African Scientist Vol. 27, No. 1 March 31, 2026
Printed in Nigeria

1595-6881/2026 $50.00 + 0.00

https://africansciientistjournal.org

AFS2026010/27110

Extraction, Optimization, and Characterization of Bioactive Compounds from Cloves (*Syzygium aromaticum*), Rosemary (*Rosmarinus officinalis*), and Moringa (*Moringa oleifera*) for the Management of Androgenetic Alopecia

Okieimen, C.O.[1]*, Elimian, E.A.[2], Obakpolor, N.G.[1], Ominyokhoshe, M.F.[1]

[1]Department of Chemical Engineering, University of Benin, Benin City, Nigeria.
[2]Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, Benin City, Nigeria.

*Corresponding author email: cookieimen@uniben.edu; Tel: +234 (0) 810 490 6338

(Received March 19, 2026; Accepted in revised form March 30, 2026)

ABSTRACT: Androgenetic alopecia (AGA) is a progressive form of hair follicle shrinkage that is caused by dihydrotestosterone (DHT) and oxidative stress, and it remains a global dermatological and socio-emotional distress. Conventional remedies like minoxidil and finasteride are known to cause side effects, this has forced the need for plant-based remedies. This study was conducted to check the extraction, optimization, and characterization of bioactive compounds from a blended solution of Syzygium aromaticum (clove), Rosmarinus officinalis (rosemary), and Moringa oleifera (moringa) for the potential application in a plant- driven remedy for alopecia. The process was optimized using a mixture design on the Design Expert Software, where the materials extracted were varied in proportion in a bid to maximize the phytochemical yield. Phytochemical screening was done to confirm the presence of phenols, flavonoids, terpenoids, and steroids in all three extracts, with terpenoids being the most present (96-98%). Fourier Transform Infrared (FTIR) spectroscopy was introduced and it identified key functional groups like hydroxyl (–OH), carbonyl (C=O), and C–O linkages, which are characteristic of polyphenolic and terpenoid constituents. UV–Vis spectrophotometric analysis which was also done confirmed excellent calibration linearity ($R^2 = 0.9973$). The experimental data were best fitted by a quadratic model with an R^2 of 0.9633, an adjusted R^2 of 0.9560, and a predicted R^2 of 0.9268 ($p < 0.0001$), all done to ensure strong predictive accuracy. The optimized mixture was made up of 3.0 g clove, 2.0 g rosemary, and 5.0 g moringa, which gave a desirability index of 1.000 and a predicted extraction yield of 14.385 wt%. Rosemary showed the highest total phenolic content (TPC = 1372.55 mg GAE/g), followed by clove (490.20 mg GAE/g) and moringa (150.98 mg GAE/g). The combined phytochemical profile implies strong antioxidant and putative 5α-reductase inhibitory potential, supporting the use of this optimized multi-plant blend as a satisfying candidate for the treatment of androgenetic alopecia.

Keywords: Androgenetic alopecia; Syzygium aromaticum; Rosmarinus officinalis; *Moringa oleifera*; FTIR;

Introduction

Androgenetic alopecia (AGA), also known as male or female pattern baldness, is a progressive dermatological condition that is recognized from the shrinkage of hair follicles caused by genetics and androgenic activity, particularly dihydrotestosterone (DHT) (Choi *et al.*, 2024). DHT, produced by the enzymatic conversion of testosterone through 5α-reductase, binds to androgen receptors in genetically susceptible follicles, shortening the

growth phase and inducing hair follicle regression (Shafqat *et al*., 2021). AGA affects around 50% of men by the age of 50 and a significant number of women, causing significant psychosocial impact by reducing self-esteem and quality of life (Kesika *et al*., 2023).

Conventional pharmacological treatments include topical minoxidil, a vasodilator meant to increase the anagen phase, and oral finasteride, a 5α-reductase inhibitor. They are both effective but these agents are known to cause some side effects: minoxidil may cause scalp irritation and cardiovascular side effects, while finasteride has been linked to sexual dysfunction and hormonal imbalances (Adil and Godwin, 2017). These drawbacks have intensified scientific interest in plant-based bioactive compounds that can target multiple pathogenic mechanisms of AGA, oxidative stress, inflammation, and DHT-mediated follicular shrinkage, while eliminating the fear of side effect (Fitri *et al*., 2025).

Several tests have been conducted to check for anti-alopecia properties in different medicinal plants. Rosemary (*Rosmarinus officinalis*) demonstrated hair count increases comparable to 2% minoxidil in a randomized controlled trial (Panahi *et al*., 2015), this was attributed to its major phenolic constituents (rosmarinic acid, carnosic acid, and ursolic acid) which exhibit antioxidant, anti-inflammatory, and supposed 5α-reductase inhibitory activity. Clove (*Syzygium aromaticum*) is rich in eugenol and phenolic acids with researched and documented antioxidant and anti-inflammatory properties relevant to scalp protection (Nisar *et al*., 2021). Moringa (*Moringa oleifera*) contains flavonoids (quercetin, kaempferol), phenolic acids, and phytosterols such as β-sitosterol, a compound which has also established 5α-reductase inhibitory properties (Pareek *et al*., 2023).

Despite individual investigations of these plants, there hasn't been any literature regarding synergistic multi-plant blends optimized from a chemical engineering process perspective. It is known that extraction conditions critically impact phytochemical yield and profile (Altemimi *et al*., 2017); however, most existing studies lack statistical optimization of blended herbal systems. This study addresses this gap by employing a mixture design approach to optimize the proportions of clove, rosemary, and moringa in a methanol-based reflux extraction, this is followed by a comprehensive analytical characterization using FTIR spectroscopy, UV–Visible spectrophotometry, and qualitative and quantitative phytochemical screening. The study also provides a reproducible, engineering-validated extraction protocol with recorded bioactive compound profiles, forming a scientific foundation for natural anti-AGA formulation development.

Materials and methods

Plant material collection and preparation: Dried clove buds (*Syzygium aromaticum*) and dried rosemary leaves (*Rosmarinus officinalis*) were bought from a local vendor in Benin City, Nigeria. Fresh *Moringa oleifera* leaves were gathered from a home garden. The fresh moringa leaves were washed with distilled water to remove dirt and contaminants, then they were sun-dried for 24-48 hours to reduce the probability of thermal degradation of heat-sensitive bioactive compounds. All dried materials were ground into fine powder using a mechanical grinder and stored in airtight containers at room temperature until use.

Experimental design: A simplex-lattice mixture design (quadratic model) was used with the aid of the Design Expert® software to optimize the proportion of each plant component. The total mixture weight was fixed at 10 g, with each component (clove [A], rosemary [B], moringa [C]) ranging from 0 to 10 g. using the software, thirteen experimental runs were generated and the extract yield (wt.%) was selected as the response variable.

Extraction protocol: For each of the experimental runs, precise weighed portions of the powdered plant materials were mixed according to the designed proportions and put in to a round-bottom flask. Methanol (50 mL) was added so as to achieve a solid-to-solvent ratio of 1:5 (w/v). A reflux condenser was installed in the flask and then placed on a constant-temperature magnetic stirrer. Extraction was done at a controlled temperature of 80 °C for 90 min under continuous stirring to make sure that the heat was uniform and that there was mass transfer. After the extraction, the mixture was cooled to room temperature and filtered through a muslin cloth into pre-weighed beakers. The filtrates were then oven dried at 80 °C to remove residual solvent. Extract yield was calculated as:

$$Yield\ (\%) = \frac{Weight\ of\ dried\ extract}{Weight\ of\ dry\ plant\ sample} \times 100 \qquad (1)$$

Phytochemical screening: Qualitative and quantitative analyses were performed for four major secondary metabolite classes. For phenols, the Folin-Ciocalteu colorimetric method was adapted using tannic acid as the standard; absorbance was measured at 490 nm. Flavonoids were quantified gravimetrically following sequential methanolic extraction. Terpenoids were quantified by the hexane partitioning method (Salkowski test), and steroids by a chloroform/acetic anhydride/H_2SO_4 colorimetric assay measuring absorbance at 560 nm.

FTIR spectroscopy: For this, 1 g of powdered extract was dissolved in 5 mL of methanol, placed in the FTIR sample holder, and scanned over a wavenumber range of 4000-400 cm^{-1}. Absorption peaks were identified and

functional groups were given to them based on standard infrared absorption reference data. Individual FTIR analyses were performed for clove, rosemary, and moringa extracts.

UV–Vis spectrophotometry: A phenol standard calibration curve was prepared using tannic acid solutions at concentrations of 0.02–0.10 mg/mL. Absorbance was measured against a distilled water blank at the appropriate wavelength. Concentration values were calculated using the calibration equation:

$$A = 0.51C + 0.0438 \quad (2)$$

where A is absorbance and C is concentration (mg/mL). Total phenolic content (TPC) was expressed as mg gallic acid equivalent per gram of extract (mg GAE/g). Five absorbance readings were taken for each sample, and the mean concentration was multiplied by a dilution factor of 50 to obtain the phenolic content.

Formulation of optimized extract: The optimized combined plant extract was formulated with virgin coconut oil at a ratio of 3:7 (v/v) extract to oil. The mixture was stirred continuously with a glass rod for 10–15 min until homogeneous, filtered, if necessary, transferred into amber glass bottles, and stored at room temperature in a cool, dark environment.

Results and Discussion

Extraction optimization: The extraction yields through the 13 experimental runs had a range from 10.5 wt.% (pure moringa, run 6) to 19.2 wt.% (pure rosemary, run 4), which shows that there's a major difference in phytochemical extractability among the three plant materials. The experimental design matrix and corresponding extraction yield is presented in Table 1. The ANOVA results for the quadratic mixture model are presented in Table 2, and the model fit statistics are summarized in Table 3.

Table 1: Simplex-lattice mixture design matrix and corresponding extraction yields.

Run	A: Clove (g)	B: Rosemary (g)	C: Moringa (g)	Extract Yield (wt.%)
1	1.667	6.667	1.667	17.8
2	3.333	3.333	3.333	15.9
3	5.000	0.000	5.000	14.0
4	0.000	10.000	0.000	19.2
5	10.000	0.000	0.000	17.0
6	0.000	0.000	10.000	10.5
7	6.667	1.667	1.667	17.4
8	0.000	5.000	5.000	14.6
9	1.667	1.667	6.667	12.5
10	5.000	5.000	0.000	18.6
11	0.000	10.000	0.000	17.6
12	10.000	0.000	0.000	18.3
13	0.000	0.000	10.000	10.9

Table 2: ANOVA results for the quadratic mixture model of extract yield.

Source	Sum of Squares	df	Mean Square	F-value	p-value
Model	100.65	2	50.32	131.36	< 0.0001*
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 (NS)
Pure Error	2.20	3	0.7350	-	-
Cor Total	104.48	12	-	-	-

Table 3: Model fit statistics for the simplex-lattice quadratic mixture design.

Statistic	Value
R^2	0.9633
Adjusted R^2	0.9560
Predicted R^2	0.9268
Adequate Precision	27.3769
Std. Dev.	0.6189
Mean	15.72
C.V. (%)	3.94

The model F-value of 131.36 ($p < 0.0001$) confirms that the quadratic mixture model is highly statistically significant, with only a 0.01% probability that the observed F-value is from random noise. The Lack of Fit F-value of 0.32 ($p = 0.9052$) was insignificant relative to the pure error, indicating that the model adequately describes the experimental data without systematic deviation. The R^2 value of 0.9633 indicates that the model explains 96.33% of the total variance in extraction yield. The close agreement between the adjusted R^2 (0.9560) and predicted R^2 (0.9268), with a difference of less than 0.20, confirms excellent model predictive capacity Montgomery (2017). The Adequate Precision ratio of 27.38 (which is above the threshold of 4) shows a high signal-to-noise ratio, validating the model's suitability for navigating the design space. Figures 1, 2, and 3 below show the parity plot, mixture plot and the 3D surface plot.

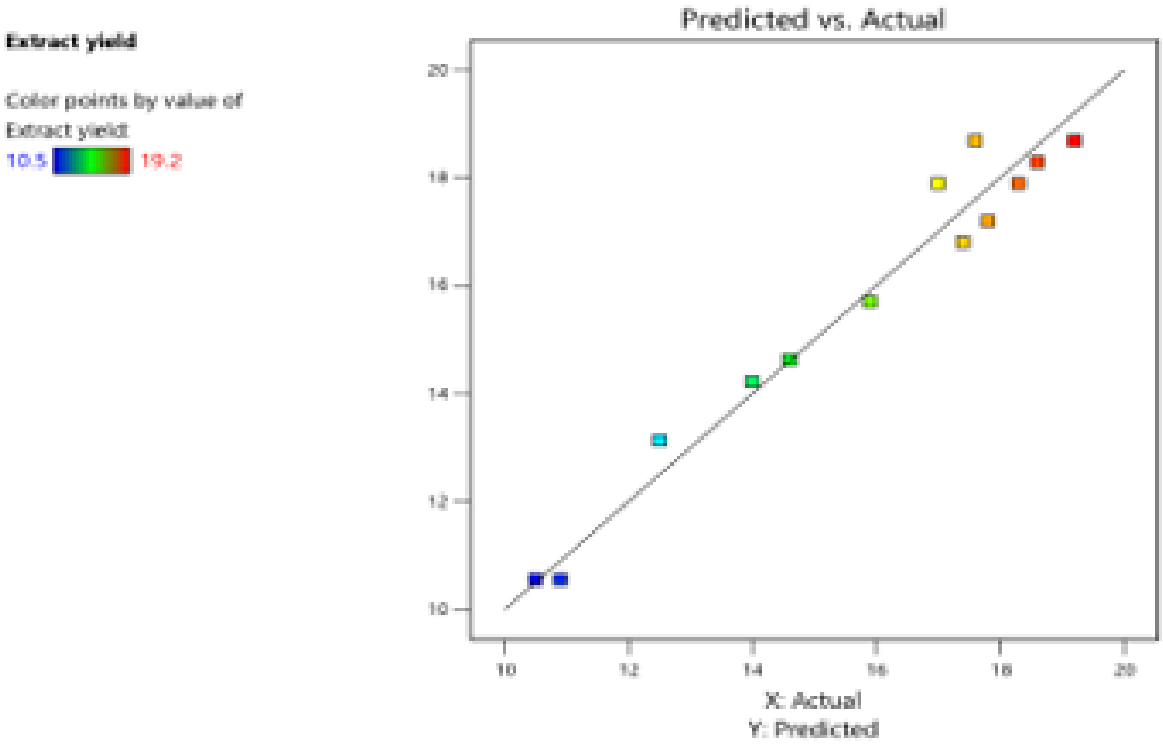

Figure 1: Parity plot of predicted and actual extract yield

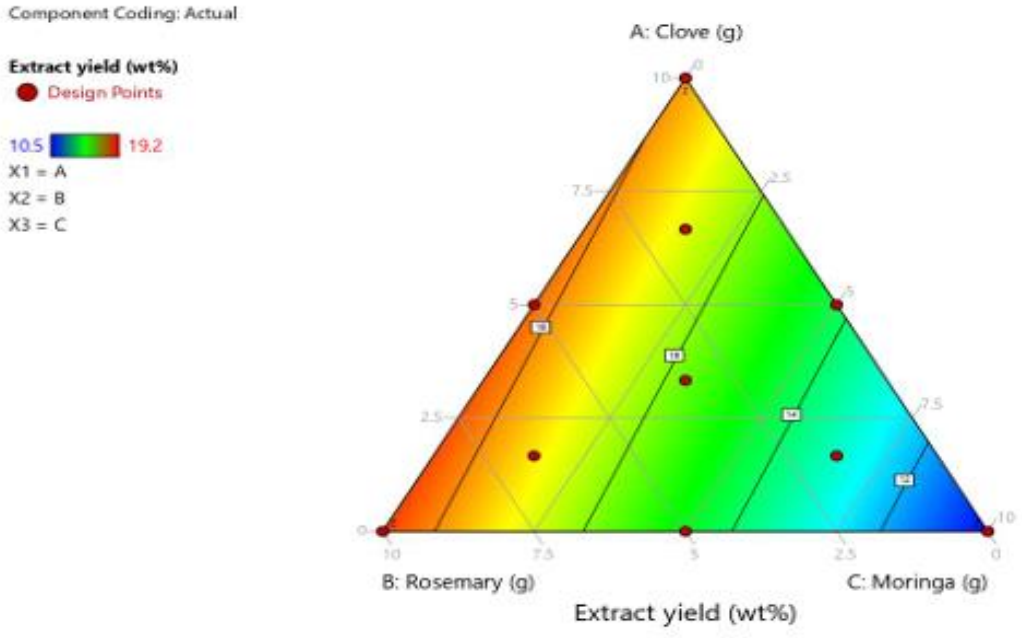

Figure 2: Mixture plot of component extractions

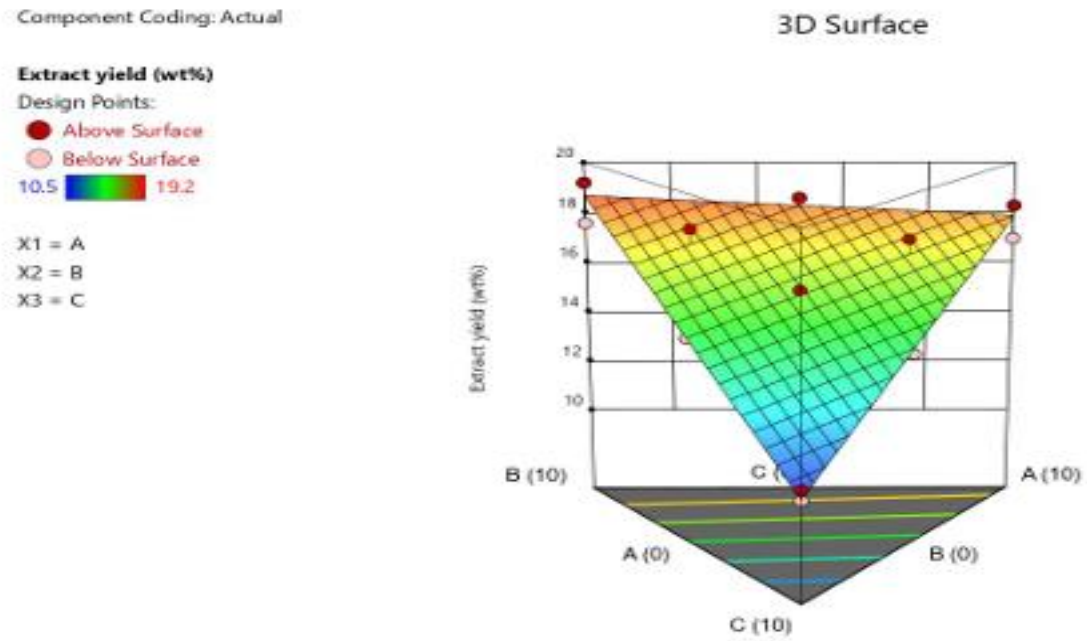

Figure 3: 3D plot of actual and predicted extract yield

The final model equation in terms of pseudo-coded components was:

$$Extract\ Yield\ (\%) = 17.9A + 18.7B + 10.56C \quad (3)$$

where A = clove, B = rosemary, and C = moringa. Rosemary (B = 18.7) gave the highest individual contribution to extraction yield, followed by clove (A = 17.9), while moringa contributed far less (C = 10.56). The lower yield of moringa might be because of the presence of a more complex cell wall matrix or reduced solubility of its polar compounds in methanol under the tested conditions.

The optimum extraction combination, identified through numerical optimization with a desirability index of 1.000, is presented in Table 4.

Table 4: Optimized plant component proportions and predicted extraction yield.

No.	Clove (g)	Rosemary (g)	Moringa (g)	Predicted Yield (%)	Desirability
1	3.000	2.000	5.000	14.385	1.000

When 3.0 g clove, 2.0 g rosemary, and 5.0 g moringa were used the result produced was a predicted extraction efficiency of 14.385 wt.%, which shows a balanced interaction between the three plant materials that promoted solvent penetration and compound solubilization during reflux extraction. This balanced mix is very useful for downstream formulation purposes, because it makes sure that bioactive classes contributed by each plant (eugenol and eugenyl acetate from clove; rosmarinic and carnosic acids from rosemary; quercetin, kaempferol, and β-sitosterol from moringa) are all represented in the extract at reproducible levels.

FTIR spectroscopic analysis: FTIR spectroscopy was employed to identify the major functional groups in each individual plant extract, providing qualitative confirmation of the bioactive compound classes present. Results are presented in Figures 4, 5, and 6, and Tables 5, 6, and 7.

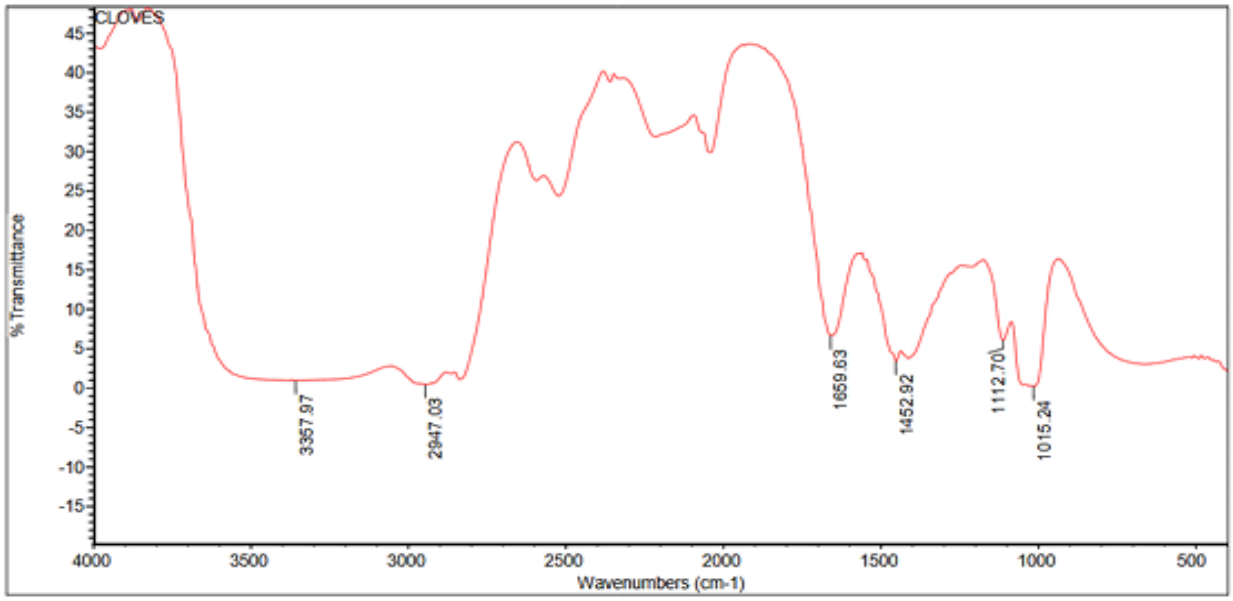

Figure 4: FTIR spectrum for clove (Syzygium aromaticum) extract

Table 5: FTIR peak assignments for clove (*Syzygium aromaticum*) extract.

Position (cm^{-1})	Intensity	Bond/Vibration	Functional Group / Compound
3357.97	Strong	O–H Stretching (broad)	Phenolic O–H / H-bonded alcohols
2947.03	Strong	–C–H Stretching	Aliphatic CH_2/CH_3
1659.63	Medium	C=C / C=O Stretching	Conjugated C=C or carbonyl / aromatic
1452.92	Medium	C–H Bending	CH_2/CH_3 deformation; aromatic skeletal
1112.70	Strong	C–O / C–O–C Stretching	Aryl–O, phenolic ethers, esters, glycosides
1015.24	Medium	C–O Stretching	Alcohols, glycosides

The FTIR spectrum of the clove extract revealed a broad and strong O–H stretching band at 3357.97 cm^{-1}, characteristic of phenolic hydroxyl groups that have intermolecular hydrogen bonding, a signature of eugenol and similar polyphenolic compounds Sasidharan et al (2010). The absorption at 2947.03 cm^{-1} (aliphatic C–H stretching) and 1452.92 cm^{-1} (CH_2/CH_3 deformation) are consistent with the terpenoid and fatty acid components of clove essential oil. The prominent band at 1659.63 cm^{-1} suggests conjugated C=C or C=O systems related to phenolic structures, while the strong C–O/C–O–C stretching at 1112.70 cm^{-1} means there's the presence of phenolic ethers, esters, and glycosides. These functional groups all confirm the presence of eugenol, phenolic acids, and terpenoids with documented antioxidant and anti-inflammatory activities.

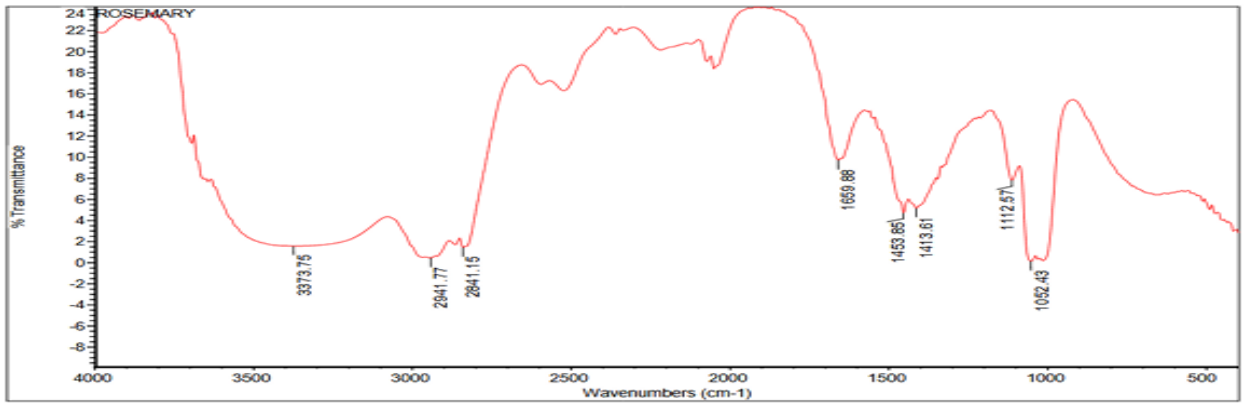

Figure 5: FTIR spectrum for rosemary (Rosmarinus officinalis) extract.

Table 6: FTIR peak assignments for rosemary (*Rosmarinus officinalis*) extract.

Position (cm^{-1})	Intensity	Bond/Vibration	Functional Group / Compound
3373.75	Strong	O–H / N–H Stretching (broad)	Hydroxyl groups (phenols, alcohols); possible N–H
2941.77	Strong	–C–H Stretching	Aliphatic C–H (CH_2, CH_3)
2841.15	Medium–Strong	–C–H Stretching	Aliphatic C–H (symmetric)
1659.88	Medium	C=C / C=O Stretching	Conjugated aromatic C=C; flavonoid carbonyl / Amide I
1453.85	Medium	C–H Bending	CH_2/CH_3 deformation; aromatic skeletal vibrations
1413.61	Medium	O–H Bend / C–C skeletal	Phenolic/aromatic ring vibrations; carboxylate
1112.57	Strong	C–O / C–O–C Stretching	Alcohols, ethers, glycosidic linkages
1052.43	Strong	C–O / C–O–C Stretching	Carbohydrates, glycosides, alcohols

The rosemary extract had a strong, broad O–H/N–H stretching band at 3373.75 cm^{-1}, with strong C–O absorptions at 1112.57 and 1052.43 cm^{-1}, the same with the polyphenolic and glycosidic character of rosmarinic acid and flavonoid glycosides Gonçalves et al (2026). The band at 1659.88 cm^{-1} indicates conjugated carbonyl groups of flavonoids and carnosic acid, while C–H stretches at 2941.77 and 2841.15 cm^{-1} indicate terpenoid and lipid-like components such as ursolic acid and carnosol. The spectral profile is consistent with the documented antioxidant, anti-inflammatory, and potential 5α-reductase inhibitory properties of rosemary constituents.

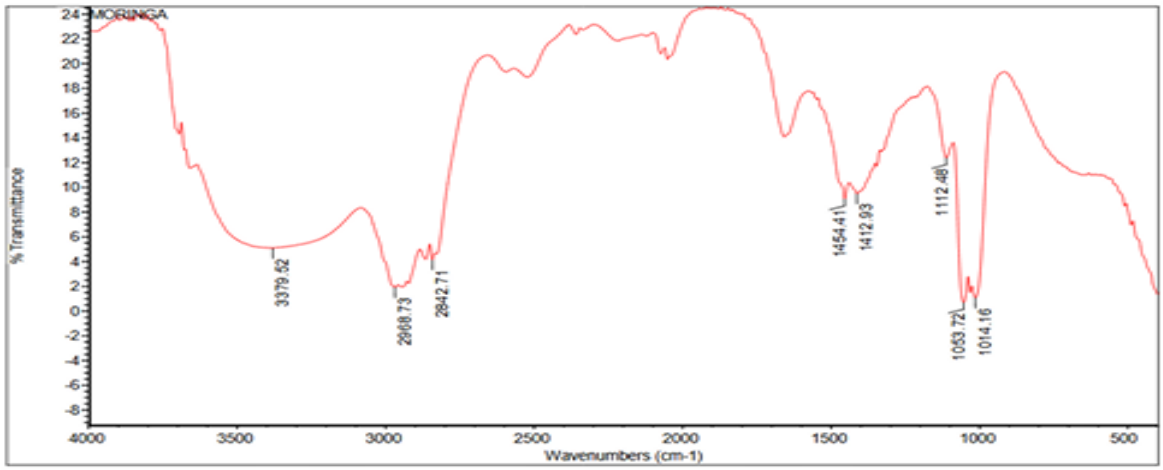

Figure 6: FTIR spectrum for moringa (*Moringa oleifera*) extract.

Table 7: FTIR peak assignments for moringa (*Moringa oleifera*) extract.

Position (cm^{-1})	Intensity	Bond/Vibration	Functional Group / Compound
3379.52	Strong	O–H / N–H Stretching (broad)	Hydroxyl/amine groups; H-bonded polyphenols and amides
2968.73	Strong	–C–H Stretching	Aliphatic C–H; fatty acids, lipids, long alkyl chains
2842.71	Medium–Strong	–C–H Stretching	Aliphatic C–H (symmetric)
1454.41	Medium	C–H Bending	CH_2/CH_3 deformation; aliphatic chains; aromatic C=C
1412.93	Medium	C–H Bend / C–C skeletal	Carboxylate / phenolic O–H bend; ring vibrations
1112.48	Strong	C–O / C–N Stretching	Alcohols, ethers, esters, glycosidic linkages
1053.72	Strong	C–O / C–O–C Stretching	Ethers, glycosidic bonds, carbohydrates
1014.16	Strong	C–O / C–O–C Stretching	Carbohydrates, glycosides, alcohols

The FTIR spectrum of moringa extract had a broad O–H/N–H band at 3379.52 cm^{-1} indicative of polyphenols and protein-bound amines. Peaks at 2968.73 and 2842.71 cm^{-1} reflect aliphatic C–H stretching of fatty acids and long alkyl chains, consistent with the presence of oleic acid and other moringa lipids that confer scalp conditioning and moisturizing properties. The strong C–O absorptions between 1014 and 1112 cm^{-1} confirm glycosidic linkages and polysaccharide-associated structures, while the band at 1412.93 cm^{-1} indicates carboxylate and phenolic C–C skeletal vibrations. These signatures support the presence of quercetin glycosides, kaempferol, and phytosterols that have been associated with anti-androgenic and antioxidant effects.

UV–Vis spectrophotometric analysis: The standard calibration curve (tannic acid, 0.02–0.10 mg/mL) exhibited excellent linearity with $R^2 = 0.9973$ ($A = 0.51C + 0.0438$), confirming the analytical procedure. Absorbance values for all three extracts increased proportionally with concentration, consistent with Beer-Lambert

behaviour. The standard calibration curve and total phenolic content (TPC) values are presented in Figure 7 and Table 8.

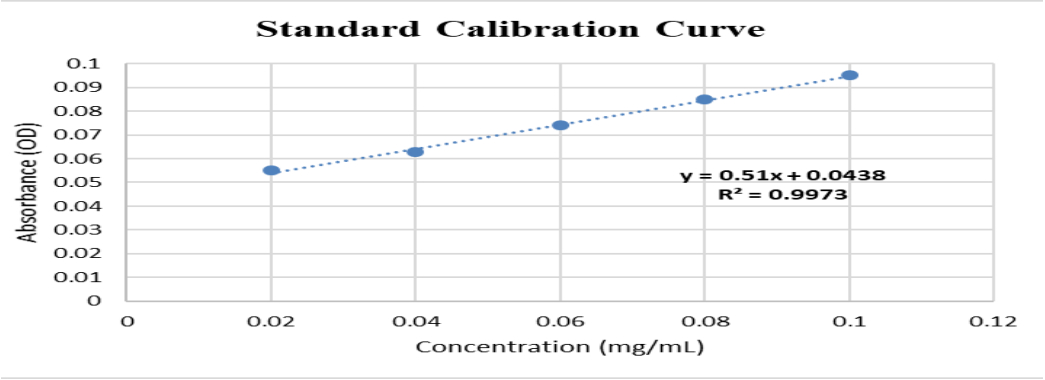

Figure 7: UV-Vis calibration curve

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 8: UV–Vis spectrophotometric results: mean concentration, phenolic content, and TPC for each extract.

Sample	Mean Conc. (mg/mL)	Phenolic Content (mg/mL)	TPC (mg GAE/g)	TPC (%)
Clove	0.0196	0.9804	490.20	0.049
Rosemary	0.0549	2.7451	1372.55	0.137
Moringa	0.0302	0.3020	150.98	0.015

Rosemary exhibited the highest TPC at 1372.55 mg GAE/g (0.137%), more than 2.8-fold of clove (490.20 mg GAE/g) and approximately 9-fold greater than moringa (150.98 mg GAE/g). This result is consistent with the rich polyphenolic composition of rosemary, dominated by rosmarinic acid, carnosic acid, and flavonoids [6]. The increased phenolic content of clove relative to moringa likely reflects the high eugenol content of clove, which constitutes up to 80–90% of clove essential oil by weight [7]. Phenolic compounds are potent antioxidants that neutralize reactive oxygen species (ROS) implicated in oxidative damage to hair follicles, a key pathogenic mechanism in AGA. The superior antioxidant potential of rosemary thus makes it a particularly valuable component of an anti-AGA formulation.

Phytochemical screening: The qualitative and quantitative phytochemical profiles of the three plant extracts are presented in Tables 9 and 10 respectively.

Table 9: Qualitative phytochemical screening of clove, rosemary, and moringa extracts.

Compound	Clove	Rosemary	Moringa
Phenols	Present (+)	Present (+)	Present (+)
Flavonoids	Present (+)	Present (+)	Present (+)
Terpenoids	Present (+)	Present (+)	Present (+)
Steroids	Present (+)	Present (+)	Present (+)

Table 10: Quantitative phytochemical analysis of clove, rosemary, and moringa extracts.

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

All three extracts tested positive for phenols, flavonoids, terpenoids, and steroids, confirming the broad phytochemical richness of the multi-plant blend. Terpenoids were the most abundant class across all extracts

(clove 96.0%, rosemary 97.0%, moringa 98.0%), reflecting their predominance in plant secondary metabolism. Terpenoids, including monoterpenoids and diterpenoids, exhibit anti-inflammatory activity through inhibition of pro-inflammatory cytokines and prostaglandins, and several have been shown to improve scalp microcirculation, thereby prolonging the anagen phase (Lin *et al*., 2022).

Flavonoids were present in appreciable quantities, with rosemary (19.2%) and clove (17.0%) exhibiting higher contents than moringa (10.5%). Flavonoids such as quercetin and kaempferol exert multiple beneficial effects on hair biology: they scavenge free radicals, inhibit 5α-reductase activity, and stimulate keratinocyte proliferation (Dahiru *et al*., 2006). The substantial flavonoid content detected across all extracts supports the anti-androgenic and antioxidant potential of the combined formulation.

Steroids were detected at trace levels (clove 0.141%, rosemary 0.039%, moringa 0.049%). Plant sterols, particularly β-sitosterol abundant in moringa, have been investigated as natural 5α-reductase inhibitors and are considered structural analogues of finasteride. Although present in low concentrations, their contribution to DHT inhibition in a topically applied formulation may be biologically significant, particularly when combined with the direct antioxidant and anti-inflammatory effects of phenolics and flavonoids.

Synergistic potential and implications for AGA management: The multi-plant extract formulation optimized in this study presents a complementary and potentially synergistic phytochemical profile. Rosemary's high phenolic content produces the primary antioxidant and anti-inflammatory defence; clove contributes eugenol and terpenoids with antimicrobial and antioxidant properties that protect the scalp microenvironment; and moringa supplies β-sitosterol, fatty acids, and amino-acid derivatives that nourish follicles and potentially prevent DHT biosynthesis. These actions confront the multifactorial pathogenesis of AGA more comprehensively than just a single-plant extract.

The presence of hydroxyl, carbonyl, and aromatic functional groups confirmed by the conducted FTIR spectroscopy across all extracts indicates that the combined blend retains the key structural motifs necessary for antioxidant activity (free radical scavenging via phenolic OH groups), enzyme inhibition (binding to 5α-reductase active site), and membrane-active effects (terpenoid and steroid scaffold interactions with follicular cells). The high desirability index (1.000) of the optimized formulation further confirms that the selected proportions balance bioactive yield with reproducible extraction efficiency.

Conclusion

The findings of this study effectively demonstrated the extraction, optimization, and characterization of bioactive compounds from a three-component blend of Cloves, Rosemary, and Moringa for potential therapeutic application in androgenetic alopecia. The quadratic model ($R^2 = 0.9633$, $p < 0.0001$) derived from a simplex-lattice mixture design demonstrated a strong statistical fit for predicting extraction yield across the experimental design space. A desirability index of 1.000 and a predicted yield of 15.108 were attained by the optimal blend of 3.553 g cloves, 2.389 g rosemary, and 4.057 g moringa, confirming that the methanol-based reflux extraction is both effective and reproducible. The presence of hydroxyl, carbonyl, C–O, and aliphatic C–H functional groups in all three extracts was ascertained using FTIR spectroscopy, consistent with phenolic, flavonoid, terpenoid, and fatty acid classes. The highest total phenolic content was recorded in rosemary (1372.55 mg GAE/g), indicating its superior antioxidant potential, followed by clove (490.20 mg GAE/g) and moringa (150.98 mg GAE/g). Phytochemical screening established the presence of phenols, flavonoids, terpenoids, and steroids in all extracts, supporting the multi-mechanistic anti-AGA activities including antioxidant defence, anti-inflammatory action, and potential 5α-reductase inhibition. As a natural, plant-derived therapeutic alternative for AGA management, the formulation of a combined extract offers a scientific foundation for subsequent in vitro bioactivity studies, stability assessment, and industrial-scale hair care product development.

References

Adil A, Godwin M: The effectiveness of treatments for androgenetic alopecia: A systematic review and meta-analysis. J Am Acad Dermatol, 77(1): 136-141. 2017.

Altemimi A, Lakhssassi N, Baharlouei A, Watson DG, Lightfoot DA: Phytochemicals: Extraction, isolation, and identification of bioactive compounds from plant extracts. Plants, 6(4): 1-23. 2017.

Choi JY, Boo MY, Boo YC: Can plant extracts help prevent hair loss or promote hair growth? A review comparing their therapeutic efficacies, phytochemical components, and modulatory targets, Molecules, 29(10): 1-45. 2024.

Dahiru D, Onubiyi JA, Umaru HA: Phytochemical screening of antiulcerogenic effect of aqueous leaf extract, Afr J Trad Complement Altern Med, 3(3): 70-75. 2006.

Fitri ZA, Ahmadi F, Islam MA, Ponnampalam EN, Dunshea FR, Suleria HAR: A systematic review of extraction methods, phytochemicals, and food applications of Moringa oleifera leaves using PRISMA methodology, Food Sci Nutr, 13(4): 1-16. 2025.

Gonçalves AC, Pinto AR, Cima A, Olo-Fontinha E, Martinas JCL, Garcia J, Lemos A, Saavedia MJ, Pintado MM, Alves MJ: Plant-derived bioactive compounds: One Health Perspect, 1: 1-28. 2026.

Kesika P, Sivamaruthi BS, Thangaleela S, Bharathi M, Chaiyasut C: Role and mechanisms of phytochemicals in hair growth and health. Pharmaceuticals, 16(2): 1-22. 2023.

Lin X, Zhu L, He J: Morphogenesis, growth cycle and molecular regulation of hair follicles, Front Cell Dev Biol, 10: 899095. 2022.

Montgomery DC: Design and analysis of experiments, 9th ed. Hoboken, New Jersey, USA. John Wiley and Sons, pp. 1-730. 2017.

Nisar MF, Khadim M, Rafiq M, Chen J, Yang Y, Wan CC: Pharmacological properties and health benefits of eugenol: A comprehensive review. Oxid Med Cell Longev, 2497354: 1-14. 2021.

Panahi Y, Taghizadeh M, Marzony ET, Sahebkar A: Rosemary oil vs minoxidil 2% for the treatment of androgenetic alopecia: A randomized comparative trial, Skinmed, 13(1): 15-21. 2015.

Pareek A, Pant M, Gupta MM, Kashania P, Ratan Y, Jain V, Aaushi P, Chuturgoon AA: *Moringa oleifera*: An updated comprehensive review of its pharmacological activities, ethnomedicinal, phytopharmaceutical formulation, clinical, phytochemical, and toxicological aspects, Int J Mol Sci, 24(3): 1-40. 2023.

Sasidharan S, Chen Y, Saravanan D, Sundram KM, Latha LY: Extraction, isolation and characterization of bioactive compounds from plants' extracts. Afr J Trad Complement Altern Med, 8(1): 1-10. 2010.

Shafqat N, Azizi A, Mumin NH: Phytochemicals with anti 5-alpha-reductase activity: A prospective for prostate cancer treatment, F1000 Res, 10: 1-18. 2021.