ORIGINAL ARTICLE

Qualitative and quantitative phytochemical screening of hair growth-promoting plants

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Abstract

Aim: The aim of this research work is to perform qualitative and quantitative phytochemical screening of hair growth-promoting plants (*Aloe barbadensis* miller, *Murraya koenigii*, *Bacopa monnieri*, *Annona squamosa*, *Nardostachys jatamansi*, *Nigella sativa*, *Trigonella foenum-graecum*). **Materials and Methods:** First, extraction was done using the Soxhlet extraction technique with two solvents, petroleum ether and methanol. Then, qualitative phytochemical screening of hair growth-promoting plants was done by several phytochemical tests, in which methanolic extracts showed the presence of various active constituents in these plants. After this, a quantitative analysis is performed by determining total phenolic content (TPC). Folin-Ciocalteu method is used to determine phenolic content. 10 mg Gallic acid was dissolved in 10 mL methanol to make a 1 mg/mL solution; various aliquots of 10–50 µg/mL were prepared in methanol. 10 mg of dried extract was dissolved in 10 mL of methanol and filtered. Two mL (1 mg/mL) of this extract was used for the estimation of phenol. 2 mL of each extract and each standard was mixed with 1 mL of Folin–Ciocalteu reagent (previously diluted with distilled water 1:10 v/v) and 1 mL (7.5 g/L) of sodium carbonate. The mixture was vortexed for 15 s and allowed to stand for 10min for color development. The absorbance was measured at 765 nm using a spectrophotometer. **Results and Discussion:** The qualitative and quantitative phytochemical analysis of leaf extracts of *A. barbadensis*, *M. koenigii*, *A. squamosa*, B. monnieri, roots of *N.* jatamansi, and seeds of *T. foenum-graecum* and *N. sativa* shows the presence of various functional chemical constituents such as alkaloids, steroids, tannins, phenolic compounds, flavonoids, and many others. **Conclusion:** All plant extracts have several essential chemical constituents and have good content of phenolic compounds. Hence, these plants could be used to develop herbal hair formulations because these plants are proven to be best for the restoration of hair.

Key words: Alopecia, hair growth, *Murraya koenigii*, phenolic content, phytochemicals

INTRODUCTION

After skin, human hair is an important part of the body, which provides protection and has a clear effect on the general personality of an individual.[1] Alopecia or hair loss is a deplorable disorder for everyone in society. Alopecia condition is associated with a wide variety of causes, the most common ones are alopecia areata, androgenetic alopecia, traction alopecia, and telogen effluvium.[2] There are synthetic and natural ways to prevent hair loss conditions. As compared to the synthetic system of medicines, humans are moving toward alternative systems of medicines such as Ayurveda, Unani, and Siddha because they involve the use of plants, which are safe, have no side effects, and have the potential to meet health care needs.[3]

From different parts (bark, leaves, flowers, roots, and seeds) of a plant, a chemical substance is collected, which is known as phytochemicals. Plants are a brilliant source of an extensive range of compounds such as polyphenols, terpenoids, with amino acids, secondary metabolites, and vitamins. Active constituents of plants have several pharmacological actions, such as antimutagenic, anticarcinogenic, antimicrobial, anti-inflammatory, antitumor, antifungal, hemolytic, diuretic, and antibacterial activities.[4]

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The objective of this research work is to perform qualitative and quantitative phytochemical screening of hair growth-promoting plants (*Aloe barbadensis* miller, *Murraya koenigii*, *Bacopa monnieri*, *Annona squamosa*, *Nardostachys jatamansi*, *Nigella sativa*, *Trigonella foenum-graecum)* as these plants are well known in the prevention of hair loss conditions.

MATERIALS AND METHODS

Leaves, roots, and seeds of *A. barbadensis* Miller, *M. koenigii, B. monnieri, A. squamosa, N. jatamansi*, *N. sativa*, and *T. foenum-graecum* were collected and authenticated.

Preparation of Crude Drug before Extraction

Leaves, roots, and seeds of *A. barbadensis*, *M. koenigii, A. squamosa,* B. monnieri, N. jatamansi, *T. foenum-graecum,* and *N. sativa* were washed with water and dried for a few days. Dried leaves, roots, and seeds were crushed to a coarse powder and stored in a bottle jar till further use, as shown in Figure 1.

Extraction

Previously prepared dried powder of plant material was extracted using the Soxhlet extraction technique, in which extraction is done using two different solvents, namely, petroleum ether and methanol. Coarse powder is loaded into the extraction equipment, then run with solvent until a clear liquid is observed. Collect, filter, and evaporate the liquid extract. Dried extract is collected in an air-tight container.[5]

Phytochemical Screening[6-9]

The extracts of each powdered part of *A. barbadensis* Miller *leaves*, *M. koenigii leaves, N. sativa* seeds*, T. foenum-graecum* seeds, *B. monnieri* leaves, *N. jatamansi* roots, and *A. squamosa* leaves were used for phytochemical tests and to identify the constituents; standard procedures were carried out. An experiment was performed to identify the presence or absence of different phytoconstituents by detailed qualitative phytochemical analysis. Tannins, carbohydrates, alkaloids, terpenoids, glycosides, and steroids were estimated following standard methods.

Figure 1: Dried powder of leaves, roots, and seeds of *Aloe barbadensis*, *Murray koenigii*, *Annona squamosa*, *Bacopa monnieri*, *Nardostachys jatamansi*, *Trigonella foenum-graecum,* and *Nigella sativa*

Tests for Alkaloids

- Dragendroff's test: 1 mL of extract was taken. Alcohol was mixed and shaken well with a few drops of acetic acid and Dragendorff's reagent. The presence of alkaloids is indicated by the presence of an orange-red precipitate
- Mayer's test: 1 mL of extract was dissolved in acetic acid with a few drops of Mayer's reagent added to it. The presence of alkaloids was indicated by the formation of a dull white precipitate
- Wagner's test: In acetic acid, 1 mL of extract was dissolved. Few drops of Wagner's reagent were added. The presence of alkaloids indicated the reddish-brown precipitate
- Hager's test: 1–2 mL of extract was dissolved in acetic acid. To it, 3 mL of Hager's reagent was added; the formation of yellow precipitates indicated the presence of alkaloids.

Test for Glycosides

- Borntrager's test: Dilute sulphuric acid was added to 3 mL of test solution dilute sulfuric acid was added. It was boiled for 5 min, and then the filtrate was obtained. To the cold filtrate, an equal amount of benzene or chloroform was added to the cold filtrate and shaken well. The separation of the organic solvent layer was obtained, and then ammonia was added to it. The presence of anthraquinone glycosides indicated the formation of a pink to red color in the ammonical layer
- Keller Killiani test: 2 mL of test solution added in a test tube, 3 mL of glacial acetic acid, and one drop of 5%ferric chloride. Add, carefully, 0.5 mL of concentrated sulphuric acid. The presence of cardiac glycosides was indicated by the formation of a blue color in the acetic acid layer.

Test for Carbohydrates

- Molisch's test: The aqueous solution of the extract to 1 mL was mixed with a few drops of Molisch reagent (naphthol) and conc. H_2SO_4 (sulphuric acid) was added dropwise along the wall of the test tube.
- It indicates the presence of carbohydrates
- Fehling's test: Equal amounts of Fehling A and Fehling B solutions were mixed (1 mL each), and 2 mL of aqueous solution of extract was added. Boil it for 5–10 min in a water bath. Formation of reddish-brown colored precipitate due to cuprous oxide formation shows the presence of reducing sugar

- Benedict's test: In a test tube, an equal amount of Benedict's reagent and extract was mixed and heated for 5–10 min in a water bath. Depending on the amount of reducing sugar present in the test solution, appears green, yellow, or red, which shows the presence of reducing sugar
- Barfoed's test: In the aqueous solution of the extract, 1 mL of Benedict solution was added and heated for boiling. In the presence of monosaccharides, a red color indication was seen due to the formation of cupric oxide.

Test for Flavonoids

- Shinoda's test: A few magnesium turnings and a few drops of concentrated hydrochloric acid to 1 mL of extract in alcohol were added. It was heated in a water bath. When the formation of red to pink color occurred, it indicated the presence of flavonoids.

Test for Tannin and Phenolic Compounds

- Ferric chloride test: The amount of extract was dissolved in distilled water. Add to it a few drops of a dilute solution of ferric chloride. The formation of dark blue color indicated the presence of tannins
- Gelatin test: The amount of extract was dissolved in distilled water. 2 mL of 1% gelatin solution containing 10% sodium chloride was added. The presence of phenolic Content was indicated by the development of white precipitate.

Test for Saponins

- Froth Test**:** 1 mL of extract was added to distilled water and shaken well. The presence of saponin was indicated by stable froth formation.

Test for Triterpenoids and Steroids

- Salkowski test: The extract was dissolved in chloroform, and an equal volume of concentrated sulphuric acid was added. The presence of steroids was indicated by the formation of a bluish red to cherry red color in the chloroform layer and green fluorescence in the acid layer.

Quantitative Studies of Phytoconstituents

Estimation of total phenol content

The total phenolic content of each extract was determined by the modified Folin–Ciocalteu method. 10 mg Gallic acid was dissolved in 10 mL methanol to make a 1 mg/mL solution; various aliquots of 10–50 µg/mL were prepared in methanol. 10 mg of dried extract was dissolved in 10 mL of methanol and filtered. 2 mL (1 mg/mL) of this extract was used for the estimation of phenol. 2 mL of each extracts and each standard was mixed with 1 mL of Folin–Ciocalteu reagent (previously diluted with distilled water 1:10 v/v) and 1 mL (7.5 g/L) of sodium carbonate. The mixture was vortexed for 15 s and allowed to stand for 10 min for colour development. The absorbance was measured at 765 nm using a spectrophotometer.[10]

RESULTS AND DISCUSSION

Calculation of Percentage Yield

Percentage yield was determined by the following formula:[11] % Yield = practical yield × 100/theoretical yield. The percentage yield is shown in Tables 1-7.

Table 1: Percentage yield of *Aloe barbadensis* miller

S. No.	Extract	% Yield (w/w)
1.	Methanol	13.50
2.	Pt. ether	12.10

Table 2: Percentage yield of *Murraya koenigii*

S. No.	Extract	% Yield (w/w)
1.	Methanol	4.57%
2.	Pt. ether	1.09%

Table 3: Percentage yield of *Nigella sativa*

S. No.	Extract	% Yield (w/w)
1.	Methanol	9.07%
2.	Pt. ether	3.51%

Table 4: Percentage yield of *Trigonella foenum-graecum*

S. No.	Extract	% Yield (w/w) (%)
1.	Methanol	16.52
2.	Pt. ether	14.12

Table 5: Percentage yield of *Bacopa monnieri*

S. No.	Extract	% Yield (w/w)
1.	Methanol	6.59%
2.	Pt. ether	3.19%

Table 6: Percentage yield of *Nardostachys jatamansi*

S. No.	Extract	% Yield (w/w)
1.	Methanol	7.05%
2.	Pt. ether	5.53%

Table 7: Percentage yield of *Annona squamosa*

S. No.	Extract	% Yield (w/w)
1.	Methanol	9.05%
2.	Pt. ether	%

Table 8: Phytochemical testing of *Aloe barbadensis* Miller leaves extract

S. No.	Experiment	Presence or absence of phytochemical test	
		Pet. Ether extract	Methanolic extract
1.	Alkaloids		
1.1	Dragendroff's test	+	+
1.2	Mayer's reagent test	+	+
1.3	Wagner's reagent test	+	+
1.3	Hager's reagent test	+	+
2.	Glycoside		
2.1	Borntrager test	+	+
2.2	Keller–Killiani test	+	+
3.	Carbohydrates		
3.1	Molisch's test	-	+
3.2	Fehling's test	-	+
3.3	Benedict's test	-	+
3.4	Barfoed's test	-	+
5.	Flavonoids		
5.1	Shinoda's test	-	+
6.	Tannin and phenolic compounds		
6.1	Ferric chloride test	-	-
+6.2	Gelatin test	-	-
7.	Saponin		
7.1	Froth test	+	+
8.	Test for triterpenoids and steroids		
8.1	Salkowski's test	+	+

Preliminary Phytochemical Screening

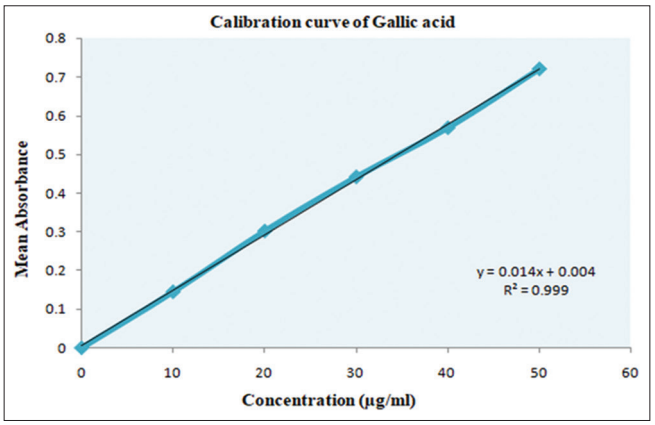
Qualitative

In the case of *A. barbadensis* Miller, *M. koenigii*, *N. sativa*, *T. foenum-graecum*, *B. monnieri*, *N. jatamansi*, and *A. squamosa* extracts described by Kokate, a series of qualitative chemical tests were conducted.The Qualitative phytochemical analysis of leaf extracts of *Aloe barbadensis, Murray koenigii, Annona squamosa, Bacopamonnieri*, roots of *Nardostachys jatamansi*, and seeds of *Trigonellafoenum-graecum* and *Nigella sativa* shows the presence of various functional chemical constituents which is beneficial for hair growth, as shown in Tables 8-14.

Qualitative phytochemical screening of the leaf extracts of *A. barbadensis* Miller revealed that alkaloids, glycosides, carbohydrates, flavonoids, phenols, saponins, terpenoids, and steroids were present in methanolic extracts.

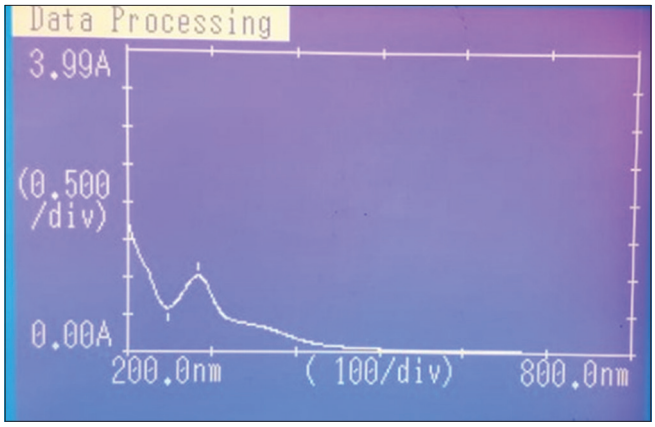
Table 9: Phytochemical testing of *Murraya koenigii* leaf extracts

S. No.	Experiment	Presence or absence of phytochemical test	
		Pet. Ether extract	Methanolic extract
1.	Alkaloids		
1.1	Dragendroff's test	-	+
1.2	Mayer's reagent test	-	+
1.3	Wagner's reagent test	-	+
1.3	Hager's reagent test	-	+
2.	Glycoside		
2.1	Borntrager test	-	+
2.2	Keller–Killiani test	-	+
3.	Carbohydrates		
3.1	Molisch's test	-	+
3.2	Fehling's test	-	+
3.3	Benedict's test	-	+
3.4	Barfoed's test	-	+
5.	Flavonoids		
5.1	Shinoda's Test	-	+
6.	Tannin and phenolic compounds		
6.1	Ferric chloride test	-	+
+6.2	Gelatin test	-	+
7.	Saponin		
7.1	Froth test	-	+
8.	Test for triterpenoids and Steroids		
8.1	Salkowski's test	+	+

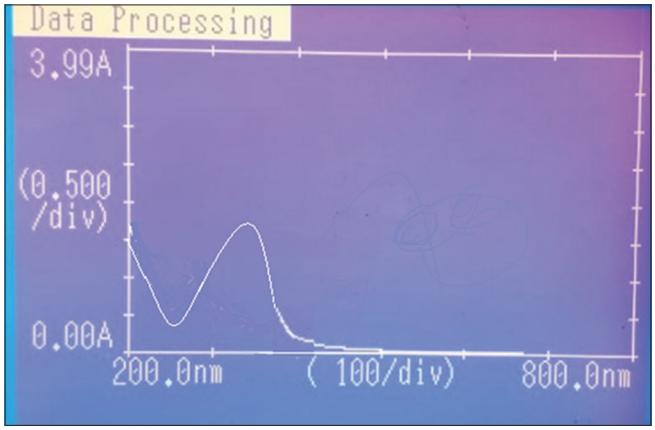


Graph 1: Graph of the calibration curve of gallic acid

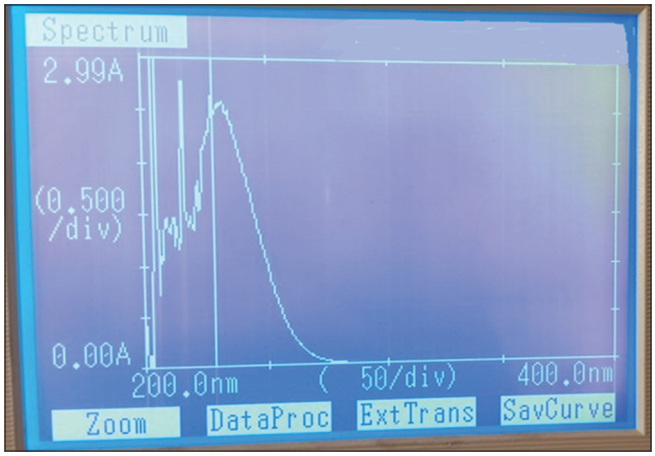
Qualitative phytochemical screening of the leaf extracts of *M. koenigii* revealed that alkaloids, glycosides, carbohydrates, flavonoids, phenols, saponins, terpenoids, and steroids were present in methanolic extracts.



Graph 2: Ultraviolet graph of gallic acid



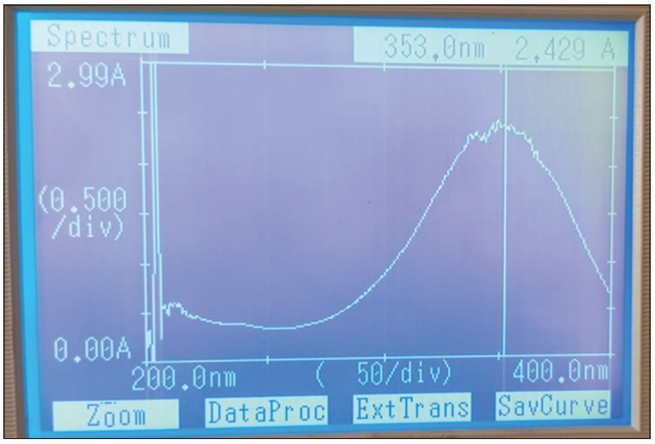
Graph 3: Ultraviolet graph of *Aloe barbadensis* Miller



Graph 4: Ultraviolet graph of *Murraya koenigii*

Qualitative phytochemical screening of the seed extracts of *N. sativa* revealed that alkaloids, glycosides, carbohydrates, flavonoids, phenols, terpenoids, and steroids were present in methanolic extracts.

Qualitative phytochemical screening of the seed extracts of *T. foenum-graecum* revealed that alkaloids, glycosides, carbohydrates, flavonoids, phenols, terpenoids, and steroids were present in methanolic extracts.



Graph 5: Ultraviolet graph of *Nigella sativa*

Table 10: Phytochemical testing of *Nigella sativa* seed extracts

S. No.	Experiment	Presence or absence of phytochemical test	
		Pet. Ether extract	Methanolic extract
1.	Alkaloids		
1.1	Dragendroff's test	+	+
1.2	Mayer's reagent test	+	+
1.3	Wagner's reagent test	+	+
1.3	Hager's reagent test	+	+
2.	Glycoside		
2.1	Borntrager test	-	+
2.2	Keller–Killiani test	-	+
3.	Carbohydrates		
3.1	Molisch's test	-	+
3.2	Fehling's test	-	+
3.3	Benedict's test	-	+
3.4	Barfoed's test	-	+
5.	Flavonoids		
5.1	Shinoda's test	-	+
6.	Tannin and phenolic compounds		
6.1	Ferric chloride test	-	+
+6.2	Gelatin test	-	+
7.	Saponin		
7.1	Froth test	-	-
8.	Test for triterpenoids and steroids		
8.1	Salkowski's test	+	+

Qualitative phytochemical screening of the leaf extracts of *B. monnieri* revealed that alkaloids, glycosides, carbohydrates, flavonoids, phenols, terpenoids, and steroids were present in methanolic extracts.

Qualitative phytochemical screening of the leaf extracts of *N. jatamansi* revealed that alkaloids, glycosides,

Table 11: Phytochemical testing of *Trigonella foenum-graecum* seed extracts

S. No.	Experiment	Presence or absence of phytochemical test	
		Pet. Ether extract	Methanolic extract
1.	Alkaloids		
1.1	Dragendroff's test	-	+
1.2	Mayer's reagent test	-	+
1.3	Wagner's reagent test	-	+
1.3	Hager's reagent test	-	+
2.	Glycoside		
2.1	Borntrager test	-	+
2.2	Keller–Killiani test	-	+
3.	Carbohydrates		
3.1	Molisch's test	-	+
3.2	Fehling's test	-	+
3.3	Benedict's test	-	+
3.4	Barfoed's test	-	+
5.	Flavonoids		
5.1	Shinoda's test	-	+
6.	Tannin and phenolic compounds		
6.1	Ferric chloride test	+	+
+6.2	Gelatin test	+	+
7.	Saponin		
7.1	Froth test	-	-
8.	Test for triterpenoids and steroids		
8.1	Salkowski's test	+	+

Table 12: Phytochemical testing of *Bacopa monnieri* leaf extracts

S. No.	Experiment	Presence or absence of phytochemical test	
		Pet. Ether extract	Methanolic extract
1.	Alkaloids		
1.1	Dragendroff's test	-	+
1.2	Mayer's reagent test	-	+
1.3	Wagner's reagent test	-	+
1.3	Hager's reagent test	-	+
2.	Glycoside		
2.1	Borntrager test	-	+
2.2	Keller–Killiani test	-	+
3.	Carbohydrates		
3.1	Molisch's test	-	+
3.2	Fehling's test	-	+
3.3	Benedict's test	-	+
3.4	Barfoed's test	-	+
5.	Flavonoids		
5.1	Shinoda's test	-	+
6.	Tannin and phenolic compounds		
6.1	Ferric chloride test	-	+
+6.2	Gelatin test	-	+
7.	Saponin		
7.1	Froth test	-	-
8.	Test for triterpenoids and steroids		
8.1	Salkowski's test	-	+

carbohydrates, flavonoids, phenols, terpenoids, and steroids were present in methanolic extracts.

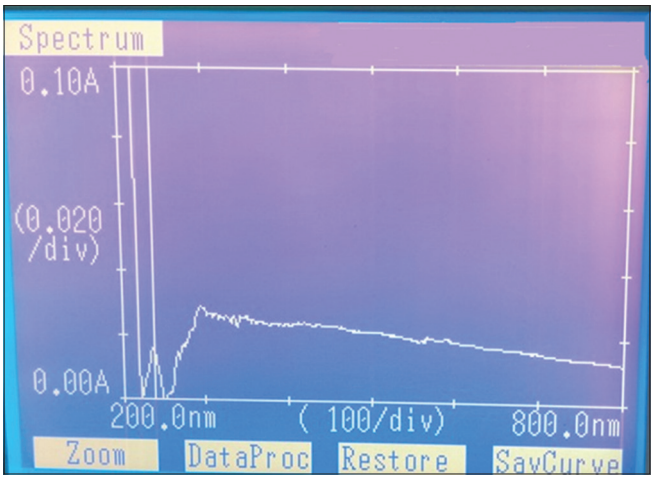
Qualitative phytochemical screening of the leaf extracts of *A. squamosa* revealed that alkaloids, glycosides, carbohydrates, flavonoids, phenols, terpenoids, and steroids were present in methanolic extracts.

Quantitative analysis

The quantitative analysis conducted in this report reveals that the extracts exhibit a notable concentration of phenols.

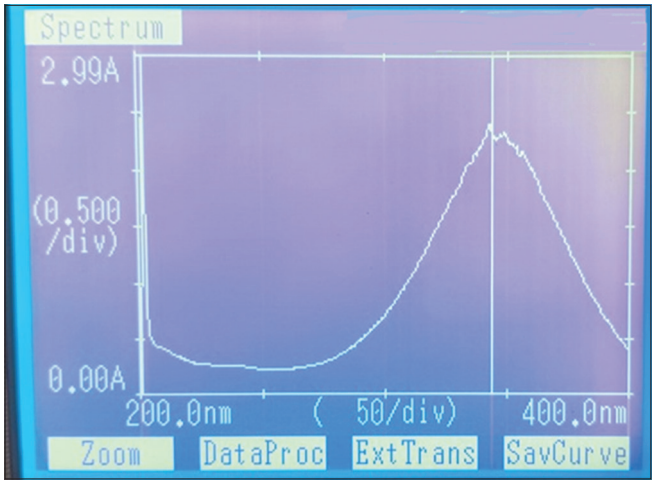
Determination of total phenolic content (TPC)

The primary contributors to the antioxidant and medicinal properties of plants are phenolics. In this study, the Folin–Ciocalteu assay was employed to determine the total phenolic content. Phenolic compounds found in plant extracts play a crucial role in their overall structure. These compounds consist of aromatic rings with one or multiple hydroxyl groups, enabling them to effectively neutralize free radicals by forming stable phenoxyl radicals through resonance stabilization. The quantification of total phenolics was achieved through the electron transfer process from



Graph 6: Ultraviolet graph of *Trigonella foenum-graecum*

phenolic compounds to the Folin–Ciocalteu reagent under alkaline conditions. This method has been identified as a straightforward and efficient approach due to its simplicity and rapidity.



Graph 7: Ultraviolet graph of *Bacopa monnieri*

Table 13: Phytochemical testing of *Nardostachys jatamansi* root extracts

S. No.	Experiment	Presence or absence of phytochemical test	
		Pet. Ether extract	Methanolic extract
1.	Alkaloids		
1.1	Dragendroff's test	-	+
1.2	Mayer's reagent test	-	+
1.3	Wagner's reagent test	-	+
1.3	Hager's reagent test	-	+
2.	Glycoside		
2.1	Borntrager test	+	+
2.2	Keller–Killiani test	+	+
3.	Carbohydrates		
3.1	Molisch's test	-	+
3.2	Fehling's test	-	+
3.3	Benedict's test	-	+
3.4	Barfoed's test	-	+
5.	Flavonoids		
5.1	Shinoda's test	-	+
6.	Tannin and phenolic compounds		
6.1	Ferric chloride test	-	+
+6.2	Gelatin test	-	+
7.	Saponin		
7.1	Froth test	-	-
8.	Test for triterpenoids and steroids		
8.1	Salkowski's test	+	+

Estimation of Total Phenolic Content (TPC)

Total phenolic compounds (TPC) were expressed as mg/100 mg of gallic acid equivalent (GAE) of dry extract sample using the equation obtained from the calibration curve: $y = 0.014 \times + 0.004$, $R^2 = 0.999$, where X is the GAE and Y is the absorbance. [Table 15, Graphs 1 and 2] and total phenolic content of hair growth-promoting plants are shown in Table 16 and Graphs 3-9.

Table 14: Phytochemical testing of *Annona squamosa* leaf extracts

S. No.	Experiment	Presence or absence of phytochemical test	
		Pet. Ether extract	Methanolic extract
1.	Alkaloids		
1.1	Dragendroff's test	-	+
1.2	Mayer's reagent test	-	+
1.3	Wagner's reagent test	-	+
1.3	Hager's reagent test	-	+
2.	Glycoside		
2.1	Borntrager test	-	+
2.2	Keller–Killiani test	-	+
3.	Carbohydrates		
3.1	Molisch's test	-	+
3.2	Fehling's test	-	+
3.3	Benedict's test	-	+
3.4	Barfoed's test	-	+
5.	Flavonoids		
5.1	Shinoda's Test	-	+
6.	Tannin and phenolic compounds		
6.1	Ferric chloride test	-	+
+6.2	Gelatin test	-	+
7.	Saponin		
7.1	Froth test	-	-
8.	Test for triterpenoids and steroids		
8.1	Salkowski's test	+	+

Table 15: Preparation of the calibration curve of Gallic acid

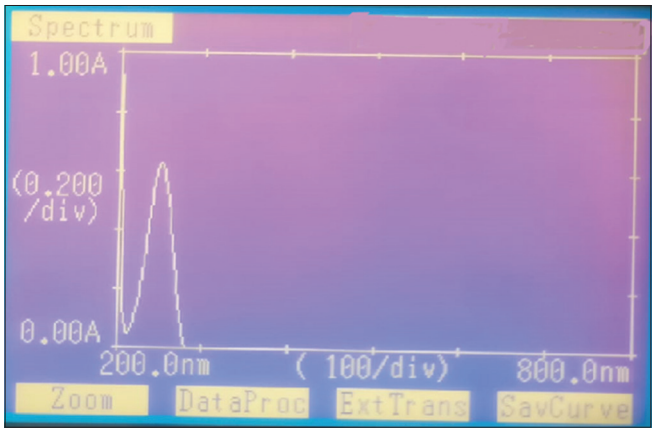
S. No.	Concentration (µg/mL)	Mean absorbance
1	10	0.145
2	20	0.302
3	30	0.442
4	40	0.569
5	50	0.721

(*n*=3, Mean±SD)

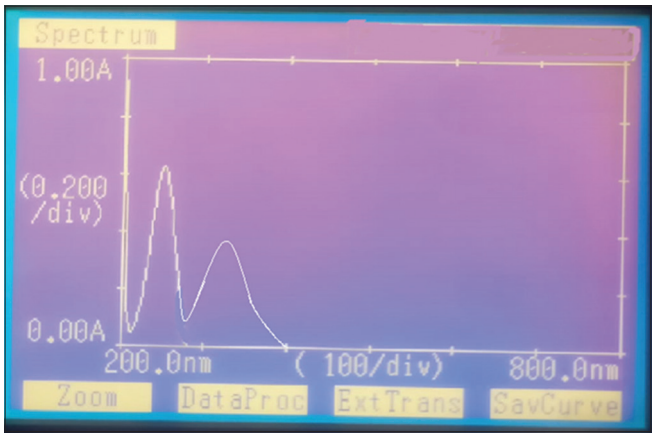
Table 16: Estimation of total phenolic compounds of different plants

S. No.	Plant	Extract	Total phenol content
1.	*Aloe barbadensis* Miller	Methanolic extract	23.73 GAE/g
2.	*Murraya koenigii*	Methanolic extract	97.67 GAE/g
3.	*Nigella sativa*	Methanolic extract	112.64 GAE/g
4.	*Trigonella foenum-graecum* seed	Methanolic extract	40.64 GAE/g
5.	*Bacopa monnieri*	Methanolic extract	9.58 GAE/g
6.	*Nardostachys jatamansi*	Methanolic extract	45.94 GAE/g
7.	*Annona squamosa*	Methanolic extract	21.58 GAE/g

GAE: Gallic acid equivalent



Graph 8: Ultraviolet graph of *Nardostachys jatamansi*



Graph 9: Ultraviolet graph of *Annona squamosa*

Calibration Curve of Gallic acid

Gallic acid (Standard) curve is constructed by preparing dilution of (10, 20,30,40,50 ug/ml) in methanol and absorbance was checked at 760 nm spectrometrically. Table 15 shows the mean absorbance of various concentrations of gallic acid and Graph 1 shows the standard gallic acid curve.

CONCLUSION

By conducting qualitative and quantitative phytochemical screening of hair growth-promoting plants using phytochemical tests and the total phenolic determination method. It was concluded that phytochemical screening of the methanolic extract shows the presence of various active constituents as compared to the petroleum ether extract of the plants. Methanolic extract of plants is further studied for phenolic content, in which plants have phenolic content such as *A. barbadensis* miller (23.75 GAE/g), *M. koenigii* (97.67 GAE/g), *N. sativa* (112.64 GAE/g), *T. foenum-graecum* (40.64GAE/g), *B. monnieri* (9.58GAE/g), *N. jatamansi* (45.94GAE/g), *A. squamosa* (21.58 GAE/g). Thus, it can be expected that these plants may be used to develop an herbal hair formulation to reduce hair loss and baldness.

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