2007)

2.6.2.4 ULTRASOUND-ASSISTED EXTRACTION (UAE) OR SONICATION EXTRACTION

UAE uses ultrasound (20–2000 kHz) to enhance solvent penetration and disrupt plant cell walls via acoustic cavitation, facilitating the release of bioactive compounds (Nn, 2015). The mechanical influence of acoustic cavitation generated by ultrasound enhances the interfacial contact between extraction solvents and sample materials while improving cellular membrane permeability. The physical and chemical characteristics of materials exposed to ultrasonic treatment undergo modification, resulting in plant cell wall disruption; this promotes the liberation of target compounds and augments solvent mass transfer into plant cellular structures (Dhanani et al., 2017). This technique is straightforward and constitutes a comparatively economical technology applicable to both small-scale and large-scale phytochemical extraction operations (Nn, 2015).

STRENGTH AND LIMITATION: UAE is low-cost, efficient, and reduces extraction time and solvent use. Excessive ultrasound energy (>20 kHz) may generate free radicals, potentially degrading active compounds (Kaufmann & Christen, 2002).

2.6.2.5 OTHER EXTRACTION METHODS

Advanced techniques such as Accelerated Solvent Extraction (ASE) and Supercritical Fluid Extraction (SFE) are also utilized. Despite high efficiency, their adoption is limited by cost (Nn, 2015).

2.6.2.5.1 ACCELERATED SOLVENT EXTRACTION (ASE)

ASE represents a more effective approach to liquid solvent extraction relative to maceration and Soxhlet methodologies, as this technique utilizes considerably reduced solvent volumes. Specimens are combined with chemically inactive substances like sand within the stainless steel extraction chamber to avoid sample clumping and obstruction of the system's conduits (Rahmalia et al., 2015). The assembled ASE chamber comprises alternating strata of sand-specimen mixtures positioned between cellulose filtration paper and sand components. This mechanized extraction apparatus enables independent regulation of temperature and pressure for individual specimens and completes extraction in under sixty minutes. Analogous to alternative solvent-based approaches, ASE performance is fundamentally influenced by solvent selection (Nn, 2015).

2.6.2.5.2 SUPERCRITICAL FLUID EXTRACTION (SFE)

A supercritical fluid (SF), alternatively termed dense-gas, represents a material exhibiting the physical characteristics of both gaseous and liquid phases when it reaches its critical threshold. The transition of a material into its critical state is governed by parameters including temperature and pressure. While SFs demonstrate gas-like behaviour, they possess liquid-like dissolving capabilities (Nn, 2015). Carbon dioxide serves as an illustrative example, achieving supercritical status when temperatures exceed 31.1°C and pressures surpass 7380 kPa.

Supercritical carbon dioxide (SC-CO_2) extraction has garnered considerable attention owing to its superior capacity to dissolve nonpolar substances, coupled with CO_2's widespread accessibility, economic affordability, and minimal toxic effects. Although SC-CO_2 demonstrates limited effectiveness in dissolving polar materials, modifications involving the introduction of trace quantities of ethanol or methanol facilitate the extraction of polar substances. Additionally, SC-CO_2 yields concentrated analyte forms since CO_2 undergoes vaporization under ambient conditions. The extractive capacity of SC-solvents can be readily adjusted through temperature or pressure manipulation, or through modifier incorporation, thereby decreasing extraction duration. However, a significant limitation of this technique involves the substantial initial investment required for equipment acquisition (Naudé et al., 1998).

2.7 CHARACTERIZATION

The detection, separation, and measurement of bioactive constituents in plant extracts are fundamental components of natural product research. These procedures depend largely on advanced analytical methodologies. This section outlines the principal characterization techniques commonly employed for the analysis of plant-derived bioactive compounds.

1. QUALITATIVE PHYTOCHEMICAL SCREENING

Preliminary qualitative assays are routinely conducted to determine the presence or absence of major groups of phytochemicals in plant extracts. These tests generally involve straightforward colour changes or precipitation reactions

- Flavonoids: Development of a yellow coloration upon treatment with strong acidic or alkaline reagents.

- Phenols: Ferric chloride assay, producing blue, black, or green coloration (Christabel Ebube Festus et al., 2024).
- Terpenoids: Salkowski's test, indicated by the formation of a reddish-brown precipitate or a red chloroform layer accompanied by greenish-yellow fluorescence in the acidic phase (Christabel Ebube Festus et al., 2024).

2. CHROMATOGRAPHIC TECHNIQUES

Chromatographic methods play a central role in separating complex mixtures present in plant extracts, enabling the identification and quantification of individual compounds.

- Thin Layer Chromatography (TLC): A rapid, inexpensive, and straightforward technique used for qualitative assessment and preliminary separation of compounds. Separation occurs due to differences in adsorption to a stationary phase, such as silica gel, and solubility in the mobile phase (Altemimi et al., 2017). TLC is widely applied in phytochemical screening, reaction monitoring, and evaluation of extract complexity, with visualization achieved under UV light or through chemical staining (Altemimi et al., 2017).

- Column Chromatography (CC): A preparative approach designed for large-scale separation and purification. The stationary phase is packed within a column, while the mobile phase passes through under gravity or applied pressure (Bajpai et al., 2016).

This method is commonly used to obtain crude fractions for subsequent purification steps.

- High-Performance Liquid Chromatography (HPLC): A highly efficient technique employed for the separation, identification, and quantification of non-volatile or thermally sensitive compounds. High pressure drives the mobile phase through a column containing finely packed stationary material, resulting in superior resolution and sensitivity (Christabel Ebube Festus et al., 2024).). HPLC is frequently coupled with UV-Vis, diode array detectors (DAD), or mass spectrometry (HPLC-MS) for enhanced analytical capability (Altemimi et al., 2017).
- Gas Chromatography (GC): Applied primarily to volatile and semi-volatile compounds, GC involves vaporizing the sample and transporting it through a column using an inert carrier gas (Christabel Ebube Festus et al., 2024). When combined with mass spectrometry (GC-MS), it enables precise compound identification and is especially important for the analysis of essential oils, terpenes, and other volatile constituents (Thamer & Thamer, 2023).
- Hyphenated Techniques: The integration of chromatographic separation with spectroscopic detection systems, such as LC-MS, GC-MS, and LC-NMR, offers powerful tools for structural characterization. These combined techniques allow rapid, sensitive, and accurate identification of bioactive compounds within complex matrices (Altemimi et al., 2017).

3. SPECTROSCOPIC TECHNIQUES

Spectroscopic approaches provide structural and functional group information by examining the interaction between bioactive compounds and electromagnetic radiation.

- Ultraviolet-Visible Spectroscopy (UV-Vis): This technique assesses the absorption of ultraviolet and visible light, offering insights into the presence of chromophores. It is commonly used for quantifying compounds with characteristic absorption profiles, such as flavonoids and polyphenols, as well as for purity evaluation and detection of conjugated systems (Marcheafave et al., 2025).
- Fourier Transform Infrared (FTIR) Spectroscopy: FTIR measures infrared absorption to generate characteristic spectra that indicate specific functional groups, including O–H, C=O, and C–H bonds (Marcheafave et al., 2025). It is employed for qualitative compound classification, structural interpretation, and monitoring of chemical modifications (Marcheafave et al., 2025).
- Nuclear Magnetic Resonance (NMR) Spectroscopy: NMR is a highly informative analytical method that elucidates molecular structure by examining the magnetic behaviour of atomic nuclei. It is indispensable for confirming the chemical structure, connectivity, and conformation of isolated compounds and is regarded as a definitive tool for structural elucidation (Ocampos et al., 2024; Valentino et al., 2020).
- Mass Spectrometry (MS): MS determines the mass-to-charge ratio of ions, yielding information on molecular weight and fragmentation behaviour. It supports molecular formula determination and structural analysis, particularly when integrated with chromatographic systems (e.g., GC-MS and LC-MS), allowing unknown compounds in complex extracts to be identified through comparison with established spectral databases (Thamer & Thamer, 2023).

2.8 SYNERGISTIC EFFECTS AND COMBINATORY FORMULATIONS

In recent years, increasing attention has been directed toward investigating the synergistic interactions of plant-derived compounds, particularly in dermatological and hair growth applications. Synergy refers to a phenomenon in which the combined effect of two or more bioactive constituents elicits a biological response that exceeds the additive effects of each component acting independently (Medicine & Products, 2016). This principle underpins many traditional medical practices, where multi-plant formulations are often favoured over single-compound therapies.

Cloves, hibiscus, moringa, neem, aloe vera, and rosemary contain a broad range of phytochemicals, including flavonoids, terpenoids, tannins, alkaloids, and phenolic acids. Individually, these compounds have been reported to support hair growth by enhancing follicular activity, improving scalp microcirculation, mitigating oxidative stress, and suppressing microbial proliferation (Kesika et al., 2023). Evidence suggests, however, that combining these plant extracts may yield superior therapeutic effects. For example, the antimicrobial activities of neem and rosemary, when applied together, may offer wide-spectrum protection against scalp infections, which are recognized contributors to hair loss (Hashem et al., 2024).

In addition, the antioxidant capacities of moringa and hibiscus may function synergistically to limit oxidative injury to hair follicle cells, thereby prolonging the anagen (growth) phase of the hair cycle (Peterle et al., 2023). Similarly, the hydrating and soothing properties of aloe vera can enhance the anti-inflammatory effects of cloves, producing a formulation that not only promotes hair growth but also supports overall scalp health (Mittal et al., 2014).

Synergy in these combined formulations extends beyond pharmacological interactions to include functional advantages. The physicochemical interactions among extracts may enhance formulation stability, scalp penetration, and retention of active compounds. Such attributes are particularly relevant in alopecia management, where sustained and consistent exposure to bioactive compound is essential for therapeutic efficacy (Thorat et al., 2009). Furthermore, combination formulations may permit the use of lower concentrations of individual extracts, thereby minimizing the risk of toxicity or irritation associated with higher single-agent doses. This approach is especially beneficial for chronic conditions such as alopecia that require prolonged treatment.

To further develop this research area, future investigations should focus on identifying optimal extract combinations, concentration ratios, and delivery systems using both in vitro and in vivo models. Advanced analytical approaches, including checkerboard assays, isobolograms analysis, and combination index methods, can quantitatively evaluate synergistic interactions and facilitate the development of safe, effective, and natural hair growth therapies.

2.9 GAPS IN LITERATURE

The use of plant extracts as therapeutic agents for managing different forms of alopecia has attracted increasing scientific interest in recent years. Evidence from contemporary studies indicates that numerous plant-derived constituents can enhance hair growth through diverse biological mechanisms. Despite these encouraging findings, several important research limitations persist, constraining the advancement of effective plant-based interventions for hair loss. This section critically discusses these gaps and outlines future research priorities, with particular focus on standardization, mechanistic elucidation, and clinical substantiation.

1) Limited Research on Synergistic Effects of Multiple Plant Extracts

The majority of existing studies concentrate on single plant extracts rather than multi-component formulations. Although recent investigations have begun to assess synergistic interactions, the evidence base remains limited. The Hair Rise™ microemulsion, which incorporates several medicinal plant extracts, illustrates that combined formulations can promote hair growth through multiple mechanisms, including antioxidant, anti-inflammatory, and anti-androgenic effects, while concurrently activating key signalling pathways such as Wnt/β-catenin, Sonic Hedgehog, and angiogenesis (Muangsanguan et al., 2024). The complementary actions of diverse plant extracts suggest that combined use may enhance therapeutic outcomes. For instance, some extracts may support cell survival, whereas others stimulate the expression of growth factors such as IGF-1, VEGF, HGF, and KGF (FGF-7), collectively initiating and prolonging the anagen phase of the hair cycle (Choi et al., 2024). Although this multi-target strategy may offer a more holistic approach to treating alopecia, systematic studies are required to determine optimal combinations and formulation parameters.

Traditional medical systems have long relied on plant mixtures derived from extensive empirical knowledge, indicating potential therapeutic value that merits scientific validation. Future research should therefore rigorously assess various extract combinations to confirm the presence and extent of synergistic effects and to identify conditions that maximize efficacy.

2) Inadequate Standardization of Extraction Processes

The absence of standardized extraction protocols remains a major barrier to reproducibility and commercialization of plant-based hair loss therapies. Variations in extraction solvents, temperatures, and processing durations result in differences in phytochemical composition, complicating comparisons across studies. While other research areas, such as pesticide analysis

in hair samples, have established highly standardized and reliable extraction methods, equivalent standards for isolating hair growth–promoting phytochemicals from plants are still lacking.

Emerging approaches, including hairy root culture techniques, have demonstrated potential for enhanced and consistent production of secondary metabolites in Solanaceous plants by allowing extraction under controlled conditions (Biswas & Chakraborty, 2023). Although promising, such techniques remain underexplored in the context of hair growth research. Variability in extraction methods directly influences the concentration and bioavailability of active constituents, thereby affecting both experimental outcomes and therapeutic potential. Defining optimal extraction conditions, such as solvent type, temperature, and processing time, would substantially advance the field by improving consistency in research findings and facilitating product development (Choi et al., 2024).

3) Lack of Characterization of Specific Compounds Responsible for Hair Growth

Although many plant extracts exhibit hair growth–stimulating properties, the precise bioactive constituents responsible are often unidentified. Notable progress has been made in some cases, such as the isolation of astilbin from *Hypericum perforatum* (St. John's Wort), which served as a lead compound for the synthesis of t-flavanone, a derivative shown to be effective against androgenetic alopecia in men and hair thinning in women. Several compound classes have been implicated in hair growth promotion, including phenolics, terpenes and terpenoids, Sulphur-containing compounds, and fatty acids (Choi et al., 2024). Studies involving hairy root cultures of Solanaceous plants have identified bioactive alkaloids (e.g., hyoscyamine, scopolamine, anisidine, atropine), polyphenols (e.g., cyanidin, peonidin, kaempferol, and quercetin derivatives), and terpenes as potential contributors (Biswas & Chakraborty, 2023).

Despite these advances, many traditionally used plant extracts remain insufficiently characterized at the molecular level. Comprehensive phytochemical profiling using advanced analytical tools such as GC–MS, HPLC, and FTIR should be more consistently applied to identify, isolate, and quantify active constituents. Such molecular-level characterization would support the development of standardized extracts with predictable and reproducible biological activity (Choi et al., 2024).

4) Scarcity of Mechanistic Studies

Elucidating the mechanisms by which plant extracts stimulate hair growth is essential for the development of targeted therapies; however, many studies focus primarily on phenotypic outcomes without investigating underlying biological processes. Evidence from formulations such as the Hair Rise™ microemulsion indicates that plant extracts can modulate multiple pathways simultaneously, including oxidative stress reduction, inflammation control, and regulation of androgen-related genes, as well as Wnt/β-catenin, Sonic Hedgehog, and angiogenesis signalling pathways (Muangsanguan et al., 2024). These pathways play critical roles in extending the anagen phase and enhancing follicular blood supply.

Advances in experimental methodologies have created new opportunities for mechanistic investigations. Co-culture systems involving dermal papilla cells and hair matrix cells enable the assessment of intercellular interactions and the screening of natural compounds for hair growth activity. Similarly, the use of induced pluripotent stem cells (iPSCs) to study hair follicle differentiation represents a promising approach for clarifying molecular mechanisms. Nonetheless, a comprehensive understanding of how plant-derived compounds interact with specific cellular and molecular targets remains incomplete. Future studies should integrate in

vitro, ex vivo, and in vivo models to systematically elucidate these mechanisms and guide the design of targeted therapeutic strategies (Choi et al., 2024).

5) Limited Focus on Specific Types of Alopecia

Alopecia encompasses several distinct disorders with differing etiologies, yet many studies treat hair loss as a single, uniform condition. This oversimplification hinders the development of tailored therapies for specific alopecia types. Some recent investigations have begun to address this limitation, including studies evaluating herbal treatments for androgenetic alopecia (AGA) in comparison with standard therapies such as 5% minoxidil. Given the diverse pathophysiological mechanisms involved, hormonal factors in AGA versus autoimmune components in alopecia areata, therapeutic responses are likely to vary across conditions. Plant extracts with anti-androgenic activity may be particularly beneficial for AGA, whereas immunomodulatory extracts may be more appropriate for alopecia areata.

6) Inadequate Clinical Evidence and Human Studies

Despite extensive preclinical research supporting the hair growth potential of plant extracts, high-quality clinical evidence remains limited. Nevertheless, existing human studies have yielded encouraging results. For example, a randomized double-blind controlled trial in Iran compared a topical herbal formulation with 5% minoxidil for the treatment of androgenetic alopecia in men, exemplifying the type of rigorous clinical investigation required. Although some reviews report that several plant extracts have demonstrated hair growth–promoting effects in clinical trials (Choi et al., 2024), many of these studies are constrained by small sample sizes, inadequate controls, or short follow-up periods, reducing their reliability and

generalizability. Bridging the gap between preclinical promise and clinical validation is therefore essential. Future research should prioritize well-designed clinical trials featuring:

- Adequate sample sizes and appropriate control groups
- Standardized and objective outcome measures
- Sufficient treatment duration to assess long-term efficacy and safety
- Stratification based on alopecia type, severity, and demographic variables

Such trials would provide the robust evidence required for plant-based therapies to transition from traditional remedies to scientifically validated treatment options.

7) Neglected Local Context and Indigenous Knowledge

Although many regions possess rich ethnobotanical traditions, scientific evaluation of locally available and traditionally used medicinal plants remains limited. In Nigeria, for instance, indigenous approaches to managing hair and scalp disorders involve various medicinal plants, chemical agents, and physical practices (Enechukwu & Ogunbiyi, 2022). These natural remedies are often affordable, accessible, and perceived as safer alternatives to commercial products. The growing global demand for natural hair care products further underscores the value of indigenous knowledge systems (Enechukwu & Ogunbiyi, 2022). However, much of this knowledge has not been systematically documented or validated using modern scientific methodologies. Integrating traditional practices with contemporary research approaches could uncover novel therapeutic candidates and support the development of culturally relevant and effective hair loss treatments.

2.10 FUTURE DIRECTIONS

To effectively bridge the gaps identified above, future investigations should focus on the following priorities:

- Formulating plant extract blends based on known phytochemical interactions and testing their efficacy and safety in comparison to single-plant extracts.
- Optimizing and standardizing extraction protocols to enhance yield, reproducibility, and sustainability.
- In-depth compound characterization and profiling using modern analytical tools to isolate and quantify hair growth-promoting agents.
- Mechanistic studies to identify the biological pathways involved in hair follicle stimulation or alopecia reversal.
- Designing clinical trials that assess efficacy in specific types of alopecia, ideally including long-term follow-up and dose-response analyses.
- Promoting indigenous knowledge integration, partnering with local communities and traditional practitioners to prioritize regionally relevant plant species.
- Developing scalable production techniques that ensure consistency, efficacy, and affordability for potential commercialization.

CHAPTER THREE

MATERIALS AND METHODS

3.1 MATERIALS AND EQUIPMENT

Table 3.1: Plant Materials

Plant	Form
Clove buds	Dried
Rosemary leaves	Dried
Moringa leaves	Dried

Source: Author's compilation

Table 3.2: General Materials

Item	Purpose
Distilled water	Dilutions
Methanol	Solvent for extraction
Coconut oil	Base oil for formulation
Labels and pen	Sample identification

Source: Author's compilation

Table 3.3: Equipment for Extraction and Sample Preparation

Item	Purpose
Reflux setup	Solvent extraction for samples
Constant temperature magnetic stirrer	Mixing and maintaining temperature during extraction
UV-VIS spectrophotometer	Measuring the absorbance of standard solutions
Beakers, conical flasks (500 mL)	Holding and mixing samples
Measuring cylinders & pipettes	Accurate measurement of liquids
Analytical balance	Measuring plant powder and extracts
Oven	Drying and concentrating extracts
Electric grinder	Pulverizing dried plants
Muslin cloth	Filtration of extracts
Glass rods	Mixing solutions
Dropper	Adding reagents drop wise

Source: Author's compilation

Table 3.4: Reagents for Phytochemical Screening

Test	Reagents
Flavonoids	Methanol + dilute Ammonia solution
Phenols	Ferric chloride
Terpenoids	Chloroform + conc. H_2SO_4
Steroids	Chloroform + conc. H_2SO_4 + acetic acid

Source: Author's compilation

3.2 COLLECTION AND PREPARATION OF PLANT SAMPLES

Dried clove buds and rosemary leaves were purchased from verified herbal markets to ensure quality and accessibility. Fresh samples of moringa leaves were harvested from home gardens.

Fresh samples were washed with water to eliminate dirt and contaminants. Fresh materials were sun-dried for 24-48 hours to minimize the loss of heat-sensitive bioactive compounds. Once completely dried, they were ground into fine powder using a mechanical grinder and stored in airtight amber containers at room temperature until used.

3.3 EXPERIMENTAL DESIGN

Table 3.5: Build Information

Design	Information
File Version	13.0.1.0
Study Type	Mixture
Design Type	Simplex Lattice
Design Model	Quadratic
Build Time (ms)	9.00
Subtype	Randomized
Runs	13.00
Blocks	No Blocks

Source: Design Expert® software

Table 3.6: Mixture Components

Name	Minimum	Maximum	Coded Low	Coded High	Mean	Std. Dev.
A: Clove (g)	0	10	+0 ↔ 0	+1 ↔ 10	3.33	3.73
B: Rosemary (g)	0	10	+0 ↔ 0	+1 ↔ 10	3.33	3.73
C: Moringa (g)	0	10	+0 ↔ 0	+1 ↔ 10	3.33	3.73
	Total =	10.00	L_Pseudo Coding			

Source: Design Expert® software

Table 3.7: Experimental Design

Run	A: Clove (g)	B: Rosemary (g)	C: Moringa (g)	Yield (wt.%)
1	1.66667	6.66667	1.66667	
2	3.33333	3.33333	3.33333	
3	5	0	5	
4	0	10	0	

5	10	0	0	
6	0	0	10	
7	6.66667	1.66667	1.66667	
8	0	5	5	
9	1.66667	1.66667	6.66667	
10	5	5	0	
11	0	10	0	
12	10	0	0	
13	0	0	10	

Source: Design Expert® software

3.4 EXTRACTION

For each experimental run, accurately weighed portions of the powdered plant materials were mixed accordingly to the designed proportions and transferred to a flat-bottom flask. 50ml of methanol was added to maintain a solid-to-solvent ratio of approximately 1:5 (w/v).

The flask was fitted with a reflux condenser and placed on a constant temperature magnetic stirrer. The mixture was maintained at a controlled temperature of 80℃ and stirred

continuously for 1 hour 30 minutes to ensure uniform heat distribution and effective mass transfer between the plant and solvent. The reflux setup helped minimize solvent loss while promoting complete extraction of the bioactive compounds. After the extraction period, the mixture was allowed to cool at room temperature and then filtered into an already weighed beaker using a muslin cloth. The filtrates were concentrated in an oven at 80℃ to remove excess solvent then measured. The concentrated extracts were stored in plastic bottles until further analysis. The yield was determined using the following formula:

$$\textbf{Yield (\%)} = \frac{weight\ of dried\ extract}{weight\ of\ dry\ plant\ sample} \text{ x } 100 \qquad \text{(eqn. 3.1)}$$

3.5 PHYTOCHEMICAL SCREENING

3.4.1 ANALYSIS OF PHENOLS

Qualitative: About 1% (w/v) of the extract was mixed with 2ml of distilled water followed by the addition of few drops of 10% ferric chloride. The formation of a blue or green color indicates the presence of phenols (Harborne, 1973).

Quantitative: 0.20 g of tannic acid was dissolved in distilled water and diluted to 200mL mark (1mg/cm^3) in preparation for phenol standard curve. Varying concentrations (0.2–1.0mg/cm^3) of the standard tannic acid solution were pipetted into five different test tubes to which 2 cm^3 of NH_3OH, 5 cm$_3$ of amyl alcohol, and 10 cm3 of water were added. The solution was made up to 100 cm^3 volume and left to react for 30 minutes for colour development. The optical density was determined at 490 nm.

3.4.2: ANALYSIS OF FLAVONOIDS

Qualitative: A portion of powdered seed in each case was heated with 10ml of ethyl acetate in a test tube over a steam bath for 3minutes. The mixture was filtered, and 4 ml of the filtrate was shaken with 1ml of dilute ammonia solution. Yellow coloration was observed that indicated the presence of Flavonoids.

Quantitative: A quantity, 10.0g of the powdered sample was repeatedly extracted with 100 ml of methanol at room temperature. The solution was shaken for 30minutes and filtrate was transferred into a weighed beaker and evaporated to dryness in an oven and weighed again. The time for the first extraction was 1 hour, 45minutes for the second extraction and 30 minutes for the third extraction. Flavonoid was determined using the following formula.

$$\text{Flavonoid (\%)} = \frac{W_2 - W_1}{W_3} \times 100 \qquad \text{(eqn. 3.2)}$$

Where,

W_1 = weight of empty beaker.

W_2 = weight of beaker + sample after drying

W_3 = weight of sample used

3.4.3: ANALYSIS OF TERPENOIDS (SALKOWSKI TEST)

Qualitative: Exactly 1.0g portion of extract was mixed in 2ml of chloroform followed by the careful addition of 3ml of concentrated H_2SO_4. A layer of reddish-brown coloration was formed at the interface thus indicating a positive result for the presence of Terpenoids (Trease and Evans, 1989).

Quantitative: About 1 g (Wi) was taken and soaked in 20 ml of ethanol (Indumathi et al., 2014). It was then mixed with 10 ml of hexane and again filtrated using separating funnel. The extract was waited for its complete drying and measurement is taken (Wf). The yield (%) of total terpenoids contents was measured by the formula:

$$\text{Total Terpenoid} = \frac{W_i - W_f}{W_i} x100 \qquad \text{(eqn. 3.3)}$$

Where,

Wi = dried plant extracts,

Wf = extracts after drying

3.4.4: ANALYSIS OF STEROIDS

Qualitative: Crude extract was mixed with 2 ml of chloroform and concentrated H_2SO_4 was added sidewise. A red colour produced in the lower chloroform layer indicates the presence of steroids. Another test was performed by mixing crude extract with 2 ml of chloroform. Then 2 ml of each of concentrated H_2SO_4 and acetic acid was poured into the mixture. The development of a greenish coloration indicates the presence of steroids.

Quantitative: A quantity, 1.0 g of the extract was dispersed in 100ml of distilled water into a conical flask, the mixture was shaken for 3 hours in an orbital shaker and allowed to stand overnight. It was then filtered and the filtrate was eluted with 10ml normal ammonium hydroxide solution. A volume, 2ml of the elute was put into a test tube and mixed with 2 ml of chloroform and 3ml acetic anhydride added to the mixture, followed by 2ml of concentrated H_2SO_4 drop wisely. The absorbance was measured in a spectrophotometer at 560 nm. The steroid concentration was determined using the following relationship.

Steroids (%) = $\frac{Abs\ x\ Path\ length}{100\ x\ weight\ of\ sample}$ (eqn. 3.4)

(Activity et al., 2018; Amin Mir et al., 2013; Dahiru et al., 2006; Ezeonu & Ejikeme, 2016; Innocent Izuchukwu Ujah et al., 2021; Krishnananda et al., 2017; Rajiv et al., 2016)

3.6 CHARACTERIZATION

3.6.1 UV-VIS SPECTROPHOTOMETRY PROCEDURE

A series of known concentrations of a standard compound was prepared. The spectrophotometer was set to the appropriate wavelength. A cuvette was filled with the solution that the absorbance is to be measured, and distilled water was used as the blank. The absorbance was measured and recorded (Padera, n.d.). A calibration curve was generated by plotting absorbance against concentration. From the curve, a calibration equation was gotten:

$A = mC + b$

Where:

A = absorbance

C = concentration (mg/mL of phenol equivalent)

m = slope of the line

b = intercept

The equation was rearranged to find concentration:

$$C = = \frac{A-b}{m} \quad \text{(eqn. 3.5)}$$

For each of the five absorbance readings of each sample (corresponding to 0.02-0.1 mg/mL), the equivalent concentration (mg/mL phenol equivalent) was calculated using that formula, giving five different concentration values. The mean concentration of the five values was calculated using:

$$\text{Mean Concentration (mg/mL)} = \frac{C1+C2+C3+C4+C5}{5} \quad \text{(eqn. 3.6)}$$

The mean concentration was then multiplied by the dilution factor, 50, to get the total phenolic mass in the extract.

Phenolic Content (mg phenol equivalent) = Mean Concentration × Dilution Factor

Since the mass of the extract dissolved was 0.2 g, the total phenolic content was calculated using:

$$\text{TPC (mg GAE/g)} = \frac{PC\left(\frac{mg}{mL}\right) \times total\ volume\ of\ extract\ (mL)}{mass\ of\ extract\ (g)}$$

$$\text{TPC (\%)} = \frac{TPC\ (mg\ GAE/g)}{10{,}000} \quad \text{(eqn. 3.7)}$$

because 1 g = 1000 mg.

3.6.2 FOURIER TRANSFORM INFRARED (FTIR) SPECTROSCOPY

Approximately 1 g of the powdered extract was measured in a bottle. 5 ml of methanol was added and the mixture was shaken till completely dissolved. The mixture was placed in the

FTIR sample holder. The FTIR scan was run in the range of 4000 to 400 cm^{-1}, and the spectrum was recorded. Functional groups were identified based on characteristic peaks.

Table 3.8: Infrared Spectroscopy Absorption Table

Wavenumber (cm^{-1})	Intensity	Shape	Bond	Vibration Type	Compound / Functional Group	Remarks
3700–3584	Medium	Sharp	O–H	Stretching	Alcohol	Free (non-H-bonded)
3550–3200	Strong	Broad	O–H	Stretching	Alcohol	Intermolecular H-bonded
3500–3400	Medium	–	N–H	Stretching	Primary amine	Two bands
3400–3300	Medium	–	N–H	Stretching	Aliphatic primary amine	–
3350–3310	Medium	–	N–H	Stretching	Secondary amine	One band
3300–2500	Strong	Broad	O–H	Stretching	Carboxylic acid	Centred ~3000 cm^{-1}
3200–2700	Weak	Broad	O–H	Stretching	Alcohol	Intramolecular bonded

3000–2800	Strong	Broad	N–H	Stretching	Amine salt	–
3333–3267	Strong	Sharp	≡C–H	Stretching	Alkyne	–
3100–3000	Medium	–	=C–H	Stretching	Alkene	–
3000–2840	Medium	–	–C–H	Stretching	Alkane	–
2830–2695	Medium	–	–C–H	Stretching	Aldehyde	Doublet (two weak bands)
2600–2550	Weak	–	S–H	Stretching	Thiol	–
2349	Strong	–	O=C=O	Stretching	Carbon dioxide	–
2275–2250	Strong	Broad	N=C=O	Stretching	Isocyanate	–
2260–2222	Weak	–	C≡N	Stretching	Nitrile	Sharp
2260–2190	Weak	–	C≡C	Stretching	Alkyne	Disubstituted
2175–2140	Strong	–	S–C≡N	Stretching	Thiocyanate	–
2160–2120	Strong	–	N=N=N	Stretching	Azide	–
2150	–	–	C=C=O	Stretching	Ketene	–
2145–2120	Strong	–	N=C=N	Stretching	Carbodiimide	–
2140–2100	Weak	–	C≡C	Stretching	Alkyne	Monosubstituted

2140–1990	Strong	–	N=C=S	Stretching	Isothiocyanate	–
2000–1900	Medium	–	C=C=C	Stretching	Allene	–
2000	–	–	C=C=N	Stretching	Ketenimine	–
2000–1650	Weak	–	C–H	Bending	Aromatic compound	Overtone region
1760–1690	Strong	Sharp	C=O	Stretching	Carbonyl compounds	Depends on type
1680–1620	Medium	–	C=C	Stretching	Alkene	–
1600–1475	Medium	–	C=C	Stretching	Aromatic ring	–
1550–1350	Strong	–	N–O	Stretching	Nitro compounds	Two strong bands
1450–1375	Medium	–	C–H	Bending	Alkane	CH_2, CH_3 deformation
1360–1290	Medium	–	O–H	Bending	Carboxylic acid	–
1300–1000	Strong	–	C–O	Stretching	Alcohol, ester, acid	–
1250–1020	Medium	–	C–N	Stretching	Amine, amide	–

900–675	Medium	Sharp	=C–H	Out-of-plane bend	Aromatic/alkene	Diagnostic for substitution pattern
700–600	Strong	–	C–Cl	Stretching	Alkyl halide	–
600–500	Strong	–	C–Br	Stretching	Alkyl halide	–
500–400	Strong	–	C–I	Stretching	Alkyl halide	–

Source: Adapted from LibreText Chemistry- Infrared Spectroscopy Absorption Table (2023) and Complementary IR Reference Data.

The peaks of each from each sample were compared with this table to know the functional groups present in them.

3.7 FORMULATION OF EXTRACTS

Each formulation was prepared as a 50 ml batch, using the ratio below:

Table 3.9: Formulation of Extract

Plant Extract Used	Ratio (Extracts: Oil)
Clove + Rosemary + Moringa	3:7 (ml extract: ml oil)

The required volume of each plant extract was measured according to the specified formulation ratios and combined in a clean, dry glass beaker. Virgin coconut oil was added to the extract

mixture in the appropriate proportion. The mixture was stirred continuously using a glass rod for 10–15 minutes to ensure uniform blending. If the final formulation appeared cloudy or contained particulate matter, it was filtered using a muslin cloth or fine sieve. It was then transferred into clean, dry amber glass bottles and appropriately labelled. The bottles were stored at room temperature in a cool, dark place until further use.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 EXTRACTION AND OPTIMIZATION

Table 4.1: Extraction Result

Run	A: Clove (g)	B: Rosemary (g)	C: Moringa (g)	Weight of empty beaker (g)	Weight empty beaker + extract (g)	Weight of extract (g)	Yield (wt.%)
1	1.66667	6.66667	1.66667	95.94	97.72	1.78	17.8
2	3.33333	3.33333	3.33333	95.33	96.92	1.59	15.9
3	5	0	5	95.66	97.06	1.40	14.0
4	0	10	0	93.88	95.80	1.92	19.2
5	10	0	0	97.83	99.53	1.70	17.0
6	0	0	10	95.24	96.29	1.05	10.5
7	6.66667	1.66667	1.66667	97.78	99.52	1.74	17.4

8	0	5	5	97.13	98.59	1.46	14.6
9	1.66667	1.66667	6.66667	95.18	96.43	1.25	12.5
10	5	5	0	95.77	97.63	1.86	18.6
11	0	10	0	95.26	97.02	1.76	17.6
12	10	0	0	96.54	98.37	1.83	18.3
13	0	0	10	95.81	96.90	1.09	10.9

Source: Author's compilation

The above table shows the recording done during the extraction of bioactive compounds from the plant samples according to the experimental design.

Table 4.2: Extract yield ANOVA for the Linear model

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	100.65	2	50.32	131.36	< 0.0001	significant
[(1)]Linear Mixture	100.65	2	50.32	131.36	< 0.0001	
Residual	3.83	10	0.3831			
Lack of Fit	1.63	7	0.2323	0.3160	0.9052	not significant
Pure Error	2.20	3	0.7350			
Cor Total	104.48	12				

Source: Design Expert® software

[(1)] Inference for linear mixtures uses Type I sums of squares.

The Model F-value of 131.36 shows that the model is significant statistically. There is only about 0.01% chance that an F-value this large could occur due to noise. P-values less than 0.0500 indicate model terms are significant. The Lack of Fit F-value of 0.32 implies the Lack of Fit is not significant relative to the pure error.

Table 4.3: Fit Statistics

R^2	0.9633
Adjusted R^2	0.9560
Predicted R^2	0.9268
Adequate Precision	27.3769
Standard Deviation	0.6189
Mean	15.72
C.V. %	3.94

Source: Design Expert® software

The Predicted R^2 value of 0.9268 is in reasonable agreement with the Adjusted R^2 value of 0.9560; i.e., the difference is less than 0.2. Adequate Precision measures the signal-to-noise ratio. A ratio greater than 4 is desirable. The ratio of 27.377 indicates an acceptable signal. This model can be used to navigate the design space.

Table 4.4: Coefficients in Terms of Coded Factors

Component	Coefficient Estimate	df	Standard Error	95% CI Low	95% CI High	VIF
A-Clove	17.90	1	0.3628	17.09	18.70	1.07
B-Rosemary	18.70	1	0.3628	17.89	19.50	1.07
C-Moringa	10.56	1	0.3628	9.75	11.36	1.07

Source: Design Expert® software

The coefficient estimate represents the expected change in response per unit change in factor value when all remaining factors are held constant. The intercept in an orthogonal design is the overall average response of all the runs. The coefficients are adjustments around that average based on the factor settings. When the factors are orthogonal the VIFs are 1; VIFs greater than 1 indicate multi-collinearity, the higher the VIF the more severe the correlation of factors. As a rough rule, VIFs less than 10 are tolerable.

Final Equation in Terms of L_Pseudo Components

$$Extract\ yield\ (\%) = 17.9A + 18.7B + 10.56C \qquad \text{(eqn. 4.1)}$$

The equation in terms of coded factors can be used to make predictions about the response for given levels of each factor. By default, the high levels of the mixture components are coded as +1 and the low levels are coded as 0. The relative impact of the factors is identified using the coded equation by comparing the factor coefficient.

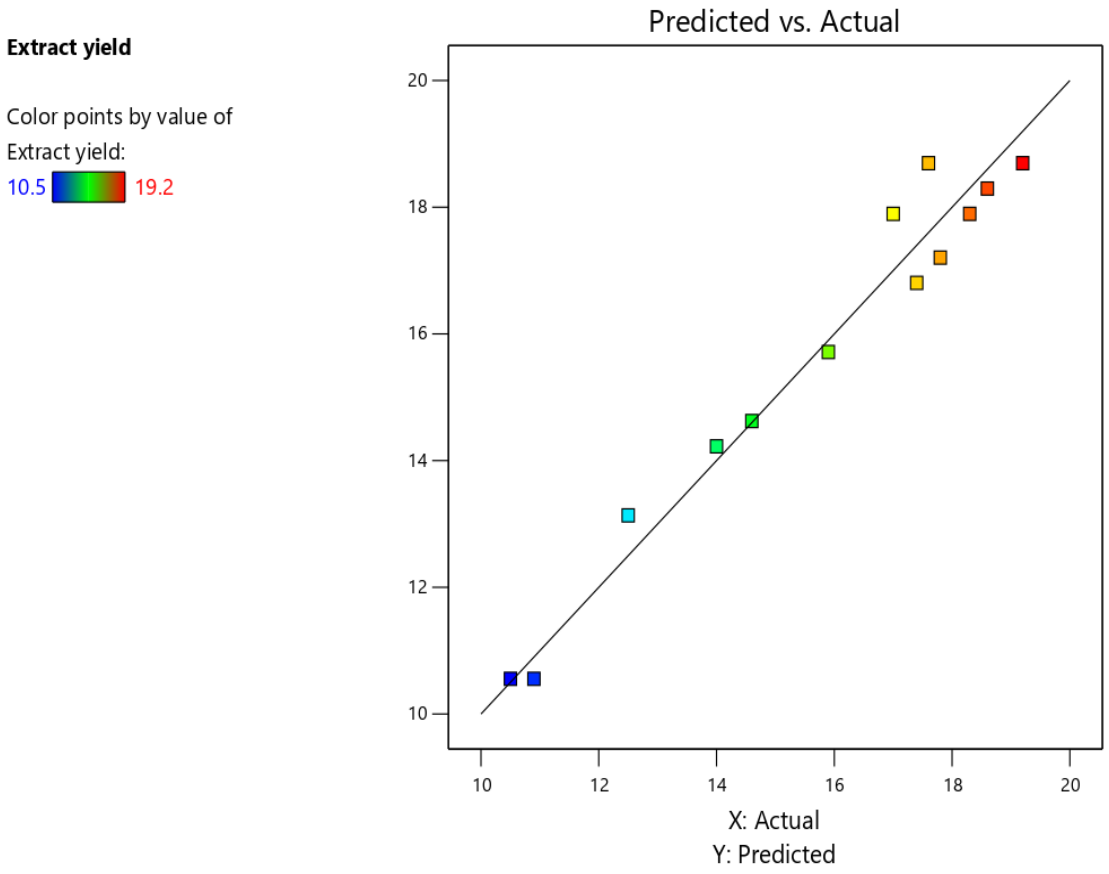

Figure 4.1: Parity Plot of Predicted and Actual Extract Yield

The parity plot of predicted versus actual extract yield in figure 4.1 shows a strong linear corelation between the experimental and model predicted extract yield values. The close alignment of data points along the 45° reference line indicates good model accuracy and minimal residual error. This confirms the suitability of the mixture model for predicting the extract yield within the studied experimental range.

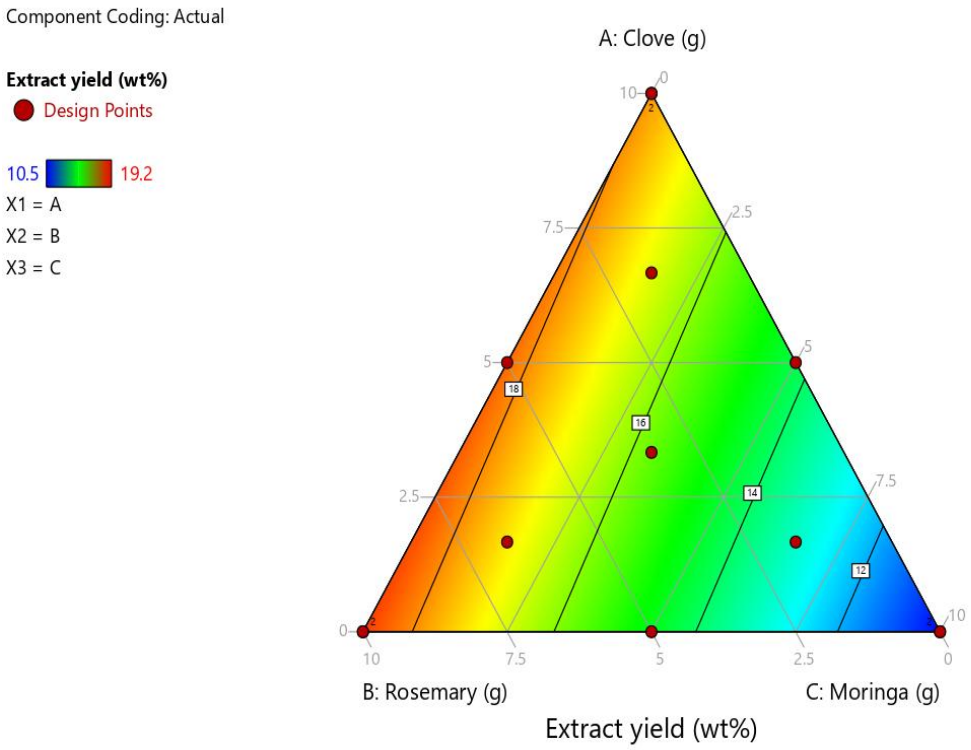

Figure 4.2: Mixture Plot of Component Extractions

Figure 4.2 shows the ternary contour plot for extract yield as a function of the proportions of clove, rosemary, and moringa. The plot shows that the extract yield is strongly influenced by the proportion of clove, with the highest yield observed in regions of elevated clove content. On the other hand, mixtures rich in moringa yielded the lowest extraction values, as evidenced by the blue region at moringa vertex. Rosemary exhibited an intermediate effect. This finding suggests that clove contains a higher concentration of extractable compounds relative to the other two samples, potentially due to its high essential oil and phenolic content.

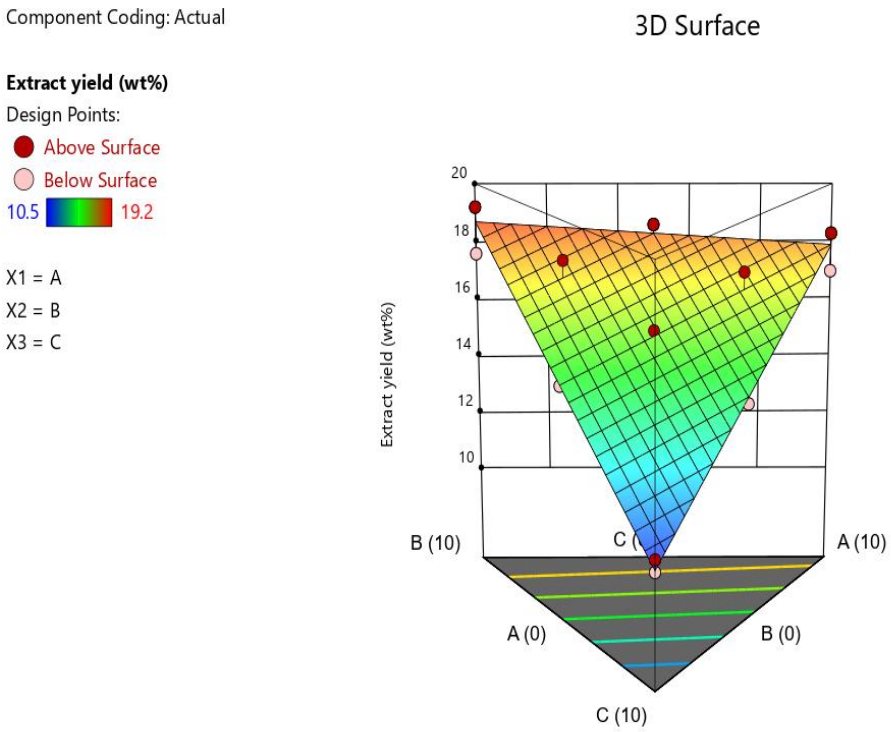

Figure 4.3: 3D Plot of Actual and Predicted Extract Yield

Figure 4.3 presents the 3D response surface plot showing the relationship between clove, rosemary, and moringa and the resulting extract yield. The curved nature of the surface confirms the presence of non-linear interactions among the components, which would not have been captured by a simple additive model. The surface achieves its maximum height in the region of high Clove proportion, corroborating the findings observed in the ternary contour plot. A pronounced decline in yield is evident as the proportion of Moringa increases, with the lowest yields recorded at the Moringa-dominant vertex. The distribution of actual design points relative to the fitted surface indicates good model agreement, as most points lie close to the surface with only minor deviations above or below. This confirms the adequacy of the fitted mixture model in predicting extract yield across the experimental design space, lending confidence to its use in identifying optimal component proportions.

4.1.1 Optimum Extraction of Components

This result suggests that the chosen ratio provided a balanced interaction among the bioactive compounds of the three plants, enhancing solvent penetration and compound solubilization during the methanol-based reflux extraction. The high desirability value reflects the effectiveness and reliability of the extraction optimization process, confirming that this combination can serve as the best formulation for obtaining potent and reproducible natural extracts for further characterization and potential hair growth applications. Table 4.5 shows the optimized combination of the plant components (clove, moringa, and rosemary) that produced the most desirable extraction outcome and an optimal and statistically satisfactory extraction condition.

Table 4.5: Optimum Extraction of Components

Number	Cloves (g)	Rosemary (g)	Moringa (g)	Yield	Desirability	
1	3.553	2.389	4.057	15.108	1.000	Selected

Source: Author's compilation

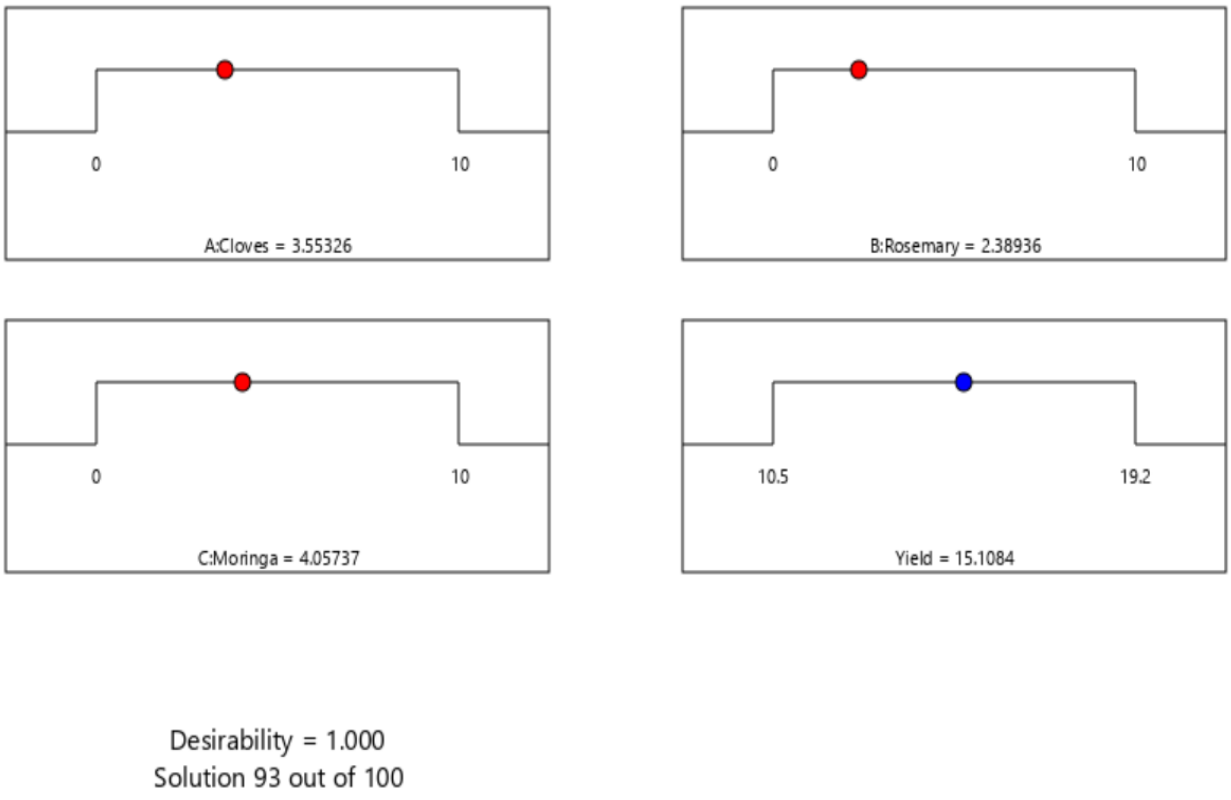

Figure 4.4: Optimum Extraction of Components

4.2 FOURIER TRANSFORM INFRARED (FTIR) SPECTROSCOPY

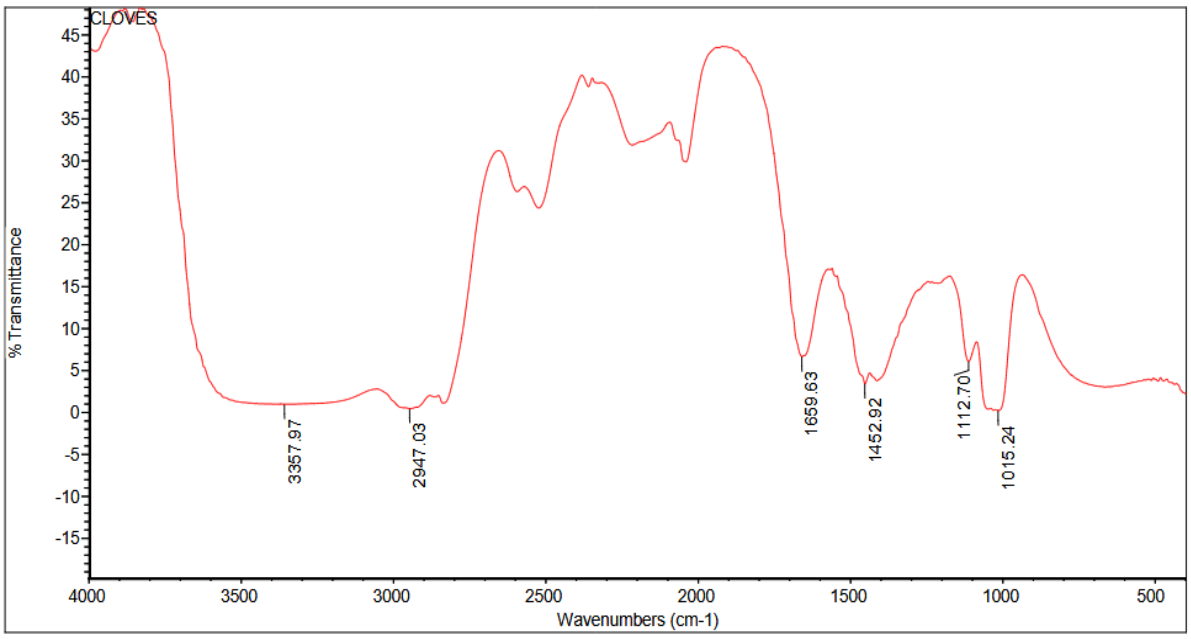

Figure 4.5: FTIR Spectrum for Clove Extract

Position: 1015.24 Intensity: 0.206

Position: 1112.70 Intensity: 6.048

Position: 1452.92 Intensity: 3.383

Position: 1659.63 Intensity: 6.629

Position: 2947.03 Intensity: 0.441

Position: 3357.97 Intensity: 0.956

Table 4.6: FTIR Result for Cloves

Position (cm^{-1})	Intensity (likely)	Shape (likely)	Bond	Vibration	Functional Group / Compound
3357.97	Strong	Broad	O–H / N–H	Stretching	Phenolic O–H / H-bonded alcohols
2947.03	Strong	Sharp/medium	–C–H (sp^3)	Aliphatic C–H stretching	Aliphatic CH_2/CH_3
1659.63	Medium	Sharp/broad	C=C (conjugated) /C=O (conjugated)	Stretching	Conjugated C=C or conjugated carbonyl / aromatic C=C
1452.92	Medium	Sharp	C–H	Bending / deformation	CH_2/CH_3 deformation; aromatic skeletal vibration
1112.70	Strong	Sharp	C–O / C–O–C	Stretching	Aryl–O, ether or C–O of phenolic ethers and esters / glycosides

Source: Author's compilation

The FTIR spectrum of clove extract indicates a strong presence of phenolic compounds, evident from the broad O–H band around 3358 cm^{-1}, confirming hydroxyl groups typical of antioxidants like eugenol. These phenolics suggest significant antioxidant potential, which is valuable in protecting hair follicles from oxidative stress linked to androgenetic alopecia. The aliphatic C–H peaks near 2947 cm^{-1} and 1453 cm^{-1} point to terpenoids and fatty acid components that can enhance scalp moisture, improve active compound delivery, and maintain follicular health. The absorption band near 1660 cm^{-1} reflects conjugated phenolic structures associated with anti-inflammatory and free radical scavenging activities, while the C–O and aryl–O peaks between 1100–1000 cm^{-1} confirm the presence of phenolic ethers contributing antimicrobial properties. Overall, the spectral data reveal a phytochemical composition rich in phenolics and terpenoids with antioxidant, anti-inflammatory, and antimicrobial activities, properties that support scalp health and could help mitigate processes underlying androgenetic alopecia.

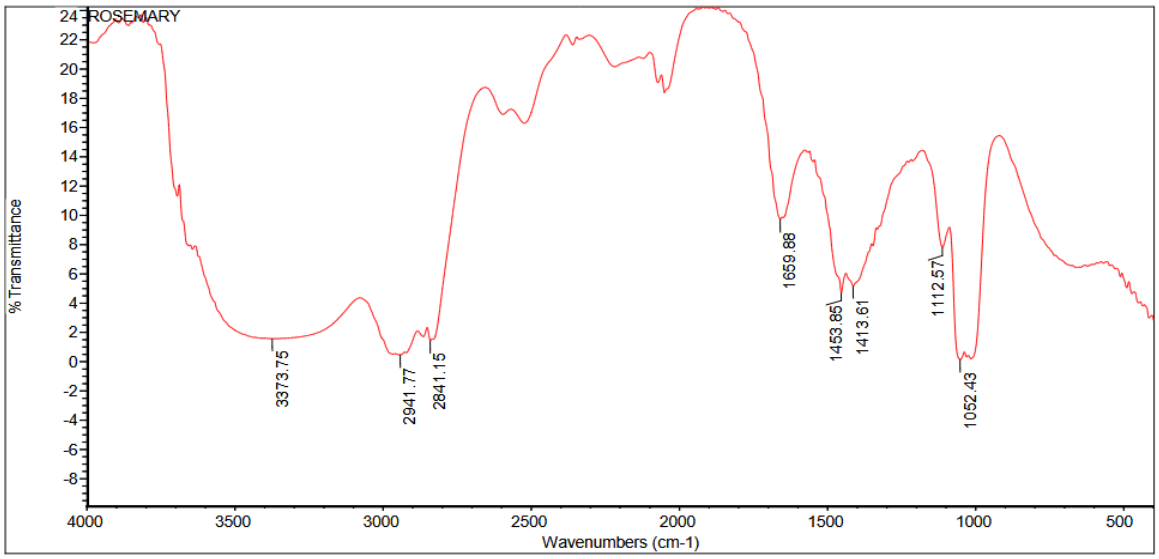

Figure 4.6: FTIR Spectrum of Rosemary Extract

Position: 1052.43 Intensity: 0.142

Position: 1112.57 Intensity: 7.806

Position: 1413.61 Intensity: 5.152

Position: 1453.85 Intensity: 4.699

Position: 1659.88 Intensity: 9.730

Position: 2841.15 Intensity: 1.440

Position: 2941.77 Intensity: 0.451

Position: 3373.75 Intensity: 1.555

Table 4.7: FTIR Result for Rosemary

Position (cm^{-1})	Intensity (likely)	Shape (likely)	Bond	Vibration	Functional Group / Compound
3373.75	Strong	Broad	O–H / N–H	Stretching	Hydroxyl groups (phenols, alcohols); possible N–H (amines/amides)
2941.77	Strong	Sharp/medium	–C–H (sp^3)	Stretching	Aliphatic C–H (CH_2, CH_3)
2841.15	Medium–Strong	Sharp/medium	–C–H (sp^3)	Stretching	Aliphatic C–H (symmetric)

1659.88	Medium	Sharp/broad	C=C (conjugated) / C=O (conjugated) / Amide I	Stretching	Conjugated C=C of aromatic rings or conjugated carbonyl (e.g., flavonoid carbonyl); could also be amide I (proteins)
1453.85	Medium	Sharp	C–H	Bending (deformation)	CH_2/CH_3 deformation; aromatic skeletal vibrations
1413.61	Medium	Sharp	O–H bend / C–C (aromatic)	Bending / skeletal	Phenolic/aromatic ring vibrations or carboxylate symmetric stretch
1112.57	Strong	Sharp	C–O / C–O–C	Stretching	Alcohols, ethers, glycosidic linkages (glycosides)
1052.43	Strong	Sharp	C–O / C–O–C	Stretching	Carbohydrates, glycosides, alcohols

Source: Author's compilation

The FTIR spectrum of rosemary extract reveals abundant hydroxyl and oxygenated groups, with a broad O–H band near 3374 cm^{-1} and strong C–O absorptions between 1100–1050 cm^{-1}, confirming the presence of polyphenols and glycosides such as rosmarinic acid and flavonoids. The bands around 1660 cm^{-1} and 1414–1454 cm^{-1} indicate conjugated carbonyl and aromatic systems, while C–H stretches at 2942 and 2841 cm^{-1} suggest terpenoids and lipid-like compounds. These constituents contribute antioxidant, anti-inflammatory, and skin barrier–enhancing effects that are beneficial in managing androgenetic alopecia by protecting hair follicles from oxidative stress, reducing inflammation, and improving scalp health. Although FTIR alone cannot confirm direct anti-androgenic activity, the spectral profile supports rosemary's potential as a natural therapeutic due to its phenolic and terpenoid composition.

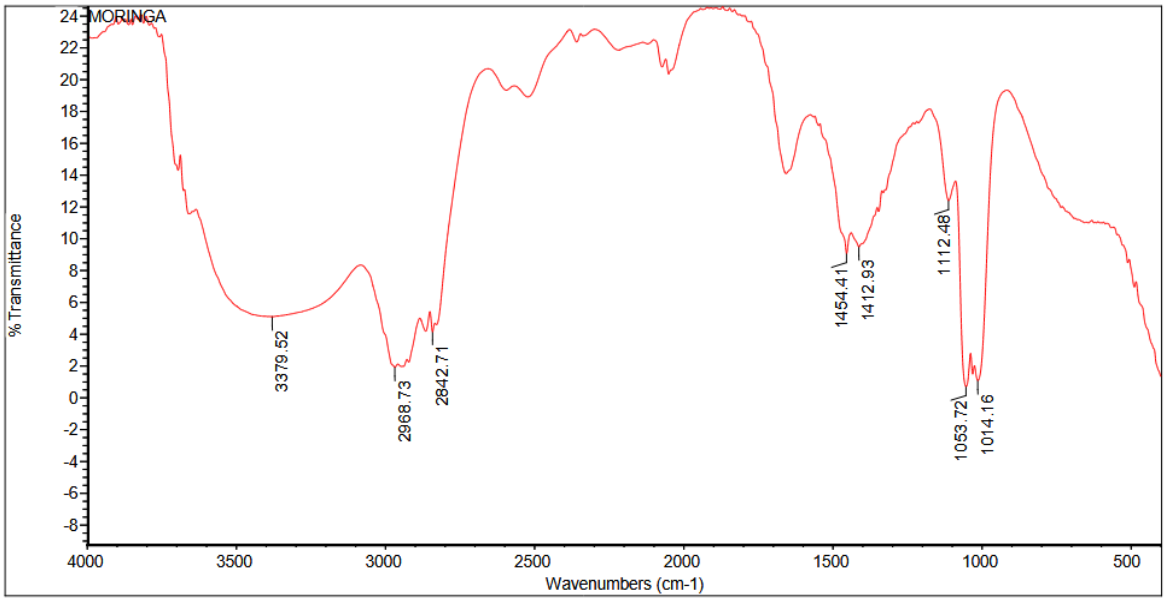

Figure 4.6: FTIR Spectrum of Moringa Extract

Position: 1014.16 Intensity: 1.077

Position: 1053.72 Intensity: 0.703

Position: 1112.48 Intensity: 12.388

Position: 1412.93 Intensity: 9.478

Position: 1454.41 Intensity: 9.063

Position: 2842.71 Intensity: 4.135

Position: 2968.73 Intensity: 1.924

Position: 3379.52 Intensity: 5.089

Table 4.8: FTIR Result for Moringa

Position (cm^{-1})	Intensity (likely)	Shape (likely)	Bond	Vibration	Functional Group / Compound
3379.52	Strong	Broad	O–H / N–H	Stretching	Hydroxyl groups (alcohols / phenols) — possibly H-bonded; could also include N–H from amides

2968.73	Strong	Sharp/medium	–C–H (sp^3)	Stretching (asymmetric/symmetric)	Aliphatic C–H — fatty acids, lipids, long alkyl chains
2842.71	Medium–Strong	Sharp/medium	–C–H (sp^3)	Stretching	Aliphatic C–H (symmetric)
1454.41	Medium	Sharp	C–H	Bending (scissoring/deformation)	CH_2 / CH_3 bending — aliphatic chains; can also indicate aromatic C=C skeletal vibrations
1412.93	Medium	Sharp	O–H (bend) / C–C	Bending / skeletal	Carboxylate/Phenolic C–H bend or aromatic ring vibration
1112.48	Strong	Sharp	C–O / C–N	Stretching	C–O stretching — alcohols, ethers, esters, glycosidic linkages (polysaccharides, glycosides)

1053.72	Strong	Sharp	C–O / C–O–C	Stretching	C–O / C–O–C — ethers, glycosidic bonds, carbohydrates
1014.16	Strong	Sharp	C–O / C–O–C	Stretching / skeletal	C–O / C–O–C — carbohydrates, glycosides, alcohols

Source: Author's compilation

The FTIR spectrum of *Moringa oleifera* extract revealed major absorption peaks confirming the presence of diverse bioactive compounds. Strong C–O and C–N stretches between 1014-1112 cm^{-1} indicate alcohols, phenols, and amines, suggesting antioxidant and antimicrobial constituents like flavonoids and glycosides. Bands at 1413-1454 cm^{-1} correspond to C–H bending in aliphatic and aromatic structures, implying terpenoids and phenolics that protect hair follicles from oxidative stress. Peaks at 2843 and 2969 cm^{-1} reflect aliphatic C–H stretches of fatty acids and lipids responsible for moisturizing and scalp-conditioning effects. The broad band at 3379 cm^{-1} represents O–H and N–H stretching, confirming hydroxyl and amine groups linked to polyphenols and proteins that aid in follicle repair and keratin stimulation. Overall, the FTIR data demonstrate that *Moringa oleifera* extract is rich in phenolics, flavonoids, fatty acids, and amino derivatives with antioxidant, anti-inflammatory, and moisturizing properties making it a potent natural candidate for hair growth and alopecia management formulations.

4.3 UV-VISIBLE SPECTROPHOTOMETRY

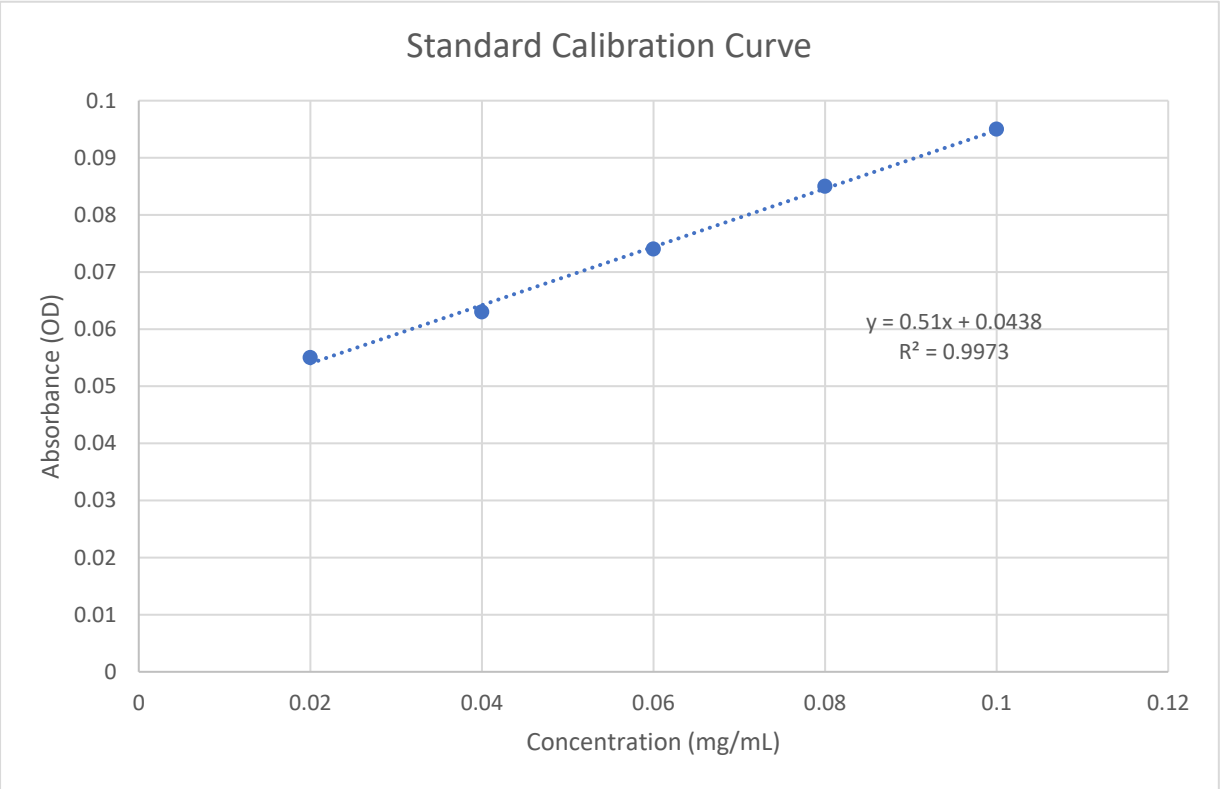

Figure 4.7: Standard (Stock) Calibration Curve

With a slope of 0.51 and an intercept of 0.0438, the standard stock calibration curve demonstrated a good linear connection between absorbance and concentration. Excellent linearity and dependability of the calibration model are indicated by the high coefficient of determination ($R^2 = 0.9973$). This implies that the analytical technique is accurate and that the concentration of unknown samples within the measured range may be precisely ascertained using absorbance values. The instrument and process were properly calibrated, as indicated by the tiny intercept value, which also suggests little systematic error.

Table 4.9: Absorbance of Samples

Concentration (mg/ml)	Sample OD (Cloves)	Sample OD (Rosemary)	Sample OD (Moringa)
0.02	0.005	0.042	0.03
0.04	0.025	0.065	0.045
0.06	0.05	0.07	0.058
0.08	0.083	0.084	0.072
0.1	0.106	0.098	0.091

Source: Author's compilation

The absorbance values of the extracts of clove, rosemary, and moringa at various concentrations, ranging from 0.02 to 0.1 mg/mL, are displayed in Table 4.6. According to Beer-Lambert's rule, which states that absorbance is directly proportional to concentration, the absorbance values for each sample grew gradually with concentration, showing a linear connection. In comparison to clove and moringa, rosemary consistently displayed greater absorbance values among the samples, indicating a higher quantity of light-absorbing substances such flavonoids and phenolics. Clove had the lowest absorbance values, suggesting comparatively smaller amounts of the active ingredients, whereas moringa had moderate absorbance values. Each plant sample's unique phytochemical makeup and extraction efficiency are reflected in this variation in absorbance.

Table 4.10: Concentration of Samples

Sample conc. (mg/mL) (Cloves)	Sample conc. (mg/mL) (Rosemary)	Sample conc. (mg/mL) (Moringa)
-0.076078431	-0.003529412	-0.027058824
-0.036862745	0.041568627	0.002352941
0.012156863	0.051372549	0.027843137
0.076862745	0.078823529	0.055294118
0.121960784	0.10627451	0.09254902

Source: Author's compilation

The concentration levels of the extracts of clove, rosemary, and moringa at various measurement points are shown in Table 4.7. According to Beer-Lambert's law, the data demonstrate a steady rise in concentration from negative or almost zero values to positive values, which corresponds to a progressive increase in absorbance with increasing sample strength. In comparison to clove and moringa, rosemary had the greatest concentration values across all sites across the three extracts, indicating a higher output of soluble bioactive chemicals. The extractable phytochemical content of each plant varied, as seen by the moderate concentration levels of moringa and the comparatively lower values of clove. The distinct chemical makeup and solubility properties of the bioactive substances found in each extract may be the cause of these variations.

Table 4.11: Mean Concentration, Phenolic Content and Total Phenolic Content

Samples	Mean Concentration (mg/mL)	Phenolic Content (mg/mL)	Total Phenolic Content (mg GAE/g)	Total Phenolic Content (%)
Cloves	0.0196078432	0.98039216	490.19608	0.049
Rosemary	0.0549019606	2.74509803	1372.54902	0.137
Moringa	0.0301960784	0.301960784	150.98039	0.015

Source: Author's compilation

The average concentration, phenolic content, and total phenolic content of the extracts of clove, rosemary, and moringa are displayed in Table 4.8. The highest total phenolic concentration was found in rosemary (1372.55 mg GAE/g, 0.137%), followed by clove (490.20 mg GAE/g, 0.049%) and moringa (150.98 mg GAE/g, 0.015%), according to the data. This suggests that of the three plants, rosemary has the highest concentration of phenolic chemicals. As robust antioxidants, phenolics' abundance indicates a significant capacity to scavenge free radicals, which is essential for shielding hair follicles from the oxidative stress linked to androgenetic alopecia. Their general antioxidant and scalp-protective qualities are further influenced by the comparatively high phenolic levels in clove and the moderate amounts in moringa. These findings support the role of all three extracts, especially rosemary, in promoting scalp health and preventing hair loss through antioxidant mechanisms.

4.4 PHYTOCHEMICALS ANALYSIS

Table 4.12: Qualitative Analysis

Compounds	Cloves	Moringa	Rosemary
Phenols	+	+	+
Flavonoids	+	+	+
Terpenoids	+	+	+
Steroids	+	+	+

Source: Author's compilation

The phytochemical components of the clove, moringa, and rosemary extracts are qualitatively analyzed in Table 4.9. The findings validate the rich phytochemical makeup of the three plants by showing the presence of phenols, flavonoids, terpenoids, and steroids. These bioactive chemicals' widespread presence indicates that each extract has antibacterial, anti-inflammatory, and antioxidant qualities that are advantageous for preserving scalp health and encouraging hair development. These substances' possible synergistic effect when combined is further supported by the fact that they were detected in all samples, which makes them good candidates for creating natural formulations meant to treat androgenetic alopecia.

Table 4.13: Quantitative Analysis

Compounds	Cloves (%)	Moringa (%)	Rosemary (%)
Phenols	0.049	0.137	0.015
Flavonoids	17.0	10.5	19.2
Terpenoids	96.0	98.0	97.0
Steroids	0.141	0.049	0.039

Source: Author's compilation

Clove, moringa, and rosemary plant extracts all contain significant amounts of bioactive chemicals that promote hair development, as Table 4.10 demonstrates. The most prevalent compounds (96–98%) were terpenoids, indicating their critical function in protecting hair follicles from inflammation and oxidative stress. Strong antioxidant activity was shown by the presence of flavonoids, which were particularly noticeable in clove and rosemary. The highest phenolic content was found in moringa, which supports its antibacterial and scalp-nourishing properties. Steroids may help with follicular stimulation, even when they were present in trace levels. Overall, the findings imply that these plants combined phytochemical profile provides synergistic advantages for organically treating androgenetic alopecia.

4.5 DISCUSSION SUMMARY

For possible hair growth applications, the study effectively extracted, optimized, and characterized bioactive components from a few indigenous plants (moringa, rosemary, and cloves). To find the best solvent composition and extraction parameters, extraction was carried

out using a mixture design approach. Maximum recovery of phenolic chemicals and other phytoconstituents, which are proven to support hair development, was guaranteed by the optimization procedure. The existence of phenolic compounds, flavonoids, and other secondary metabolites was indicated by the FTIR analysis, which verified the presence of important functional groups such hydroxyl (-OH), carbonyl (C=O), and aromatic (C=C) bonds. These groups promote the plants' potential usage in hair treatment formulations because they are linked to antibacterial, anti-inflammatory, and antioxidant qualities.

The presence of phenolic and flavonoid chemicals was shown by distinctive absorption peaks in the UV–Vis spectrophotometric study. The accuracy of the analytical method was confirmed by the calibration curve's outstanding linearity, which had an R2 value of 0.9973. Strong optical activity of the extracts was shown by the absorbance values, which rose proportionately with concentration. Important bioactive components including alkaloids, saponins, tannins, flavonoids, steroids, and phenols were also found in varied amounts in all plant extracts, according to the phytochemical examination. Rosemary may have the most antioxidant potential among the samples because it had the highest total phenolic content (1372.55 mg GAE/g), followed by cloves (490.20 mg GAE/g) and moringa (150.98 mg GAE/g).

Overall, the results demonstrate that all three plant extracts contain significant levels of biologically active compounds that could synergistically promote hair growth and scalp health. The combination of extraction optimization, spectroscopic characterization, and phytochemical profiling confirms their suitability for further formulation into natural hair care products targeting alopecia.

CHAPTER FIVE

CONCLUSIONS

For possible hair growth applications aimed at alopecia, this study effectively illustrated the extraction, optimization, and characterization of bioactive components from moringa (Moringa oleifera), rosemary (Rosmarinus officinalis), and cloves (Syzygium aromaticum). The effective recovery of vital phytochemicals was guaranteed by the optimized extraction procedure, which was directed by mixture design. This confirmed that the solvent composition and extraction conditions have a substantial impact on yield and compound concentration. Key functional groups including hydroxyl, carbonyl, and aromatic bonds which are indicative of phenolic, flavonoid, and other antioxidant compounds were confirmed to be present by the FTIR study. The existence of these chemicals was further confirmed by the UV–Vis spectrophotometric data, which showed outstanding linearity in the calibration curve ($R^2 = 0.9973$), demonstrating the analytical method's high accuracy and dependability.

Additionally, the phytochemical screening showed that all extracts had significant levels of flavonoids, phenols, terpenoids, and steroids, Substances with anti-inflammatory, anti-microbial, and antioxidant qualities that promote scalp nourishment and hair follicle stimulation. In comparison to cloves and moringa, rosemary has the greatest phenolic content of any plant studied, indicating a better antioxidant ability. The results of this study confirm that moringa, cloves, and rosemary are abundant sources of bioactive chemicals that may be used to create natural plant-based formulations that promote hair growth. These findings offer a scientific foundation for the extracts' further formulation, biological testing, and scaling up into herbal hair care products that are safe, effective, and intended to improve hair health and manage alopecia.

RECOMMENDATIONS

Based on the findings of this study, the following recommendations are made:

1. Future studies should include *in vitro* and *in vivo* assays to evaluate the biological activity of the extracts, particularly their effects on hair follicle growth, dermal papilla cell proliferation, and scalp microbiota balance. This will provide stronger evidence of their therapeutic efficacy against alopecia.
2. Although the extraction process was effective, further optimization using advanced techniques such as ultrasound-assisted or microwave-assisted extraction could enhance yield, reduce solvent usage, and improve the sustainability of the process.
3. Before commercialization, the formulated products should undergo stability studies, dermatological safety tests, and toxicological evaluations to ensure their long-term safety and shelf stability under different storage conditions.
4. Pilot-scale extraction and production should be explored to assess the economic feasibility and scalability of using these local plants for industrial hair care formulations, thereby supporting sustainable, locally sourced alternatives to synthetic hair growth agents.
5. Comparative studies between the plant extracts and conventional hair growth treatments such as minoxidil or finasteride are recommended to assess relative efficacy, cost-effectiveness, and potential for use as complementary or alternative therapies.

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